# Human immune and metabolic biomarker levels, and stress-biomarker associations, differ by season: Implications for biomedical health research

Jeffrey Gassen , Summer Mengelkoch , George M. Slavich [*]

*Department of Psychiatry and Biobehavioral Sciences, University of California, Los Angeles, CA, USA*

ABSTRACT

Although seasonal changes in physiology are well documented, little is known about how human immune and metabolic markers vary across seasons, and no studies have examined how stress → health biomarker associations differ across the year. To investigate these issues, we analyzed data from 2118 participants of the Midlife in the United States (MIDUS) study to determine whether there were differences in (a) levels of 19 immune and metabolic markers, and (b) the association between perceived stress and each biomarker across the year. Results of component-wide boosted generalized additive models revealed seasonal patterning for most biomarkers, with immune proteins generally peaking when days were shorter. Moreover, whereas levels of hemoglobin A1C rose from late fall to spring, triglycerides were elevated in the summer and fall, and high-density lipoprotein decreased steadily from January to December. Urinary cortisol and cortisone exhibited opposite patterns, peaking at the beginning and end of the year, respectively. Most critically, we found that the effects of perceived stress on 18 of the 19 health biomarkers assessed varied by month of measurement. In some cases, these differences involved the magnitude of the stress → biomarker association but, in other cases, it was the direction of the effect that changed. Studies that do not account for month of biomarker assessment may thus yield misleading or unreproducible results.

Decades of animal model research has found that immune, hormonal, and metabolic markers vary seasonally, and that these seasonal changes are largely mediated by photoperiod (Nelson, 2004; Nelson et al., 2002). For example, early pioneering work that experimentally manipulated photoperiods to simulate shorter or longer day lengths was able to alter immune cell counts and effector functions in Siberian hamsters and other small mammals (Nelson and Demas, 1996). This research showed heightened immune activity when photoperiods (i.e., days) were shorter, an effect that has also been found in other animal species (Walton et al., 2011). Some studies have further suggested that elevated immunity in the context of shorter photoperiods is accompanied by changes in glucose and lipid metabolism as indexed by increased circulating levels of glucose and lipids, independent of food intake (Mariné-Casadó et al., 2018; Otsuka et al., 2014).

A complete review of the evolutionary theory behind seasonal changes in immune and endocrine function is beyond the scope of this article (see Nelson et al., 2002). Briefly, it is hypothesized that in many climates, animals use day length as a reliable signal of impending harsh winter conditions characterized by low food availability and

thermoregulatory stress (i.e., in non-tropical climates), each of which compromise immune function. Accordingly, energetic investment is shifted away from functions not immediately essential to survival (e.g., reproduction), and toward somatic maintenance and immunity.

Human environments are unique in that many of us are fortunate to be, in large part, buffered from winter harshness. Therefore, humans might not be expected to exhibit seasonal immune, endocrine, and metabolic responses. On the other hand, given the animal research reviewed above (Nelson et al., 2002; Nelson and Demas, 1996; Walton et al., 2011), it is possible that human immune and metabolic activity also varies as a function of day length or other seasonally-varying factors (e.g., temperature). Further, human environments differ across the year in a number of additional ways that could contribute to seasonal physiology. For example, holiday activities (Ceballos et al., 2015; Jones et al., 2014), travel (Falkenbach and Sedlmeyer, 1997; Hruska et al., 2020), and transient academic stress (Uchakin et al., 2001) have each been found to alter levels of various biomarkers, and they also vary seasonally. The existence of these links between modern environmental factors and biomarker levels, however, does not provide an alternative

explanation to seasonality. Instead, these associations highlight that, if seasonal changes in human biomarker levels do occur, the set of variables that mediate and attenuate such patterns among humans is likely wider and more diverse than for non-human animals. Below, we review growing evidence for seasonal variation of human immune function and metabolism.

## 1. Seasonal effects on human biomarkers

Consistent with the possibility that human biomarkers vary across the year, observational research examining seasonal shifts in human immunity found that shorter day lengths were associated with higher circulating inflammatory markers, and greater peripheral blood mononuclear cell proliferation and cytotoxicity (Gassen et al., 2019). Corroborating results were found using data from both children and adults who participated in the National Health and Nutrition Examination Survey (Liu and Taioli, 2015), as well as UK Biobank participants (Wyse et al., 2021). In accord with findings from animal research, these studies found that certain white blood cell counts and C-reactive protein (CRP) were higher in the winter-spring compared to summer-fall months.

Longitudinal multi-omics work has produced similar findings. One study identified >1000 immune, metabolic, and other multi-omic markers that varied seasonally (Sailani et al., 2020). Separate research that combined data on transcriptomics and blood cell components from individuals living in several geographical regions found evidence for over 4000 markers that vary seasonally, with opposite patterns of change observed in the Southern and Northern hemispheres (Dopico et al., 2015). Such seasonal shifts in human biology, in turn, appear to be clinically relevant. For example, multiple studies have found that glucose metabolism among patients with diabetes mellitus is affected by season, with levels of HbA1c and insulin resistance peaking in the winter months (Gikas et al., 2009; Kershenbaum et al., 2011; Tsujimoto et al., 2014). Moreover, several human communicable and non-communicable diseases exhibit seasonal patterns of incidence and severity, although the causal roles that seasonal changes to immunometabolism play in such processes is unknown (Nelson, 2004).

These studies provide evidence that human immunity and metabolism vary seasonally. However, little attention has been paid to the larger implications that this seasonal variation may have for biomedical health research. For example, we are not aware of any studies that have investigated the extent to which there are individual differences in the magnitude of seasonal changes in human biomarker levels and, if so, what factors contribute to this heterogeneity. In other words, it is unclear whether time of year interacts with social, environmental, and other variables that have been previously shown to influence immunity and metabolism. An important practical implication of such interactions is that the strength of risk factor → biomarker/outcome associations might also vary seasonally. Accordingly, the seasonal timing of data collection could impact the direction or magnitude of these effects, which may not fully be redressed by merely including season or day length as covariates (Wysocki et al., 2022). In the next section, we discuss the implications of seasonal variation in the effects of perceived stress on immune and metabolic markers, given the well-known impact of stress on health and disease biology (Slavich, 2016; Slavich et al., 2023).

## 2. Stress × Season interactions on health biomarker levels

Research has found that greater perceived stress is consistently associated with higher inflammation and poorer metabolic function (Gowey et al., 2019; Jurgens et al., 2023, 2023, 2023; Knight et al., 2021; Lehrer et al., 2020; Liu et al., 2017; Martínez de Toda et al., 2019; Mauss and Jarczok, 2021; Sribanditmongkol et al., 2015). Moreover, the effects of stress also appear to manifest in human biorhythms, which have implications for health (James, 1991; Knight et al., 2021; Koch

et al., 2016; Lovell et al., 2011; Wu et al., 2022). Although it is not uncommon for studies on stress and humanity immunity to adjust for season in some capacity (e.g., Cohen et al., 2012; Corallo et al., 2021; Gassen et al., 2021, 2022; Malarkey et al., 1995; Murphy et al., 2017; Pereira et al., 2019), simply including a measure of season as a covariate—as is often done—assumes that the strength and direction of stress → biomarker associations are consistent across the year. However, this adjustment may be insufficient, or even inappropriate, when the effects of stress vary seasonally (Attia et al., 2022; Cohen et al., 2021; Laubach et al., 2021; Vander Weele, 2012; Vetter and Mascha, 2017; Wysocki et al., 2022), or when stress modifies seasonal biorhythms, as has been found for diurnal and circadian rhythms (James, 1991; Knight et al., 2021; Koch et al., 2016; Lovell et al., 2011; Wu et al., 2022).

Consider a situation involving perceived stress (i.e., a risk factor) and an outcome where the strength and/or sign of the association varies substantively as a function of season. If data are only collected during one season, the effects of stress found may not then generalize to the rest of the year and there would be no indication otherwise. Even if data are collected over the course of the full year, simply including season or day length as a covariate—especially if included in the incorrect mathematical form (e.g., falsely assuming linearity)—is unlikely to provide a precise and accurate estimate of stress's effects, as there is heterogeneity across levels of the covariate (Cohen et al., 2021; Laubach et al., 2021; Robertson et al., 2021; Vander Weele, 2012; Wysocki et al., 2022). Instead, introducing statistical interaction terms or another strategy should be pursued to assess the effects of stress across the year (Chesnaye et al., 2021; Gruber et al., 2015; Robertson et al., 2021; Robins et al., 2000). Although the best strategy for dealing with moderation by time of year will depend on causal associations between the exposure, effect modifier or interacting variable (for distinction, see e.g., Attia et al., 2022), and outcome, as well as the type and magnitude of modification that occurs (Vander Weele, 2012; VanderWeele and Robins, 2007), this example illustrates the need for a more in-depth analysis into the implications of seasonality for biomedical health research.

