Case Studies: Effects of Beef, Whey and Carbohydrate Supplementation in Female Master Triathletes

Kosha J. Mehta[1], Marco Seijo[2], Eneko Larumbe-Zabala[3], Nadia Ashrafi[2], Tatiana Christides[2*], Bettina Karsten[2], Birthe V. Nielsen[2] and Fernando Naclerio[2]

1. School of Population Health & Environmental Sciences, Faculty of Life Sciences & Medicine, King's College London, UK
2. Department of Life and Sport Sciences, Faculty of Engineering and Science, University of Greenwich, **UK**
3. Clinical Research Institute, Texas Tech University Health Sciences Center, Lubbock, TX, USA

***Corresponding Author**

Fernando Naclerio, Department of Life and Sport Sciences, Faculty of Engineering and Science, University of Greenwich, **UK. Email:** f.j.naclerio@gre.ac.uk **Phone: +44(0)208331 8441**

Accepted for publication at the Journal of Human Sport & Exercise on 21st June 2018

Short title: Nutrient supplementation in endurance female athletes

Abstract

Appropriate nutritional supplementation is crucial for athletic performance, particularly for female endurance athletes as their numbers steadily increase. This report involves a set of six case studies examining the effects of ingesting a post-workout supplement containing beef, or whey or carbohydrate on iron status, blood indices, muscular thickness, peak oxygen consumption (VO_2 max) and body composition in six female masters-age (> 35 years old) triathletes. Over a 10-week training period, a 20 g supplement was ingested immediately post workout or during breakfast on the non-training days. Of the six analyzed cases, two ingested protein powder from beef, two consumed whey, and two consumed maltodextrin. Data showed that concomitant with increased dietary iron ingestion, levels of the iron-storage protein ferritin increased in beef-consumers (by 56% and 74 %) and carbohydrate-consumers (by 71% and 27 %), but decreased in whey-consumers (by 55% and 36%). Contrastingly, the effect on transferrin levels was highly variable between participants in each supplementation case. The whey-consumers showed reduced RBC count (by 6%), hematocrit (by 8%) and red blood cell distribution width (by 14% and 5%). While one beef-consumer showed a remarkable 34% increase in platelets, the whey and carbohydrate-consumers showed reduced platelets, but increased neutrophil:lymphocyte ratio. Vastus medialis thickness reduced in carbohydrate-consumers (by 6% and 5%), unlike the beef and whey-consumers. Females consuming beef increased iron stores and platelets, while those ingesting whey were unable to maintain specific RBC indices. Only the four athletes ingesting protein-containing supplements were able to maintain muscle thickness, thereby averting muscle loss.

Key words: nutrition, iron, athletic performance

Introduction

Nutrients such as iron, proteins and carbohydrates promote metabolic adaptations that delay the onset of fatigue (Alaunyte, Stojceska, & Plunkett, 2015; Aoi, Naito, & Yoshikawa, 2006; Beck, Thomson, Swift, & von Hurst, 2015; Lambert, Hawley, Goedecke, Noakes, & Dennis, 1997). Therefore, their dietary inclusion and additional supplementation is pivotal for resilience and enhancing performance in athletes. In the female athletes, maintenance of iron levels is often challenging, partly due to menstruation, gastrointestinal bleeding, sweating, hemolysis and footstrike. While non-heme iron from plant-based sources has poor bioavailability due to the inhibitory effects of phenols and phytates in the plant-based foods, heme-iron from animal sources is highly bioavailable, and therefore would be the preferred first line of action to prevent iron deficiency in athletes (Alaunyte et al., 2015; Sharp & Srai, 2007). In addition to iron, increased intake of high-quality protein is important for muscle health as the significance of meat proteins in countering age-related muscle loss is well-recognized (Phillips, 2012). Due to age-related anabolic resistance, older athletes (>40 years old) demonstrate impairments in protein remodeling in skeletal muscle (Doering, Reaburn, Phillips, & Jenkins, 2016). Thus, the consumption of post-workout high quality BCAA-rich protein supplements can enhance protein synthesis and repair in muscles (Stark, Lukaszuk, Prawitz, & Salacinski, 2012). Similarly, the significance of carbohydrates in sustaining muscle energy reserves and replenishing glycogen stores during and after training has been established (Hawley & Leckey, 2015). Such recovery practices can positively influence the outcomes of subsequent training sessions, while avoiding training-induced nutritional deficiencies and muscle loss (Naclerio, Larumbe-Zabala, Cooper, Jimenez, & Goss-Sampson, 2014). Supplementation case studies are therefore required to better understand the impact of different nutritional strategies to help maximize athletic performance.

In this set of novel case studies, the post-workout effects of ingesting beef or whey protein extracts or carbohydrates (CHO) were examined in female endurance athletes during a 10-week endurance-training program, which has not been reported before. The supplements were protein-rich and heme-iron-rich beef, protein-rich and low-iron whey, and non-protein and non-iron carbohydrate. Pre and post supplementation observations of systemic levels of the iron-storage and iron-transport proteins, ferritin and transferrin, respectively, blood indices, muscle thickness and maximal aerobic power were noted.

