Within-person variation in the postprandial lipemic response of healthy adults¹–³

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ABSTRACT

Background: The response to dietary fat plays a key role in metabolic health. Although this can vary widely between individuals, variation within an individual and the associated contribution of phenotypic and genotypic factors to this variation are less defined.

Objectives: The objectives were to quantify within-person variation in triacylglycerol response by means of a novel variation score (Sv) and to explore the phenotypic and genotypic factors associated with this score.

Design: Two consecutive 5-h oral-lipid-tolerance tests (OLTTs) were conducted in 51 healthy adults aged 18–60 y with a BMI (in kg/m²) of 18.5 to 49.8. Detailed body composition, physical function, biochemistry, and genotype data were gathered.

Results: The postprandial triacylglycerol response profile did not differ (P = 0.64) across OLTTs for the group; nor did average concentrations of functional markers apolipoprotein C2 (P = 0.73) and apolipoprotein C3 (P = 0.74). Sv was low in most (82%) of the adults and was significantly (P < 0.05) associated with age, fasting triacylglycerol, triacylglycerol AUC, and fasting nonessential fatty acids. Significant associations were also observed between Sv and single nucleotide polymorphisms in 7 genes (APOA1, IL1α, IL1β, TLR4, TCF7L2, CCK1Rec, and STAT3) after correction for phenotypic differences.

Conclusions: This work showed that the within-person variability in postprandial lipemic response is low in most healthy adults. It also showed that variability in this response is associated with a defined set of phenotypic and genotypic characteristics. This trial was registered at clinicaltrials.gov as NCT01172951. Am J Clin Nutr 2013;97:261–7.

INTRODUCTION

Disturbed lipid metabolism in response to diet is implicated in the development of many key metabolic diseases (1–5). Endeavors to ameliorate the situation through the use of standardized dietary interventions appear thwarted, however, with research indicating that the lipemic response to a given food can vary hugely from person to person, even within an apparently similar group of individuals (6–9). Meanwhile, within-person variation is suggested to be much lower (7). Robust knowledge on reproducibility within the area of postprandial lipid metabolism is generally lacking because of small cohort sizes (7, 10–12). Quantifying variation in the lipemic response to dietary intake within and between individuals and elucidating where variation is greatest are essential if diets are to be successfully targeted to the specific needs of an individual or group, which minimizes disease risk—a primary goal of personalized nutrition.

A great deal of our understanding of the link between lipids, lipid metabolism, and disease is based on fasting measures. In Westernized countries where individuals typically eat 5 times daily, most of the time is spent in a nonfasting, postprandial state during which lipoprotein composition and concentrations vary. Measuring these variations by means of an oral-lipid-tolerance test (OLTT)⁴ can determine the efficiency with which the individual uses lipid components such as triacylglycerol, which accumulate during the postprandial phase in a bid to return to homeostasis. This is important if early metabolic disturbances predictive of disease are to be identified. Lipemic response between people is typically greater postprandially than at fasting, because metabolic disturbance may become apparent only when metabolism is stressed (6, 13). Lipemic spikes after a high-fat dietary intake are known to adversely affect several areas of metabolic regulation (14, 15), and elevated triacylglycerol concentrations, both fasting and postprandially, present an independent cardiovascular disease risk factor (1, 16). Moreover, postprandial hyperlipidemia and premature coronary artery disease have been observed in individuals with clinically “normal” fasting lipemia (17), which adds further weight to the importance of postprandial assessment.

Lipemic response is complex and is known to be influenced by many environmental and genetic factors (5). Literature links aspects of an individual’s phenotype with adverse lipid response profiles such as advancing age, being male, and overweight (18–21), and biochemical variables such as elevated fasting triacylglycerol and insulin concentrations are known to affect response profile (21–24).

Single nucleotide polymorphisms (SNPs) in a number of candidate genes are established modulators of postprandial lipid metabolism.

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⁴Abbreviations used: OLTT, oral-lipid-tolerance test; SNP, single nucleotide polymorphism; Sv, variability score.