## 3. The present research

In the present study, we analyzed associations between perceived stress, month of data collection (i.e., seasonality), and 19 immune and metabolic markers to test the hypothesis that both health biomarker levels, as well as *association between* perceived stress and these biomarkers, vary seasonally. To test this hypothesis, we use data from the Midlife in the United States study (MIDUS; Radler, 2014), which is a large, nationally representative study of health and wellbeing in midlife in the U.S. First, to examine whether seasonal changes to biomarkers reliably occurred and were consistent with prior research (Dopico et al., 2015; Gassen et al., 2019; Liu and Taioli, 2015; Sailani et al., 2020), we used component-wise gradient boosted generalized additive models (GAMs; Hofner et al., 2014). Second, we tested the predictive utility of interactions between month and perceived stress for levels of each biomarker. Based on prior research (Dopico et al., 2015; Gassen et al., 2019; Liu and Taioli, 2015; Sailani et al., 2020), we expected that markers of inflammatory activity and insulin resistance would peak during periods when days were shortest (e.g., December). Finally, we tested the hypothesis that associations between perceived stress levels and participants' health biomarker levels would vary across seasons, but had no *a priori* prediction about the shape of these interactions. If this last hypothesis was confirmed, it would provide novel evidence that accounting for month of biomarker measurement could be critical not just in stress studies, but in any research examining associations between an established risk factor for poor health and markers of stress or disease risk.

## 4. Method

### 4.1. Procedure

Data were obtained from 2118 MIDUS participants (Refresher, completed in 2016: $n = 863$; MIDUS 2, completed in 2009: $n = 1255$). We also used longitudinal follow-up data from 747 MIDUS 2 participants who completed MIDUS 3 (in 2023), for a total of 2865 observations. Procedures for MIDUS data collection are described online (https://midus.wisc.edu/). Briefly, for each wave, participants provided demographic information and completed the Perceived Stress Scale (PSS; Cohen et al., 1983), among other scales (see Table 1 for characteristics of the sample). Additionally, blood, urine, and saliva were collected for biomarker analysis. MIDUS participants provided informed consent prior to participation. The present study falls under institutional review board approval for MIDUS, which extends to research on publicly available data.

### 4.2. Measures

Perceived stress levels in MIDUS were assessed using the PSS, a commonly used and well-validated measure of overall perceived stress levels (Cohen et al., 1983). Responses to each PSS item were summed to compute a composite score PSS, per convention (Cohen, 1988).

Methods for the biological assay data—including reference ranges, intra- and inter-assay coefficients of variation, and other key details—are available online (https://www.midus.wisc.edu/midus2/project4/). In brief, serum assays were conducted on fasting blood samples, and urinary assays were conducted using 12-h overnight urine samples. Biomarkers used for the current research included (a) serum immune markers: interleukin-6 (IL-6), IL-8, IL-10, tumor necrosis factor-alpha (TNF-α), CRP, fibrinogen, E-selectin, intercellular adhesion molecule 1 (I-CAM), and soluble IL-6 receptor (sIL-6r); (b) serum metabolic markers: hemoglobin A1c (HbA1c), glucose, insulin, Homeostatic Model Assessment of Insulin Resistance (HOMA-IR), triglycerides, high- and low-density lipoproteins (HDL, LDL), insulin-like growth factor-1 (IGF-1); and (c) urinary cortisol and cortisone adjusted for creatinine levels. Rates of missing biomarker data were low (0.6–3.4%).

Age, sex, and body mass index (BMI) measured at the biomarker portion of each study wave were included as linear covariates in all analyses. We also included year of collection as a predictor and modeled this using spline base-learners to capture non-linearity. Seasonal

patterns of biomarker levels were modeled using month of biological sample collection. The number of observations per month was relatively consistent across years (see Fig. S1 in Supplementary File 1).

### 4.3. Data analysis plan

All analyses were conducted using R, and all of our code is available on the Open Science Framework (DOI:10.17605/OSF.IO/W5FCS) (R Core Team, 2021). For all analyses, we included random effects of participant ID to adjust for observations nested within participants. First, we used the R package *mboost* (Hofner et al., 2014) to perform component-wise gradient boosted GAMs to explore non-linear relations between month of data collection and log-transformed biomarker levels. Component-wide boosting is a multi-faceted machine learning technique that applies gradient descent techniques to perform feature selection and optimize predictive accuracy of an outcome given a set of covariates (Hothorn et al., 2010). Specifically, the models leverage input of distributional assumptions about the outcome (and associated loss function, specified by choice of *family*) and structural assumptions about predictors (specified as *base-learner* formulas) to independently fit each base-learner to the negative gradient vector of the loss function across $m$ iterations, and select the base-learner that best fits the vector at each step. In a stagewise manner across iterations, the estimate of the negative gradient vector is updated, and base-learner selection is repeated until the final specified iteration ($m_{stop}$). The primary tuning parameter for boosted models is $m_{stop}$, which must be optimally selected to prevent model overfitting and optimize generalizability (Bartlett and Traskin, 2007; Hofner et al., 2014). We performed 25-fold bootstrap cross-validation for each model, and updated the model with the optimal stopping iteration (Hothorn et al., 2010). For the simpler models without PSS-month interaction effects, we searched for the optimal stopping point for up to 5,000 iterations; we increased this to 10,000 for the more complex interaction models.

The main advantage of component-wide boosting for GAMs is the algorithm's ability to modify smoothness and flexibility of spline base-learners as iterations proceed when non-linearity is expected (Tutz and Binder, 2006). With the present data, this method supports identifying an optimal smooth function of month that is most predictive of biomarker levels. For the initial seasonal models, we included P-spline base-learners with eight interior knots and four starting degrees of freedom (package default) for the effects of month (Hofner et al., 2014). Although seasonal changes are periodic, we elected to exclude cyclic constraints as shifts in biomarker levels from the beginning of December to end of January may be biologically plausible (for cyclic effects, see e.g., Hofner et al., 2016). Next, we used bivariate tensor products to examine the interaction surface between month and PSS scores for all biomarkers (Wood et al., 2013). These models also included P-splines for month and PSS (knots = 8 each) to test whether the main effects of these variables yielded better predictive utility than their interaction. A final set of follow-up models examined linear effects of PSS on each biomarker across seasons after months were binned into a factor variable: spring, summer, winter, and fall (for results, see Supplementary File 2). These final analyses provided insights into information loss that may occur when categorizing month of the year into discrete seasons.

## 5. Results

### 5.1. Seasonal patterns of health biomarkers

#### 5.1.1. Immune markers

As shown in Table 2, consistent with prior research, pro-inflammatory cytokine levels (e.g., IL-6, IL-8, TNF-α) were higher in months with shorter day lengths (Fig. 1). Specifically, levels of IL-6 (Model $R^2$: 63.2%) and IL-8 (Model $R^2$: 57.7%) rose from the beginning of the year to their peak in the fall and remained elevated through the rest of the year. TNF- α levels (Model $R^2$: 52.3%) were low through

**Table 1**
Characteristics of the sample.

| Variable (Range) | M (SD) or Frequency (%) | |
| --- | --- | --- |
| | MIDUS 2 (n = 1255) | MIDUS Refresher (n = 863) |
| Years of Biomarker Collection | 2004–2009; 2017–2022 | 2012–2016 |
| Sex | | |
| Male | 542 (43.2%) | 413 (47.9%) |
| Female | 713 (56.8%) | 450 (52.1%) |
| Race | | |
| White | 978 (77.9%) | 600 (69.5%) |
| Black/African American | 27 (2.2%) | 56 (6.5%) |
| Asian | 3 (0.2%) | 12 (1.4%) |
| Other | 43 (3.4%) | 73 (8.5%) |
| Missing/Refused | 204 (16.3%) | 122 (14.1%) |
| Age (25–84) | 54.52 (11.71) | 50.84 (13.41) |
| Body Mass Index (14.99–77.58) | 29.77 (6.63) | 30.40 (7.65) |
| Perceived Stress Scale (10–48) | 22.24 (6.34) | 22.49 (6.36) |

*Note.* MIDUS = Midlife in the United States. For repeated-measures participants, characteristics reported here for first visit.

**Table 2**
Statistics and selection frequencies for month in seasonal models.

| Outcome | Optimal Stopping Iteration | Relative Selection Frequency | Model $R^2$ |
|---|---|---|---|
| Hemoglobin A1C | 5000 | 12.12% | 60.1% |
| Triglycerides | 5000 | 7.55% | 60.0% |
| High-Density Lipoprotein | 5000 | 7.41% | 66.6% |
| Low-Density Lipoprotein | 3726 | Not Selected | 44.3% |
| Glucose | 2959 | Not Selected | 40.8% |
| Insulin | 5000 | Not Selected | 65.7% |
| Insulin Resistance | 5000 | Not Selected | 66.3% |
| Insulin-like Growth Factor 1 | 5000 | 16.00% | 60.0% |
| Cortisol | 3748 | 4.47% | 56.4% |
| Cortisone | 1719 | 3.57% | 26.8% |
| Interleukin-6 | 5000 | 12.50% | 63.2% |
| Interleukin-8 | 5000 | 6.67% | 57.7% |
| Interleukin-10 | 4367 | 7.69% | 47.8% |
| Tumor Necrosis Factor-Alpha | 4294 | 10.19% | 52.3% |
| C-Reactive Protein | 5000 | Not Selected | 63.8% |
| Fibrinogen | 4380 | 3.81% | 56.1% |
| E-selectin | 5000 | 7.55% | 60.7% |
| Intercellular Adhesion Molecule-1 | 5000 | Not Selected | 57.2% |
| Soluble Interleukin-6 Receptor | 5000 | 14.81% | 60.3% |

the spring and early summer before increasing steadily to their zenith in December. Similar patterns were observed for the anti-inflammatory/regulatory cytokine IL-10 (Model $R^2$: 47.8%) and soluble IL-6 receptor (Model $R^2$: 60.3%; which mediates inflammatory activity via the trans-signaling pathway; Rose-John et al., 2023). Although IL-10 levels increased sharply from the summer to winter, soluble IL-6 receptor levels rose from their lowest point at the beginning of the year to their peak in the fall where they largely remained the rest of the year, much like IL-6. Levels of E-selectin (Model $R^2$: 60.7%), on the other hand, reached their nadir in late summer and increased rapidly to December; E-selectin levels stayed somewhat elevated through spring. Finally, fibrinogen was low from the beginning of the year to summer (Model $R^2$: 56.1%), rising thereafter to its peak in December. Seasonal patterns were not observed for CRP or ICAM-1.