Materials and Methods

Participants

Female triathletes aged 40-55 years were recruited. They had consistently trained between 6-10 hours per week for the last 3 years. These athletes had no musculoskeletal limitations or metabolic conditions and agreed to refrain from other supplements and non-prescription medications that may affect the parameters examined in this study. All experimental procedures were conducted in accordance with the Declaration of Helsinki, and approved by the Research Ethics Committee. Trial Registration: ClinicalTrials.gov, U.S. National Institutes of Health (Identifier: NCT02675348). The participants provided written permission for publication of the case study after having read the paper.

Experimental design and nutritional supplementation

After the preliminary assessments two participants were randomly allocated into the following three treatment conditions: beef protein, whey protein or carbohydrate. Each athlete consumed a 20 g sachet of powder of the allocated supplement mixed with ~300 mL plain water once a day. Beef hydrolyzed protein powder is a protein-rich and heme-iron-rich commercially available supplement (100% All Beef, Crown® Sport Nutrition, Spain), Whey isolate (Isolac, Carbery) is a protein-

replete non-iron supplement containing higher concentrations of BCAAs, which are essential for supporting muscle protein synthesis following intense exercise (Naclerio & Larumbe-Zabala, 2016). Compliance with supplement intake (determined by individual follow-up) was evaluated continuously during the supplementation. For dietary monitoring, a qualified nutritionist collected the information on the dietary habits of the participants and explained the correct procedures for recording dietary intake. Several parameters were assessed before and after a 10-week endurance-training period (referred to as pre and post supplementations).

Hematological and iron-related measurements

To examine hematological and iron-related parameters, blood was collected one day before and one day after completion of the supplementation period to assess red blood cell (RBC) concentration (10^6/mm^3), hemoglobin (HGB) concentration (g/dl), hematocrit (%) (HCT), mean corpuscular volume (MVC) (mm^3), mean corpuscular hemoglobin mass (MCH) (pg), mean corpuscular hemoglobin concentration (MCHC) (g/dl) and red cell distribution width (RDW) (%) and platelets (10^3/mm^3) using a fully automated hematology analyzer (ABX Pentra 60C+, Horiba Medical, Montpellier, France). Ferritin (ng/mL) and transferrin (µg/mL) levels were analyzed using ELISA, as per manufacturer's instructions (Abcam, UK).

Measurement of muscle thickness

Right-side vastus medialis muscle thicknesses were measured using a Diasus diagnostic ultrasound imaging unit (Dynamic Imaging, Livingston, UK) (Forrester, 2014). Thickness was calculated as the distance between superficial and deep aponeuroses measured at the ends and middle region of each 3.8 cm-wide sonograph. Data were considered for descriptive analyses only if the effect was the same (increase or decrease) in both participants and if the alterations were 4% and above in both participants.

Determination of Peak Oxygen Consumption (VO_2 max):

Following a standardized warm-up, participants completed a maximal incremental laboratory exercise test to exhaustion on a Cyclus2 ergometer (RBM Electronics, Leipzig, Germany). The test commenced at a work rate of 90 W. Thereafter, intensity increased at a step rate of 25Watts every minute. Participants were instructed to maintain a cadence between 70 and 80 rev/min throughout the test. When cadence dropped by more than 10 rev min^{-1} for more than 10 s despite strong verbal encouragement, tests were terminated. Expired gases were collected continuously during the test using a Cortex MetaLyzer 3B gas analyzer (Cortex Biophysik, Leipzig, Germany). Additionally, heart rate (HR) was continuously monitored using a Polar Sporttester (Polar Electro, Finland). VO_2 max as calculated as the highest mean oxygen consumption over a 30-s period (Karsten, Jobson, Hopker, Stevens, & Beedie, 2015).

Analysis of dependent variables

While each case was studied separately, in reporting results from a particular supplement (beef, whey or maltodextrin), data were considered for analysis only if the effect was the same (increase or decrease) in both participants and if the alterations were 4% and above in both participants.

Results

Dietary iron, ferritin and transferrin

Table 1 shows the daily consumption of carbohydrate, protein, fat, energy and iron before and during the study. Pre-supplementation, all the athletes consumed similar levels of proteins, carbohydrates, fats and iron, except athlete 4 who ingested the lowest amount of total iron (7 $mg \cdot d^{-1}$). However, post-supplementation analyses showed variability in ingestion of these nutrients between participants consuming the same supplement. For example, while the beef and

carbohydrate-consumers showed elevated total iron and protein ingestion, the whey-consumers showed decreased dietary iron and protein ingestion. The post-supplementation dietary carbohydrate ingestion reduced in all the participants.

Table 1. Diet composition of the participants.