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Exploration of the genetic association with variability in this lipemic response itself is now warranted, as is assessment of the relative contribution of genotype and phenotype to this response. The current study aimed to quantify within-person variation in lipemic response to a repeated OLTT using a scoring system and to explore the contribution of phenotype and genotype to this variation.

SUBJECTS AND METHODS

Recruitment

Ethical approval for this study was obtained from the local university (University College Dublin) and hospital (St Vincent’s University Hospital) Human Research Ethics committees. Adults aged 18–60 y with good general health were recruited between 2008 and 2010 and provided their informed written consent. Good health was defined as the absence of a known chronic or infectious disease and supported by a series of fasting blood tests. Individuals with a BMI (in kg/m²) <18.5, a low hemoglobin concentration (<12 g/dL), an elevated fasting plasma glucose concentration (≥7 mmol/L), an elevated cholesterol concentration (>7.5 mmol/L), an elevated triglyceride concentration (>3.8 mmol/L), or elevated enzyme indicators of liver or kidney function—any of which warranted pharmaceutical treatment—were excluded. Body weight (kg), height (m), and hip and waist circumferences (cm) were recorded.

Study design

The sample used in the study was drawn from a larger clinical registered study of 214 individuals investigating the metabolic effect of oral lipid and oral glucose postprandial test challenges. This sub-study included only individuals who had been randomly assigned to a group undergoing an OLTT on 2 occasions (n = 65) and for whom a complete data set for all variables tested had been collected (n = 51). A third and final test visit consisted of a series of physical fitness and function tests conducted at the University College Dublin Institute for Sport and Health.

OLTT

Participants arrived at the clinical research unit at 0800 after fasting overnight. An indwelling cannula was placed in the forearm. A total of 18 mL blood was drawn into EDTA-coated and lithium-heparin–containing tubes for plasma isolation, and 6 mL was drawn into an evacuated tube for serum isolation. Serum samples were allowed to clot for 30 min at room temperature. EDTA and lithium heparin tubes were placed directly on ice. Participants consumed the test drink within 5 min after initial blood sampling. Subsequent samples were then taken at hourly intervals over 5 h, centrifuged (1800 × g, 15 min), and stored at −80°C. Participants remained resting throughout and were asked not to eat or drink anything except water, which was given in 50-mL quantities each hour. The OLTT given consisted of a mixture of 100 mL Calogen (Numico, Ireland) and 50 mL Liquid Duocal (SHS Nutrition) providing 533 kcal energy, 54 g fat (7 g SFAs, 31 g MUFAs, and 16 g PUFAs), and 11 g carbohydrate, given in the form of a blended drink.

Anthropometric, physical fitness, and function testing

On arrival and after fasting overnight, the subjects’ body weights were measured to within 0.1 kg on a flatbed-weighing scale and heights to within 0.1 cm with a wall-mounted stadiometer. Resting metabolic rate was measured by indirect calorimetry with the use of a ventilated-hood system (Cosmed Quark B2; H Evans) in accordance with best practice guidelines (27). Fat mass, fat-free mass, and bone mineral density were assessed at both a whole-body and segmental level by using a combination of dual-energy X-ray absorptiometry scanning (Lunar iDXA; GE Health Care) and air-displacement plethysmography (BOD-POD GS system) in accordance with the manufacturer’s instructions. After these measurements were completed, the participants received a standard breakfast (~550 kcal, 5 g fat, 108 g carbohydrate, and 72 g protein). Hand and forearm muscular strength were then measured to the nearest 1 kg with a hand hydraulic dynamometer (Sh5001; Saehan Corp). Knee extensor and flexor muscle force, a measure of lower body muscle strength, were analyzed by using an isokinetic dynamometer (Cybex NORM system, HUMAC, software V.9.1.67) set at an angular velocity of 60 degrees/s, as detailed elsewhere (28). Aerobic fitness was assessed by using a randomized load submaximal oxygen consumption test by using an electronically braked cycle ergometer (Ergoline 500; Bosch). Blood pressure was measured to the nearest 1 mm Hg (Omron Intellisense) while the subjects were sitting.