### 5.1.2. Metabolic markers

See Table 2 for statistics. Among the biomarkers of glucose metabolism, results revealed no seasonal patterns for glucose, insulin, or insulin resistance. Levels of HbA1c (Model $R^2$: 60.1%), however, reached their nadir in the fall and then rose steadily through winter to their peak in the spring (Fig. 2). Further, IGF-1 levels were highest in the winter and at their lowest in early fall (Model $R^2$: 60.0%). For lipids, although LDL levels did not appear to vary seasonally, HDL was highest at the beginning of the year and decreased steadily to its lowest point around late fall and early winter (Model $R^2$: 66.6%). Levels of triglycerides (Model $R^2$: 60.0%), on the other hand, peaked in the summer and then decreased to the end of the year. Finally, urinary cortisol and cortisone levels exhibited opposite patterns of seasonal change. Although cortisol levels dropped from their peak in January to their lowest levels around late summer through the rest of the year (Model $R^2$: 56.4%), cortisone remained low from January to July before increasing to its highest levels in December (Model $R^2$: 26.8%).

### 5.2. Perceived stress → health biomarker associations across seasons

Smooth-smooth interactions between PSS scores and month were selected as features in final models for 18 of the 19 outcomes (i.e., all but LDL). As shown in Table 3, this suggests that associations between participants' perceived stress and health biomarker levels consistently varied by month of data collection.

### 5.2.1. Immune markers

With respect to the immune biomarkers (Fig. 3), higher perceived stress scores were related to higher IL-6 levels in the late fall and early winter (Model $R^2$: 70.1%). However, the magnitude of this association was weaker during the rest of the year, particularly during late winter and early spring. Similar results were found for CRP (Model $R^2$: 69.8%) and fibrinogen (Model $R^2$: 54.0%). Levels of IL-10 (Model $R^2$: 47.0%) and TNF-α (Model $R^2$: 57.3%) appeared to vary positively with stress in late fall and early winter, but negatively (albeit weakly) in late winter. For TNF-α, the simple effects of perceived stress in early winter were relatively weak, possibly because of the prominent seasonal elevation of TNF-α levels during this period. Unexpectedly, higher perceived stress levels were associated with lower levels of IL-8 in the first few months of the year (Model $R^2$: 70.2%), with seasonal increases after this time seeming to overshadow any effects of stress the rest of the year. For the remaining immune proteins, stress scores were positively related to I-CAM (Model $R^2$: 71.8%) and E-selectin (Model $R^2$: 78.2%) in late winter/early spring—but less so the rest of the year—and negatively related to levels of soluble IL-6 receptor across seasons (Model $R^2$: 80.5%).

### 5.2.2. Metabolic markers

As shown in Fig. 4, for markers of glucose metabolism, associations between perceived stress and the outcomes assessed tended to be weakest in the first half of the year, with the strongest associations occurring in late fall to early winter (Model $R^2$: 38.6–78.4%). During this period, higher perceived stress levels were related to higher levels of glucose, HbA1C, insulin, and insulin resistance. For lipid levels, higher stress levels predicted higher triglycerides irrespective of season (Model $R^2$: 78.5%). Higher levels of perceived stress were also related to lower HDL levels across the year (Model $R^2$: 82.8%), but seasonal variation remained evident such that even among those with high PSS scores, HDL levels were lowest toward the end of the year. For the remaining metabolic markers, IGF-1 levels were high in the late winter and early spring regardless of PSS scores (Model $R^2$: 69.4%); in early winter, however, higher perceived stress was associated with lower levels of IGF-1. Finally, higher perceived stress scores were related to lower levels of cortisol from the beginning of the year through spring (Model $R^2$: 57.3%), and lower levels of cortisone around late fall/early winter (Model $R^2$: 31.4%).

### 5.2.3. Linear models

Finally, we binned months into seasons (spring, summer, fall, winter) and examined both main effects of this categorical variable and interactions with PSS scores on participants' biomarker levels using linear mixed-effects models (see Supplementary File 2 for full results and plots of interactions). For the most part, seasonal effects were attenuated by categorizing months into discrete seasons, which is to be expected as the results of the GAMs revealed sometimes stark differences between months within the same season (e.g., early vs. late winter). Levels of certain immune proteins (e.g., IL-10, TNF-α, E-selectin) were nonetheless significantly higher during winter compared to other months in the linear models, but null results were found for most other biomarkers. Furthermore, interactions between participants' perceived stress scores and season were only statistically significant for a small portion of the biomarkers, even though consistent seasonal effects were found for month of year in the boosted models. Simple slopes analyses revealed that, even in the case of a non-significant interaction between perceived stress levels and season, the direction of season-specific perceived stress effects was consistent with the boosted models in cases when associations were similar between the three months in a given season, but inconsistent when this was not the case. Together, these results indicate that the extent to which health biomarkers and their associations with stress vary seasonally may not be captured in models that omit the non-linear effects of month.
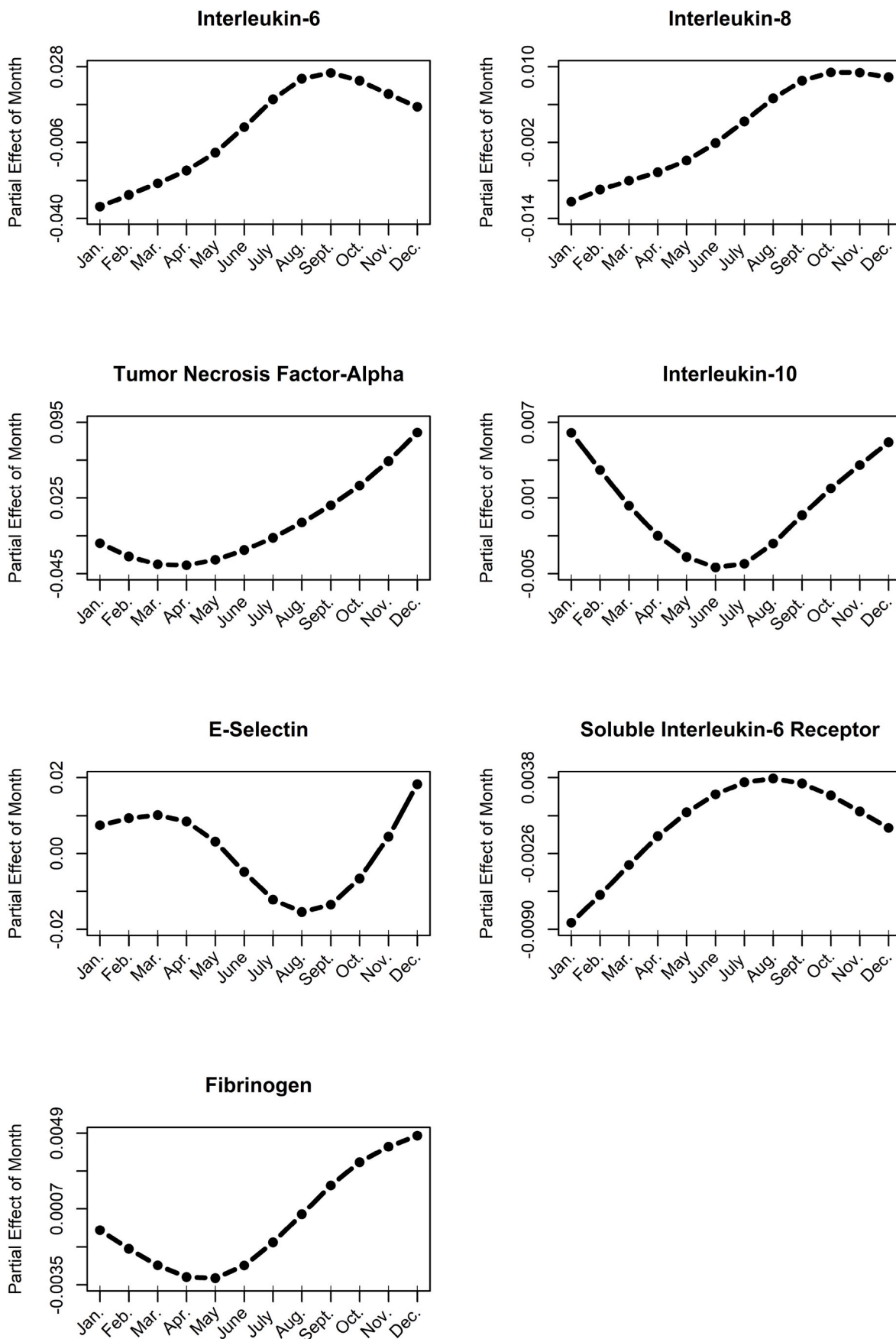
**Fig. 1.** Main effects of month on immune biomarkers from component-wide gradient-boosted generalized additive models. Month of measurement is displayed on the x-axis, whereas the y-axis represents the partial effect of month.
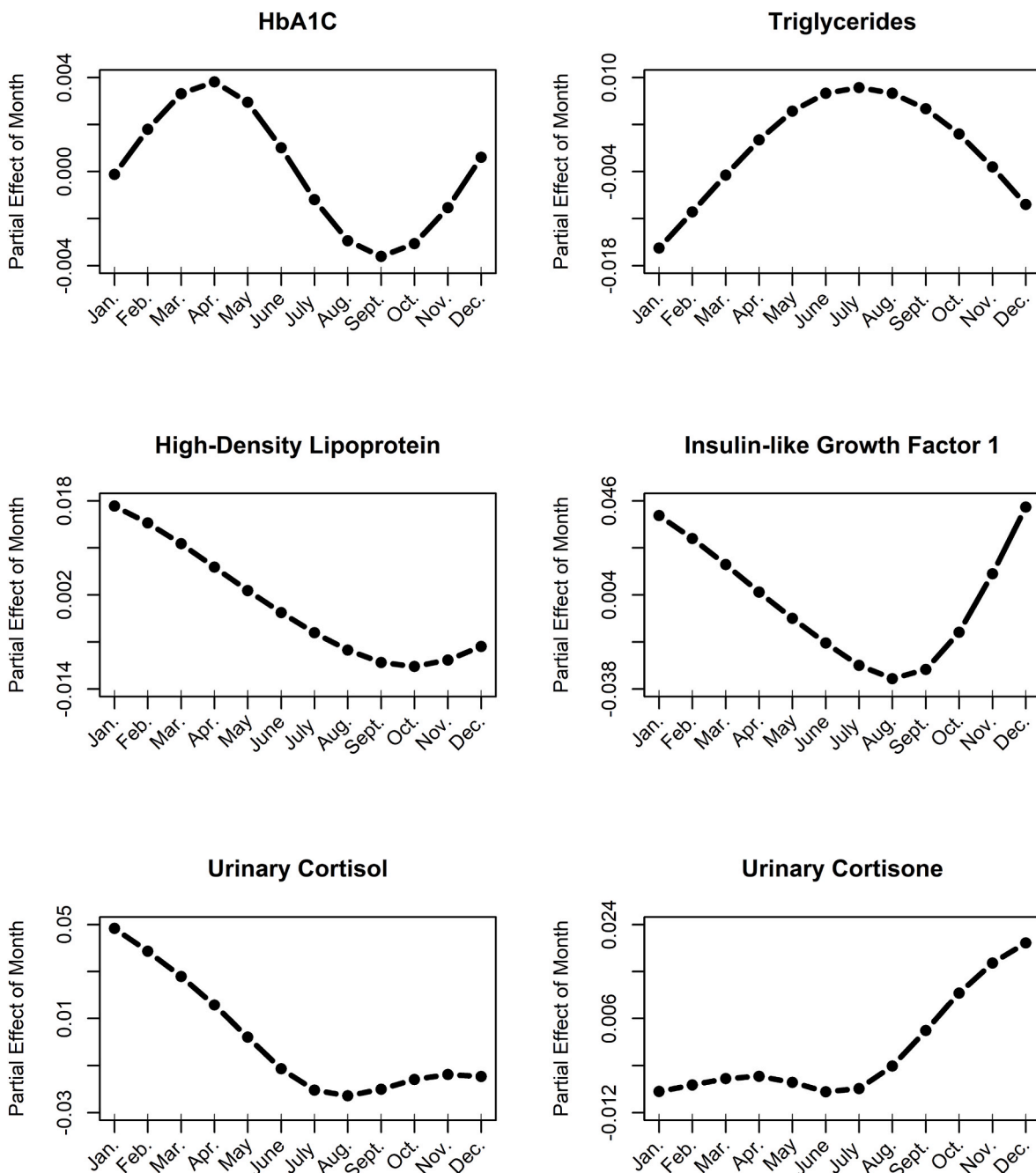
**Fig. 2.** Main effects of month on metabolic biomarkers from component-wide gradient-boosted generalized additive models. Month of measurement is displayed on the x-axis, whereas the y-axis represents the partial effect of month.

