ORIGINAL RESEARCH

Implications of Airway Resistance and Conductance on the Respiratory Rate in individuals With Various Nutritional States Exposed To Exercise

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Purpose: To determine how airway resistance (RAW) and airway conductance (GAW) affect inspiratory time (iT) and expiratory time (eT) in subjects with different nutritional states using the step test.

Methods: Forty-eight participants were recruited and divided into three groups: 16 normal weight (NW), 16 overweight (OW), and 16 obese (OB). A lung function test and anthropometric evaluation were performed. iT and eT were measured using a sonographic device before and after the step test.

Results: Body Mass Index (BMI) and Body Fat Percentage (BF%) were significantly higher (P<0.0001; P<0.0001, respectively) in OB group. RAW was significantly higher in the OB group (P=0.004), and GAW (P=0.004) was significantly lower in the same group. At rest, the Respiratory Rate (RR) was significantly higher in OB group (P<0.05), while iT and eT showed no significant differences. On the other hand, after the step test, eT was significantly lower ($P=0.016$), with the multiple linear regression model being the best predictor of post-exercise eT, including BF%/GAW and BF%/sGAW (explained variability of 15.7% and 14.6%, respectively).

Conclusion: Nutritional status can significantly impact lung function; at rest, there was a decrease in lung volumes and an increase in RR in OB subjects. In addition, there was a significant decrease in eT of OB subjects post-exercise. Finally, a significant relationship of BF%/GAW and BF%/sGAW with post-exercise eT was noted.

Keywords: airway resistance, airway conductance, respiratory time, nutritional states

Introduction

Obesity is a complex multifactorial chronic disease that is considered a worldwide epidemic. In 2012, the WHO estimated that 2.3 billion people were overweight (OW) and 700 million were obese (OB).^{[1](#page-8-0)} In Chile, the last National Health Survey (NHS) applied in 2016–2017 to a sample of 6,233 people aged 15 years and older showed that about 70% of the population had problems with their body weight. More specifically, it was observed that 39.8% had OW, and 31.2% were classified as OB.[2](#page-8-1)

Among the various disorders of obesity, excess adipose tissue causes a series of problems for the respiratory system. Here, proinflammatory cytokines and hormones have a direct effect on the airways. Leptin, tumor necrosis factor-alpha (TNF-a), and various interleukins (IL-6 and IL-8) have receptors in the walls of the airways, which, when activated, contribute to increased airway resistance (RAW) .^{[3](#page-8-2)} In addition, the distribution of fatty tissue in the abdomen and thorax

limits the action of the ventilatory muscles, 4 decreasing respiratory volumes and affecting radial traction on the airways.^{[5](#page-8-4)} This situation increases RAW and decreases airway conductance (GAW).

Although the direct impact of obesity on the respiratory system has been documented,^{[6,](#page-8-5)[7](#page-8-6)} there is a paucity of research on respiratory rate (RR) during exercise in the context of obesity. In this regard, it is known that OB subjects produce a compensatory mechanism, increasing RR and reaching up to 21 breaths per minute, almost 40% higher than in subjects with normal weight (NW).⁷ In addition, Chlif et al described obesity as also causing changes in the RR pattern, making it faster and shallower.⁸ Such changes in RR would inevitably be associated with a change in the inspiratory time (iT)/ expiratory time (eT) ratio; hence, it would be reasonable to know what happens to this ratio in obese people who exercise.

Obesity burdens the respiratory system, reducing functional residual capacity (FRC), which ultimately alters respiratory mechanics during exercise.^{[9](#page-8-8),10} One of the most critical obesity-induced changes in lung function is the reduction in end-expiratory lung volume (EELV) at rest and also during exercise. The adaptation of the EELV is an essential process of the ventilatory response to exercise, and its alteration would be reflected in the limitations of the respiratory system to cope with this "extra load" that exercise would involve.^{[11,](#page-8-10)[12](#page-8-11)}

In this light, it would be interesting to ascertain the responses of the respiratory system to an increased ventilatory workload and whether the morphological and functional changes of obesity would alter these. Therefore, it is hypothesized that the increase in RAW characteristic of obesity directly affects iT and eT, causing them to decrease. This study explored the implications of RAW and/or GAW on iT /eT post-exercise of subjects with different nutritional states.

