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# Day length predicts investment in human immune function: Shorter days yield greater investment

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ABSTRACT

Winter is characterized by stressful conditions which compromise health and render animals more vulnerable to infection and illness than during other times of the year. Organisms are hypothesized to adapt to these seasonal stressors by increasing investment in immune function in response to diminished photoperiod duration. Here, we examined this hypothesis in a sample of healthy human participants. Using several functional immune assays in vitro, as well as by utilizing measures of in vivo proinflammatory cytokine levels, we predicted that shorter day length would be associated with greater investment in immunological function. Results revealed that shorter days predicted significant upregulation of several facets of immune function, including natural killer cell cytotoxicity, peripheral blood mononuclear cell proliferation (in response to, and in the absence of stimulation), and plasma levels of interleukin-6, as well as lower rates of *Staphylococcus aureus* growth in serum *ex vivo*. Further, consistent with the hypothesis that these trade-offs would be offset by decreased investment in mating effort, shorter day length also predicted lower levels of total testosterone in men. These results suggest that ambient photoperiod may be a powerful regulator of human immunological activity, providing some of the first evidence of seasonal changes in multiple facets of human immune function.

## 1. Introduction

Winter is characterized by shorter days, lower temperatures, diminished food availability, and increased psychological and somatic stress (Demas and Nelson, 1998a,b,c; Enders and Nunney, 2012; Wingfield and Farner, 1993; Wunder, 1992). This constellation of factors can compromise immune function, rendering animals more susceptible to infection and illness (Berczi, 1986; Kelley, 1985). For example, research finds that experimentally inducing thermoregulatory and dietary stress leads to reductions in immunological activity (Demas and Nelson, 1998a,b,c; Franci et al., 1996; Messmer et al., 2014), effects which are largely mediated by increased glucocorticoid production (Demas and Nelson, 1998a,b,c; Martin et al., 2007; Nelson and Demas, 1996).

Although the onset of winter is marked by a variety of changes in the environment (e.g., temperature changes, changes in vegetation), one of the most reliable predictors of its impending arrival is the shortening of days. As winter approaches, the days get shorter. Given the increased somatic stress incurred by organisms in the winter months, researchers have hypothesized that animals should increase investment in immunological activity in anticipation of stressful winter conditions that compromise health and immune function (Nelson, 2004; Nelson et al., 2002; Nelson and Demas, 1996). Consistent with this hypothesis, research finds that many species upregulate immune function as days become shorter (Bilbo et al., 2002; Brown et al., 2016; Nelson, 2004; Nelson et al., 2002; Nelson and Demas, 1996). For example, Siberian hamsters (*Phodopus sungorus*) experimentally exposed to shorter photoperiods had higher numbers of total leukocytes, lymphocytes, T cells, and natural killer (NK) cells than those exposed to comparatively longer photoperiods (Bilbo et al., 2002). Similarly, deer mice (*Peromyscus maniculatus*) exposed to shorter (compared to longer) photoperiods exhibited increased splenocyte proliferation in response to mitogen stimulation (i.e., concanavalin A; Demas and Nelson, 1998a,b,c). Together, these results suggest that day length may play an important role in regulating investment in immunological activity, with greater investments being made at times when the days are shorter.

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Moreover, such seasonal shifts in immunological investment may promote survival in the face of stressful winter conditions that render animals more vulnerable to infection compared to other times of the year (Nelson et al., 2002; Sinclair and Lochmiller, 2000).

Although seasonal changes in immune function have been documented in several species of non-human animals (Bilbo et al., 2002; Brown et al., 2016; Nelson, 2004; Nelson et al., 2002; Nelson and Demas, 1996), much less is known about the impact of photoperiod on human immune system activity. Given that research in non-human animals suggests that seasonal shifts in immune function are mediated by changes in photoperiod, per se, there is reason to predict that a similar pattern might be found in humans, even among those inhabiting environments where the impact of winter stressors are typically mitigated through the use of technology (e.g., temperature-controlled homes). Some evidence supports this possibility. For example, researchers have linked shorter photoperiods to elevated proinflammatory immune profiles (Dopico et al., 2015; Liu and Taioli, 2015; Sung, 2006). Nonetheless, little research to date has examined whether specific components of immunological function vary based on seasonal variations in day length in healthy humans (i.e., without diagnosed physical or psychological disorders), or in other continuously breeding species.

