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ORIGINAL ARTICLE *Reproductive epidemiology*

Dairy food intake in relation to semen quality and reproductive hormone levels among physically active young men

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STUDY QUESTION: Is increased consumption of dairy foods associated with lower semen quality?

SUMMARY ANSWER: We found that intake of full-fat dairy was inversely related to sperm motility and morphology. These associations were driven primarily by intake of cheese and were independent of overall dietary patterns.

WHAT IS KNOWN ALREADY: It has been suggested that environmental estrogens could be responsible for the putative secular decline in sperm counts. Dairy foods contain large amounts of estrogens. While some studies have suggested dairy as a possible contributing factor for decreased semen quality, this finding has not been consistent across studies.

STUDY DESIGN, SIZE, DURATION: The Rochester Young Men's Study ($n = 189$) was a cross-sectional study conducted between 2009 and 2010 at the University of Rochester.

PARTICIPANTS/MATERIALS, SETTING, METHODS: Men aged 18–22 years were included in this analysis. Diet was assessed via food frequency questionnaire. Linear regression was used to analyze the relation between dairy intake and conventional semen quality parameters (total sperm count, sperm concentration, progressive motility, morphology and ejaculate volume) adjusting for age, abstinence time, race, smoking status, body mass index, recruitment period, moderate-to-intense exercise, TV watching and total calorie intake.

MAIN RESULTS AND THE ROLE OF CHANCE: Total dairy food intake was inversely related to sperm morphology (P-trend $= 0.004$). This association was mostly driven by intake of full-fat dairy foods. The adjusted difference (95% confidence interval) in normal sperm morphology percent was −3.2% (−4.5 to −1.8) between men in the upper half and those in the lower half of full-fat dairy intake ($P < 0.0001$), while the equivalent contrast for low-fat dairy intake was less pronounced [−1.3% (−2.7 to −0.07; $P = 0.06$)]. Full-fat dairy intake was also associated with significantly lower percent progressively motile sperm ($P = 0.05$).

LIMITATIONS, REASONS FOR CAUTION: As it was a cross-sectional study, causal inference is limited.

WIDER IMPLICATIONS OF THE FINDINGS: Further research is needed to prove a causal link between a high consumption of full-fat dairy foods and detrimental effects on semen quality. If verified our findings would mean that intake of full-fat dairy foods should be considered in attempts to explain secular trends in semen quality and that men trying to have children should restrict their intake.

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Key words: diet / dairy / environmental effects / semen quality

Introduction

The secular decline in semen quality, most consistently for sperm counts (Swan, 2000; Rolland et al., 2013) but also for morphology (Rolland et al., 2013), is an ongoing controversy in male reproductive health (Swan, 2000; Axelsson et al., 2011; Jorgensen et al., 2012; Rolland et al., 2013). Several potential culprits have been suggested as explanations for this putative secular decline including concurrent trends in decreased physical activity and increase in sedentary behavior (Gaskins et al., 2013), obesity (Sermondade et al., 2012) and diet quality (Attaman et al., 2012; Gaskins et al., 2012). Most of the attention, however, has focused on the role of environmental contaminants, particularly, environmental estrogens (Sharpe, 2003) and anti-androgens (Fisher, 2004).

In modern dairy farming practice, around 75% of commercial milk originates from pregnant cows (Davaasambuu et al., 2001; Davaasambuu et al., 2004). Pregnancy is a physiological state characterized by an increase in many naturally occurring hormones such as progesterone, insulin, and estradiol (E2), some of which readily cross from the cow's plasma into the milk (Pape-Zambito et al., 2008). As a result, dairy products contain measurable amounts of estrogens (Daxenberger et al., 2001) and account for 60–80% of intake of estrogens from food in Western countries (Hartmann et al., 1998). Rodent models have been inconsistent in identifying reproductive or estrogenic effects of milk intake (Davaasambuu et al., 2004; Li et al., 2005; Dolgin, 2012). In boys, intake of milk and other dairy foods has been associated with higher levels of circulating prepubertal growth hormone, insulin-like growth factor 1 (IGF-1) and the ratio of IGF-1 to IGF-binding protein 3 (Rich-Edwards et al., 2007) and increased urinary excretion of estrone, estriol, E2 and pregnanediol (Maruyama et al., 2010). In men, dairy food intake has been related to decreased secretion of LH, FSH and testosterone (Maruyama et al., 2010).

Specific literature on the relation between dairy food intake and semen quality is scarce. While some studies have suggested that dairy is a possible contributing factor for decreased semen quality (Davaasambuu et al., 2001; Mendiola et al., 2009; Eslamian et al., 2012), this finding has not been consistent across studies (Vujkovic et al., 2009). Thus, the objective of this study was to examine the relation between dairy food intake and semen quality in a group of young, physically active men.

Methods

Study population

The Rochester Young Men's Study (RYMS) is a cross-sectional study that enrolled men at the University of Rochester (New York) between 2009 and 2010 through flyers and newspapers as described elsewhere (Swan et al., 2007). RYMS is part of a multi-center international study (USA, Spain, Finland and Denmark) aimed at evaluating the role of environmental contaminants on semen quality. Subjects were eligible to participate in RYMS if they were born in the USA after 31 December 1987, able to read and speak English and able to contact their mother and ask her to complete a questionnaire. A total of 389 potential participants contacted the study coordinator between spring 2009 and spring 2010. Of these, 305 met all eligibility criteria. Among eligible men, 83 did not join the study because they were no longer interested after learning about the details of the study, were unable to arrange a study visit due to scheduling or transportation difficulties or manifested interest but never scheduled a study visit. The remaining 222 (73%) men participated in the study. A food frequency questionnaire (FFQ) was introduced in the fall of 2009, after enrollment had started. All men after this point ($n = 194$) completed the FFQ. Among them, three had missing data on sperm morphology and two had implausible calorie intake (>10 000 or <600 kcals/day) leaving a final sample size of 189 men.

Height and weight were measured by trained personnel during the physical examination. Reproductive disorders such as varicocele, hydrocele and surgical scars were also noted during the physical examination. We asked participants to fill out a brief questionnaire on lifestyle (physical activity, TV watching and smoking), demographics, psychological stress, as well as medical and reproductive history. Participants' mothers also filled out questionnaire on demographics, lifestyle and smoking during their pregnancy. Participants received $75 upon study completion. The study was approved by the University of Rochester Research Subjects Review Board and written informed consent was obtained from all men prior to their participation.

