Sleep and Breathing (2019) 23:95–101
https://doi.org/10.1007/s11325-018-1666-y

SLEEP BREATHING PHYSIOLOGY AND DISORDERS • ORIGINAL ARTICLE

# Biochemical markers of cardiac dysfunction in children with obstructive sleep apnoea (OSA)

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Received: 13 December 2017 / Revised: 17 April 2018 / Accepted: 19 April 2018 / Published online: 9 May 2018


## Abstract

**Objectives** We explored relationships between biochemical markers and cardiac responses of children with and without obstructive sleep apnoea (OSA) during exercise. We hypothesised that serum markers of sympathetic nervous system activity and low-grade inflammation would correlate with cardiac responses to exercise in children with or without OSA.

**Methodology** The study included 40 of 71 children with previously characterised responses to cardiopulmonary exercise testing. Measures included serum cytokine levels using a multiplex bead-based assay (interleukins IL-1β, IL-2, IL-4, IL-6, IL-8, IL-10, TNF-α and IFN-γ). Serum amyloid A (SAA) was quantified by nephelometry, and metanephrine/normetanephrine levels were measured by liquid chromatography, mass-spectroscopy. Comparisons were made between children with and without OSA, and with and without obesity. Relationships between biomarkers and various cardiac parameters were explored by linear regression.

**Results** Amongst the 40 children in this study, OSA was present in 23. Compared to the 17 children without OSA, those with OSA had higher resting serum IL-6 levels compared to those without (median 3.22 pg/ml vs. 2.31, $p < 0.05$). Regarding correlations with cardiac function after adjusting for OSA, IL-8 negatively correlated to heart rate (HR) response following exercise ($p = 0.03$) and IFN-γ negatively correlated with Stroke Volume Index (SVI) ($p = 0.03$). Both metanephrine and normetanephrine levels positively correlated with SVI ($p = 0.04$, $p = 0.047$; respectively) and QI ($p = 0.04$, $p = 0.04$; respectively) during exercise when adjusting for OSA.

**Conclusions** Children with OSA have raised morning levels of serum IL-6. Separately, higher levels of IFN-γ and IL-8 and lower levels of metanephrine and normetanephrine related to poorer cardiac function during exercise.

**Keywords** Biochemical markers · Cardiac responses · Obstructive sleep apnoea · Cardiopulmonary exercise testing (CPET)

## Abbreviations

| | |
|---|---|
| BMI | Body mass index |
| BP | Blood pressure |
| CRP | C-reactive protein |
| CPET | Cardiopulmonary exercise testing |
| GM-CSF | Granulocyte-macrophage colony-stimulating factor |
| HR | Heart rate |
| IFN-γ | Interferon-gamma |
| IL-1β | Interleukin-1-beta |
| IL-2 | Interleukin-2 |
| IL-4 | Interleukin-4 |
| IL-6 | Interleukin-6 |
| IL-8 | Interleukin-8 |
| IL-10 | Interleukin-10 |
| OSA | Obstructive sleep apnoea |
| Q | Cardiac output |
| QI | Cardiac Output Index |
| SAA | Serum amyloid A |
| SV | Stroke volume |
| SVI | Stroke Volume Index |
| TNF-α | Tumour necrosis factor-alpha |

**Electronic supplementary material** The online version of this article (https://doi.org/10.1007/s11325-018-1666-y) contains supplementary material, which is available to authorized users.

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## Introduction

OSA is associated with significant cardiovascular morbidity and mortality in adults [1–3]. It is also associated with structural and functional changes to the cardiovascular system, including a decreased cardiac output in response to exercise—even in the early stages of disease [4]. Children with OSA also show evidence of cardiovascular changes, including higher BP parameters [5, 6], HR measurements, [6–8] and impaired Q at peak exercise independent of BMI [9].

Two proposed mechanisms to explain these cardiovascular changes are persistent inflammation and autonomic dysfunction, both of which relate to characteristic biochemical changes. Supporting the inflammation theory, children with OSA have a pro-inflammatory cytokine profile resembling that seen in heart failure [10, 11] and coronary artery disease in adults [12, 13]. This includes reports of raised circulating levels of CRP, TNF-α, IFN-γ, IL-6 and IL-8 [14–17], and decreased levels of the anti-inflammatory cytokine IL-10 [15]. Of interest, serum amyloid A (SAA) is a conserved acute phase reactant and adipokine with an established relationship to cardiac dysfunction [18, 19] and inflammation in adults [20] that is yet to be explored in children with OSA.