Participant and condition	Total Iron ($mg \cdot d^{-1}$)		Non-heme Iron ($mg \cdot d^{-1}$)		Heme-Iron ($mg \cdot d^{-1}$)		Proteins ($g \cdot kg^{-1} \cdot d^{-1}$)		Carbohydrate ($g \cdot kg^{-1} \cdot d^{-1}$)		Fats ($g \cdot kg^{-1} \cdot d^{-1}$)		Energy ($kcal \cdot kg^{-1} \cdot d^{-1}$)	
	Pre	**Post**	**Pre**	**Post**	**Pre**	**Post**	**Pre**	**Post**	**Pre**	**Post**	**Pre**	**Post**	**Pre**	**Post**
1 (Beef)	10.93	13.39	7.53	6.89	3.40	6.50	1.30	1.45	3.5	2.68	1.05	1.01	28.21	26.41
2 (Beef)	13.34	18.9	10.01	10.89	3.33	8.01	1.25	2.03	4.01	3.77	0.9	2.21	27.33	41.10
3 (Whey)	11.69	10.35	8.28	7.34	3.41	3.01	1.31	1.12	3.9	2.67	0.7	1.17	26.50	26.07
4 (Whey)	9.96	9.56	6.95	6.79	3.01	2.77	1.32	1.52	4.1	3.96	1.2	1.25	30.21	33.72
5(Carbohydrate)	7.20	16.01	5.76	7.96	1.44	8.05	1.25	2.1	4.01	3.6	0.75	1.12	28.50	33.45
6(Carbohydrate)	11.48	19.43	8.5	8.56	2.98	8.56	1.28	2.14	4.1	3.7	1.12	1.23	31.62	35.01

The table shows the pre and post supplementation dietary intake of iron, proteins, carbohydrates and fats in all the participants.

The levels of dietary iron ingestion increased by 23% and 42 % in the beef consumers (Table 1). This was topped with heme-iron from beef supplementation and their ferritin (iron storage protein) levels markedly increased by 56% and 74 % (Fig 1A). Similarly, as the levels of dietary iron ingestion (from meat sources) remarkably increased in the carbohydrate-consumers by 178% and 118% (Table 1), ferritin levels were elevated by 71% and 27 % (Fig 1C). In contrast, whey-consumers showed decreased ferritin levels by 55% and 36%, as the levels of dietary iron dropped by 11% and 4% (Fig 1B and Table 1). Transferrin (iron transport protein) levels consistently increased (37% and 8%) in carbohydrate-consumers (Fig 1F), unlike the beef and whey-consumers (Figs 1D and 1E).

Specifically, pre-supplementation, athlete 2 (beef-consumer) showed a high iron intake of 13 $mg \cdot d^{-1}$, while athlete 5 (carbohydrate-consumer) ingested only 7 $mg \cdot d^{-1}$, which was 46% lower than athlete 2 (Table 1). However, their pre-supplementation ferritin levels were similar (Figs 1A and 1C). Moreover, during the training period, athlete 2 further increased iron intake via the allocated

beef supplement (with approximately 16 mg iron) and consumed approximately 29 $mg \cdot d^{-1}$ that resulted in the expectedly increased ferritin levels post-supplementation (Fig 1A). However, in athlete 5, despite the initial low iron intake and consumption of the iron-deprived carbohydrate supplement, the increased ingestion of meat during the training period increased her dietary iron intake similar to athlete 2 (Table 1), leading to similar ferritin levels post-supplementation (Figs 1A and 1C).

RBCs and blood indices

Before and after the supplementation , most athletes presented values within the normal reference ranges (Camaschella, 2015; Wakeman et al., 2007) for RBC count, HGB, HCT, MCV, MHC, MCHC and RDW (data not shown for brevity). However, pre-supplementation, athlete 2 showed HGB at 11.5 g/dL and HCT at 32.4%, athlete 6 with HCT at 33.7% and athletes 1, 4, 5 and 6 showed RDW of 10.8%, 9.9%, 11% and 9.6 %, respectively; the values being slightly lower than the lower end of the reference range.

Following the supplementation, athletes 1, 2, 3 and 4 showed lower HCT of 35.8%, 32.4%, 35.7% and 33.7%, respectively. Likewise, the RDW was slightly below the reference range for all the athletes (8.8% to 10.8%). Beef and carbohydrate-consumers did not show any consistent alterations in RBC count (Figs 2A and 2C), HGB, HCT, MCV, MHC, MCHC and RDW, except the 4% decreases in HCT in carbohydrate-consumers (athlete 5: 40.9% to 39.2% and athlete 6: 33.7% to 32.2%). In contrast, both the whey-consumers showed consistent 6% decreases in RBC count (Fig 2B) (athletes 3 and 4: 4.3 to 4 x $10^6/mm^3$), 8% decreases in HCT (athlete 3: 38.5% to 35.5% and athlete 4: 36.5% to 33.7%) (Fig 3A), and 14% and 5% decreases in RDW (athlete 3: 11.8% to 10.1% and athlete 4: 9.9% to 9.4%) (Fig 3B).

Platelets

The six participants showed normal ranges of platelets at both pre (162 to 295 $x10^3/mm^3$) and post (155 to 318 x $10^3/mm^3$) supplementation. Beef consumption increased platelets by 4% (295 to 308 x $10^3/mm^3$) in athlete 1 and by 36 % (238 to 318 $x10^3/mm^3$) in athlete 2. However, platelets decreased in whey-consumers [by 14 % (292 to 250 $10^3/mm^3$) in athlete 3 and by 4% (152 to 165 x $10^3/mm^3$) in athlete 4] and in carbohydrate-consumers (by 15% (247 to 209 x $10^3/mm^3$) in athlete 5 and 11% (291 to 259 x $10^3/mm^3$) in athlete 6] (Fig 4).