Here, we examine the relationship between day length and multiple measures of human immune system activity, predicting that day length will be negatively related to immunological function. Additionally, because the expensive activation of the immune system is often traded off with investment in the tremendously costly processes of mating and gestation (Demas and Nelson, 1998a,b,c; Nelson et al., 2002; Walton et al., 2011), we assessed whether shorter day lengths would also be associated with decreased total testosterone, suggesting seasonal shunting of energy investments toward immune function during the winter months.

## 2. Material and methods

### 2.1. Participants

Participants included 159 (80 men, 79 women; $M_{age}$ = 20.17 years, $SD$ = 2.75) who were either students at Texas Christian University or members of the surrounding community. Eligible participants were 1) without a history of chronic medical problems, including depression and other mental illnesses, 2) non-obese (body mass index [BMI] below 30), 3) free from acute illness for the two weeks prior to participation, 4) not on hormonal contraceptives (females), 5) willing to abstain from steroidal and non-steroidal anti-inflammatory medications, exercise, and alcohol consumption for two days prior to participation, and 6) willing to fast the morning of participation. Characteristics of the current sample are published elsewhere (Gassen et al., 2019). Women participated 4–7 days after the initiation of their most recent menstrual cycle (e.g., early follicular phase). Participants were given the choice of partial course credit or a $50 gift card as compensation.

### 2.2. Procedure

Written informed consent was obtained from all participants and the research was approved as compliant with ethical standards by the Texas Christian University Institutional Review Board (Approval #: 1411-117-1606). Participants completed online demographic and lifestyle questionnaires prior to their laboratory sessions. For these sessions, participants arrived to the laboratory at 7:30 AM, provided informed consent, and were asked additional study compliance questions (e.g., not feeling ill, fasting for eight hours, abstaining from drugs and alcohol). For the purpose of a larger study, additional questionnaires and behavioral tasks were completed before participants were led into an adjoining room (in groups of 2–6) where 85 mL of blood was collected via venipuncture into heparinized (or EDTA-containing) Vacutainer® tubes (Becton-Dickinson, Franklin Lakes, NJ).

Next, peripheral blood mononuclear cells (PBMCs) were isolated from whole blood using density gradient centrifugation in Ficoll® Paque Plus (Sigma-Aldrich, St. Louis, MO [GE Healthcare Life Sciences]) to assess 1) PBMC proliferation in response to, and the in the absence of, stimulation, 2) NK cell lysis of target tumor cells, and 3) PBMC phagocytosis of fluorescent dye-labeled *E. coli* bioparticles. For each assay, PBMCs were brought to the correct plating density in RPMI-1640 supplemented with 10% heat-inactivated fetal bovine serum (FBS), 2 mM L-glutamine, 1 mM sodium pyruvate, 100 U of penicillin/mL, 100 µg of streptomycin/mL, and 0.25 µg of amphotericin B/mL (Caisson Labs, Logan, UT). Additionally, a separate tube of whole blood was taken for hematology analysis. Finally, plasma and serum were collected and frozen at −80 °C until thawed and assayed for IL-6 and TNF-α (plasma), total testosterone (serum), and for use in a live *Staphylococcus aureus* (*S. aureus*) growth assay (serum).

### 2.3. Materials

#### 2.3.1. Day length

Blood samples were collected from participants between August 2016 and February 2017 in Fort Worth, TX, USA ($Range_{day\ length}$ = 10 h 4 min to 13 h 16 min, $M$ = 11 hs 26 min, $SD$ = 51 min). Day length was measured as the amount of time between sunrise and sunset at the location of data collection on the date of the laboratory session (Fort Worth, TX, USA). Day length data were gathered from TimeAndDate.com ("Sunrise and Sunset for USA, Fort Worth, TX," Timeanddate, 2018), a website commonly utilized as a source of such data in empirical research (e.g., Goodman et al., 2014, 2012; Rusnak et al., 2018). Day length was converted to a decimal representing % of 24 h for all analyses.