Semen collection and analysis

Men produced semen samples by masturbation into a specimen cup at the clinic on the day of the physical examination. They had been asked to abstain from ejaculation for 48 h prior to the clinic visit (but they were not excluded if this was not the case) and to report the time of their previous ejaculation. Abstinence times >240 h ($n = 7$) were truncated at 240 h. Samples were processed within 30 min of collection.

Ejaculate volumes were estimated by specimen weight, assuming a semen density of 1.0 g/ml. Sperm concentration was evaluated by hemocytometer (Improved Neubauer; Hausser Scientific, Inc., Horsham, PA, USA). Two chambers of the hemocytometer were counted and we used the average in this analysis. Motility was assessed using the World Health Organization 2010 criteria (Cooper et al., 2010) and classified as both progressive (A + B) and total (A + B + C). Smears for morphology were made, air-dried, fixed and shipped to the University Department of Growth and Reproduction at the Rigshospitalet (Copenhagen, Denmark). The slides were Papanicolaou stained and assessed using Kruger strict criteria (Menkveld et al., 1990).

Reproductive hormone measurement

Blood samples were drawn from a cubital vein of each participant, centrifuged and the serum was separated, stored and frozen at −80°C. Serum samples were then shipped to Copenhagen, Denmark on dry ice and stored at −20°C until hormone analysis was performed at the Rigshospitalet. The methods used have been described previously (Asklund et al., 2007). Briefly, hormone assessments were done simultaneously to reduce intra-laboratory variations. Serum levels of FSH, LH and sex hormone-binding globulin (SHBG) were determined using time-resolved immunofluorometric assays (DELFIA; PerkinElmer, Skovlund, Denmark). Intra- and inter-assay variations were both <5% in each of the three assays. Serum testosterone levels were determined using a time-resolved fluoroimmunoassay (DELFIA; PerkinElmer) with intra- and inter-assay variation <8%. E2 was measured by radioimmunoassay (Pantex, Santa Monica, CA, USA) with an intra-assay variation of <8% and an inter-assay variation of <13%. Inhibin B levels were determined by a specific two-sided enzyme immunometric assay (Oxford Bio-Innovation Ltd, Bicester, UK) with intra- and inter-assay variation of 13 and 18%, respectively. The free androgen index was calculated as total testosterone × 100/SHBG. We calculated the free testosterone (FT) concentration using the equation of Vermeulen et al. (1999).

Dietary assessment

Diet was assessed using a previously validated 131-item FFQ (Rimm et al., 1992). Men reported how often, on average, they consumed specified amounts of each food, beverages and supplements, on average, during the previous year. The nine categories for food frequency options ranged from never to six or more times per day. The selected frequency category for

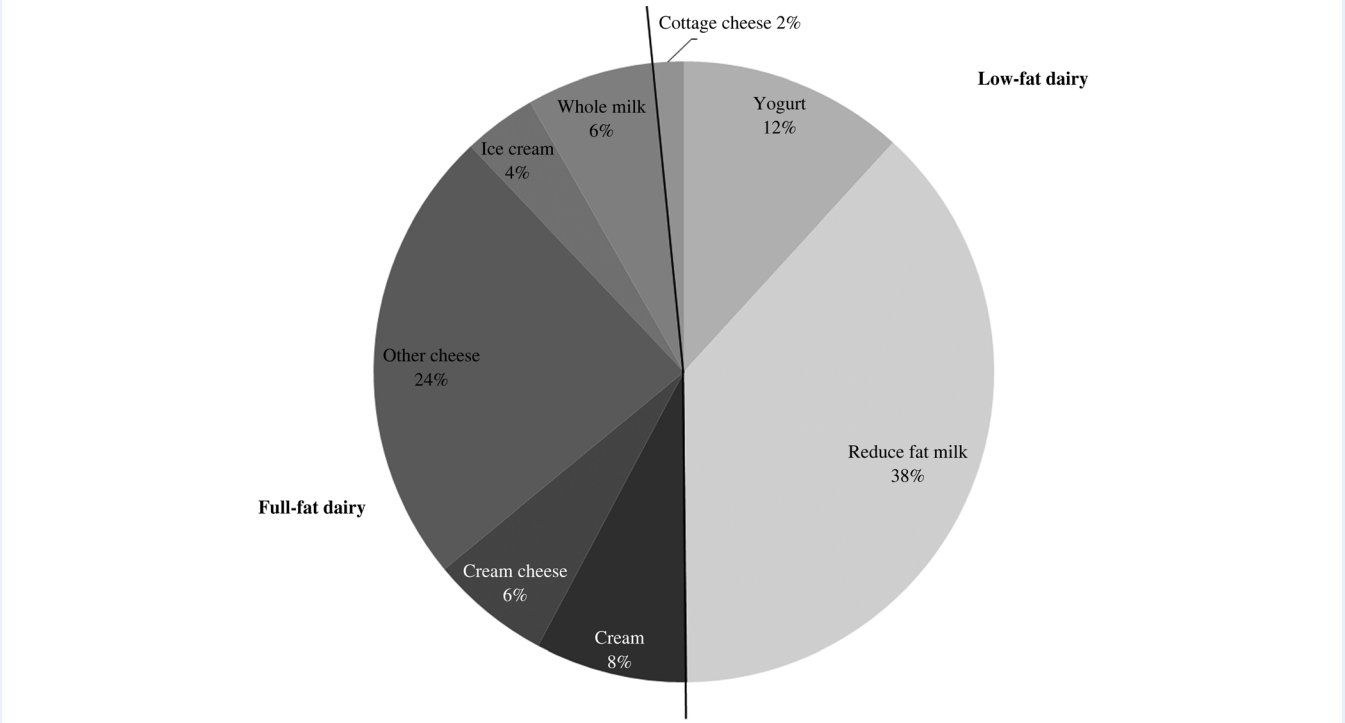

Figure 1 Contribution of individual dairy foods.