## 6. Discussion

Although several studies examining immune and metabolic health biomarkers in the context of stress have adjusted for seasonal effects (e. g., Cohen et al., 2012; Corallo et al., 2021; Gassen et al., 2021, 2022; Malarkey et al., 1995; Murphy et al., 2017; Pereira et al., 2019), the existence of interactions between stress and time of year may render the current convention of including a categorical season variable as a co-variate inadequate. With this in mind, we investigated seasonal changes in 19 immune and metabolic biomarkers, and, additionally, whether associations between perceived stress and these biomarkers changed across months of the year. The results provide robust evidence of sea-sonal variation for most of the biomarkers assessed. Moreover, both the strength *and* direction of the perceived stress → health biomarker effect

changed depending on biomarker assessment month. Studies that do not account for precisely when biomarkers are assessed may thus lead to misleading or unreproducible results.

With regard to differences in biomarker levels across the year, we found that immune biomarkers tended to peak toward the end of the year in months when days were shortest. These results are consistent with prior research finding that certain white blood cell populations and their effector functions, cytokines and other immune proteins, and pro-inflammatory transcriptional signatures are elevated in the winter months (Dopico et al., 2015; Gassen et al., 2019; Liu and Taioli, 2015; Sailani et al., 2020). Transcriptional changes and alterations to cell populations both provide potential explanations—that are not mutually exclusive—for short-day increases in immune biomarkers. With respect to the glucose metabolism markers assessed, levels of HbA1C peaked in

**Table 3**
Statistics and selection frequencies for perceived stress × month interactions.

| Outcome | Optimal Stopping Iteration | Relative Selection Frequency | Model $R^2$ |
|---|---|---|---|
| Hemoglobin A1C | 10,000 | 12.20% | 78.4% |
| Triglycerides | 10,000 | 22.67% | 78.5% |
| High-Density Lipoprotein | 10,000 | 10.53% | 82.8% |
| Low-Density Lipoprotein | 3619 | Not Selected | 43.5% |
| Glucose | 2686 | 10.34% | 38.6% |
| Insulin | 6652 | 5.26% | 72.2% |
| Insulin Resistance | 7566 | 10.29% | 75.5% |
| Insulin-like Growth Factor 1 | 7181 | 8.70% | 69.4% |
| Cortisol | 3888 | 6.63% | 57.3% |
| Cortisone | 2185 | 3.33% | 31.4% |
| Interleukin-6 | 6578 | 11.90% | 70.1% |
| Interleukin-8 | 7860 | 9.86% | 70.2% |
| Interleukin-10 | 4219 | 17.86% | 47.0% |
| Tumor Necrosis Factor-Alpha | 5150 | 10.29% | 57.3% |
| C-Reactive Protein | 6447 | 10.61% | 69.8% |
| Fibrinogen | 4004 | 7.48% | 54.0% |
| E-selectin | 9998 | 4.64% | 78.2% |
| Intercellular Adhesion Molecule 1 | 8283 | 13.50% | 71.8% |
| Soluble Interleukin-6 Receptor | 10,000 | 24.32% | 80.5% |

the spring and were lowest in the fall, whereas insulin, glucose, and insulin resistance did not appear to vary seasonally, contrary to our predictions. Among the lipid biomarkers, triglycerides were highest in late summer and early fall, and levels of HDL decreased steadily from the

beginning to end of the year. Seasonal effects were not observed for LDL.

Finally, for all but one of the biomarkers assessed (i.e., LDL), we observed seasonal variation in the strength—and in certain cases, direction—of stress-biomarker associations. For example, inflammatory biomarkers (i.e., IL-6, CRP, fibrinogen, IL-10, TNF-α) varied positively with perceived stress in late fall/early winter, but these associations were attenuated (i.e., IL-6, CRP, fibrinogen) or switched directions (i.e., IL-10, TNF-α) later in the year. Similar patterns were observed for markers of glucose metabolism (e.g., HbA1C, glucose, insulin, HOMA-IR), even though the main effects of month were not selected for many of these outcomes in the initial models that excluded month by PSS interactions. Further, higher perceived stress scores were consistently associated with higher triglycerides and lower HDL levels across the year. However, for the latter lipid marker, a rise in HDL from the beginning to the end of the year was prominent even for those with high PSS scores.