WBCs

In the beef-consumers, total WBC count markedly decreased by 20% (athlete 1: 8.2 x $10^3/mm^3$to 6.6 x $10^3/mm^3$ and athlete 2: 6.6 x $10^3/mm^3$to 5.3 $x10^3/mm^3$), with particularly decreased lymphocytes levels (7% and 20%) (athlete 1: 28.3% to 26.4% and athlete 2: 28.1%to 22.6%). However, an 11% and 50% increases in monocytes was observed (athlete 1: 5.4% to 6% and athlete 2: 6.6% to 9.9%). The normal neutrophil: lymphocyte ratio is between 0.78 and 3.53 (Forget et al., 2017) and all the athletes presented the ratio within this range (data not shown for brevity). Interestingly, the neutrophil:lymphocyte ratio increased in beef and whey consumers, unlike the carbohydrate-consumers (Fig. 4)

Body composition, muscle thickness and oxygen consumption

The beef and whey-consumers did not show altered vastus medialis thickness (Figs 6A and 6B), whereas the carbohydrate-consumers showed decreased vastus medialis thickness (6% and 5%) (Fig 6C). Athlete 1, the beef-consuming participant showed 11 % increase in VO_2 max (47 to 52 $ml{\cdot}kg^{-1}$), whereas the others showed no consistent changes. No consistent alterations were observed in athletes of the three treatment conditions for body composition (body weight, body mass index, fat

mass, fat-free mass). Relevant parameters examined in all participants and the corresponding observations have been summarized in Table 2.

Table 2 Summary of parameters and effects in the six participants

Participants	Supplement consumed	Ferritin	Transferrin	RBC	HGB	HCT	RDW	Platelets	Neutrophil/ lymphocyte ratio	VM thickness	V02 max
1 (Beef)	Beef	56% increase	15% decrease	13% decrease	10% decrease	14% decrease	No change	4% increase	6% increase	4% increase	11% increase
2 (Beef)	Beef	75 % increase	62% increase	2% increase	2% increase	No change	21% decrease	34% increase	29% increase	1% increase	7 % decrease
3 (Whey)	Whey	55 % decrease	7% decrease	6% decrease	1% decrease	8% decrease	14% decrease	14% decrease	11% increase	No change	No change
4 (Whey)	Whey	36% decrease	104% increase	6% decrease	8% decrease	8% decrease	5% decrease	4% decrease	73% increase	4% decrease	6% decrease
5 (Carbohydrates)	CHO	71% increase	37% increase	1% decrease	3% increase	4% decrease	20% decrease	15% decrease	2% decrease	6% decrease	No change
6 (Carbohydrates)	CHO	27% increase	8 % increase	3% decrease	6% decrease	4% decrease	2% decrease	11% decrease	17% decrease	5% decrease	10% decrease

Legend: RBC: CHO: carbohydrate, Red Blood Cell (count), HGB: hemoglobin, HCT: hematocrit, RDW: Red cell distribution width, VM: vastus medialis.

Discussion

The participants ingesting the heme-iron-rich beef supplement markedly increased ferritin levels (Fig 1A). Thus, an additional intake of iron in the form of hydrolyzed beef powder increased iron stores over 10 weeks of training in the two analyzed female triathletes. We expected concomitant elevations in hematological indices (that represent iron utilization) such as RBC count and HGB because exercise training can increase RBC and HGB due to elevated erythropoietin levels (Hu & Lin, 2012). Overall, the two beef-consumers did not show consistent alterations in these indices (Fig 2A). This could be partly because the normal turnover of RBCs is 120 days (Clark, 1988) whereas the post-supplementation analysis was conducted after 10 weeks (70 days). Thus, the duration of our study may have provided an early window to observe notable increments in these specific RBC indices. In contrast, decreased ferritin in the two whey-consumers (Fig 1B) was accompanied by a reduced RBC count (Fig 2B), HCT and RDW (Fig 3). This implies that in these two females, increased iron stores did not cause the expected elevation in RBC count and HGB within 70 days. However, decreased iron stores certainly had a negative impact, as the RBC indices

were not maintained in the absence of supplemental iron, thereby demonstrating the significance of iron in maintaining RBC indices. This could be due to the regulatory mechanisms that control complex iron signaling and utilization pathways, involving hormones such as erythropoietin that is the main regulator of RBC production (Adamson, 1994). Measurement of erythropoietin levels in these athletes would have explained the reason for these observations. Despite the remarkable increase in dietary iron from the habitual diet in the two carbohydrate-consumers, particularly when compared to the beef-consumers, their serum ferritin levels were similar (Figs 1A and 1C). This highlights the regulatory mechanisms that govern iron absorption from the duodenal enterocytes to prevent both, iron deficiency and excessive iron absorption under normal physiological conditions.