Autonomic dysfunction has been demonstrated in children with OSA during sleep [8, 21] and in response to physiological stress [22–24]. Biochemically, children (and adults) with OSA have raised overnight plasma noradrenaline [25] and morning urine noradrenaline [26, 27], independent of BMI [27, 28]. This is often considered a marker of increased sympathetic activity. Metanephrine and normetanephrine are catecholamine metabolites [29] that may serve as alternative markers of sympathetic nervous system activity.

To date, the relationship between impaired cardiac function in OSA and the biochemical changes expected with chronic low-grade inflammation and autonomic dysfunction has not been investigated in the same cohort. In 2014, Evans et al. showed that a population of children with OSA had lower cardiac output measures during exercise when compared to healthy controls, independent of BMI [9]. The current study characterises the biochemical profiles of these children and explores the relationships between biochemical markers and those cardiac parameters. We hypothesise that serum markers of sympathetic nervous system activity and low-grade inflammation relate to an impaired cardiac response during exercise in children with OSA.

## Material and methods

This was a cross-sectional study with ethics approval from the Sydney Children's Hospitals Network Human Ethics Committee. Methods pertaining to recruitment, anthropometry, polysomnography, spirometry, and cardiopulmonary exercise testing (CPET) have been published previously [9]. Informed consent was obtained from all individuals who participated in the study.

### Recruitment, anthropometry and sleep study

Between February 2009 and June 2011, children aged 7–12 years who attended the Sleep Clinic or Weight Management Service at the Children's Hospital at Westmead, NSW, Australia, were approached to be in the study. Age- and weight-matched controls were recruited from the community. Subjects in both groups were included if they were healthy weight or obese and were taller than 135 cm in order to perform CPET. Healthy weight was characterised by a BMI between the 5th and 85th centile, and obesity by a BMI ≥ 95th centile, for age and sex [30]. Exclusion criteria included a BMI between the 85th and 95th centile for age and sex, developmental delay, any underlying syndrome, a diagnosed sleep disorder other than OSA or OSA already on treatment.

Height and waist circumference were measured to the nearest 0.1 cm. Weight was measured to the nearest 0.01 kg. BMI centiles and *z*-scores were determined using the US CDC reference values [30].

Polysomnography was performed at the Children's hospital at Westmead, NSW, Australia, according to the 1997 American Thoracic Society (ATS) guidelines [31], recording a minimum of 9 continuous hours of data. Studies were analysed according to the Australasian Sleep Association addendum to the 2007 American Academy of Sleep Medicine guidelines [32]. OSA was diagnosed if the Obstructive Apnoea Hypopnoea Index (OAHI) was ≥ 1/h [33] .

### Cardiopulmonary exercise testing

CPET was performed using a cycle ergometer (Excalibur Sport Cycle ergometer, the Medgraphics CPX/D Breath-by-Breath Exchange system and BreezsSuite version 6.4.1 software, Medgraphics Corporation). Children were cycled at 60 revolutions per minute (rpm) against increasing resistance until physically exhausted or unable to maintain 60 rpm, or until the maximum predicted heart rate was reached (estimated as 220-age). Satisfactory tests had a respiratory quotient (RQ) > 1.00. Children who did not achieve peak exercise were excluded. All testing and analysis were in accordance with the ATS and American College of Chest Physician guidelines [34].

Ultrasonic Cardiac Output Monitor Doppler was used to measure Q, SV, and HR. QI and SVI were then calculated by adjusting Q and SV, respectively, to the participants body surface area in meters-squared.

### Blood collection and analysis

Fasting bloods were collected by venepuncture between 6 and 7am in the morning after participants' sleep study. Bloods

were centrifuged between 7 and 7:30am, and serum samples were stored at – 80 °C until the time of analysis.