The models tested explained large portions of biomarker variance in the present sample (see Table 3, $R^2$: 38.6%–82.8%), which is also attributable to the additive effects of covariates selected for each model (i.e., age, sex, BMI, and year), in addition to the stress-month tensor interactions. The follow-up linear models that involved binning months into a categorical season variable provided additional evidence that for certain biomarkers, annual timing of sample collection influenced both whether statistically significant effects of stress on biomarker levels were found and, in addition, the direction of those associations (see Supplementary File 2). Together, these results suggest that not only do levels of biomarkers vary seasonally as has been shown in prior research (Dopico et al., 2015; Gassen et al., 2021; Liu and Taioli, 2015; Sailani et al., 2020; Wyse et al., 2021) but, in addition, that *associations* between perceived stress and health biomarker levels differ by month of data
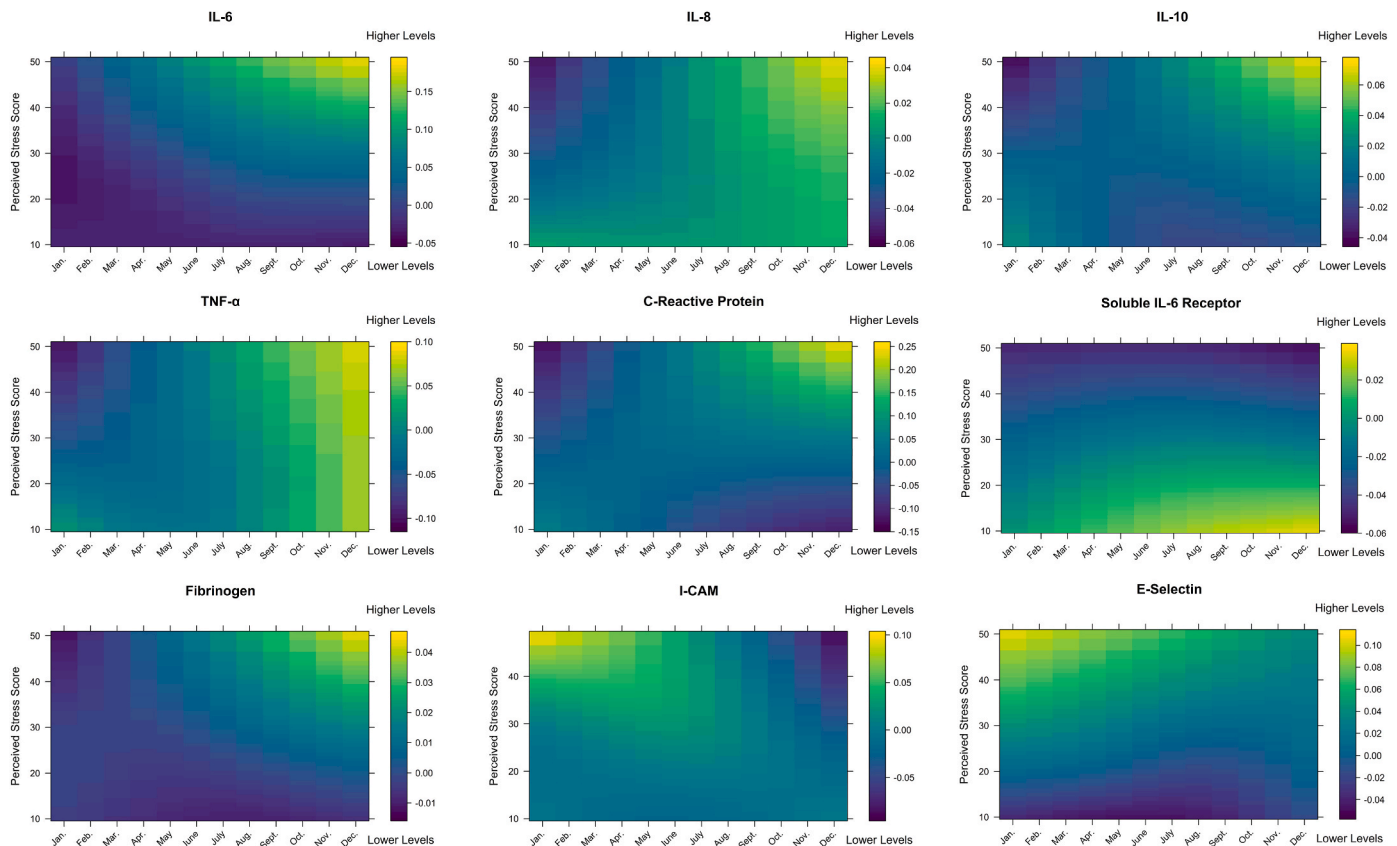


**Fig. 3.** Tensor product P-spline interactions between perceived stress scale (PSS) scores and month on metabolic biomarkers from component-wide gradient-boosted generalized additive models. Month of measurement is displayed on the x-axis, whereas the y-axis represents participants' perceived stress scores. The color gradient reflects effects that change from positive (yellow) to negative (purple). HbA1C = hemoglobin A1C, HOMA-IR = homeostatic model assessment for insulin resistance, IGF-1 = insulin-like growth factor 1, HDL = high-density lipoprotein.
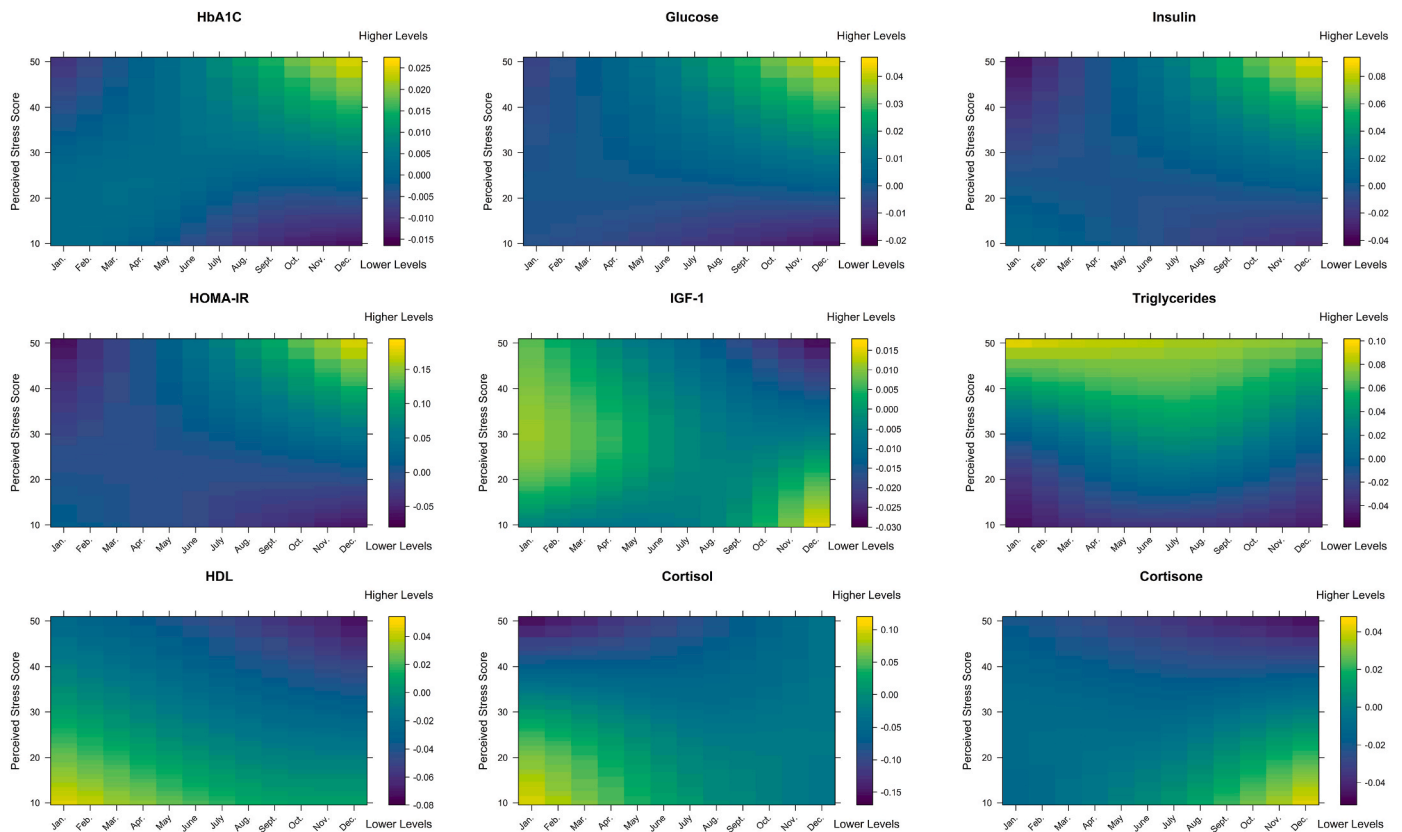
**Fig. 4.** Tensor product P-spline interactions between perceived stress scale (PSS) scores and month on metabolic biomarkers from component-wide gradient-boosted generalized additive models. Month of measurement is displayed on the x-axis, whereas the y-axis represents participants' perceived stress scores. The color gradient reflects effects that change from positive (yellow) to negative (purple). HbA1C = hemoglobin A1C, HOMA-IR = homeostatic model assessment for insulin resistance, IGF-1 = insulin-like growth factor 1, HDL = high-density lipoprotein.

collection. Accordingly, the present results indicate that seasonal timing of data collection may influence researchers' ability to identify and estimate stress-biomarker associations, and render conventional methods of adjusting for seasonality (e.g., categorical season covariate) inadequate. These findings could have important implications for research examining links between stress, immune, function, and metabolism, as well as biomedical health research more broadly.

### 6.1. Limitations and future research

This research has several limitations. First, although longitudinal measurements were available for a subset of participants, these data were otherwise cross-sectional. Combining cross-sectional and panel data likely reduced the precision of seasonal estimates, and although we do not believe this procedure introduced any systematic bias, the possibility remains. Further, seasonal patterns estimated using between-subjects data may not reflect the shape or magnitude of biomarker change across the year within individuals. Future studies involving repeated biomarker measurements for the same participants, several times over the course of the year, are necessary to precisely determine the consistency and magnitude of seasonal variation in human biology. Such research may also lend novel insights into moderators (i.e., in addition to perceived stress) of seasonal effects that will help identify if and why some individuals are more sensitive to seasonal changes than others. Second, this study did not investigate environmental mediators of seasonal effects. Whereas experimental animal work on seasonal variation in physiology has focused mostly on the mediating effects of day length, food availability, and temperature, human environments are characterized by several other features that may contribute to changes in biomarkers across the seasons, such as seasonal variation in infectious

diseases, work or academic stress, and holiday schedules, among others. Further research is needed to determine how different environmental factors mediate or attenuate changes in biomarker levels across the year.