#### 2.3.2. PBMC proliferation assay

Isolated PBMCs were plated into Falcon® 96-well tissue culture plates (Corning, Corning, NY) at a density of $2.5 \times 10^5$ cells/well in a 200 µL final volume. PBMCs were plated in triplicate for each of four conditions: 1) with media only (i.e., unstimulated, spontaneous proliferation), 2) with 1 µg/mL of lipopolysaccharide (LPS) obtained from *Escherichia coli* (*E. coli*; serotype 026:B6, Sigma-Aldrich, St. Louis, MO), 3) with 5 µg/mL of phytohaemagglutinin (PHA; Sigma-Aldrich, St. Louis, MO), and 4) with 50 µg/mL of polyinosinic:polycytidylic acid (poly [I:C]; high molecular weight; InvivoGen, San Diego, CA). PBMCs were incubated at 37 °C, 5% $CO_2$, and 100% humidity.

PBMC density for each participant was assessed at three time-points: 24, 48, and 72 h post-plating, using the CellTiter 96® AQueous Non-Radioactive Cell Proliferation Assay (Promega, Madison, WI). Plates were read at 490 nm on a plate reader (BMG LabTech FLUOstar™ Omega, Cary, NC). PBMC proliferation assays were performed immediately after blood collection.

#### 2.3.3. NK cell cytotoxicity

NK cell cytotoxicity was measured using the classic $^{51}$Cr-release assay. Per convention, target cells were K-562 tumor cells (ATCC® CCL-24™, Manassas, VA) grown inside T-25 flasks (ThermoFisher Scientific, Waltham, MA) in RPMI-1640 growth medium supplemented with 10% FBS, 2 mM L-glutamine, 1 mM sodium pyruvate, 100 U of penicillin/mL, 100 µg of streptomycin/mL, and 0.25 µg of amphotericin B/mL (Caisson Labs, Logan, UT), at incubation conditions of 37 °C, 5% $CO_2$, and 100% humidity. Target cells were labelled with 1 µCi $^{51}$Cr (PerkinElmer, Waltham, MA) for one hour, and plated into Corning® V-bottom plates (Corning, Corning, NY) at a density of $1 \times 10^4$ cells/well in a 200 µL final volume. PBMCs were plated together with target cells in triplicate at four effector:target cell (E:T) ratios: 100:1 ($1 \times 10^6$ PBMCs/well), 50:1 ($5 \times 10^5$ PBMCs/well), 25:1 ($2.5 \times 10^5$ PBMCs/well), and 12.5:1 ($1.25 \times 10^5$ PBMCs/well). The spontaneous/background lysis controls (target cells plated in media only, i.e., with no PBMCs) and maximal lysis controls (target cells plated in with 1%

Triton X-100 [Sigma-Aldrich, St. Louis, MO]) were plated in sextuplicate. Effector and target cells were incubated together at 37 °C, 5% $CO_2$, and 100% humidity for four hours.

Following brief centrifugation of the V-bottom plate, supernatants were collected into glass scintillation vials, and $^{51}Cr$ release was quantified on a CAPRAC®-t gamma counter (Capintec, Inc., Ramsey, NJ). Percent maximal lysis of target cells by participant NK cells for each E:T ratio was calculated by dividing $^{51}Cr$ release at each E:T ratio by maximal release after spontaneous release had been subtracted from both, and multiplying by 100. The NK cell cytotoxicity assay was performed immediately after blood collection.

#### 2.3.4. Phagocytosis assay

Phagocytic capability of participant PBMCs was assessed using fluorescent pHrodo™ Green *E. coli* BioParticles™ (ThermoFisher Scientific, Waltham, MA). PBMCs were plated with opsonized *E. coli* bioparticles (1 mg/mL; using manufacturer-provided opsonization buffer) into BrandTech® black, flat-bottom microplates (BrandTech Scientific, Essex, CT) in triplicate, at a density of $5 \times 10^5$ cells/well in a 200 µL volume. Negative controls (bioparticles plated with media only in 200 µL volume) and positive controls (bioparticles plated with pH 4.5 Intracellular Calibration Buffer [ThermoFisher Scientific, Waltham, MA]) were plated in triplicate. Plates were incubated at 37 °C, 5% $CO_2$, and 100% humidity for two hours, and then read using a fluorescence plate reader (BMG LabTech FLUOstar™ Omega, Cary, NC) at FITC dye settings of 490 nm excitation/ 520 nm emission. Percent maximal fluorescence (e.g., bioparticles taken up into the acidic environment of the phagosome) for each participant was computed by dividing participant experimental fluorescence by fluorescence in the positive control (maximal fluorescence) condition after negative control fluorescence had been subtracted from both, and multiplying by 100. The phagocytosis assay was performed immediately after blood collection.

