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Supplementary Material 2

Supplementation details

The supplement was independently verified in triplicate by adding 209 mg to 1 L of deionized water (20.9 mg/dl). Total calcium concentration was then determined with a photometric technique (Modular P, Roche Diagnostics Ltd., West Sussex, UK). The calcium concentration was 1.25 ± 0.05 mmol/L (given an atomic mass of 40.078 g/mol this equates to 5.02 ± 0.18 mg/dl). Thus, the milk-extracted calcium powder was $24.1 \pm 0.9\%$ calcium, and the addition of 4 g resulted in an increase in the calcium content of the milkshake from 400 mg in CON to 1363 ± 35 mg in CAL (difference = 963 ± 35 mg).

The powder was completely soluble in water, and quantities used to elevate the calcium content of the test drinks resulted in negligible increases in protein, carbohydrate and fat (all < 0.5 g) and sodium, magnesium, chloride and potassium (all < 90 mg). The addition of calcium powder did not affect the appearance or taste of the milkshake which was confirmed by asking participants to rate test-drink palatability with VAS and by asking the participants to guess which supplemental period was the CON or CAL. Participants were correct on 9 out of a possible 26 occasions (below the 50% considered random chance). Supplemental compliance was evaluated by pre-packaged bag counts. Participants were initially provided more bags than necessary (ie. > 14) and asked to return unused bags. Participants were asked to maintain their usual, habitual exercise habits during supplementation; confirmed by training diaries. During supplementation, participants were informed to consume only 1 serving (eg. 250 ml glass of milk) of dairy/d in an attempt to limit dairy calcium intake. Compliance was assessed verbally, and by 3-day food diaries. Only 1 participant completed the full weighed food diary. Therefore, it was not possible to estimate energy intake, however, all participants completed food items consumed and approximate portion sizes (eg. medium bowl). Accordingly, food diaries were still deemed

appropriate for assessing compliance 1 serving of dairy per day, which all participants adhered to ($n = 13$). Furthermore, all participants consumed all milkshakes, although 1 participant admitted to missing one day and therefore consumed two on a subsequent day, during the first week of supplementation. A food frequency questionnaire, previously validated and used in similar populations^{1,2}, was completed to estimate habitual calcium intake.

References

1. Bescos Garcia R, Rodriguez Guisado FA. Low levels of vitamin D in professional basketball players after wintertime: relationship with dietary intake of vitamin D and calcium. *Nutr Hosp* 2011; **26**(5): 945-951. doi: 10.1590/S0212-16112011000500004
2. Taylor C, Lamparello B, Kruczek K, Anderson EJ, Hubbard J, Misra M. Validation of a food frequency questionnaire for determining calcium and vitamin D intake by adolescent girls with anorexia nervosa. *Journal of the American Dietetic Association* 2009; **109**(3): 479-485, 485 e471-473. doi: 10.1016/j.jada.2008.11.025