each food item was converted to a daily intake and the nutrient content of each food and the specific portion size or dose were calculated using the nutrient database from the US Department of Agriculture (USDA) (Gebhardt *et al.*, 2008) with additional information from manufacturers when necessary. Intake of dairy foods has been previously validated in a separate population using this questionnaire (Feskanich *et al.*, 1993). In particular, the de-attenuated correlation coefficient (Pearson correlation corrected for within-subject variation) between dairy food intakes assessed with the FFQ and the 1 year average of prospectively collected diet records ranged from 0.52 for cottage cheese to 0.88 for skim milk (Feskanich *et al.*, 1993). Total dairy food intake was defined as the sum of whole milk, 1 and 2% milk, skim milk, cream cheese, cottage cheese, other cheese, frozen, plain and flavored yogurt, cream and ice cream intake. Full-fat dairy intake was defined as the sum of whole milk, cream, ice cream, cream cheese and other cheese; while low-fat dairy intake was defined as the sum of skim milk, 1 and 2% milk, total yogurt and cottage cheese. Two previously described data-derived dietary patterns in the same population, the 'Prudent Pattern' and the 'Western Pattern' (Gaskins *et al.*, 2012) were considered to summarize overall food choices. The Prudent pattern was characterized by high intakes of fish, chicken, fruits, vegetables and whole grains, whereas the Western pattern by high processed and red meat intakes, full-fat dairy, butter, refined grains, snacks, pizza, mayonnaise, high-energy drinks and sweets (Gaskins *et al.*, 2012).

Statistical analysis

We first summarized participant characteristics and compared them across quartiles of dairy food intake, using the Kruskal–Wallis test for continuous measures and an extended Fisher's exact test for categorical variables. We used linear regression to assess the association of dairy foods with conventional semen quality parameters (total sperm count, sperm concentration, progressive motility, morphology and ejaculate volume) by comparing semen parameter levels in men in higher intake levels to those in the lowest quartile of intake (reference) while adjusting for potential confounders. Robust estimators of the variance (White, 1980) were used in the computation of 95% confidence intervals. Total sperm count, sperm concentration and FSH were log-transformed to more closely approximate a normal distribution as required for linear regression. An alternate normalizing transformation (cube root transformation) was also explored for total sperm count and sperm concentration yielding comparable results and the same conclusions to analyses based on log-transformation. For consistency with previous literature we chose to present results based on log-transformed sperm count and concentration. Results for these parameters were back-transformed to allow presentation of results in the original scale. Population marginal means (Searle *et al.*, 1980) were utilized to present marginal population averages adjusted for the covariates in the model. Tests for linear trend were performed by assigning the median dairy intake within each quartile of intake and modeling it as a continuous variable.

We considered as potential confounders baseline characteristics that were associated with dairy intake and semen analysis. Based on these criteria, all models were adjusted for age, body mass index (BMI), abstinence time, smoking status, physical activity, TV watching, race, recruitment period (2009 versus 2010), alcohol intake and total caloric intake. In addition, sperm motility models were adjusted for time from semen collection to start of semen analysis. We further adjusted for the 'Prudent' and 'Western' dietary pattern scores as continuous variables (Gaskins *et al.*, 2012) to determine whether any observed association was specific to a particular dairy food or whether the overall food pattern was driving the association. Finally, since saturated fat intake has been previously related to lower semen quality (Attaman *et al.*, 2012) and full-fat dairy is a large source of saturated fat, we further adjusted models for fat intake to explore whether any observed association was mediated through fat intake. The same set of covariates was used for adjustment of semen quality parameters and reproductive hormone levels with two exceptions: (i) hormones were not adjusted for

Table I Participants' characteristics according to quartiles of dairy food intake.

	Quartiles of total dairy food intake				
	Q1 (lowest)	Q2	Q3	Q4 (highest)	P[a]
n	46	48	48	47	
Range, servings/day	0–1.65	1.67–3.24	2.98–4.29	4.3–13.26	
	Median (IQR) or *n* (%)				
Demographics					
Age, years	19.6 (18.9–20.6)	19.6 (18.8–20.6)	19.5 (19.0–20.6)	19.2 (18.7–20.4)	0.66
Race/ethnicity, *n* (%)					0.73
Hispanic or Latino	2 (4.3)	2 (4.1)	1 (2.1)	2 (4.2)	
White, not Hispanic	33 (71.7)	41 (85.4)	41 (85.4)	41 (87.2)	
Black	5 (10.8)	2 (4.1)	3 (6.2)	2 (4.2)	
Asian	4 (8.7)	2 (4.1)	1 (2.1)	0 (0.0)	
Other	2 (4.3)	1 (2.1)	2 (4.1)	2 (4.2)	
BMI, kg/m^2	25.0 (22.8–26.5)	24.3 (22.8–28.3)	23.8 (22.2–25.4)	24.8 (23.4–26.7)	0.24
Moderate-to-vigorous physical activity, hours/week	7.0 (4.0–11.0)	10.0 (6.0–14.0)	7.0 (4.0–11.2)	10.0 (7.0–18.0)	0.004
TV watching, hours/week	7.0 (0.0–14.0)	14.0 (10.0–20.0)	14.0 (4.0–14.0)	14.0 (4.0–20.0)	0.005
Stress (yes/no)[b], *n* (%)	16 (34.8)	21 (43.8)	22 (45.8)	19 (40.4)	0.72
Current smoker, *n* (%)	12 (26.0)	14 (29.1)	12 (25.0)	5 (10.6)	0.11
Maternal smoking during pregnancy[c], *n* (%)	1 (3.1)	2 (5.0)	3 (7.0)	4 (10.0)	0.73
Abstinence time, hours	71.5 (62.0–110.8)	69.8 (48.7–91.3)	71.1 (62.1–110.4)	70.1 (54.6–94.0)	0.56
Self-reported reproductive history					
Undescended testes at birth, *n* (%)	1 (2.1)	0 (0.0)	2 (4.1)	2 (4.2)	0.82
Varicocele, *n* (%)	3 (6.5)	1 (2.0)	0 (0.0)	1 (2.1)	0.20
Hydrocele, *n* (%)	0 (0.0)	1 (2.0)	0 (0.0)	2 (4.2)	0.42
Inguinal hernia repair, *n* (%)	3 (6.5)	1 (2.0)	3 (6.3)	3 (6.3)	0.73
History of genital disease, *n* (%)[d]	2 (4.3)	2 (4.1)	5 (10.4)	1 (2.1)	0.39
Physical examination findings					
Testes high in scrotum, *n* (%)	4 (8.7)	3 (6.2)	4 (8.3)	4 (8.5)	0.96
Varicocele, *n* (%)	9 (19.5)	3 (6.2)	3 (6.2)	7 (14.8)	0.11
Hydrocele, *n* (%)	0 (0.0)	1 (2.0)	1 (2.0)	2 (4.2)	0.80
Surgical scars, *n* (%)[e]	1 (2.1)	2 (2.0)	1 (2.0)	5 (10.6)	0.19
Diet					
Total energy intake, kcal/day	2108.2 (1615.1–2619.4)	2771.4 (2269.7–3350.0)	2985.4 (2361.8–3698.3)	3695.3 (2951.8–4783.7)	<0.0001
Multivitamin supplement, *n* (%)[f]	13 (28.3)	13 (7.0)	11 (22.9)	16 (34.0)	0.70
Vitamin D supplement, *n* (%)	3 (6.5)	6 (12.5)	4 (8.3)	1 (2.1)	0.27
Alcohol intake, g/day	8.5 (3.0–25.6)	15.4 (3.5–32.5)	17.9 (5.3–28.2)	11.3 (2.9–22.4)	0.25
Saturated fat, % energy	9.6 (8.2–11.3)	10.4 (9.0–11.5)	10.5 (9.2–11.9)	10.6 (9.8–12.2)	0.05
Mono-unsaturated fat, % energy	11.5 (10.1–12.6)	11.6 (9.9–12.8)	11.6 (10.3–12.9)	11.0 (10.2–12.8)	0.56
Poly-unsaturated fat, % energy	5.6 (5.0–6.5)	5.5 (4.8–6.1)	5.3 (5.0–5.9)	5.1 (4.4–5.8)	0.10
Trans fat, % energy	1.3 (1.0–1.6)	1.2 (0.9–1.5)	1.2 (1.1–1.5)	1.1 (1.0–1.4)	0.69
Protein intake, % energy	15.1 (13.5–18.0)	15.4 (14.1–16.8)	16.1 (14.7–17.6)	17.6 (15.2–19.4)	0.003
Prudent pattern score	−0.5 (−0.9, −0.04)	−0.4 (−0.7, 0.2)	−0.21 (−0.5, 0.3)	0.1 (−0.3, 1.2)	<0.0001
Western pattern score	−0.6 (−0.9, −0.2)	0.06 (−0.4, 0.6)	−0.01 (−0.6, 0.6)	0.1 (−0.4, 1.3)	<0.0001