Materials and Methods

This observational cross-sectional study was conducted between October and November 2019 in the Respiratory Function-Dysfunction Laboratory of the Kinesiology Department of the Universidad Católica del Maule.

Participants

The sample size (N) was calculated with a power of 95%, a significance level of 0.05, and an effect size of 2.1 using G*Power software version 3.1.9.4. The calculation of the effect size (Cohen's *d*) was carried out considering the difference between the respiratory cycle between normal weight and obese subjects in the study by Chlif et al 2009. According to the sample size calculation, a minimum of 13 participants are needed, taking into account a dropout rate of approximately 20% during the experimental trial, 3 more subjects will be added per group.⁸ To guarantee the significance of the data, 48 participants were evaluated: 16 NW, 16 OW, and 16 OB. The inclusion criteria were: i) adults between 18 and 40 years of age, ii) be from the city of Talca and iii) NW, OW, and O status according to their body fat percentage (BF%). The exclusion criteria were: i) acute or chronic respiratory diseases (Asthma, allergy and cystic fibrosis), ii) history of smoking, and iii) skin alterations (burns, scars, wounds, etc). that prevented the correct measurement of skin folds. This research was approved by the Scientific Ethics Committee of the University Catholic of Maule (File N° 59/ 2018). All participants read and signed the informed consent.

Measurements

The measurements were in two phases and on different days: i) on the first day, anthropometry and lung function were performed, lasting approximately 1 hour each. Here we sought to divide the sample into the different nutritional categories according to BF%. In addition, the pulmonary function evaluation was intended to rule out respiratory pathologies. And ii) on the second day, the iT and eT were measured at rest, the exercise test was performed and the iT and eT were measured after exercise. The iT and eT evaluations were randomized ([Figure 1](#page-2-0)).

First Phase

Anthropometry

The percentage of BF% was determined with the Siri equation: $BF\% = [(4.95/Density) - 4.5] \times 100$, and the density was obtained from the equation by Durnin and Womersly¹³ from the skin folds of the triceps, biceps, subscapularis and iliac crest). The skinfold measurements were taken according to the protocols of The International Society for the

Figure I Scheme of the method used. The two phases of the investigation are observed. The first phase was evaluation to classify the participants. And the second phase was the measurement of the inspiratory and expiratory time before and after exercise. **Abbreviations**: BF%, body fat percentage; MIP, Maximal inspiratory Pressure; MEP, maximal expiratory pressure; NW, normal weight; OW, overweight; OB, obese.

Advancement of Kinanthropometry $(ISAK)$,¹⁴ while nutritional status was classified as follows, female: NW: 20–30%, OW: 30–35% and OB: ≥35%. Male: NW: 10–20%, OW: 20–25% and OB: ≥25%.[15](#page-8-14)

Pulmonary Function

It was divided into spirometry, lung volumes and maximal inspiratory/expiratory pressure (MIP/MEP).

Spirometry

The spirometry was performed with a Mediagraphics plethysmograph (Platinum Elite DL® model, St. Paul, MN, USA). The highest Forced Vital Capacity (FVC) value of the 3 attempts that met the acceptability and reproducibility criteria established by the American Thoracic Society (ATS) was recorded. The variables used were FVC, Forced Expiratory Volume in the First Second (FEV₁), FEV₁/FVC, and Forced Expiratory Flow between 25 and 75% of Forced Vital Capacity (FEF_{25-75%}).^{[16](#page-8-15)}

Lung Volumes

The tests were performed on a body plethysmograph (Medgraphics PlatinumElite DL® Model). Briefly, the pneumotachograph was adjusted to the height of the mouth. The subject placed the nose clip and hands on the facial musculature to block its use in the test. Then, the cabin was closed, and they were instructed to perform four breaths at tidal volume. The subject was asked to "puff gently" attempting to move volumes between 50 and 60 mL and at a rate close to 60 per minute (1 Hz). The shutter was then activated for 2–3 seconds, and after that, a maximal inspiration and maximal expiration up to residual volume was indicated. The variables obtained were slow vital capacity (SVC), inspiratory capacity (IC), expiratory reserve volume (ERV), residual volume (RV), functional residual capacity (FRC), total lung capacity (TLC), RAW and GAW.¹⁷

Maximal Inspiratory Pressure and Maximal Expiratory Pressure (MIP-MEP)