Biochemical analysis

Plasma biochemistry values were assessed by using a clinical bioanalyzer (Rx Daytona; Randox Laboratories). Details of the methods used are as follows: triacylglycerol (lipase/glycerol kinase colorimetric); total cholesterol (cholesterol oxidase); HDL (direct clearance); glucose (glucose oxidase); apolipoprotein A-I, apolipoprotein B, apolipoprotein C2, apolipoprotein C3, and apolipoprotein E (immunoturbidimetric); nonesterified fatty acids (colorimetric); and C-reactive protein (immunoturbidimetric). Concentrations of cytokines and hormones (TNF-α, IL-6, leptin, insulin, C-peptide, and resistin) in plasma specimens were measured by using a biochip array system (Evidence Investigator; Randox laboratories). Specimens were run in duplicate, and the concentration of each cytokine was calculated from a calibration curve. Standard quality-control procedures were followed on both test systems to ensure data integrity. The HOMA-IR was used as a proxy estimate of insulin sensitivity and was calculated as fasting insulin concentration (µU/mL) × fasting glucose concentration (mmol/L)/22.5.

Genotyping

Buffy coat was collected from EDTA-containing evacuated tube tubes after removal of plasma and was stored at −80°C. An autopure system was used to extract DNA from frozen samples by using a Gentra Autopure LS robotic workstation (Qiagen) to give a purified sample in a rehydration volume of 100 µL in Tris/EDTA buffer. DNA purity was assessed by ratio 260/280, and the DNA was quantified by using a Nanodrop system conducted in duplicate. Values with a CV <10% across duplicate samples were retained for further analysis. SNP genotyping was then conducted by Khbiosciences (www.khbioscience.co.uk) by using their proprietary KASPar polymerase chain reaction technique. A total of 129 SNPs from 82 candidate genes, some of known associations with lipoprotein metabolism, were assessed.

Statistical analysis

The analysis was conducted by using the statistical packages PASW for Windows, V.18.0.0 (SPSS), GraphPad Prism 5, and
RESULTS

An approximately equal number of males and females (n = 25 and 26, respectively) were assessed. No significant differences in age, race, BMI, body composition, physical function, or fasting metabolic profile were found between the group of 51 individuals selected for the current analysis and the rest of the larger cohort (n = 163). Participants had a mean (±SD) age of 29.9 ± 11.9 y, body weight of 72.4 ± 11.9 kg, and BMI of 24.5 ± 2.7. Most (92%) of the individuals were European-white. OLTTs were conducted an average of 25 d apart, and most (78%) were conducted within one calendar month.

Plasma triacylglycerol response

Repeated-measures ANOVA showed that triacylglycerol concentrations significantly (P < 0.0005) increased after ingestion of both OLTTs; however, no difference in fasting triacylglycerol (P = 0.13), average triacylglycerol concentration over 300 min (P = 0.13), or time × visit interaction (P = 0.64; Figure 1) were observed between visits. Similarly, neither the peak triacylglycerol concentration attained (P = 0.67) nor the triacylglycerol AUC differed across visits (P = 0.67 and P = 0.83, respectively). Plasma concentrations of apolipoprotein C2 and apolipoprotein C3, surrogate functional markers of postprandial triacylglycerol metabolism, also did not differ significantly across tests (apolipoprotein C2, P = 0.73; apolipoprotein C3, P = 0.74) (Figure 1). The fasting triacylglycerol concentration correlated with both fasting concentrations of apolipoprotein markers (apolipoprotein C2: r = 0.43 P = 0.002; apolipoprotein C3: r = 0.48, P = 0.001) and with peak triacylglycerol concentrations attained during the OLTTs (r = 0.78, P = 0.001). To further examine the stability of the triacylglycerol measurements between visits, Sv was assessed as a generalized measure of the cross-visit difference in OLTT response. Within the cohort there was a high positive skew, and cluster analysis indicated that most individuals (82%) exhibited a low Sv (<3.48), with a wide range from 0.0016 (almost no variation) to 10.36 mmol/L (high intraindividual variation; Figure 2). Given this skewed distribution, a natural log transformation was applied. Sv was correlated (r = 0.45, P = 0.001) with the triacylglycerol AUC. Within- and between-visit variances in triacylglycerol concentration were compared to assess whether most of the variability arose from variation between individuals or from visit to visit. At 180 min (closest to the average time of peak triacylglycerol concentration), no significant difference (F[100, 50] = 1.201, P = 0.24) was found. No visit effect was detected at any time point during the OLTTs.