Research on seasonality is limited to observational studies and, therefore, it is difficult to estimate causal effects. Although few variables are likely to confound relations between time of year and an outcome specifically, there may be many measured and unmeasured confounds of mediators and moderators of seasonality. For example, shift work may increase both perceived stress and markers of inflammation (Christensen et al., 2021; Matre et al., 2021; Velazquez-Kronen et al., 2023). Travel across time zones (Doane et al., 2010) and other factors that affect biorhythms not measured in the present study likely also impact patterns of seasonal change in biomarkers. Future research is needed to replicate the current results and investigate social and environmental mediators of seasonality while keeping potential confounds in mind. Simulation studies may lend further insights into the implications that season-risk factor interactions have for observational and experimental research.

In the present research, we focused primarily on interpreting the moderating effects that month of data collection have on those of perceived stress given the practical implications of such patterns for stress-biomarker studies. However, because the data were observational and both variables involved in the focal interaction were continuous, the analyses were agnostic as to whether effect modification (one factor varies across level of the other) or interaction (each factor varies across levels of the other) occurred (Attia et al., 2022; Bours, 2021; Vander-Weele and Robins, 2007). This distinction may be important for research aiming to adjust for or estimate seasonal effects. Although not directly tested, our results do provide some preliminary evidence in favor of interaction. For example, interaction requires that the joint effect of both

variables is different from the sum of the individual effects of each (Bours, 2021; Corraini et al., 2017). For nearly all biomarkers, the perceived stress × month interaction offered predictive utility above the additive effects of each variable individually. Further, plots of results for most biomarkers suggest that both the effects of perceived stress and month vary across values of the other. Experimental stress studies conducted at different times of the year may shed further light on whether season truly moderates biological responses to stress.

Finally, we did not perform external validation of our prediction models as our primary aim was to investigate the presence and patterns of stress-season interactions. Accordingly, as with any study, it is possible that the seasonal patterns found here may not generalize well to other populations. Future MIDUS waves will provide important opportunities to evaluate the reproducibility of the present findings and test for possible differences between periods of sample collection.

### 6.2. Study design recommendations and conclusions

Notwithstanding these limitations, the present data indicate that researchers investigating immune or metabolic biomarkers—as well as stress-health biomarker associations—should not only account for the seasonal timing of data collection to prevent seasonality from unknowingly biasing or reducing the precision of inferences, but also consider the possibility of seasonal effect modification or interaction. Using the present research as a simple but striking example, if we had used MIDUS data collected in only one season, for many of the biomarkers assessed, the effects of stress on these biomarkers would *not* have generalized to the rest of the year. In addition to leading to imprecise or incorrect inferences, failing to account for moderation by biomarker assessment month would also preclude the examination of mechanisms underlying season-specific links between stress and biomarker levels, whether they be dietary, social, biological, or even just due to unmeasured confounding. Given that time of year appears to have sweeping associations with human physiology, regardless of the underlying mediating mechanisms (Dopico et al., 2015; Gassen et al., 2019; Liu and Taioli, 2015; Sailani et al., 2020), biomedical health researchers are urged to carefully consider and assess the potential impacts of seasonality even if they do not study stress.

Ultimately, the consequences of seasonality for studies of health will depend on the research questions asked and the type of model estimated. For example, if a researcher aims to study seasonality itself, our results suggest that simply binning months into four seasons is insufficient to capture the non-linearity, timing, and within-season variation of changes to biomarker levels across the year. Therefore, if adequate power is available, granular measurements of seasonality (e.g., days, months, weeks of year) are preferred over categorical season variables. Careful consideration should also be given to the mathematical form of the variable representing the season construct, as incorrect specification is common when dealing with non-linear effects, itself a potential source of bias (Cui et al., 2009; Laubach et al., 2021; Wysocki et al., 2022).

If and how seasonality should be introduced into models to "control" for its effects in observational data is less straightforward. One consideration is whether seasonality is actually a confound in the association between a predictor and outcome (i.e., influences both X and Y) (Greenland and Morgenstern, 2001). If not, adjusting for time of year may not even be necessary (although could be beneficial to increase power) when it does not moderate the effects of the target predictor (Kahan et al., 2014). For example, in the present study, perceived stress levels did not vary substantively as a function of month and there is not a well-established causal path from season to perceived stress scores that we are aware of (although there likely is for specific stressors, like academic stress). Accordingly, there is not an obvious need to adjust for the effects of month when examining perceived stress-biomarker associations in general populations (Wysocki et al., 2022). However, our results do suggest that even if time of year is not a true confound, a second consideration should be whether there are statistical interactions

between the focal predictor and season on the target outcome. Depending on the nature of the interaction, main effects of the predictor could be biased or imprecise because they differ seasonally (Attia et al., 2022; Cohen et al., 2021; Kievit et al., 2013; Vander Weele, 2012). These considerations also apply to situations where time of year *does* confound relations between the target X and Y. That is, controlling for seasonality may introduce bias if the season variable is improperly modeled (e.g., neglecting to include appropriate non-linear effects) or if an interaction between season and the predictor exists, but is not included in the model (Laubach et al., 2021).

Similar care should be exercised when thinking about other potential covariates in analyses involving the effects of seasonality. In addition to considering the form of the variable and its potential as a moderator, researchers should also evaluate its necessity in the model at all (Middleton et al., 2016; Schisterman et al., 2009; van Zwieten et al., 2022). Instead of decreasing bias, controlling for highly correlated variables, colliders, proxies, and even mediators (e.g., sleep) in associations between season and outcomes can have the opposite effect (Wysocki et al., 2022). With season and day length, there are far more variables that meet these criteria than that of a confound. Nonetheless, research seeking to estimate seasonal effects should still consider the possibility of effect modification or interaction, even if confounding is not a primary concern.

In conclusion, the present results suggest that biomedical health researchers should not only consider the effects of seasonality, but also potential interactions between season and other risk factors of interest. Doing so may lead to novel insights into the interplay of environments, health, and chronobiology. It could also help to improve the precision of inferences about associations between a variety of risk factors, health-relevant biomarkers, and human health and wellbeing. Herein, we demonstrate the importance of accounting for the impact of seasonality on biomarkers levels in the context of stress research, but the implications of this work extend to all studies examining links between an establish risk factor for poor health and the immune and metabolic markers assessed here. Put simply, studies that do not account for month of biomarker assessment may potentially yield misleading or unreproducible results.

### Funding

### CRediT authorship contribution statement

**Jeffrey Gassen:** Conceptualization, Data curation, Formal analysis, Investigation, Visualization, Writing – original draft, Writing – review & editing. **Summer Mengelkoch:** Conceptualization, Investigation, Project administration, Writing – original draft, Writing – review & editing. **George M. Slavich:** Conceptualization, Funding acquisition, Investigation, Resources, Supervision, Writing – original draft, Writing – review & editing.

## Declaration of competing interest

The authors declare no conflicts of interest with respect to this work.

## Data availability

Data are publicly available

## Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.bbih.2024.100793.

## References

Attia, J., Holliday, E., Oldmeadow, C., 2022. A proposal for capturing interaction and effect modification using DAGs. Int. J. Epidemiol. 51 (4), 1047–1053. https://doi.org/10.1093/ije/dyac126.

Bartlett, P., Traskin, M., 2007. AdaBoost Is Consistent, vol. 8, pp. 2347–2368.

Bours, M.J.L., 2021. Tutorial: a nontechnical explanation of the counterfactual definition of effect modification and interaction. J. Clin. Epidemiol. 134, 113–124. https://doi.org/10.1016/j.jclinepi.2021.01.022.

Ceballos, N.A., Sharma, S., Patterson, T.L., Graham, R., Howard, K., 2015. Stress, immune function and Collegiate holiday Drinking: a Pilot study. Neuropsychobiology 72 (1), 8–15. https://doi.org/10.1159/000438757.

Chesnaye, N.C., Stel, V.S., Tripepi, G., Dekker, F.W., Fu, E.L., Zoccali, C., Jager, K.J., 2021. An introduction to inverse probability of treatment weighting in observational research. Clinical Kidney Journal 15 (1), 14–20. https://doi.org/10.1093/ckj/sfab158.

Christensen, J.O., Nilsen, K.B., Hopstock, L.A., Steingrímsdóttir, Ó.A., Nielsen, C.S., Zwart, J.-A., Matre, D., 2021. Shift work, low-grade inflammation, and chronic pain: a 7-year prospective study. Int. Arch. Occup. Environ. Health 94 (5), 1013–1022. https://doi.org/10.1007/s00420-020-01626-2.

Cohen, J.B., McGowan, L.D., Jensen, E.T., Rigdon, J., South, A.M., 2021. Evaluating sources of bias in observational studies of angiotensin-converting enzyme inhibitor/angiotensin II receptor blocker use during COVID-19: beyond confounding. J. Hypertens. 39 (4), 795–805. https://doi.org/10.1097/HJH.0000000000002706.

Cohen, S., 1988. Perceived stress in a probability sample of the United States. In: The Social Psychology of Health. Sage Publications, Inc, pp. 31–67.

Cohen, S., Janicki-Deverts, D., Doyle, W.J., Miller, G.E., Frank, E., Rabin, B.S., Turner, R.B., 2012. Chronic stress, glucocorticoid receptor resistance, inflammation, and disease risk. Proc. Natl. Acad. Sci. USA 109 (16), 5995–5999. https://doi.org/10.1073/pnas.1118355109.