#### 2.3.5. Staphylococcus aureus serum growth assay

To measure growth rates of the human pathogen *S. aureus* (strain Newman) in media supplemented with participant serum *ex vivo*, bacteria were first grown overnight for approximately 18 h at 37 °C in 1 mL Lysogeny broth (LB; CulgGenex, Santa Maria, CA) in open air, with continuous shaking. The next day, bacteria were diluted in 3 mL LB to an optical density of less than .01 (600 nm [$OD_{600}$]), grown an additional two hours until reaching the mid-log phase of growth (i.e., $OD_{600}$ of .4), and measured utilizing a spectrophotometer (Spectronic 20D+, Thermo Fisher Scientific, Waltham, MA). Via serial dilution and plating on tryptic soy agar plates, this $OD_{600}$ value was determined to correspond to approximately $7 \times 10^8$ colony forming units (CFU)/mL. Next, 1 mL of the culture was centrifuged, after which LB was removed and the pellet re-suspended in 1 mL RPMI-1640 growth medium. Bacteria were further diluted 1:100 in RPMI-1640, subsequently plated 1:1 in triplicate with participant serum (200 µl final volume) in Falcon® 96-well tissue culture plates (Corning, Corning, NY), and incubated for 24 hs at 37 °C, 5% $CO_2$, and 100% humidity. Optical density (OD) values were measured every hour for the first eight hours, and then again at 12 h and 24 h at a wavelength of 600 nm using a plate reader (BMG LabTech FLUOstar™ Omega, Cary, NC). The *S. aureus* growth assay was performed using thawed participant serum that had been frozen at −80 °C immediately after collection and centrifugation.

#### 2.3.6. Proinflammatory cytokine assays

Plasma samples were immediately frozen at −80 °C after blood collection and centrifugation. These samples were thawed and assayed for levels of IL-6 and TNF-α using commercially-available high-sensitivity enzyme-linked immunosorbent assay (ELISA) kits (R&D Systems, Minneapolis, MN). Samples were assayed in duplicate and read at 490 nm, with a correction wavelength set at 650 nm (BMG LabTech FLUOstar™ Omega, Cary, NC). Intra-assay coefficients of variation (CVs) were 3.58% (IL-6) and 4.68% (TNF-α). Inter-assay CVs were 13.91% (IL-6) and 7.92% (TNF-α).

#### 2.3.7. Total testosterone assay

Serum samples were immediately frozen at −80 °C after blood collection and centrifugation. Samples were thawed and assayed in duplicate for total testosterone levels using commercially-available ELISAs (Abcam, Cambridge, UK). Plates were read on a plate reader (BMG LabTech FLUOstar™ Omega, Cary, NC) at a wavelength of 450 nm. The intra-assay CV was 2.48% and the inter-assay CV was 13.45%.

#### 2.3.8. Alternative explanations

Several additional variables that may influence the relationship between day length and immune function were collected. These included sex, race, age, exercise, sleep, body mass index (BMI), childhood and adult socioeconomic status, current stress, loneliness, recent illness. Total white blood cell count was also measured, to help rule out potential confounding effects of current infection and allergy (see supplementary materials for more information), despite exclusion criteria that eliminated individuals who reported any recent/ongoing sickness or disease.