[a]From Kruskal–Wallis test for continuous variables and Fisher's exact test for categorical variables.
[b]Responding yes to ≥2 stress-related questions such as losing a job, dropping out of school, experiencing serious difficulties with a family member, etc.
[c]Sample size for maternal smoking status across quartiles of dairy intake: *n* = 32, 40, 43, 40.
[d]Self-report of any of the following: infection of epididymis, testicle, prostate, urinary tract infection, gonorrhea, genital warts or herpes, chlamydia or other diseases of the penis, testicles, urinary tract or scrotum.
[e]From hernia repair, appendectomy, orchidopexy or other lower abdomen/inguinal procedures.
[f]Use of any multivitamin supplement (not necessarily daily supplement).

Table II Adjusted[a] semen quality parameters [mean (95% confidence interval)] according to intake of dairy foods.

	n	Total sperm count (million)	Sperm concentration (million/ml)	Progressive motility[d] (% motile)	Sperm morphology (% normal)	Ejaculate volume (ml)
Quartiles of total dairy food intake [range, servings/day]						
Q1 [0–1.65]	46	162 (120–219)	50.9 (38.6–67.2)	61.3 (57.5–64.9)	10.4 (9.2–11.7)	3.5 (3.1–3.9)
Q2 [1.67–2.96]	48	148 (109–200)	50.9 (38.1–67.8)	58.6 (54.8–62.4)	8.9 (7.6–10.3)	3.4 (2.9–3.9)
Q3 [2.98–4.29]	48	146 (108–198)	45.3 (34.4–59.7)	56.2 (52.6–60.0)	7.7 (6.4–9.1)*	3.6 (3.2–3.9)
Q4 [4.33–13.26]	47	107 (78–148)	35.3 (26.2–47.5)	57.5 (52.4–62.7)	7.5 (6.2–8.8)*	3.4 (2.9–3.8)
P-trend		0.09	0.07	0.31	0.004	0.93
Quartiles of full-fat dairy food intake[b] [range, servings/day]						
Q1 [0–0.71]	44	163 (120–223)	52.3 (39.0–70.1)	62.3 (57.9–66.7)	10.0 (8.7–11.4)	3.3 (2.9–3.7)
Q2 [0.73–1.16]	49	175 (131–233)	54.4 (41.8–70.7)	60.8 (57.3–64.1)	10.4 (9.0–11.8)	3.7 (3.2–4.2)
Q3 [1.22–1.94]	45	117 (87–157)	39.1 (30.7–49.8)	55.3 (51.0–59.5)*	7.1 (6.1–8.1)*	3.4 (2.9–4.0)
Q4 [2.00–7.51]	51	114 (85–153)	37.6 (28.8–49.3)	55.4 (51.2–59.5)*	7.1 (5.8–8.4)*	3.4 (3.0–3.7)
P-trend		0.08	0.08	0.04	0.002	0.72
Quartiles of low-fat dairy food intake[c] [range, servings/day]						
Q1 [0–0.56]	47	153 (111–209)	49.7 (37.4–65.8)	58.7 (55.1–62.4)	9.1 (7.8–10.3)	3.4 (3.0–3.7)
Q2 [0.57–1.04]	47	136 (104–177)	46 (36.6–57.9)	57.8 (53.7–61.8)	9.5 (8.2–10.8)	3.3 (2.9–3.7)
Q3 [1.08–2.66]	49	156 (118–207)	47.3 (35.7–62.7)	56.7 (53.2–60.2)	7.9 (6.6–9.2)	3.7 (3.2–4.2)
Q4 [2.7–12.08]	46	116 (84–161)	38.3 (28.7–51.1)	60.4 (55.8–65.0)	8.1 (6.6–9.5)	3.4 (2.9–3.8)
P-trend		0.29	0.22	0.51	0.18	0.83

[a]Adjusted for age, abstinence time, race, smoking status, BMI, recruitment period, moderate-to-intense exercise, TV watching, alcohol intake, prudent and western dietary patterns and total calorie intake.
[b]Includes whole milk, cream, ice cream, cream cheese and other cheese.
[c]Includes skim/low-fat milk, yogurt, frozen yogurt and cottage cheese.
[d]Additionally adjusted for time from current ejaculation to start of semen analysis.
*P-value for trend <0.05 compared with men in the lowest quartile of intake.

abstinence time and (ii) hormones were adjusted for time of blood sampling to take into consideration circadian variation in blood levels of some hormones. We also adjusted all models for alcohol intake as some studies have found lower testosterone levels among men with high alcohol intake (La Vignera *et al.*, 2013).