These were measured according to ATS regulations. During the MIP, the subject placed the nose clips and breathed at tidal volume for five respiratory cycles through the pneumotachograph, and from RV, a maximum inspiration against the

closed valve was requested. For the MEP, under the same conditions, the subject was instructed to perform a maximal exhalation from total lung capacity. The best test was selected from a minimum of three acceptable and reproducible maneuvers in both cases according to ATS standards.^{[18](#page-8-17)}

Second Phase

Ventilatory Times

iT and eT were measured at rest and after the exercise test. In both situations, the subject was positioned on a gurney in a semi-upright position (60°) , with the lower limbs in a neutral position and the upper limbs aligned to the body. Here, the measurement was taken with a microphone (lapel type) connected to the pneumotachograph of the body plethysmograph (Medgraphics Model PlatinumElite DL®). The audio and video recording of the RR was taken. The audio recording of the participants' RR was transformed to OGG audio format and entered into the Octave 4.4.0® program, which is compatible with Matlab® and provides a free, scientifically oriented programming environment. Octave 4.4.0® made it possible to represent the audio data graphically in time periods, identifying the iT and eT periods ([Figure 2\)](#page-3-0). All the analyses were performed from the first full inspiration recorded on the audio until the end of the expiration.^{[19](#page-8-18)}

Exercise Test

The step test was performed as follows: i) 2 minutes rest in a chair, ii) the participant facing a 30 cm box must perform one step up and one step down consecutively for 3 minutes, with the step rate being indicated by a metronome set at 96 repetitions per minute (4 clicks = one step cycle) for a step rate of 24 steps per minute, and iii) once the test was completed, the participant stopped and recovered for 2 minutes in a chair.^{[20](#page-8-19)}

Statistical Analysis

GraphPad Prism statistical software (version 6.0®, San Diego, USA) was used. The descriptive management of the variables was using average ± standard deviation. Data normality was determined with the Shapiro–Wilk test. To observe differences in the behavior of airway resistance and conductance (RAW, GAW, sRAW, sGAW), spirometry, lung volumes, iT, eT, and fat percentage of the NW, OW, and OB participants, the ANOVA or Kruskal–Wallis test was used according to the data distribution. The effect of RAW and GAW on post-exercise eT was evaluated using

multivariate linear regression. eT was used as the dependent variable, and BF%, RAW, GAW, sRAW, sGAW, and age as independent variables. The level of statistical significance was set at $p<0.05$.

Results

Sixteen participants in each group were evaluated. There were no significant differences in age (P=0.915) or height (P=0.357). BMI and BF% were significantly higher (P<0.0001; P<0.0001, respectively) in the obese subjects [\(Table 1\)](#page-4-0).

FVC and FEV_1 showed no significant differences (P=0.622; P=0.379, respectively) between the different groups. The OB group's $FEV₁/FVC$ ratio was significantly lower ($P=0.01$). No significant differences were observed among the different groups in MIP (P= 0.889) and MEP (P= 0.259) ([Table 1\)](#page-4-0).

The IC was significantly higher in the OB group (P=0.023). The ERV (P=0.018), RV (P=0.007), and FRC (P<0.0001) were significantly lower in the OB group ([Table 1](#page-4-0)). The RAW was significantly higher in the OB group ($P=0.004$), and GAW (P=0.004) was significantly lower in this same group [\(Table 1\)](#page-4-0).

At rest, RR was significantly higher in the OB group $(P< 0.05)$. iT and eT showed no significant differences at rest. On the other hand, post-exercise, eT was significantly lower (P=0.016) [\(Table 2](#page-5-0), [Figure 3C](#page-5-1)).

Exploration of several models revealed that the multiple linear regression model that best predicts post-exercise eT includes BF%/GAW and BF%/sGAW (explained variability of 15.7% and 14.6%, respectively), ie, that the effect of BF%