### 2.4. Data analytic plan

Data were examined for normality prior to model testing. To improve model fit, outliers across all biological measures were excluded from data analysis (see supplementary materials for details) (Genser et al., 2007; Kline, 2016). Values for total white blood cell count, IL-6, and TNF-α were positively skewed and were thus log-transformed prior to analysis, per convention (Genser et al., 2007). Values for testosterone levels in women were also positively skewed, but this skew was not corrected by log-transformation. Thus, we used the untransformed variable in analysis. All models were estimated using MPlus statistical software (MPlus 7.4; Muthén and Muthén, 2012; see supplementary materials for more information about data analysis). Four participants who reported being sick the day of the session were excluded from analysis; however, including these participants did not change the pattern or significance of the results. For each analysis, we report unstandardized parameter estimates obtained with maximum likelihood estimation. We also report standardized parameter estimates, credibility intervals, and effect sizes for each effect obtained with Bayes estimation in MPlus, a Markov chain Monte Carlo process (see e.g., Asparouhov and Muthén, 2010), as an additional measure of parameter reliability.

Because day length at the time of participation was, on average, slightly greater for male ($M$ = .49, $SD$ = .04) compared to female participants ($M$ = .46, $SD$ = .03), Wald test follow-ups examined whether the relationship between day length and each outcome was sex-differentiated. In cases for which significant between-sex differences were found, effects are reported separately for each sex (in addition to results reported for both sexes combined). Results are reported for models adjusted for potential covariates (including sex, but also e.g., age, body mass index, white blood cell count, etc.; see 'Alternative Explanations' for full list). The pattern and significance of the results were generally unchanged when these variables were not controlled for; the results of the models without covariates, as well as model comparison statistics, can be found in the supplementary materials.

In addition, in a follow-up analysis, we also controlled for participants' perceptions about the cleanliness of their environments, their perceived ability to control exposure to disgusting aspects of the environment, and their reported contact with potential sources of infection (e.g., animals and individuals who are suffering from an illness). This analysis helped rule out the possibility that relationships between day length and immune function could be explained by seasonal differences in pathogen exposure, rather than ambient photoperiod, per se. Importantly, the pattern and significance of the results reported remained unchanged when controlling for these variables. Finally, we also tested an alternative model to examine whether seasonal changes

**Table 1**
Descriptive Statistics for Hormonal and Immunological Measures.

| | *M* (*SD*) | |
|---|---|---|
| Variable | Men | Women |
| Unstimulated Proliferation | .65 (.14) | .71 (.16) |
| Proliferation in Response to LPS | 1.02 (.19) | 1.01 (.19) |
| Proliferation in Response to PHA | 1.07 (.17) | 1.10 (.18) |
| Proliferation in Response to Poly (I:C) | .79 (.16) | .82 (.14) |
| NK Cell Lysis of Tumor Cells (%) | 38.55 (13.69) | 36.59 (16.55) |
| Phagocytosis of *E. coli* Bioparticles (%) | 13.55 (6.40) | 11.64 (5.21) |
| Plasma TNF-α | 1.01 (.33) | .91 (.28) |
| Plasma IL-6 | 1.32 (1.20) | 1.91 (1.57) |
| Testosterone Levels | 469.99 (177.78) | 24.57 (28.72) |

*Note.* Peripheral blood mononuclear cell proliferation values shown here as absorbance reading at 490 nm collapsed across time-points (24 h, 48 h, 72 h) for each plating condition. Values for natural killer cell lysis of K-562 tumor cells (i.e., NK cell cytotoxicity) shown here collapsed across effector:target cell ratios (100:1, 50:1, 25:1, 12.5:1). Testosterone levels displayed as ng/dL. Cytokine levels displayed as pg/mL. LPS = lipopolysaccharide, PHA = phytohaemagglutinin, poly (I:C) = polyinosinic:polycytidylic acid, NK = natural killer, *E. coli* = *Escherichia coli*, TNF-α = tumor necrosis factor-alpha., IL-6 = interleukin-6.

in immune function were mediated through changes in testosterone. This possibility, however, was not supported (see supplementary materials for results of these models).