We explored whether self-reported or physician-diagnosed reproductive disorders modified the association of dairy intake and semen quality. We dichotomized men into having any reproductive disorder (whether self-reported or physician diagnosed) and those without a reproductive disorder. We also assessed effect modification by BMI (<25 kg/m^2 and ≥ 25 kg/m^2) and smoking status (current and never/former smokers) using cross-product terms. We analyzed the data using SAS (version 9.2; SAS Institute Inc., Cary, NC, USA), and two-sided P-values ≤ 0.05 were considered statistically significant.

Results

The 189 men were young [mean (SD) = 19.7 (1.0) years], predominantly Caucasian (83%), highly physically active [mean (SD) = 10.5 (8.2) h of moderate-to-vigorous activity/week], primarily non-smoking (77%) and without history of relevant reproductive disease (<6%). Forty-one percent were overweight or obese (BMI ≥ 25 kg/m^2). The median sperm concentration was 53.0×10^6/ml [interquartile range (IQR) = 20.5 to 95.5×10^6/ml], percent progressively motile sperm was 60.5% (IQR = 49.5 to 69.5%) and percent morphologically normal sperm was 8.5% (IQR = 5.0 to 12.0%). Men with any reproductive disorder had lower sperm concentration (mean = 36.6 million/ml, SD = 3.0) than men without any reproductive disorder (mean = 49.4 million/ml, SD = 2.5), $P = 0.048$. All other semen parameters were similar between these two groups. History of reproductive diseases, either self-reported or documented during physical examination, was not related to intake of dairy foods, however.

Milk and cheese were the most commonly consumed dairy foods (Fig. 1). Dairy food intake was positively related to physical activity, hours of TV watching per week, total energy intake, fats and protein intakes and the Prudent and Western food patterns (Table I). There was also a suggestion of an inverse relation between dairy food intake and smoking. Black and Asian men were less likely to have high dairy intakes. Dairy food intake was unrelated to other subject characteristics.

Total dairy food intake was inversely related to sperm morphology (Table II). Compared with men in the lowest quartile of total dairy food intake (0–1.65 servings/day), normal sperm morphology (95% confidence intervals) was 1.5% (−0.3 to 3.3), 2.7% (0.9 to 4.5) and 3.0% (1.0 to 4.9) percentage lower for men in the second, third and highest quartiles of intake, respectively (4.33 to 13.26 servings/day for men in the highest quartile), after adjustment for potential confounders (P-trend = 0.004). In addition, there was a suggestion of an inverse association between total dairy intake and total sperm count (P-trend = 0.09) and sperm concentration (P-trend = 0.07). Total dairy food intake was unrelated to the remaining semen quality parameters.

Table III Adjusted[a] mean values (95% CIs) of semen quality parameters associated with intake of specific dairy foods.

Tertiles of dairy food intake [range, servings/day]	*n*	Total sperm count (million)	Sperm concentration (million/ml)	Progressive motility[e] (% motile)	Sperm morphology (% normal)	Ejaculate volume (ml)
Cheese[b]						
Q1 [0–0.51]	60	158 (120–207)	50.9 (39.7–65.2)	60.8 (56.5–65.2)	9.1 (8.0–10.2)	3.4 (3.0–3.7)
Q2 [0.57–1.08]	66	133 (103–174)	44.1 (35.1–55.5)	58.5 (55.9–61.2)	9.2 (8.1–10.4)	3.5 (3.1–3.8)
Q3 [1.14–6.43]	63	129 (101–166)	41.1 (33.0–51.3)	55.9 (52.4–59.4)	7.5 (6.5–8.5)*	3.5 (3.1–3.9)
P-trend		0.38	0.27	0.08	0.02	0.63
Cream						
Q1 [0]	76	130 (103–166)	42.0 (33.6–52.5)	57.3 (54.3–60.3)	8.2 (7.2–9.2)	3.5 (3.1–3.8)
Q2 [0.08]	48	176 (135–231)	55.9 (44.2–70.8)	62.1 (59.0–65.3)*	10.5 (9.0–11.9)*	3.5 (3.1–3.9)
Q3 [0.14–6]	65	126 (98–163)	41.8 (33.6–52.0)	56.9 (53.2–60.5)	7.8 (6.7–8.9)	3.4 (3.0–3.7)
P-trend		0.53	0.62	0.48	0.21	0.64
Whole milk						
Q1 [0]	131	148 (125–176)	48.3 (41.4–56.4)	57.7 (55.2–60.2)	8.6 (7.8–9.4)	3.4 (3.2–3.7)
Q2 [0.08]	20	113 (66–194)	40.6 (26.4–62.5)	57.2 (52.4–61.9)	8.4 (6.2–10.6)	3.1 (2.5–3.8)
Q3 [0.14–6]	38	125 (91–172)	37.6 (27.2–51.8)	61.3 (56.9–65.8)	8.7 (7.4–10.1)	3.6 (3.2–4.0)
P-trend		0.39	0.18	0.17	0.91	0.39
Reduced fat milk[d]						
Q1 [0–0.43]	71	146 (114–186)	46.8 (38.0–57.5)	58.0 (54.8–61.3)	8.9 (7.9–10.0)	3.4 (3.1–3.7)
Q2 [0.51–1.08]	56	149 (118–188)	50.2 (39.6–63.6)	57.8 (54.8–60.8)	8.4 (7.2–9.6)	3.4 (3.0–3.7)
Q3 [1.14–7]	62	124 (94–163)	39.3 (30.5–50.6)	59.3 (55.6–63.0)	8.5 (7.3–10.0)	3.6 (3.1–4.0)
P-trend		0.34	0.24	0.57	0.70	0.60
Yogurt[c]						
Q1 [0–0.08]	64	148 (115–191)	47.3 (37.0–60.5)	56.8 (53.0–60.6)	8.0 (7.0–9.0)	3.5 (3.1–3.9)
Q2 [0.14–0.28]	61	108 (83–141)	37.1 (29.7–46.4)	59.1 (55.8–62.5)	8.9 (7.8–10.0)	3.3 (3.0–3.7)
Q3 [0.30–5.08]	64	166 (128–215)	51.8 (41.1–65.3)	59.2 (55.3–63.2)	9.0 (7.6–10.4)	3.5 (3.1–3.9)
P-trend		0.19	0.26	0.56	0.43	0.75