For the presented case studies, anemia was defined as HGB concentration of <12 g/dl. Accordingly, athlete 2 (beef-consumer) and athlete 6 (carbohydrate-consumer) with post-supplementation HGB levels of 11.7 g/dL and 11.6 g/dL, respectively, were theoretically anemic. This was in tandem with their elevated transferrin levels (Figs 1D and 1F), as transferrin levels increase during iron deficiency (Akin et al., 2014). While athlete 2 (beef consumer) showed no major alteration in HGB levels, athlete 6 (carbohydrate consumer) showed a 6% decrease in HGB levels. Such anemic condition in the two aforementioned athletes, despite their remarkably high dietary iron intake and elevated ferritin stores (Table 1 and Figs 1A and 1C) reflect functional iron deficiency and not iron deficiency with anemia. The described changes suggest iron sequestration in ferritin and lack of availability of sufficient iron in the systemic circulation to be utilized for elevating RBC indices in these two athletes. The observed effects may be secondary to endurance training inflammation that is associated with iron sequestration and a functional iron deficiency (Latunde-Dada, 2013). Further observations in the two whey-consumers clearly show the significance of iron stores in RBC indices. In these athletes, reduced RBC count, HGB levels, hematocrit and red blood cell distribution width can be attributed to their reduced ferritin levels (Table 2).

Strenuous exercise induces several alterations in immune function. A high neutrophil: lymphocyte ratio immediately post exercise (acute response) is expected and is an indicator of a post exercise stress, as reviewed by Gunzer et al. (Gunzer, Konrad, & Pail, 2012). On the other hand, increased ratio observed after a long period (provided the neutrophil and lymphocyte levels are within the normal range) can be interpreted as a long-term positive adaptation associated with performance enhancement in athletes (Gleeson, 2002). In the present investigation, measurements of leukocytes were taken before (pre) and after 10 weeks of study-duration (post). Therefore, in this instance, the increased ratio of neutrophil: lymphocyte cannot be negatively interpreted as an increase in the exercise-induced stress, but as a positive change associated with endurance performance. Accordingly, the elevated neutrophil: lymphocyte ratio in beef and whey-consumers and its decrement in carbohydrate-consumers (specifically in athlete 6) can be attributed to the supplementation. This reiterates the significance of nutrient supplementation, particularly the protein content in beef and whey in supporting exercise performance in these athletes.

Consumption of high quality protein post-workout has been proposed to facilitate muscle repair and remodeling in the athletes. Only athlete 3 (whey consumer) ingested less than 1.2 $g \cdot kg^{-1} \cdot d^{-1}$ of protein, which is the minimum amount of daily protein intake for endurance athletes (Thomas, Erdman, & Burke, 2016). The other five participants were within the recommended range of daiy protein intake for endurance athlees (>1.2 to 1.8 $g \cdot kg^{-1} \cdot d^{-1}$). Indeed, the two carbohydrate consumers ingested >2 $g \cdot kg^{-1} \cdot d^{-1}$, whch is well above the recently recommended average daily protein intake of 1.65 $g \cdot kg^{-1}$ to satisfy the metabolic demands of endurance training (Kato, Suzuki, Bannai, & Moore, 2016)] (Table 2). Although, the carbohydrate-consumers increased meat in their diet during the training period, their post-workout protein intake would have remained lower than the beef and whey-consumers. Therefore, the observed responses reinforce the potential positive effects of ingesting high-quality protein supplements for supporting muscle repair and remodelling after exercise (Kerksick et al., 2017). The overall maintenance of vastus medialis thickness in beef and

whey consumers (Figs 6A and 6B) and its decrease in carbohydrate-consumers (Fig 6C) indicates the impact of high quality protein consumption in preserving muscle mass in endurance athletes. The presented set of case studies are novel. Here, the effect of a specific nutritional strategy was assessed in master female endurance athletes during the time when regular training program was integrated in their regular lifestyle. Essentially, the data supports usage of this novel form of meat, while information on how this new food can affect endurance performance and iron metabolism in female athletes is scarce. Moreover, while the general recommendation for protein consumption by athletes is based on the outcomes of performance and body composition, very little data is available examining the effect of supplements on other variables such as the iron status. Herein, our set of case studies examines several iron-related parameters such as ferritin, transferrin, RBC and HGB levels. Overall, the manuscript will help to bridge the gap between the industry, which often markets supplements from different protein sources based on the current literature, and those who practice sport using said supplements based on various claims for performance and health. Data from this sets of cases studies and further such studies can aid in understanding the exercise physiology of the growing numbers of female athletes, thereby aiding in formulating better nutritional procedures to maximize their performance.

Acknowledgements

We thank Kelly Cooper, Julia Stent, Carmen Dina Harmse, Alexandros, Anagnostou, Carlo Poggiali and Richard Jeffrey for their support during data collection.

Author contributions

The study was conceived and designed by FN and EZ. Data were collected by MS, NA, BK, and BN. KM, TC and FN analyzed and interpreted the data. KM prepared (designed and wrote) the manuscript. All authors approved the final version of the paper.

Funding

Funding Crown Sports Nutrition and the University of Greenwich provided joint funding for the completion of this project.

Conflict of Interest

The authors declare no conflict of interest.

References

Adamson, J. W. (1994). The relationship of erythropoietin and iron metabolism to red blood cell production in humans. *Seminars in Oncology*, *21*(2 Suppl 3), 9–15.