Cytokine levels were quantified by a multiplex bead-based assay using the Human Ultrasensitive Cytokine 10-plex Panel as per the manufacturer's instructions (Invitrogen, catalog no. LHC6004) at the Children's Hospital at Westmead. All cytokine assays were batched in a single run to minimise inter-assay variability. Included in this panel were IL-1β, IL-2, IL-4, IL-6, IL-8, IL-10, TNF-α, IFN-γ and GM-CS. SAA was quantified by nephelometry as per the protocol at the Children's Hospital Westmead. The lower limit of detection was 4 mg/l.

Serum metanephrine and normetanephrine levels were quantified at the NSW government South Eastern Area Laboratory Services, north clinical chemistry department, according to their liquid-chromatography/mass spectrometry protocol [29].

### Statistical analysis

Data are presented as mean (SD) unless otherwise stated. Data analysis was performed using SPSS version 22 (IBM SPPS statistics, Armonk, New York). Categorical variables are shown as frequencies and compared using the chi-squared test. Descriptive and continuous variables are shown as mean (SD). Means were compared using independent two-tailed *t* tests.

For the cytokine markers, data are represented as median with interquartile range and were compared using non-parametric independent samples median test.

Cardiac parameters were compared to serum biomarkers by linear regression for any associations (relationships were adjusted for OSA status). Cardiac parameters were used as the dependent variables. No adjustment was made for multiple statistical comparisons. $P < 0.05$ was considered statistically significant.

## Results

### Demographics, anthropology, polysomnography and CPET

For the study, 115 children were approached, 71 participated in CPET and morning blood samples were obtained in 40 children after their overnight sleep study. Thus, 40 children were included in the current study. This group included 23 children with OSA (mean age 10.0 ± 1.7 years) and 17 without OSA (mean age 10.7 ± 1.2 years). There were no differences in age, gender or BMI *z*-score between the groups (Supplementary Table 1).

Cardiopulmonary testing for this subgroup supported the original findings and is shown in Supplementary Tables 2 and 3. We were able to confirm that, as in the original study, children with OSA had impaired responses to exercise compared to those without OSA. Peak exercise was achieved at comparable workloads and respiratory quotients (RQ) and pulmonary function was also comparable between the groups. However, children with OSA had lower levels of expired oxygen for their weight (measured as $VO_2$ peak in ml/kg/min) during exercise compared to those without OSA. In addition, children with OSA had lower mean Q, QI and SVI at peak exercise, in keeping with an impaired ability of the heart to meet the demands of the body (Supplementary Table 3).

We also confirmed that obese children achieved peak exercise at comparable workloads and respiratory quotients to healthy weight children, but obese children demonstrated lower levels of expired oxygen during exercise when correcting for weight (as seen with children with OSA) and had lower ventilatory equivalents for $CO_2$ and $O_2$ during exercise. Compared to healthy weight participants, obese children had higher baseline Q and HR, and a lower SVI. During exercise, they demonstrated lower QI and SVI when compared to healthy weight participants (Supplementary Table 3).

The only variation compared to the larger study group was that there was no difference in peak HR during exercise between those with and without OSA. However, the change in HR (HR difference) in response to exercise was lower in children with OSA.

### Inflammatory markers

Children with OSA had higher median morning serum IL-6 levels compared to those without OSA (Table 1). No cytokine differences were noted between groups when analysed by weight category (Table 1).

All 40 samples were assayed for metanephrine and normetanephrine on the same day. Neither serum metanephrine nor normetanephrine levels differed between children with or without OSA (Table 2). However, obese children had lower metanephrine levels than healthy weight children ($p = 0.03$, Table 2).

Detectable levels of SAA were found in 5 of 23 children with OSA and 5 of 17 children without OSA ($p = 0.72$). Similarly, when stratified by weight category, there was no difference in the number of detectable SAA samples between groups ($p = 0.26$) (Table 2).

### Regression analyses

IL-8 negatively correlated to HR difference ($p = 0.03$, Table 3), such that higher levels of IL-8 predicted a smaller increase in HR during exercise. Additionally, higher IFN-γ levels associated with lower SVI measures ($p = 0.03$, Table 3).