Phenotypic variables were associated with Sv

The relation between Sv and phenotypic variables was assessed by using regression analysis. Age was significantly associated in a negative manner with Sv (Table 1). The plasma fasting and peak triacylglycerol concentration and triacylglycerol AUC were positively associated with Sv, whereas the fasting nonesterified fatty acid concentration was negatively associated (Table 2). No other significant difference in markers of body composition, body strength, fitness, insulin sensitivity, or biochemical, hormonal, or inflammatory profile was observed.

Association between SNP genotype and Sv

In terms of defining the relative contribution of genetic compared with physiologic metrics on postprandial triacylglycerol variation, a detailed SNP association was performed on Sv, as a quantitative trait. Linear regression analysis including adjustments for age, sex, fasting triacylglycerol concentration, and waist circumference as covariates demonstrated important associations.
between $S_v$ and SNPs in 7 genes: APOA1, IL1α, IL1β, TLR4, TCF7L2, CCK1Rec, and STAT3 ($P < 0.05$) for the whole group (Table 3). All results for genes demonstrating a $P$ value $<0.1$ for any SNP associated with that gene are listed elsewhere (see Supplemental Table S1 under “Supplemental data” in the online issue).

**DISCUSSION**

The primary hypothesis that this study addressed was that within-person variability in the response of plasma triacylglycerol to a repeated high intake of dietary fat is low. This hypothesis was prompted by a previous study of 3 individuals (7). The current work with 51 individuals clearly upholds this hypothesis, which indicates that for most metabolically healthy adults, lipemic response to a high intake of dietary fat is reproducible, given that no difference in the response of plasma triacylglycerol, apolipoprotein C2, or apolipoprotein C3, surrogate functional markers of triacylglycerol metabolism, was observed across meal challenges for the group. However, the use of a constructed variability score ($S_v$) in this study allowed triacylglycerol response reproducibility to be viewed at an individual level, which also indicated that a minority of subjects ($n = 9$) deviated from this norm, which suggests that a small subset of this healthy population could yield further biological insight into a dynamic triacylglycerol response to an OLTT.

Robust knowledge on the reproducibility of the lipemic response to a fat load has been lacking to date because of limited cohort sizes, wherein the average study sample equals 10 (7, 10–12). Identifying reproducible and measurable phenotypes in healthy individuals has the potential to predict lipid-related disease patterns. The current regression analysis showed that triacylglycerol (fasting, peak, and AUC values) was positively associated with within-person variability in postprandial triacylglycerol ($S_v$). The plasma triacylglycerol concentration is known to rise steadily through the day after repeated intake of fat from various meals, with concentrations typically reaching their maximum after midnight (1). Apolipoproteins such as apolipoprotein C2 and apolipoprotein C3 affect this rise primarily through their influence on lipoprotein lipase (29–32). The average response of all 3 of these predictive disease markers was found to be highly reproducible across OLTTs, despite tests being conducted several weeks apart. Whether or not a variable response to an identical fat load is truly a problem in terms of future metabolic health for an individual is uncertain. The fasting triacylglycerol concentration correlated with postprandial peak triacylglycerol, with those with higher fasting concentrations showed higher peaks and more variable triacylglycerol responses. This potential of fasting triacylglycerol to predict postprandial response was previously reported (21, 22).