Dairy foods are presented in descending order of intake within high-fat/low-fat groupings.
[a]Adjusted for age, abstinence time, race, smoking status, BMI, recruitment period, moderate-to-intense exercise, TV watching, alcohol intake, prudent and western dietary patterns and total calorie intake.
[b]Includes cream cheese, cottage cheese and other cheese.
[c]Includes frozen yogurt, plain yogurt and flavored yogurt.
[d]Includes skim milk and 1 and 2% milk.
[e]Additionally adjusted for time from current ejaculation to start of semen analysis.
*P-value for trend <0.05 compared with men in the lowest tertile of intake.

The association between dairy and sperm morphology was stronger for intake of full-fat dairy foods than for intake of low-fat dairy (Table II). The adjusted difference in normal sperm morphology (95% confidence intervals) was −3.2% (−4.5 to −1.8) between men in the upper half (1.22 to 7.51 servings/day) and those in the lower half (0 to 1.16 servings/day) of full-fat dairy intake ($P < 0.0001$). The same contrast for low-fat dairy was −1.3% unit (−2.7 to −0.07; $P = 0.06$). Full-fat dairy intake was also associated with lower progressive motility. Compared with men in the lower half of full-fat dairy intake, those in the upper half of full-fat dairy intake had 6.0% (1.5 to 10.4; $P = 0.009$) lower percent progressively motile sperm. The results for total motility closely mirrored the results for progressive motility. In addition, there were inverse associations of full-fat dairy intake with total sperm count (P-trend = 0.08) and concentration (P-trend = 0.08) that did not reach conventional levels of statistical significance. Further adjustment for intake of major types of fat (saturated, mono-unsaturated, poly-unsaturated and *trans*) and protein did not change the results (Supplementary data, Table SI). No other significant relations were identified.

We investigated which specific dairy foods were driving the association between dairy food groupings and sperm parameters (Table III). Intake of cheese was inversely related to sperm morphology ($P = 0.02$). Cheese was also the dairy food most strongly related to progressive motility ($P = 0.08$) while whole milk was the food most strongly related to sperm concentration albeit not significantly ($P = 0.18$). All other individual dairy foods (cream, skim/low-fat milk, yogurt and ice cream) were not associated with semen parameters (Table III). The results did not change when we adjusted for fat and protein intake (Supplementary data, Table SII).

To gain insights into the mechanisms underlying the observed associations, we investigated whether dairy foods were associated with reproductive hormone levels (Table IV). There was an association between higher intake of total dairy foods with higher FSH levels

Table IV Adjusted[a] mean values (95% CIs) of hormones according to intake of dairy foods.

	N	LH (IU/l)	FSH (IU/l)	E2 (pmol/l)	Testosterone (nmol/l)	Inhibin B (pg/ml)	SHBG (nmol/l)
Quartiles of total dairy food intake [range, servings/day]							
Q1 [0–1.65]	46	3.8 (3.4–4.3)	2.3 (2.0–2.7)	91.6 (81.8–101.4)	20.2 (17.9–22.5)	204.2 (182.9–225.5)	29.3 (26.2–32.4)
Q2 [1.67–2.96]	48	3.8 (3.4–4.3)	2.4 (2.1–2.7)	95.1 (87.7–102.6)	21.4 (19.0–23.8)	196.1 (181.9–210.2)	33.0 (28.7–37.3)
Q3 [2.98–4.29]	48	3.8 (3.4–4.2)	2.6 (2.3–3.0)	90.4 (84.5–96.4)	20.5 (19.9–22.1)	196.8 (180.2–213.5)	31.0 (27.8–34.2)
Q4 [4.33–13.26]	47	3.4 (3.0–3.8)	2.9 (2.4–3.5)	87.8 (81.0–94.6)	19.4 (17.4–21.4)	182.9 (164.1–201.7)	28.1 (24.9–31.4)
P-trend		0.17	0.05	0.32	0.39	0.16	0.28
Quartiles of full-fat dairy food intake[b] [range, servings/day]							
Q1 [0–0.71]	44	4.0 (3.5–4.4)	2.2 (1.9–2.6)	93.8 (84.3–103.2)	21.1 (18.6–23.6)	199.6 (177.8–221.4)	31.6 (28.0–35.1)
Q2 [0.73–1.16]	49	3.7 (3.3–4.1)	2.6 (2.3–3.0)	91.9 (84.9–99.0)	20.4 (18.6–22.3)	198.0 (183.7–212.3)	28.0 (25.2–30.7)
Q3 [1.22–1.94]	45	3.7 (3.3–4.1)	2.5 (2.2–2.8)	86.4 (81.0–91.9)	20.0 (17.9–22.0)	190.3 (172.6–208.0)	32.3 (28.1–36.5)
Q4 [2.00–7.51]	51	3.5 (3.1–4.0)	2.8 (2.3–3.3)	92.7 (85.5–99.9)	20.1 (18.2–22.0)	192.1 (175.4–208.9)	30.0 (27.0–32.9)
P-trend		0.21	0.20	0.94	0.60	0.61	0.83
Quartiles of low-fat dairy food intake[c] [range, servings/day]							
Q1 [0–0.51]	46	3.5 (3.1–3.9)	2.5 (2.1–3.0)	87.7 (78.7–96.7)	19.1 (16.9–21.2)	194.0 (173.6–214.4)	28.7 (25.6–31.8)
Q2 [0.56–1.04]	47	3.7 (3.4–4.1)	2.4 (2.1–2.8)	94.5 (86.4–102.2)	21.4 (18.8–23.9)	203.4 (186.0–220.8)	32.0 (27.8–36.2)
Q3 [1.08–2.6]	49	4.0 (3.6–4.5)	2.5 (2.2–2.9)	96.3 (89.9–102.6)	21.5 (19.8–23.1)	189.3 (174.8–203.9)	32.9 (29.7–36.0)
Q4 [2.7–12.08]	47	3.5 (3.1–4.0)	2.7 (2.3–3.1)	86.3 (79.5–93.1)	19.6 (17.5–21.7)	193.3 (175.5–211.1)	27.8 (24.6–30.9)
P-trend		1.00	0.56	0.51	0.88	0.69	0.43
	N	**Testosterone/LH**	**FT/LH**	**E2/testosterone**	**Inhibin B/FSH**		
Quartiles of total dairy food intake [range, servings/day]							
Q1 [0–1.65]	46	5.8 (5.0–6.7)	139.5 (118.9–160.1)	4.8 (4.3–5.2)	116.0 (84.8–147.3)		
Q2 [1.67–2.96]	48	6.1 (5.2–7.1)	137.3 (115.0–159.6)	4.8 (4.3–5.2)	91.9 (75.7–108.2)		
Q3 [2.98–4.29]	48	6.5 (5.5–7.5)	150.4 (127.9–172.9)	4.6 (4.3–4.9)	91.5 (72.2–110.8)		
Q4 [4.33–13.26]	47	6.6 (5.6–7.5)	157.4 (135.7–179.1)	4.9 (4.4–5.3)	80.0 (57.2–102.8)		
P-trend		0.26	0.15	0.84	0.10		
Quartiles of full-fat dairy food intake[b] [range, servings/day]							
Q1 [0–0.71]	44	5.8 (4.8–6.7)	131.5 (112.0–151.0)	4.7 (4.3–5.21)	108.3 (80.8–135.9)		
Q2 [0.73–1.16]	49	6.2 (5.4–7.0)	153.6 (132.6–174.7)	4.8 (4.4–5.3)	90.4 (69.2–111.5)		
Q3 [1.22–1.94]	45	6.1 (5.2–7.0)	136.7 (117.9–155.4)	4.5 (4.2–4.8)	92.2 (72.8–111.6)		
Q4 [2.00–7.51]	51	6.8 (5.8–7.8)	160.0 (137.2–182.7)	5.0 (4.5–5.4)	89.4 (67.9–111.0)		
P-trend		0.16	0.14	0.35	0.48		