Variable	NW	OW	OB	p value
N (male/female)	16(8/8)	16(8/8)	16(8/8)	
Age (years)	22.06±2.35	22.13 ± 1.99	22.44 ± 3.5	0.915
Weight (kg)	56.68 ± 7.94 [#]	72.53 ± 13.73 [#]	$90.16 \pm 15.86^{\#}$	< 0.0001
Height (m)	1.66 ± 0.08	1.64 ± 0.08	1.66 ± 0.08	0.3573
BMI $(Kg/m2)$	21.04 ± 1.72	26.66±3.63∞	32.44±4.9∞	< 0.0001
Body Fat (%)	21.09±6.42+	26.93 ± 4.56	32.38±4.85+	< 0.0001
FVC (L)	4.57 ± 0.99	4.35 ± 0.86	4.54 ± 1.04	0.622
VEF ₁ (L)	3.99 ± 0.81	3.63 ± 0.61	3.80 ± 0.76	0.379
$VEF1/FVC$ (%)	89.25 ± 6	86.06 ± 5.22	84.38 ± 4.06	0.010
FEF $_{25-75}$ (L/sec)	4.64 ± 1.03	3.73 ± 0.76	4.09 ± 0.71	0.027
MEP ($cmH2O$)	115.1 ± 25.50	107.1 ± 21.94	110.3 ± 28.85	0.679
MIP -(cm H_2O)	-106.4 ± 28.75	-122.6 ± 37.71	-127.3 ± 46.24	0.259
SVC (L)	4.21 ± 0.90	4.12 ± 1.16	4.29 ± 1.01	0.889
IC (l)	2.32 ± 0.47	2.76 ± 0.88	3.16 ± 0.83	0.023
ERV(L)	1.89 ± 0.84	1.35 ± 0.57	1.13 ± 0.56	0.018
RV(L)	1.88 ± 0.58	1.35 ± 0.57	1.36 ± 0.34	0.007
TLC (L)	6.01 ± 1.02	5.47 ± 1.34	5.66 ± 1.22	0.453
FRC (L)	3.77 ± 0.91	2.712 ± 0.60	2.501 ± 0.70	< 0.0001
RAW (cmH ₂ O/L/s)	0.79 ± 0.29 ^T	1.12 ± 0.45	$1.30 \pm 0.55^{\mathrm{T}}$	0.004
GAW (L/s/cmH ₂ O)	1.41 ± 0.48^{2}	1.01 ± 0.39	0.93 ± 0.50^{t}	0.004
sRAW (cmH ₂ O*s)	3.14 ± 0.95	3.36 ± 1.07	3.60 ± 1.18	0.489
$sGAW$ ($I/cmH2O*s$)	0.34 ± 0.10	0.33 ± 0.14	0.30 ± 0.10	0.443

Table 1 Summary of Anthropometric Values and Ventilatory Function Values in NW, OW, and OB Subjects

Abbreviations: FVC, Forced Vital Capacity; FEV₁, Forced Expiratory Volume in the First Second, VEF₁ /FVC, Ratio between Forced Expiratory Volume in the First Second and Forced Vital Capacity; %, Percentage; FEF ₂₅₋₇₅, Forced Expiratory Flow between 25 and 75% of Forced Vital Capacity; FEFmax, Peak Forced Expiratory Flow; MIP, Maximum Inspiratory Pressure; cmH₂O, centimeters of water; MEP, Maximum Expiratory Pressure; SVC, Slow Vital Capacity; IC, Inspiratory Capacity, ERV, Expiratory Reserve Volume; RV, Residual Volume; TLC, Total Lung Capacity; RAW, Airway resistance; GAW, Airway conductance; sRAW, Specific airway resistance; sGAW, Specific airway conductance; cmH2O/L/s, centimeters of water per liter per second; L/s/cmH₂O, Liters per second per centimeters of water; cmH₂ O*s, centimeters of water per second; 1/cmH2O*s, one per centimeter of water per second. #, NW vs OW vs OB. ∞, Significant differences in BMI variable NW vs OW vs OB. +, Significant differences, NW vs OB. Ŧ, Significant differences, NW vs OB. £, Significant differences, NW vs OB.