Higher levels of serum metanephrine correlated with higher SVI and QI measures at peak exercise ($p = 0.04$, $p =$

**Table 1** Inflammatory cytokines by OSA and by weight category

| | *n* OSA, non-OSA | OSA median (IQR) | Non-OSA median IQR | *p* | *n* Ob, healthy wt | Obese median (IQR) | Healthy wt median (IQR) | *p* |
|---|---|---|---|---|---|---|---|---|
| IL-1b (pg/ml) | 23, 17 | 0.34 (0.23–0.63) | 0.33 (0.14–0.46) | 1 | 24, 16 | 0.33 (0.17–0.57) | 0.34 (0.57–0.18) | 0.75 |
| IL-2 (pg/ml) | 23, 17 | 2.13 (1.46–4.84) | 1.43 (1.16–2.52) | 0.2 | 24, 16 | 2.28 (1.28–4.68) | 1.53 (1.2–2.75) | 0.11 |
| IL-4 (pg/ml) | 23, 17 | 4.87 (2.46–8.14) | 5.48 (2.79–8.39) | 0.52 | 24, 16 | 5.7 (2.55–9.5) | 4.76 (2.79–6.56) | 0.75 |
| IL-5 (pg/ml) | 22, 16 | 0.63 (0.44–1.08) | 0.45 (0.37–0.82) | 0.27 | 23, 15 | 0.49 (0.39–1.33) | 0.45 (0.43–0.81) | 0.42 |
| IL-6 (pg/ml) | 23, 15 | **3.22 (2.25–4.21)** | **2.31 (0.97–2.95)** | **0.046** | 23, 15 | 2.95 (2.31–4.08) | 1.56 (0.81–3.95) | 0.18 |
| IL-8 (pg/ml) | 23, 17 | 4.07 (3.48–6.41) | 3.46 (2.63–5.71) | 0.52 | 24, 16 | 4.49 (3.20–8.01) | 3.49 (2.54–5.15) | 0.33 |
| IL-10 (pg/ml) | 23, 16 | 2.09 (1.26–2.91) | 1.72 (1.13–2.08) | 0.85 | 23, 16 | 1.86 (1.5–3.02) | 1.59 (1.13–2.38) | 0.85 |
| TNF-α (pg/ml) | 21, 11 | 0.97 (0.36–1.67) | 1.35 (0.48–2.09) | 0.46 | 19, 13 | 1.02 (0.39–1.8) | 1.21 (0.43–1.83) | 1 |
| IFN-γ (pg/ml) | 17, 12 | 2.68 (1.13–4.71) | 2.55 (1.27–3.76) | 1 | 17, 12 | 3.3 (1.13–4.71) | 2.24 (1.4–3.0) | 0.26 |
| GM-CSF (pg/ml) | 21, 15 | 0.57 (0.32–1.21) | 0.36 (0.23–0.57) | 0.18 | 22, 14 | 0.54 (0.31–1.4) | 0.42 (0.22–0.58) | 0.73 |

Values shown in bold have significant differences

*OSA* obstructive sleep apnoea, *Ob* obese, healthy wt healthy weight, *IL* interleukin, *IFN-γ* interferon-gamma, *TNF-α* tumour necrosis factor-alpha, *GM-CSF* granulocyte-macrophage colony-stimulating factor

0.04; respectively, Table 4). The same was true for normetanephrine ($p = 0.047$, $p = 0.04$; respectively, Table 4).

## Discussion

This study is the first to explore biochemical correlations with cardiac dysfunction in response to exercise in a paediatric population with OSA. Higher morning plasma levels of IFN-γ and IL-8 correlated with poorer cardiac function during exercise, while higher serum metanephrine and normetanephrine levels were, contrary to our hypothesis, associated with better cardiac function during exercise. Children with OSA demonstrated higher morning levels of serum IL-6.

We first confirmed that this subgroup also showed the original abnormalities of lower Q, QI and SVI, and a smaller increase in heart rate during exercise for children with OSA. In addition, As with the original cohort studied by Evans et al. [9], children with OSA, and separately obesity, demonstrated changes in cardiac parameters consistent with an impaired ability of the heart to meet the demands of the exercising body.

In this subgroup, the impaired cardiac responses correlated with higher serum IL-8 levels. This finding has not been previously reported and may represent an inflammation-related haemodynamic compromise. Other studies have demonstrated raised IL-8 levels in children with OSA [16, 35]. Li et al. also found raised IL-8 levels in children with moderate/severe disease [35]. The current finding may offer an additional functional association to the previously demonstrated inflammatory abnormalities.