A sustained elevation in triacylglycerol concentration, whether fasting (33) or postprandially (34, 35), represents one of the most powerful biomarkers of cardiovascular disease risk. Although the number of days between visits did not statistically relate to $S_v$, a change in diet between OLTTs (ie, to one of lower fat) could not be ruled out. A recent review (1) refers to a “second meal effect,” wherein triacylglycerol concentrations during an OLTT are influenced by the macronutrient composition of the previous meal (36). The results of the current study do not support this given that average triacylglycerol concentrations were almost identical for most of the individuals, despite the meals not being standardized on the evenings before
testing. Age was negatively associated with the variable triacylglycerol response in the current study. Age was previously shown to independently influence the magnitude of triacylglycerol response after a fat load (21). Overweight and increased abdominal fat have also been implicated in the elevated postprandial triacylglycerol response (18, 19). However, in the current study, BMI per se did not influence response reproducibility, similar to the finding of Weiss et al (10), who reported the triacylglycerol response to be reproducible in lean and obese individuals alike.

A significant body of literature currently exists on the effect of gene-diet and gene-environment interactions on lipemic response (8, 26, 37). Meanwhile, the potential for a genetic driver of consistency in this response per se has not been explored to our knowledge. SNPs in a wide range of genes were included in the current study to test for potential novel associations. Significant associations with the triacylglycerol variability score were found in genes thought to predispose individuals to the metabolic syndrome or type 2 diabetes. APOA1 gene polymorphisms (rs2727784) are well known to disturb lipid metabolism (8, 26), and the current study suggests that such polymorphisms may also influence the consistency of this disturbance across eating occasions. The IL-1 pathway is an important mediator of inflammatory reactions with polymorphisms in known adiposity-sensitive genes may modify the reproducibility of response to a high intake of dietary fat across eating occasions. Identifying these variants is necessary for a more complete understanding of the development of lipid-related disease. Given that the primary objective of this study was to quantify lipemic response variation, for which the sample size is sufficient, it is acknowledged that the numbers are small for thorough genetic assessment, with conclusions on associations limited to SNPs on individual genes. The current

<table>
<thead>
<tr>
<th>Table 1</th>
<th>Associations between phenotypic characteristics of the group (n = 51) and S(2,3)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Characteristic</td>
<td>Mean ± SD</td>
</tr>
<tr>
<td>Sex</td>
<td>-0.016</td>
</tr>
<tr>
<td>Age (y)</td>
<td>0.001</td>
</tr>
<tr>
<td>Weight (kg)</td>
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</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>0.009</td>
</tr>
<tr>
<td>Waist circumference (cm)</td>
<td>0.001</td>
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<tr>
<td>Waist-to-hip ratio</td>
<td>2.529</td>
</tr>
<tr>
<td>Total body fat (%)</td>
<td>0.022</td>
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<tr>
<td>Trunk fat mass (%)</td>
<td>0.033</td>
</tr>
<tr>
<td>Leg fat mass (%)</td>
<td>0.006</td>
</tr>
<tr>
<td>Total bone mineral density (g/cm²)</td>
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</tr>
<tr>
<td>Trunk bone mineral density (g/cm³)</td>
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</tr>
<tr>
<td>Body density (kg/L)</td>
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</tr>
<tr>
<td>Resting metabolic rate (kcal/kg FFM)</td>
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</tr>
<tr>
<td>Peak handgrip strength (kg)</td>
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</tr>
<tr>
<td>VO₂ max (mL · kg FFM⁻¹ · min⁻¹)</td>
<td>0.002</td>
</tr>
<tr>
<td>Systolic blood pressure (mm Hg)</td>
<td>0.014</td>
</tr>
<tr>
<td>Diastolic blood pressure (mm Hg)</td>
<td>0.013</td>
</tr>
</tbody>
</table>

1 FFM, fat-free mass; Sv, triacylglycerol variability score; VO₂, volume of oxygen consumed.
2 Statistically significant association (P < 0.05) from linear regression analysis.