Akin, M., Atay, E., Oztekin, O., Karadeniz, C., Karakus, Y. T., Yilmaz, B., & Erdogan, F. (2014). Responsiveness to parenteral iron therapy in children with oral iron-refractory iron-deficiency anemia. *Pediatric Hematology and Oncology*, *31*(1), 57–61. https://doi.org/10.3109/08880018.2013.829540

Alaunyte, I., Stojceska, V., & Plunkett, A. (2015). Iron and the female athlete: a review of dietary treatment methods for improving iron status and exercise performance. *Journal of the International Society of Sports Nutrition*, *12*, 38. https://doi.org/10.1186/s12970-015-0099-2

Aoi, W., Naito, Y., & Yoshikawa, T. (2006). Exercise and functional foods. *Nutrition Journal*, *5*, 15. https://doi.org/10.1186/1475-2891-5-15

Beck, K. L., Thomson, J. S., Swift, R. J., & von Hurst, P. R. (2015). Role of nutrition in performance enhancement and postexercise recovery. *Open Access Journal of Sports Medicine*, *6*, 259–267. https://doi.org/10.2147/OAJSM.S33605

Camaschella, C. (2015). Iron-deficiency anemia. *The New England Journal of Medicine*, *372*(19), 1832–1843. https://doi.org/10.1056/NEJMra1401038

Clark, M. R. (1988). Senescence of red blood cells: progress and problems. *Physiological Reviews*, *68*(2), 503–554.

Doering, T. M., Reaburn, P. R., Phillips, S. M., & Jenkins, D. G. (2016). Postexercise Dietary Protein Strategies to Maximize Skeletal Muscle Repair and Remodeling in Masters Endurance Athletes: A Review. *International Journal of Sport Nutrition and Exercise Metabolism*, *26*(2), 168–178. https://doi.org/10.1123/ijsnem.2015-0102

Forget, P., Khalifa, C., Defour, J.-P., Latinne, D., Van Pel, M.-C., & De Kock, M. (2017). What is the normal value of the neutrophil-to-lymphocyte ratio? *BMC Research Notes*, *10*. https://doi.org/10.1186/s13104-016-2335-5

Forrester, A. (2014). Atlas of musculoskeletal ultrasound anatomy. By M Bradley and P O'Donnell, pp. xi + 260, 2002 (Greenwich Medical Media, London, UK), £22.50 ISBN 184110 118 4. *The British Journal of Radiology*. https://doi.org/10.1259/bjr.77.918.770541b

Gleeson, M. (2002). Biochemical and Immunological Markers of Over-Training. *Journal of Sports Science & Medicine*, *1*(2), 31–41.

Gunzer, W., Konrad, M., & Pail, E. (2012). Exercise-Induced Immunodepression in Endurance Athletes and Nutritional Intervention with Carbohydrate, Protein and Fat—What Is Possible, What Is Not? *Nutrients*, *4*(9), 1187–1212. https://doi.org/10.3390/nu4091187

Hawley, J. A., & Leckey, J. J. (2015). Carbohydrate Dependence During Prolonged, Intense Endurance Exercise. *Sports Medicine (Auckland, N.Z.)*, *45*(Suppl 1), 5–12. https://doi.org/10.1007/s40279-015-0400-1

Hu, M., & Lin, W. (2012). Effects of exercise training on red blood cell production: implications for anemia. *Acta Haematologica*, *127*(3), 156–164. https://doi.org/10.1159/000335620

Karsten, B., Jobson, S. A., Hopker, J., Stevens, L., & Beedie, C. (2015). Validity and reliability of critical power field testing. *European Journal of Applied Physiology*, *115*(1), 197–204. https://doi.org/10.1007/s00421-014-3001-z

Kato, H., Suzuki, K., Bannai, M., & Moore, D. R. (2016). Protein Requirements Are Elevated in Endurance Athletes after Exercise as Determined by the Indicator Amino Acid Oxidation Method. *PloS One*, *11*(6), e0157406. https://doi.org/10.1371/journal.pone.0157406

Kerksick, C. M., Arent, S., Schoenfeld, B. J., Stout, J. R., Campbell, B., Wilborn, C. D., … Antonio, J. (2017). International society of sports nutrition position stand: nutrient timing. *Journal of the International Society of Sports Nutrition*, *14*, 33. https://doi.org/10.1186/s12970-017-0189-4

Lambert, E. V., Hawley, J. A., Goedecke, J., Noakes, T. D., & Dennis, S. C. (1997). Nutritional strategies for promoting fat utilization and delaying the onset of fatigue during prolonged exercise. *Journal of Sports Sciences*, *15*(3), 315–324. https://doi.org/10.1080/026404197367326

Latunde-Dada, G. O. (2013). Iron metabolism in athletes--achieving a gold standard. *European Journal of Haematology*, *90*(1), 10–15. https://doi.org/10.1111/ejh.12026

Naclerio, F., & Larumbe-Zabala, E. (2016). Effects of Whey Protein Alone or as Part of a Multi-ingredient Formulation on Strength, Fat-Free Mass, or Lean Body Mass in Resistance-Trained Individuals: A Meta-analysis. *Sports Medicine (Auckland, N.Z.)*, *46*(1), 125–137. https://doi.org/10.1007/s40279-015-0403-y