IFN-γ is a pro-inflammatory cytokine that is raised in adults with congestive cardiac failure [36] and has been causally linked to markers of cardiac dysfunction in animals [37–39]. Higher IFN-γ levels correlated with lower SVI and a lower QI. Given that SVI and QI reflect the ability of the heart to supply blood to the body, this finding is in keeping with inflammation-related haemodynamic compromise. While reports linking IFN-γ to reduced systolic function and cardiomyocyte contractility are restricted to animals [38, 39], they are consistent with our associations with lower SVI and QI in the current cohort.

**Table 2** Catecholamine metabolites and SAA

| | OSA ($n = 21$) | Non-OSA ($n = 14$) | *p* | Obese ($n = 23$) | Healthy wt ($n = 12$) | *p* |
|---|---|---|---|---|---|---|
| MET (nmol/l) | 0.2 (0.06) | 0.2 (0.11) | 0.95 | **0.18 (0.06)** | **0.24 (0.11)** | **0.03** |
| NORMET (nmol/l) | 0.44 (0.16) | 0.43 (0.19) | 0.88 | 0.40 (0.14) | 0.50 (0.2) | 0.12 |
| N:NM ratio | 0.48 (0.16) | 0.45 (0.11) | 0.63 | 0.46 (0.16) | 0.48 (0.09) | 0.75 |
| Detectable SAA ($n^a$) | OSA ($n = 23$) | Non-OSA ($n = 17$) | | Obese ($n = 24$) | Healthy wt ($n = 16$) | |
| | 5 | 5 | 0.72 | 8 | 2 | 0.08 |

Values shown in bold have significant differences

*MET* metanephrine, *NORMET* normetanephrine, *N:NM ratio* normetanephrine to normetanephrine ratio, *SAA* serum amyloid A

[a] The number of samples in which serum amyloid A was detectable (> 4 mg/l) is shown

**Table 3** Relationships between cytokines and cardiac function

| | IL-8 Slope | *p* | IFN-γ Slope | *p* |
|---|---|---|---|---|
| CO rest | 0.08 | 0.10 | − 0.07 | 0.43 |
| CO peak | 0.01 | 0.89 | − 0.19 | 0.16 |
| QI rest | − 3.32 | 1.00 | − 0.14 | 0.07 |
| QI peak | − 0.12 | 0.16 | **− 0.36** | **0.05** |
| SV rest | 0.05 | 0.91 | − 0.39 | 0.65 |
| SV peak | 0.41 | 0.34 | − 1.28 | 0.21 |
| SVI rest | − 0.63 | 0.10 | − 1.25 | 0.15 |
| SVI peak | − 0.52 | 0.29 | **− 2.29** | **0.03** |
| HR rest | 1.40 | 0.05 | − 0.33 | 0.81 |
| HR peak | − 0.96 | 0.22 | 0.32 | 0.86 |
| HR diff | **− 0.24** | **0.02** | 0.66 | 0.73 |

Values shown in bold have significant differences

*IL-8* interleukin-8, *IFN-γ* interferon-gamma, *CO* cardiac output, *QI* Cardiac Output Index, *SV* stroke volume, *SVI* Stroke Volume Index, *rest* values at rest, *peak* values at peak exercise, *HR diff* (HR difference) change in HR in response to exercise

The current finding of significantly raised IL-6 levels in morning samples of children with OSA is consistent with the findings of transient elevations in IL-6 (but not TNF-α) in piglets submitted to acute intermittent hypercapnic hypoxia in an animal model of OSA in children [40]. Since IL-6 is a key inducer of inflammatory responses [41], including the production of CRP and SAA, if repeated on a nightly basis, this transient elevation could commence the cascade of pro-inflammatory responses leading to chronic low-grade inflammation and possible changes in cardiovascular function. Raised inflammatory cytokines are variably reported in children with OSA. As an example, raised levels of IL-6 [15, 42] and lower levels of IL-10 [15] are reported in some OSA cohorts but not others [43], and some findings depend on disease severity [42]. They are also hard to disentangle from the effects of obesity, for example Gaines et al. recently attributed 42% of the association of obesity and OSA to IL-6 [44].