## 3. Results

Descriptive statistics separated by sex are displayed in Table 1. Full statistics are shown in Table 2. Bivariate correlations between the immunological outcomes are shown in Table 3. Regression analyses revealed that longer day lengths predicted lower spontaneous PBMC proliferation across all time-points (all *ps* < .001). Longer day lengths also significantly predicted lower PBMC proliferation in response to LPS and PHA at the later time-points (LPS: 72 h; PHA: 48 and 72 hs; *ps* < .05), as well as a lower increase in proliferation from 24 to 72 h (slope *ps* < .03), and reduced proliferation in response to poly (I:C) at all time-points (all *ps* < .02). Additionally, longer day lengths predicted diminished NK cell cytotoxicity at each E:T ratio (all *ps* < .03), as well as a steeper decrease in cytotoxicity from the 100:1 ratio to the 12.5:1 ratio (slope $p = .008$). There was no significant effect of day length on our measure of phagocytosis ($p = .86$), nor were there sex differences in any of these effects (all *ps* > .10).

Results also revealed that longer day lengths were associated with greater initial *S. aureus* growth in participants' serum through the first 6 h (*ps* < .04). The slope of growth over time (i.e., 1 h to 24 h) was more gradual at longer day lengths (compared to shorter day lengths), but this was due to the higher initial growth in those whose samples were collected at longer day lengths (see Fig. 1 for growth curves graphed on log scale, per convention: e.g., Cross et al., 2015; Samant et al., 2008). No sex differences in these effects were found ($p = .99$).

In addition to day length effects on the in vitro measures described above, longer day lengths also significantly predicted lower plasma levels of IL-6 ($p = .001$), but not TNF-α ($p = .39$), and there were no sex differences in these effects (*ps* > .37). Lastly, for men, longer day lengths were associated with significantly higher levels of total testosterone ($p = .01$). For women, longer day lengths were negatively associated with testosterone levels, but this relationship did not reach significance ($p = .10$).

## 4. Discussion

The results of the current study provide some of the first evidence demonstrating that humans make seasonal adjustments in specific aspects of immunological function based on day length. Specifically, we found that shorter days were associated with increased PBMC proliferation, both spontaneous and in response to stimulation with mitogens and bacterial/viral mimetics (e.g., media-only, LPS, PHA, and poly [I:C]), NK cell cytotoxicity, plasma levels of IL-6, and also decreased *S. aureus* growth in serum. Conversely, we found no evidence of upregulated investment in phagocytic capability of PBMCs or plasma levels of TNF-α. Although these results were not anticipated in advance, this pattern likely reflects a tendency for individuals to strategically allocate their investment to aspects of immune function that offer the largest payoff at the smallest cost in the winter months, rather than unilaterally increasing their investment in all aspects of immune function (for review, see Walton et al., 2011). Consistent with this interpretation, the current results found that there were no season-based differences in phagocytosis, which is an activity mediated primarily by neutrophils (e.g., Dale et al., 2008). Neutrophils are significantly more energetically costly than, e.g., NK cells, due to their larger numbers and rapid regeneration rate (Tak et al., 2013). Future research would benefit from examining energetic cost as a factor in a test of season-based changes in immune function.

The results of the current study are consistent both with research in the evolutionary sciences that finds that photoperiod plays an important role in regulating investment in immunological activity (Bilbo et al., 2002; Brown et al., 2016; Nelson et al., 2002), as well as the growing body of research demonstrating the role that ecology plays in modulating life history trade-offs (Blackwell et al., 2016, 2010; Ellison, 2003; McDade et al., 2016; Muehlenbein and Bribiescas, 2005; Reiches et al., 2009; West-Eberhard, 2003). In addition to its contribution to evolutionary theories on health, the current research has much practical value as it may have important implications for health, patterns of illness transmission, and clinical treatment decisions. For example, many human diseases – both communicable (e.g., viral illnesses) and non-communicable (e.g., autoimmune disorders, cancer, SAD) – show seasonal patterns of occurrence and severity (for a review, see e.g., Nelson, 2004). The current research lends support for the hypothesis that photoperiod-mediated changes in endocrine and immune function might help explain seasonal variation in disease risk. Although most of the research exploring this possibility to date has focused on the role photoperiod plays in SAD (see e.g., Leu et al., 2001; Levandovski et al., 2013), seasonal shifts in immune function might contribute to an individual's risk for a much broader range of negative health outcomes (e.g., Dopico et al., 2015; Dowell, 2001; Nelson, 2004). Moreover, the present results suggest that managing artificial light exposure could be a useful tool for treating a variety of diseases and promoting overall health even in non-polar climes, an idea supported by recent research (Cho et al., 2015; Dowell, 2001; Haim and Zubidat, 2015; Stevens et al., 2007).