Naclerio, F., Larumbe-Zabala, E., Cooper, R., Jimenez, A., & Goss-Sampson, M. (2014). Effect of a carbohydrate-protein multi-ingredient supplement on intermittent sprint performance and muscle damage in recreational athletes. *Applied Physiology, Nutrition, and Metabolism = Physiologie Appliquee, Nutrition Et Metabolisme*, *39*(10), 1151–1158. https://doi.org/10.1139/apnm-2013-0556

Phillips, S. M. (2012). Nutrient-rich meat proteins in offsetting age-related muscle loss. *Meat Science*, *92*(3), 174–178. https://doi.org/10.1016/j.meatsci.2012.04.027

Sharp, P., & Srai, S.-K. (2007). Molecular mechanisms involved in intestinal iron absorption. *World Journal of Gastroenterology*, *13*(35), 4716–4724.

Stark, M., Lukaszuk, J., Prawitz, A., & Salacinski, A. (2012). Protein timing and its effects on muscular hypertrophy and strength in individuals engaged in weight-training. *Journal of the International Society of Sports Nutrition*, *9*, 54. https://doi.org/10.1186/1550-2783-9-54

Thomas, D. T., Erdman, K. A., & Burke, L. M. (2016). American College of Sports Medicine Joint Position Statement. Nutrition and Athletic Performance. *Medicine and Science in Sports and Exercise*, *48*(3), 543–568. https://doi.org/10.1249/MSS.0000000000000852

Wakeman, L., Al-Ismail, S., Benton, A., Beddall, A., Gibbs, A., Hartnell, S., … Munro, R. (2007). Robust, routine haematology reference ranges for healthy adults. *International Journal of Laboratory Hematology*, *29*(4), 279–283. https://doi.org/10.1111/j.1365-2257.2006.00883.x

FIGURES

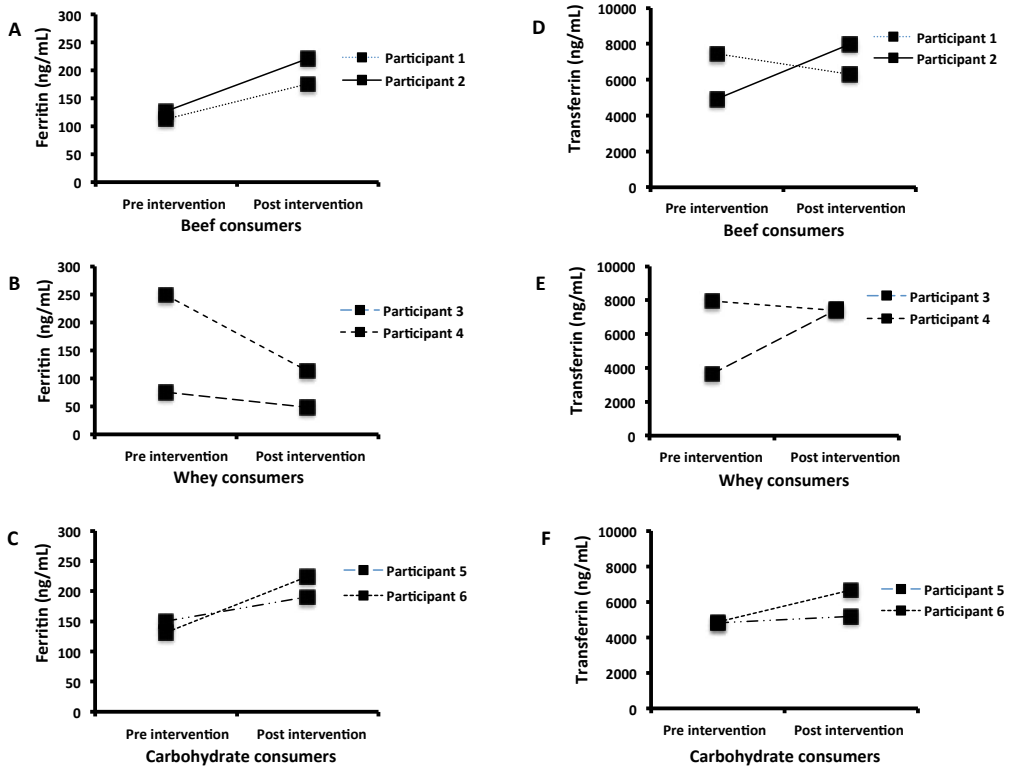

Fig 1. Effect of nutrient supplementation on ferritin and transferrin levels

The figure shows pre and post supplementation levels of ferritin (A, B, C) and transferrin (D,E,F) for each participant under corresponding nutrient supplementation: beef (A, D) whey (B, E) and carbohydrate (CHO) (C, F).