Although we were unable to demonstrate relationships between IL-6 and cardiac parameters, animal studies have causally linked IL-6 to reduced myocardial contractility [45], with downstream reductions in myocardial nitric oxide synthase 2 in papillary muscle [46] and reduced expression of contractile proteins in rat cardiac myocytes [47]. Our findings would support exploring the role of IL-6 on cardiac function in a larger paediatric OSA cohort.

SAA is a conserved acute phase reactant and adipokine that was investigated because of its established relationship to cardiac dysfunction [18, 19] and to inflammation relating to cardiovascular disease in adults [20]. No prior studies have looked as SAA in children with OSA, and we found no differences in the amount of detectable SAA samples between disease groups. The same was true when comparing children by weight category. In adults, SAA seems to share a closer relationship to obesity than to OSA [48], but in children, further inquiry with a larger group is needed. SAA had low statistical power in this cohort partly because detection rates were lower than those of we expected based upon an existing study [18].

Serum metanephrine and normetanephrine levels were chosen as markers of sympathetic nervous system activity due to their long half-lives in the blood despite limited evaluation in existing studies [49], with the hypothesis that levels of both would be elevated as markers of sympathetic dysfunction in children with OSA. No other study has examined associations amongst these markers, OSA and cardiac dysfunction in children. The metanephrine levels were within expected ranges for age and sex, while normetanephrine levels were higher than those expected [50, 51]. While we found no correlations with the presence or absence of OSA, both metanephrine and normetanephrine levels correlated positively to SVI and QI during exercise. SVI and QI reflect the ability of the heart to meet the demands of the exercising body and higher morning serum metanephrine levels predicted better cardiac function during exertion. Consistent with this, obese children had lower levels of serum metanephrine and worse cardiac function than healthy weight controls. Since autonomic dysregulation amongst children with OSA is typically independent of BMI [27, 28], this finding was unexpected.

We acknowledge that the main limitation of this study is the small sample size, because it limited our ability to stratify and evaluate the relative contributions of obesity and OSA to our findings.

**Table 4** Regression analysis of catecholamine metabolites

| | Metanephrine | | Normetanephrine | |
|---|---|---|---|---|
| | Slope | *p* | Slope | *p* |
| CO rest | − 1.83 | 0.48 | − 1.35 | 0.29 |
| CO peak | 0.35 | 0.92 | 1.77 | 0.27 |
| QI rest | 0.49 | 0.42 | − 0.07 | 0.94 |
| QI peak | **8.89** | **0.04** | **4.12** | **0.05** |
| SV rest | − 14.72 | 0.5 | − 10.63 | 0.32 |
| SV peak | 3.96 | 0.86 | 10.1 | 0.34 |
| SVI rest | 26.45 | 0.18 | 4.84 | 0.62 |
| SVI peak | **51.78** | **0.03** | **23.15** | **0.05** |
| HR rest | − 14.58 | 0.7 | − 8.87 | 0.63 |
| HR peak | − 13.11 | 0.74 | − 3.03 | 0.88 |
| HR diff | 1.46 | 0.98 | 5.83 | 0.81 |

Values shown in bold have significant differences

*HR diff* (HR difference) the change in HR in response to exercise, *CO* cardiac output, *QI* Cardiac Output Index, *SV* stroke volume, *SVI* Stroke Volume Index, *rest* values at rest, *peak* values at peak exercise

## Conclusions

In this study, children with OSA had impaired cardiac function in response to exercise and raised levels of IL-6. Correlations between serological markers and poorer cardiac function during exercise included higher morning serum levels of IL-8 and IFN- $\gamma$, and lower morning serum levels of metanephrine and normetanephrine.

**Acknowledgements** The authors thank the staff from the David Read Sleep Unit, Respiratory Function Unit, The Children's Hospital Institute of Sport's Medicine and Weight Management Service and the Immunology laboratory at The Children's Hospital at Westmead for contributing and supporting this study. Also, we thank the children and families who agree to participate in this study.

**Funding** The National Health and Medical Research Council (NHMRC) provided financial support in the form of a postgraduate scholarship for Dr. Evans. The sponsor had no role in the design or conduct of this research.

## Compliance with ethical standards

**Conflict of interest** The authors declare that they have no conflict of interest.

**Ethical approval** All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and/or national research committee (name the institution/committee) and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards.

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