Cohen, S., Kamarck, T., Mermelstein, R., 1983. A global measure of perceived stress. J. Health Soc. Behav. 24 (4), 385–396. https://doi.org/10.2307/2136404.

Corallo, K.L., Lyle, S.M., Murphy, M.L.M., vanDellen, M.R., Ehrlich, K.B., 2021. Recalled Neighborhood environments, Parental control, and cytokine-mediated response to Viral challenge. Psychosom. Med. 83 (8), 843–851. https://doi.org/10.1097/PSY.0000000000000991.

Corraini, P., Olsen, M., Pedersen, L., Dekkers, O.M., Vandenbroucke, J.P., 2017. Effect modification, interaction and mediation: an overview of theoretical insights for clinical investigators. Clin. Epidemiol. 9, 331–338. https://doi.org/10.2147/CLEP.S129728.

Cui, X., Guo, W., Lin, L., Zhu, L., 2009. Covariate-adjusted nonlinear regression. Ann. Stat. 37 (4), 1839–1870. https://doi.org/10.1214/08-AOS627.

Doane, L.D., Kremen, W.S., Eaves, L.J., Eisen, S.A., Hauger, R., Hellhammer, D., Levine, S., Lupien, S., Lyons, M.J., Mendoza, S., Prom-Wormley, E., Xian, H., York, T.P., Franz, C.E., Jacobson, K.C., 2010. Associations between Jet Lag and cortisol diurnal rhythms after Domestic travel. Health Psychol. : Official Journal of the Division of Health Psychology, American Psychological Association 29 (2), 117–123. https://doi.org/10.1037/a0017865.

Dopico, X.C., Evangelou, M., Ferreira, R.C., Guo, H., Pekalski, M.L., Smyth, D.J., Cooper, N., Burren, O.S., Fulford, A.J., Hennig, B.J., Prentice, A.M., Ziegler, A.-G., Bonifacio, E., Wallace, C., Todd, J.A., 2015. Widespread seasonal gene expression reveals annual differences in human immunity and physiology. Nat. Commun. 6 (1) https://doi.org/10.1038/ncomms8000. Article 1.

Falkenbach, A., Sedlmeyer, A., 1997. Travel to sunny countries is associated with changes in immunological parameters. Photodermatol. Photoimmunol. Photomed. 13 (4), 139–142. https://doi.org/10.1111/j.1600-0781.1997.tb00217.x.

Gassen, J., Nowak, T.J., Henderson, A.D., Muehlenbein, M.P., 2022. Dynamics of temperature change during experimental respiratory virus challenge: Relationships with symptoms, stress hormones, and inflammation. Brain Behav. Immun. 99, 157–165. https://doi.org/10.1016/j.bbi.2021.10.001.

Gassen, J., Proffitt Leyva, R.P., Mengelkoch, S., White, J.D., Peterman, J.L., Prokosch, M.L., Bradshaw, H.K., Eimerbrink, M.J., Corrigan, E.K., Cheek, D.J., Boehm, G.W., Hill, S.E., 2019. Day length predicts investment in human immune function: shorter days yield greater investment. Psychoneuroendocrinology 107, 141–147. https://doi.org/10.1016/j.psyneuen.2019.05.011.

Gassen, J., White, J.D., Peterman, J.L., Mengelkoch, S., Proffitt Leyva, R.P., Prokosch, M.L., Eimerbrink, M.J., Brice, K., Cheek, D.J., Boehm, G.W., Hill, S.E., 2021. Sex differences in the impact of childhood socioeconomic status on immune function. Sci. Rep. 11 (1), 9827. https://doi.org/10.1038/s41598-021-89413-y.

Gikas, A., Sotiropoulos, A., Pastromas, V., Papazafiropoulou, A., Apostolou, O., Pappas, S., 2009. Seasonal variation in fasting glucose and HbA1c in patients with type 2 diabetes. Primary Care Diabetes 3 (2), 111–114. https://doi.org/10.1016/j.pcd.2009.05.004.

Gowey, M.A., Khodneva, Y., Tison, S.E., Carson, A.P., Cherrington, A.L., Howard, V.J., Safford, M.M., Dutton, G.R., 2019. Depressive symptoms, perceived stress, and metabolic health: the REGARDS study. Int. J. Obes. 43 (3), 615–632. https://doi.org/10.1038/s41366-018-0270-3.

Greenland, S., Morgenstern, H., 2001. Confounding in health research. Annu. Rev. Publ. Health 22, 189–212. https://doi.org/10.1146/annurev.publhealth.22.1.189.

Gruber, S., Logan, R.W., Jarrín, I., Monge, S., Hernán, M.A., 2015. Ensemble learning of inverse probability weights for marginal structural modeling in large observational datasets. Stat. Med. 34 (1), 106–117. https://doi.org/10.1002/sim.6322.

Hofner, B., Kneib, T., Hothorn, T., 2016. A unified framework of constrained regression. Stat. Comput. 26 (1), 1–14. https://doi.org/10.1007/s11222-014-9520-y.

Hofner, B., Mayr, A., Robinzonov, N., Schmid, M., 2014. Model-based boosting in R: a hands-on tutorial using the R package mboost. Comput. Stat. 29, 3–35. https://doi.org/10.1007/s00180-012-0382-5.

Hothorn, T., Bühlmann, P., Kneib, T., Schmid, M., Hofner, B., 2010. Model-based boosting 2.0. J. Mach. Learn. Res. 11.

Hruska, B., Pressman, S.D., Bendinskas, K., Gump, B.B., 2020. Vacation frequency is associated with metabolic syndrome and symptoms. Psychol. Health 35 (1), 1–15. https://doi.org/10.1080/08870446.2019.1628962.

James, G.D., 1991. Race and perceived stress independently affect the diurnal variation of blood pressure in women. Am. J. Hypertens. 4 (4 Pt 1), 382–384. https://doi.org/10.1093/ajh/4.4.382.

Jones, A.G., McDonald, T.J., Hattersley, A.T., Shields, B.M., 2014. Effect of the holiday season in patients with diabetes: Glycemia and lipids increase postholiday, but the effect is small and transient. Diabetes Care 37 (5), e98–e99. https://doi.org/10.2337/dc13-2353.

Jurgens, S.M., Prieto, S., Hayes, J.P., 2023. Inflammatory biomarkers link perceived stress with metabolic dysregulation. Brain, Behavior, & Immunity - Health 34, 100696. https://doi.org/10.1016/j.bbih.2023.100696.

Kahan, B.C., Jairath, V., Doré, C.J., Morris, T.P., 2014. The risks and rewards of covariate adjustment in randomized trials: an assessment of 12 outcomes from 8 studies. Trials 15, 139. https://doi.org/10.1186/1745-6215-15-139.

Kershenbaum, A., Kershenbaum, A., Tarabeia, J., Stein, N., Lavi, I., Rennert, G., 2011. Unraveling seasonality in population averages: an examination of seasonal variation in glucose levels in diabetes patients using a large population-based data set. Chronobiol. Int. 28 (4), 352–360. https://doi.org/10.3109/07420528.2011.560315.

Kievit, R.A., Frankenhuis, W.E., Waldorp, L.J., Borsboom, D., 2013. Simpson's paradox in psychological science: a practical guide. Front. Psychol. 4, 513. https://doi.org/10.3389/fpsyg.2013.00513.

Knight, E.L., Jiang, Y., Rodriguez-Stanley, J., Almeida, D.M., Engeland, C.G., Zilioli, S., 2021. Perceived stress is linked to heightened biomarkers of inflammation via diurnal cortisol in a national sample of adults. Brain Behav. Immun. 93, 206–213. https://doi.org/10.1016/j.bbi.2021.01.015.

Koch, C.E., Leinweber, B., Drengberg, B.C., Blaum, C., Oster, H., 2016. Interaction between circadian rhythms and stress. Neurobiology of Stress 6, 57–67. https://doi.org/10.1016/j.ynstr.2016.09.001.

Laubach, Z.M., Murray, E.J., Hoke, K.L., Safran, R.J., Perng, W., 2021. A biologist's guide to model selection and causal inference. Proc. Biol. Sci. 288 (1943), 20202815 https://doi.org/10.1098/rspb.2020.2815.

Lehrer, H.M., Steinhardt, M.A., Dubois, S.K., Laudenslager, M.L., 2020. Perceived stress, psychological resilience, hair cortisol concentration, and metabolic syndrome severity: a moderated mediation model. Psychoneuroendocrinology 113, 104510. https://doi.org/10.1016/j.psyneuen.2019.104510.

Liu, B., Taioli, E., 2015. Seasonal variations of complete blood count and inflammatory biomarkers in the US population—analysis of NHANES data. PLoS One 10 (11), e0142382. https://doi.org/10.1371/journal.pone.0142382.

Liu, Y.-Z., Wang, Y.-X., Jiang, C.-L., 2017. Inflammation: the common pathway of stress-related diseases. Front. Hum. Neurosci. 11, 316. https://doi.org/10.3389/fnhum.2017.00316.

Lovell, B., Moss, M., Wetherell, M.A., 2011. Perceived stress, common health complaints and diurnal patterns of cortisol secretion in young, otherwise healthy individuals. Horm. Behav. 60 (3), 301–305. https://doi.org/10.1016/j.yhbeh.2011.06.007.

Malarkey, W.B., Pearl, D.K., Demers, L.M., Kiecolt-Glaser, J.K., Glaser, R., 1995. Influence of academic stress and season on 24-hour mean concentrations of ACTH, cortisol, and beta-endorphin. Psychoneuroendocrinology 20 (5), 499–508. https://doi.org/10.1016/0306-4530(94)00077-n.