Table 2 | Associations between fasting biological characteristics of the group (n = 51) and S(2,3) |
<table>
<thead>
<tr>
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<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Characteristic</td>
<td>Mean ± SD</td>
</tr>
<tr>
<td>TAG (mmol/L)²</td>
<td>0.91 ± 0.29</td>
</tr>
<tr>
<td>Peak TAG (mmol/L)²</td>
<td>1.54 ± 0.50</td>
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<tr>
<td>TAG AUC (mmol/L)²</td>
<td>96.6 ± 54.4</td>
</tr>
<tr>
<td>NEFA (mmol/L)²</td>
<td>0.58 ± 0.30</td>
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<tr>
<td>Total cholesterol (mmol/L)</td>
<td>4.44 ± 0.99</td>
</tr>
<tr>
<td>HDL cholesterol (mmol/L)</td>
<td>1.59 ± 0.40</td>
</tr>
<tr>
<td>Apolipoprotein A-I (mg/dL)</td>
<td>133.1 ± 29.2</td>
</tr>
<tr>
<td>Apolipoprotein B (mg/dL)</td>
<td>74.3 ± 19.6</td>
</tr>
<tr>
<td>Apolipoprotein C2 (mg/dL)</td>
<td>3.6 ± 1.3</td>
</tr>
<tr>
<td>Apolipoprotein C3 (mg/dL)</td>
<td>6.7 ± 2.4</td>
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<tr>
<td>Apolipoprotein E (mg/dL)</td>
<td>2.0 ± 0.8</td>
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<tr>
<td>Glucose (mmol/L)</td>
<td>5.09 ± 0.41</td>
</tr>
<tr>
<td>High-sensitivity CRP (mg/L)</td>
<td>1.23 ± 0.63</td>
</tr>
<tr>
<td>Insulin (µIU/mL)</td>
<td>6.43 ± 2.91</td>
</tr>
<tr>
<td>C-peptide (ng/mL)</td>
<td>1.88 ± 1.13</td>
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<tr>
<td>HOMA-IR index</td>
<td>1.47 ± 0.69</td>
</tr>
<tr>
<td>Resistin (ng/mL)</td>
<td>4.47 ± 1.95</td>
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<tr>
<td>Leptin (ng/mL)</td>
<td>0.85 ± 3.2</td>
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<tr>
<td>IL-6 (pg/mL)</td>
<td>1.67 ± 1.94</td>
</tr>
</tbody>
</table>

1 CRP, C-reactive protein; NEFA, nonessential fatty acid; Sv, triacylglycerol variability score; TAG, triacylglycerol.
2 Statistically significant association (P < 0.05) from linear regression analysis.
3 Nonfasting.
results are instead intended for use as a discovery sample to identify potential candidate genes that may influence the consistency of the lipemic response and aid disease pattern prediction to repeatedly high intakes of dietary fat. Replication of associations with the SNPs reported here should be attempted as part of larger independent samples and pooled data sets, which require a focus on more stringent tests of the genes identified to remove potential false-positive findings. Testing for interactions between the SNPs reported here should also be conducted, because the detection of any significant interaction may indicate whether a joint effect of several SNPs or genes on triacylglycerol response variation has a greater effect than any one SNP individually.

The existing literature clearly indicates that the postprandial lipemic response, as typically measured by AUC, is associated with age and fasting plasma triacylglycerol concentrations. The current study showed that such phenotypic characteristics are also associated with the level of within-person variation in postprandial lipemic response—a response that is also associated with genotypic characteristics. Future research will now need to address whether a highly variable postprandial lipemic response is, in itself, detrimental to health and also to ascertain the true extent of genotype-by-phenotype interactions in this postprandial variation.

The authors’ responsibilities were as follows—MJG, LB, HMR, ERG, MFR, and MCW: designed the research; MFR, MCW, COG, and CM: conducted the research; MFR and RS: performed the statistical analysis; and MFR and MJG: wrote the manuscript; and MFR: had primary responsibility for the final content of the paper. All authors read and approved the final manuscript. The authors had no conflicts of interest.

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