	Rest				Post-Exercise			
	NW	OW	OB	p Value	NW	OW	OB	p Value
RR (cpm)	$15(9 - 24)$	$15(9 - 27)$	$17(8-24)$	< 0.05	$20(12-31)$	$21(14-32)$	$22(11-32)$	0.616
iT (sec)	1.34 ± 0.39	1.40 ± 0.41	1.34 ± 0.38	0.669	1.16 ± 0.21	1.16 ± 0.25	1.20 ± 0.36	0.111
eT (sec)	1.65 ± 0.48	1.75 ± 0.62	1.70 ± 0.51	0.148	1.49 ± 0.3^{2}	1.51 ± 0.30	1.43 ± 0.3^{2}	< 0.01

Table 2 Results of Ventilatory Times in Resting and Post-Exercise Conditions in NW, OW, and OB Subjects

Abbreviations: NW, normal weight; OW, overweight; OB, obese; RR, respiratory rate; iT, inspiratory time; eT, expiratory time. cpm, cycles per minute. sec, seconds. £, Significant differences, NW vs OB.

and L/s/cmH₂O and BF% and 1/cmH₂O*s on post-exercise eT is noted. Thus, for each additional second in post-exercise eT, the BF% decreases by 0.022% and GAW by 0.208 L/s/cmH2O. Similarly, for each additional second in post-exercise eT, the BF% decreases by 0.017% and sGAW 0.7460 1/cmH₂O ([Table 3](#page-6-0)).

Discussion

This study aimed to determine the relationship between iT/eT and RR, and RAW in subjects with different nutritional statuses. The results confirm that the anthropometric condition in terms of weight can significantly impact ventilatory function, specifically in RR, ERV, FRC, RAW, and GAW. In addition, there was a significant decrease in post-exercise eT in OB subjects. Finally, a significant relationship of BF%/GAW and BF%/sGAW with post-exercise eT was observed. Recent studies have reported the usefulness of acoustic monitoring of the respiratory rate. Specifically, sonographic measurement has positively correlated with dental and surgical procedures. However, the findings are still modest, and the clinical significance is still developing.^{[19](#page-8-18)}

Concerning lung function values, specifically lung volumes behaved according to the available evidence, ie, a significant decrease in ERV, RR, and FRC in the OB group compared to the NW group ([Table 1](#page-4-0), [Figure 3C](#page-5-1)). In this regard, one of the earliest and most commonly reported effects of obesity on static lung volumes is a reduction in ERV, which is usually accompanied by a normal RR, ultimately resulting in reduced FRC.²¹ Similarly, Brock et al indicate that there is a small decrease in FEV_1 , FVC , and RR (a decrease of approximately 10%) and a large reduction in ERV and FRC (up to 34% of the expected value) linked to obesity.^{[7](#page-8-6)} Here, the accumulation of fat in the thorax and abdomen typical of obesity brings about a series of changes in respiratory and chest wall mechanics: i) reduced downward movement of the diaphragm, ii) decreased lung compliance, iii) weakness of the expiratory muscles, and iv) decreased expiratory flow and closure of small caliber airways.^{[3](#page-8-2)[,4,](#page-8-3)9} In addition to these mechanical changes, there is the behavior of

Figure 3 (**A**). Respiratory rate; (**B**). Post-exercise inspiratory and expiratory time; (**C**). Pulmonary volumes at rest. Two phenomena are seen to occur in parallel at rest: a decrease in lung volumes and an increase in respiratory rate in obese participants at rest. After exercise, there is a decrease in expiratory time.

Note: Bold values denote $p < 0.05$.

Abbreviations: 95% CI, 95% Confidence Interval; B, unstandardized beta coefficients; LL, lower limit; UL, Upper limit; SE, Standard Error.

RR and lung volumes at rest ([Figure 3A,C\)](#page-5-1), which, as indicated in the literature, is accentuated during exercise, $10-12$ thus explaining the decrease in post-exercise eT found in this study [\(Figure 3B\)](#page-5-1).

Another result obtained in this study was a significant increase in RAW (p=0.004) and a significant decrease in GAW (p=0.004) in the OB compared to the NW group ([Table 1\)](#page-4-0). This agrees with the report by de Sant'Anna et al, who evaluated the different components of RAW and their impact on the occurrence of expiratory flow limitation (EFL) in morbidly obese patients when sitting. Their results showed a significant increase in central $(p=0.001)$ and peripheral ($p=0.0001$) RAW in the OB group compared to the NW group. Complementarily, no patient exhibited EFL.^{[22](#page-8-21)} Similarly, Muñoz-Cofré et al reported that a higher BF% was associated with a higher risk of increased RAW (OR = 14.04; $p =$ 0.030) and sRAW (OR = 4.14; $p = 0.028$), thereby concluding that an increase in BF% is linked to an increase in EFL in small caliber airways.²³ Likewise, Chan and Lipworth (2023) observed obese and morbidly obese patients were associated with significantly worse heterogeneity of peripheral resistance (between 5 and 20 Hz) compared to normal weight patients.²⁴ Finally, the FEF $_{25-75}$, in the present investigation, was significantly lower in overweight and obese participants [\(Table 1](#page-4-0)). In this context, airway narrowing and consequent increased resistance to expiratory flow are now considered a definitive characteristic of people with obesity despite a normal FEV1/FVC ratio,^{[7](#page-8-6),20} so in the absence of assessment of the RAW it is recommended to request the measurement of the FEF 25–75, considered a parameter that measures small caliber airways, with the purpose of not losing the diagnosis of EFL.^{[25](#page-8-24)}