Continued

Table IV *Continued*

	N	Testosterone/LH	FT/LH	E2/testosterone	Inhibin B/FSH
Quartiles of low-fat dairy food intake[c] [range, servings/day]					
Q1 [0–0.51]	46	6.1 (5.2–7.0)	145.0 (123.4–166.7)	4.9 (4.5–5.4)	103.2 (73.2–133.3)
Q2 [0.56–1.04]	47	6.2 (5.3–7.1)	141.1 (122.3–159.8)	4.7 (4.3–5.1)	99.5 (78.2–120.9)
Q3 [1.08–2.6]	49	6.2 (5.3–7.1)	141.1 (119.7–162.5)	4.7 (4.4–5.1)	88.7 (70.4–107.0)
Q4 [2.7–12.08]	47	6.7 (5.5–7.6)	157.6 (134.5–180.8)	4.7 (4.2–5.1)	87.9 (66.6–109.2)
P-trend		0.50	0.36	0.47	0.37

BMI, body mass index; CI, confidence interval; LH, luteinizing hormone; FSH, follicle-stimulating hormone; SHBG, sex hormone-binding globulin; testosterone/LH, ratio of testosterone (nmol/l) to LH (IU); FT/LH, ratio of calculated free testosterone (pmol/l) to LH (IU); E2/testosterone, ratio of E2 (pmol/l) to testosterone (nmol/l); inhibin B/FSH, ratio of inhibin B (pg/ml) to FSH (IU).
[a]Adjusted for age, race, smoking status, BMI, recruitment period, moderate-to-intense exercise, TV watching, alcohol intake, prudent and western dietary patterns, total calorie intake and hour of blood sampling.
[b]Includes cream cheese and other cheese.
[c]Includes frozen yogurt, plain yogurt and flavored yogurt.
*P-value for trend <0.05 compared with men in the lowest tertile of intake.

(*P*-trend = 0.05). This association was stronger among men with at least one semen analysis abnormality ($n = 50$; *P*-trend = 0.003) than among men with no semen analysis abnormality ($n = 139$; *P*-trend = 0.61, Fig. 2). We did not find evidence of an association between dairy foods and any other of the measured hormones (Table IV).

Lastly, we examined whether the association of dairy foods with semen quality differed by smoking, BMI or history of reproductive disorders. Among men with no history of reproductive disorders, full-fat dairy intake was related to lower total sperm count ($P = 0.05$), sperm concentration ($P = 0.02$), progressive motility ($P = 0.02$) and morphology ($p = 0.004$) (Supplementary data, Table SIII). Among men with at least one reproductive disorder, full-fat dairy intake, was related to lower sperm morphologically ($P = 0.02$). Formal tests of heterogeneity, however, suggested that the apparent differences between these groups were not statistically significant. A similar pattern was observed for cheese intake (Supplementary data, Table SIV). BMI and smoking did not modify the observed relations.

Discussion

We evaluated the association between dairy food intake and semen parameters in a cohort of young, physically active men and found that dairy food intake was inversely related to sperm morphology and progressive motility. This association was stronger for full-fat dairy foods, particularly cheese. Full-fat dairy intake of men in the top quartile (consuming 2.00–7.51 servings/day) was within the USDA healthy plate recommended intake of dairy foods of 3 servings/day (USDA and U.S. Department of Health and Human Services, 2010).

Since a population-wide increase in dairy food intake (USDA, 2003) has coincided with a secular decline in semen quality (Swan, 2000; Rolland *et al.*, 2013), the primary motivation of this study was to evaluate, at the level of individuals, whether consumption of dairy foods was associated with semen quality. Our finding of a strong inverse association between full-fat dairy foods and lower sperm morphology is consistent with the hypothesis that dairy foods may have contributed to a secular decline in sperm morphology (Rolland *et al.*, 2013). One limitation of our study to examine this hypothesis is that, while intake of full-fat dairy has increased in the USA and that the range of dairy intake in our population is particularly wide, intake of whole milk (the dairy food most strongly related to lower concentration) has decreased (USDA, 2003) limiting the observed range of intake in this population. It would be important to follow-up on this finding in populations with a wider range of whole milk intake.