Mariné-Casadó, R., Domenech-Coca, C., Del Bas, J.M., Bladé, C., Arola, L., Caimari, A., 2018. The exposure to different photoperiods strongly Modulates the glucose and lipid metabolisms of Normoweight Fischer 344 Rats. Front. Physiol. 9, 416. https://doi.org/10.3389/fphys.2018.00416.

Martínez de Toda, I., Miguélez, L., Siboni, L., Vida, C., De la Fuente, M., 2019. High perceived stress in women is linked to oxidation, inflammation and immunosenescence. Biogerontology 20 (6), 823–835. https://doi.org/10.1007/s10522-019-09829-y.

Matre, D., Christensen, J.O., Mork, P.J., Ferreira, P., Sand, T., Nilsen, K.B., 2021. Shift work, inflammation and musculoskeletal pain-The HUNT Study. Occupational Medicine (Oxford, England) 71 (9), 422–427. https://doi.org/10.1093/occmed/kqab133.

Mauss, D., Jarczok, M.N., 2021. The streamlined allostatic load index is associated with perceived stress in life—findings from the MIDUS study. Stress 24 (4), 404–412. https://doi.org/10.1080/10253890.2020.1869935.

Middleton, J.A., Scott, M.A., Diakow, R., Hill, J.L., 2016. Bias Amplification and bias Unmasking. Polit. Anal. 24 (3), 307–323. https://doi.org/10.1093/pan/mpw015.

Murphy, M.L.M., Cohen, S., Janicki-Deverts, D., Doyle, W.J., 2017. Offspring of parents who were separated and not speaking to one another have reduced resistance to the common cold as adults. Proc. Natl. Acad. Sci. USA 114 (25), 6515–6520. https://doi.org/10.1073/pnas.1700610114.

Nelson, R.J., 2004. Seasonal immune function and sickness responses. Trends Immunol. 25 (4), 187–192. https://doi.org/10.1016/j.it.2004.02.001.

Nelson, R.J., Demas, G.E., 1996. Seasonal changes in immune function. Q. Rev. Biol. 71 (4), 511–548. https://doi.org/10.1086/419555.

Nelson, R.J., Demas, G.E., Klein, S.L., Kriegsfeld, L.J., 2002. Seasonal Patterns of Stress, Immune Function, and Disease. Cambridge University Press. https://doi.org/10.1017/CBO9780511546341 xv, 291.

Otsuka, T., Kawai, M., Togo, Y., Goda, R., Kawase, T., Matsuo, H., Iwamoto, A., Nagasawa, M., Furuse, M., Yasuo, S., 2014. Photoperiodic responses of depression-like behavior, the brain serotonergic system, and peripheral metabolism in laboratory mice. Psychoneuroendocrinology 40, 37–47. https://doi.org/10.1016/j.psyneuen.2013.10.013.

Pereira, S.M.P., Merkin, S.S., Seeman, T., Power, C., 2019. Understanding associations of early-life adversities with mid-life inflammatory profiles: evidence from the UK and USA. Brain Behav. Immun. 78, 143–152. https://doi.org/10.1016/j.bbi.2019.01.016.

R Core Team, 2021. R: A Language and Environment for Statistical Computing. R Foundation for Statistical Computing [Computer software].

Radler, B.T., 2014. The midlife in the United States (MIDUS) Series: a national longitudinal study of health and well-being. Open Health Data 2 (1), e3. https://doi.org/10.5334/ohd.ai.

Robertson, S.E., Leith, A., Schmid, C.H., Dahabreh, I.J., 2021. Assessing heterogeneity of treatment effects in observational studies. Am. J. Epidemiol. 190 (6), 1088–1100. https://doi.org/10.1093/aje/kwaa235.

Robins, J.M., Hernán, M.Á., Brumback, B., 2000. Marginal structural models and causal inference in epidemiology. Epidemiology 11 (5), 550.

Rose-John, S., Jenkins, B.J., Garbers, C., Moll, J.M., Scheller, J., 2023. Targeting IL-6 trans-signalling: Past, present and future prospects. Nat. Rev. Immunol. 23 (10), 666–681. https://doi.org/10.1038/s41577-023-00856-y.

Sailani, M.R., Metwally, A.A., Zhou, W., Rose, S.M.S.-F., Ahadi, S., Contrepois, K., Mishra, T., Zhang, M.J., Kidziński, Ł., Chu, T.J., Snyder, M.P., 2020. Deep longitudinal multiomics profiling reveals two biological seasonal patterns in California. Nat. Commun. 11 (1) https://doi.org/10.1038/s41467-020-18758-1. Article 1.

Schisterman, E.F., Cole, S.R., Platt, R.W., 2009. Overadjustment bias and Unnecessary adjustment in Epidemiologic studies. Epidemiology 20 (4), 488–495. https://doi.org/10.1097/EDE.0b013e3181a819a1.

Slavich, G.M., 2016. Life stress and health: a review of conceptual issues and recent findings. Teach. Psychol. 43, 346–355. https://doi.org/10.1177/0098628316662768.

Slavich, G.M., Roos, L.G., Mengelkoch, S., Webb, C.A., Shattuck, E.C., Moriarity, D.P., Alley, J.C., 2023. Social Safety Theory: conceptual foundation, underlying mechanisms, and future directions. Health Psychol. Rev. 17, 5–59. https://doi.org/10.1080/17437199.2023.2171900.

Sribanditmongkol, V., Neal, J.L., Patrick, T.E., Szalacha, L.A., McCarthy, D.O., 2015. Effect of perceived stress on cytokine production in healthy college students. West. J. Nurs. Res. 37 (4), 481–493. https://doi.org/10.1177/0193945914545658.

Tsujimoto, T., Yamamoto-Honda, R., Kajio, H., Kishimoto, M., Noto, H., Hachiya, R., Kimura, A., Kakei, M., Noda, M., 2014. Seasonal variations of severe hypoglycemia in patients with type 1 diabetes mellitus, type 2 diabetes mellitus, and non-diabetes mellitus: clinical analysis of 578 hypoglycemia cases. Medicine 93 (23), e148. https://doi.org/10.1097/MD.0000000000000148.

Tutz, G., Binder, H., 2006. Generalized additive modeling with implicit variable selection by likelihood-based boosting. Biometrics 62 (4), 961–971. https://doi.org/10.1111/j.1541-0420.2006.00578.x.

Uchakin, P.N., Tobin, B., Cubbage, M., Marshall, G., Sams, C., 2001. Immune responsiveness following academic stress in first-year medical students. J. Interferon Cytokine Res.: The Official Journal of the International Society for Interferon and Cytokine Research 21 (9), 687–694. https://doi.org/10.1089/107999001753124426.

van Zwieten, A., Tennant, P.W.G., Kelly-Irving, M., Blyth, F.M., Teixeira-Pinto, A., Khalatbari-Soltani, S., 2022. Avoiding overadjustment bias in social epidemiology through appropriate covariate selection: a primer. J. Clin. Epidemiol. 149, 127–136. https://doi.org/10.1016/j.jclinepi.2022.05.021.

Vander Weele, T.J., 2012. Confounding and effect modification: Distribution and measure. Epidemiol. Methods 1 (1), 55–82. https://doi.org/10.1515/2161-962X.1004.

VanderWeele, T.J., Robins, J.M., 2007. Four types of effect modification: a classification based on directed acyclic graphs. Epidemiology 18 (5), 561–568. https://doi.org/10.1097/EDE.0b013e318127181b.

Velazquez-Kronen, R., MacDonald, L.A., Akinyemiju, T.F., Cushman, M., Howard, V.J., 2023. Shiftwork, long working hours and markers of inflammation in a national US population-based sample of employed black and white men and women aged ≥45 years. Occup. Environ. Med. 80 (11), 635–643. https://doi.org/10.1136/oemed-2023-108902.

Vetter, T.R., Mascha, E.J., 2017. Bias, confounding, and interaction: Lions and Tigers, and Bears, Oh My! Anesth. Analg. 125 (3), 1042–1048. https://doi.org/10.1213/ANE.0000000000002332.

Walton, J.C., Weil, Z.M., Nelson, R.J., 2011. Influence of photoperiod on hormones, behavior, and immune function. Front. Neuroendocrinol. 32 (3), 303–319. https://doi.org/10.1016/j.yfrne.2010.12.003.

Wood, S.N., Scheipl, F., Faraway, J.J., 2013. Straightforward intermediate rank tensor product smoothing in mixed models. Statistical Computing 23, 341–360. https://doi.org/10.1007/s11222-012-9314-z.

Wu, X., Lu, Y., Xie, X., Chen, R., Zhang, N., Zhou, C., Ye, Z., 2022. Association between circadian rhythm and sleep quality among nursing interns: a latent profile and moderation analysis. Front. Neurosci. 16, 995775 https://doi.org/10.3389/fnins.2022.995775.

Wyse, C., O'Malley, G., Coogan, A.N., McConkey, S., Smith, D.J., 2021. Seasonal and daytime variation in multiple immune parameters in humans: evidence from 329,261 participants of the UK Biobank cohort. iScience 24 (4), 102255. https://doi.org/10.1016/j.isci.2021.102255.

Wysocki, A.C., Lawson, K.M., Rhemtulla, M., 2022. Statistical control requires causal Justification. Advances in Methods and Practices in Psychological Science 5 (2), 25152459221095823. https://doi.org/10.1177/25152459221095823.