Regarding the hypothesis posited, a notable result was the significant decrease in post-exercise eT in the OB group [\(Table 2\)](#page-5-0) and the impact of BF%/GAW and BF%/sGAW on this [\(Table 3\)](#page-6-0). In this respect, Bhammar et al observed the existence of differences in ventilatory responses to exercise in obese adults, highlighting gender-related particularities. Their results showed that as minute ventilation increases in the peak exercise test, RR increases, which is significantly higher in women than in men. At the same time, they also observed a significant correlation between EFL/fat mass and EFL/BF% ($r=0.483$; $p=0.042$ and $r=0.479$; $p=0.045$, respectively), in men.^{[10](#page-8-9)} In complement to this, Chan et al, observed in a group of 100 female patients that lower paraspinal muscle density PSMD was significantly associated with older age, higher FeNO and worse small airway disease, evidenced by the heterogeneity of the peripheral resistance between 5 and 20 Hz (R5-R20).²⁶ While the results of this study show the relationship between BF%/GAW, BF%/sGAW and postexercise eT, this correlation also exists between BF% and an altered respiratory pattern. It could be argued that these differences are due to: (i) the age of the sample; the sample is relatively young, so the levels of adipokines and proinflammatory cytokines in the systemic circulation typical of obesity have not yet had an impact on the sRAW, and ii) the location of the fat deposits, which would be in the mediastinum, abdomen and thoracic cavities, decreasing the distensibility of the thoracic wall due to the increase in intra-abdominal and pleural pressure, restricting the movement of the diaphragm and the thoracic wall. $3,4,22$ $3,4,22$ $3,4,22$

One of the limitations of this study was the absence of body fat distribution patterns, which could have contributed to a more accurate analysis. In this context, Lo Mauro et al studied how high levels of obesity affect respiratory function. Their results indicated that the location of fat as abdominal volume occupied 41% and 31% in the OB and NW groups, respectively (p<0.001), indicating an accumulation mainly of abdominal fat. In addition, they noted that at rest and in supine position, obese subjects breathed with higher minute ventilation (11.9 L/min) and lower contribution of the rib cage (5.7%) compared to NW subjects (7.5 L/min, $p=0.001$ and 31.1%, $p=0.003$), thus indicating a restrictive pattern in 17% of the obese sample studied. 27

Conclusion

Anthropometric condition referring to weight may have a significant impact on ventilatory function, specifically in: RV, ERV, FRC, RAW, and GAW. There was also a significant decrease in post-exercise eT among the OB subjects. Finally, a significant relationship of BF%/GAW and BF%/sGAW with post-exercise eT was noted. Such results show a complex relationship between obesity and ventilatory function, highlighting the prolongation of eT as a key marker that may have implications in the clinic and in the design of interventions to improve ventilatory function in OB subjects.

Data Sharing Statement

The datasets used and/or analyzed during the current study available from the corresponding author on reasonable request.

Ethics Approval and Informed Consent

The study was conducted in accordance with the Declaration of Helsinki, and approved by the Scientific Ethics Committee of the Universidad Católica del Maule (Resolution N° 59/2018). All participants provided written informed consent to participate in the assessment.

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Author Contributions

All authors made a significant contribution to the work specifically in conception, study design, execution, data acquisition, analysis and interpretation. They also participated in writing the writing and critically revising the article. They agreed on the journal to which the article was submitted. In addition. They reviewed and agreed on all versions of the article before submission, during revision, the final version accepted for publication and on any significant changes introduced at the review stage. Finally, they agreed to take responsibility and account for the content of the article.

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Disclosure

The authors declare no competing interests in this work.

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