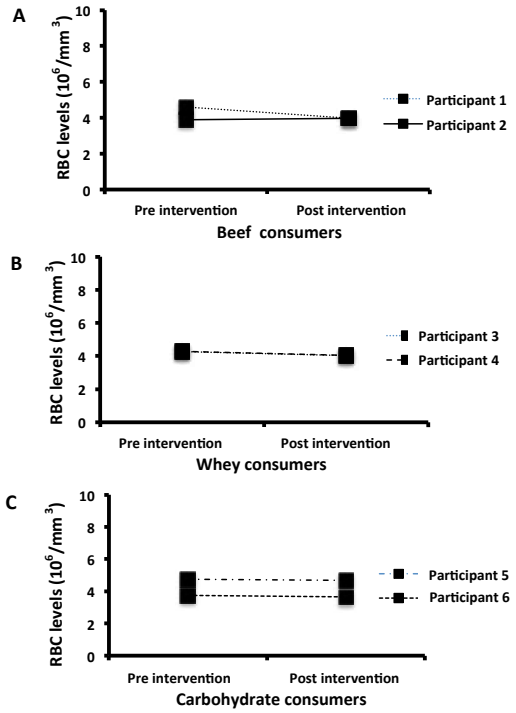

Fig 2. Effect of nutrient supplementation on RBC levels

The figure shows pre and post supplementation RBC levels in each participant under nutrient supplementation with (A) beef, (B) whey and (C) carbohydrate (CHO).

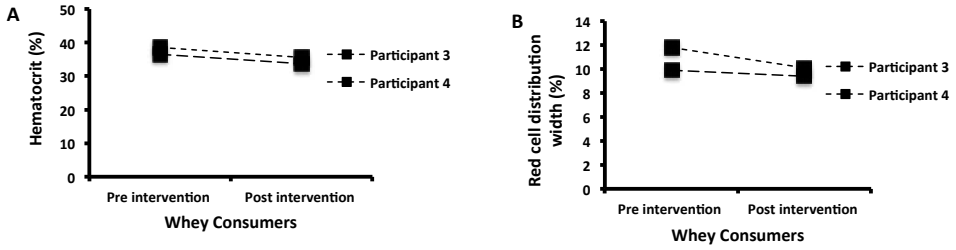

Fig 3. Effect of nutrient supplementation on HCT and RDW

The figure shows pre and post supplementation hematocrit (HCT) (A) and red cell distribution width (RDW) (B) in whey consumers.

Fig 4. Effect of nutrient supplementation on platelet levels

The figure shows pre and post supplementation platelets levels in each participant under nutrient supplementation with (A) beef, (B) whey and (C) carbohydrate (CHO).

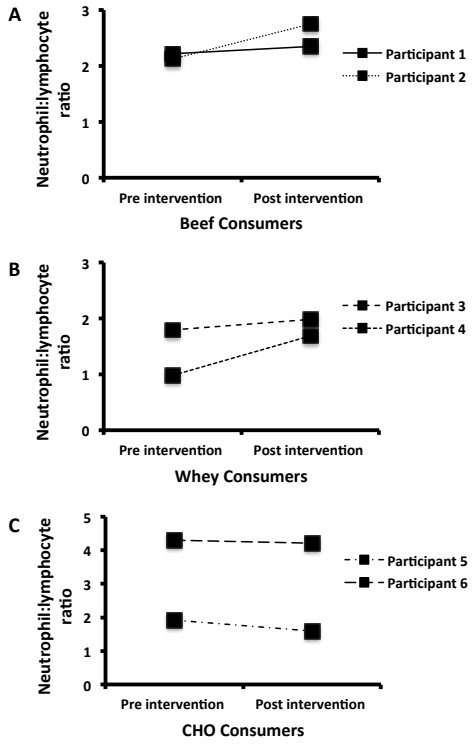

Fig 5. Effect of nutrient supplementation on neutrophil:lymphocyte ratio

The figure shows pre and post supplementation neutrophil:lymphocyte ratio in each participant under nutrient supplementation with (A) beef, (B) whey and (C) carbohydrate (CHO).

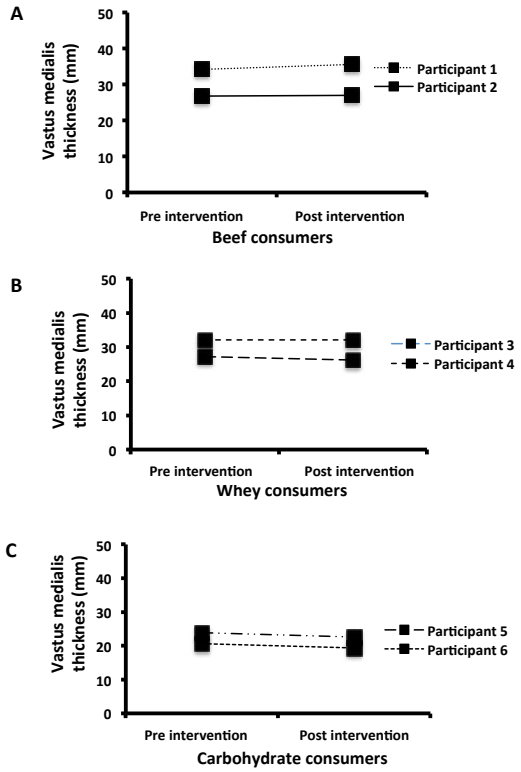

Fig 6. Effect of nutrient supplementation on vastus medialis

The figure shows pre and post supplementation value of vastus medialis thickness for each participant under nutrient supplementation with (A) beef, (B) whey and (C) carbohydrate (CHO).