We found that intake of full-fat dairy was inversely related to sperm motility and morphology. These associations were driven primarily by intake of cheese and were independent of overall food choices as captured by dietary patterns. While it is not possible to identify the underlying mechanism linking dairy food intake to lower sperm motility and morphology, our findings are not consistent with our initial hypothesis of an estrogenic effect of dairy. Commercial milk is a mixture of milk from cows at different stages of pregnancy and non-pregnant cows (Daxenberger *et al.*, 2001; Davaasambuu *et al.*, 2004) with ~75% of the mixture coming from pregnant cows. Naturally occurring estrogens of placental origin are present in the milk obtained from pregnant cows (Pape-Zambito *et al.*, 2008; Maruyama *et al.*, 2010). In the USA, dairy cows, unlike cattle for meat production, are not administered exogenous growth-promoting sex hormones (Andersson and Skakkebaek, 1999). In

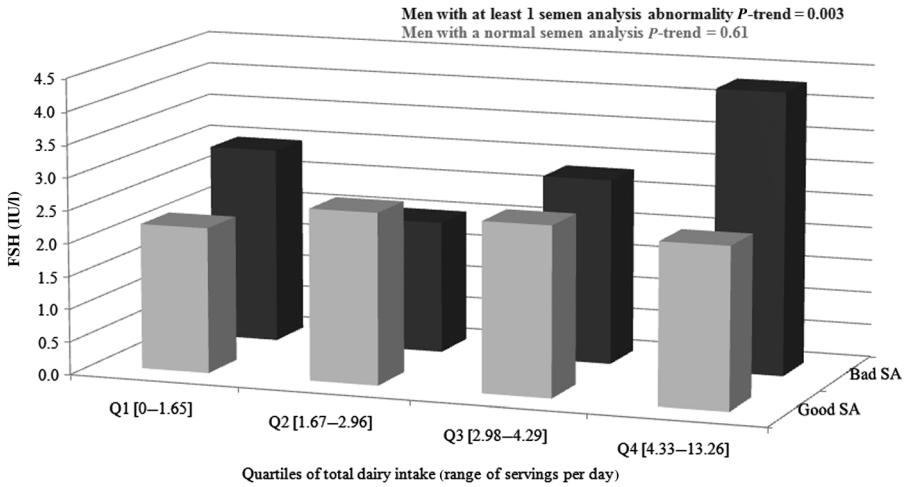

Figure 2 Adjusted mean values of FSH levels associated with total dairy food intake. Adjusted for age, race, smoking status, BMI, recruitment period, moderate-to-intense exercise, TV watching, alcohol intake, prudent and western diary patterns, total calorie intake and hour of blood sampling. SA = semen analysis. $P = 0.09$ for interaction between total diary intake and semen abnormality.

theory, estrogens derived from dairy (or other food sources) could contribute to a negative feedback loop on LH and FSH ultimately decreasing sperm production. Instead, our findings on the relation between dairy intake and reproductive hormones suggest that dairy intake may be implicated in direct testicular damage. Dairy food intake was positively related to FSH levels and this association was strongest among men with at least one abnormality in the semen analysis. While this may be a chance finding, a plausible alternative explanation could be that the presence of environmental contaminants in dairy such as pesticides and chlorinated pollutants (Schaum *et al.*, 2003), which have been associated with lower sperm quality (Rozati *et al.*, 2002; Meeker and Hauser, 2010) and with higher FSH levels (Aguilar-Garduño *et al.*, 2012) may be responsible for the observed relations. Further research is necessary to clarify the biology underlying the observed associations.

Studies investigating dairy intake and men's reproductive potential are scarce. In a case–control study comparing dietary habits of oligoasthenoteratospermic versus normospermic fertility clinic patients in Spain, Mendiola *et al.* (2009) observed that cases had higher intakes of full-fat dairy products (yogurt, whole milk, cheese and semi-skimmed milk) and lower intakes of skimmed milk than controls. However, because semen quality was dichotomized as poor versus normal, it is not possible to identify which of the individual semen parameters (or if multiple parameters) was driving the association. In a different case–control study of asthenozoospermic men in Iran, the odds of asthenozoospermia were marginally significantly higher with intake of total dairy products (P-trend = 0.06), but significantly lower with intake of skim milk (P-trend = 0.02) (Eslamian *et al.*, 2012). A third cross-sectional study, however, found that dairy intake was unrelated to semen quality among men attending a fertility clinic in the Netherlands (Vujkovic *et al.*, 2009).

While this study contributes to the emergent literature on this topic, it does have several limitations. As it was a cross-sectional study, we cannot determine causality of the observed associations. However, we were able to adjust for multiple important determinants of semen quality such as BMI, abstinence time, hours per week of physical activity and TV watching and alcohol intake. More importantly, since participants were unaware of their fertility potential, it is unlikely that they would have made any changes to their diet based on knowledge of their semen quality; a sharp contrast with the existing literature which is limited to men presenting at fertility centers. Secondly, although our population was homogenous and increased our study's internal validity, these findings may not generalize to a clinical population of subfertile men. For example, these men were very physically active, spending, on average 10 h/week on moderate-to-vigorous activities, whereas 52% of USA men do not meet the recommendation of engaging in 2.5 h/week of moderate physical activity (Macera, *et al.*, 2005). Thirdly, we cannot exclude the possibility that residual confounding was driving the association of full-fat dairy with semen quality parameters. However, we collected data on known predictors of semen quality and adjusted our results for these factors. Results were independent of prudent and western dietary patterns and intakes of alcohol and major types of fat and protein. Strengths of the study are the use of a previously validated diet questionnaire (Rimm *et al.*, 1992), the physical examination and the wide dairy food intake range observed in this population which allowed us to make more extreme comparisons than in the existing literature.

In summary, we examined the relation between dairy intake and semen quality in a population of physically active young men and found that full-fat dairy foods were inversely related to sperm progressive motility and morphology. Given the paucity of literature on this topic, it is important that this relation is further examined, ideally in prospective studies and randomized trials.

Supplementary data

Supplementary data are available at http://humrep.oxfordjournals.org/.

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Authors' roles

S.H.S. was involved in study concept and design. J.M., J.E.C., S.H.S. and N.J. contributed to the acquisition of data. M.A. performed statistical analysis. M.A., P.L.W., N.J., A.J.G., J.E.C. and S.H.S. contributed to the analysis and interpretation of the data. M.A. and J.E.C. drafted the manuscript. M.A., J.M., P.L.W., N.J., A.J.G., S.H.S. and J.E.C. were involved in a critical revision of the manuscript for important intellectual content.

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Conflict of interest

The authors have no financial relationships relevant to this article to disclose.

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