Also of note, the results of the current research suggest that seasonality is an important variable to consider in immunological research. It is possible, for example, that promising research results from areas as diverse as immunology, endocrinology, psychoneuroimmunology, and psychoneuroendocrinology may have been diluted or masked by seasonality effects. Given that photoperiodic information is relatively easy to obtain, controlling for this factor (or testing for this factor as a moderator of immunological outcomes) could increase the predictive power of experiments being conducted at present, and might also shed new light on ambiguous results obtained in the past. This often ignored, but important, variable could play an important role in clarifying the results of studies conducted in several important research areas.

The results of the current research also lend some insight into the trade-offs that may be made to afford greater investment in immunological activity. For example, in men, we found that the increased investment in immunological activity that occurs in the context of shorter days co-occurs with concomitant reductions in total testosterone. This is consistent with the idea that men may be trading off investment in mating effort for increased investment in immune function as daylight wanes and the shadow of winter approaches. This

**Table 2**
Impact of Day Length on Biological Outcomes Across Models (Controlling for Covariates).

| Dependent Variable | *B* (*SE*) | *t* | *p* | *β* | *CIs* | Unique $R^2$ | Total $R^2$ |
|---|---|---|---|---|---|---|---|
| TNF-α Levels | −.41 (.47) | −.86 | .39 | −.09 (.10) | [-.25, .14] | .01 | .19 |
| IL-6 Levels | −2.76 (.85)*** | −3.24 | .001 | −.28 (.08) | [-.44, -.13] | .08 | .37 |
| Serum Testosterone – Men | 8.01 (3.24)** | 2.47 | .01 | – | – | .05 | .41 |
| Serum Testosterone – Women | −1.73 (1.05) | −1.65 | .10 | – | – | .06 | .27 |
| Phagocytosis | −3.39 (19.33) | −.18 | .86 | −.03 (.10) | [-.27, .17] | .00 | .30 |
| *PBMC Proliferation* | | | | | | | |
| Media (24 hrs) | −1.69 (.30)*** | −5.67 | < .001 | −.30 (.07) | [-.42, -.18] | .11 | .23 |
| Media (48 hrs) | −2.17 (.29)*** | −7.38 | < .001 | −.35 (.06) | [-.46, -.23] | .14 | .26 |
| Media (72 hrs) | −2.60 (.41)*** | −6.38 | < .001 | −.43 (.06) | [-.54, -.30] | .19 | .32 |
| Media Slope | −.45 (.21)* | −2.10 | .04 | −.17 (.08) | [-.33, -.02] | .03 | .29 |
| LPS (24 hrs) | −.30 (.34) | −.89 | .37 | −.05 (.07) | [-.19, .08] | .01 | .20 |
| LPS (48 hrs) | −.71 (.44) | −1.62 | .11 | −.10 (.06) | [-.22, .01) | .02 | .20 |
| LPS (72 hrs) | −1.17 (.59)* | −2.00 | .046 | −.16 (.07) | [-.28, -. 03] | .03 | .22 |
| LPS Slope | −.46 (.20)* | −2.29 | .02 | −.24 (.09) | [-.42, -.08] | .06 | .29 |
| PHA (24 hrs) | −.39 (.47) | −.82 | .41 | −.04 (.08) | [-.21, .09] | .02 | .20 |
| PHA (48 hrs) | −.94 (.42)* | −2.23 | .03 | −.14 (.07) | [-.27, -.02] | .03 | .23 |
| PHA (72 hrs) | −1.57 (.46)*** | −3.39 | .001 | −.23 (.08) | [-.38, -.08] | .05 | .26 |
| PHA (Slope) | −.61 (.20)** | −3.04 | .002 | −.31 (.11) | [-.51, -.08] | .10 | .45 |
| Poly I:C (24 hrs) | −.87 (.35)** | −2.47 | .01 | −.14 (.07) | [-.29, -.01] | .03 | .19 |
| Poly I:C (48 hrs) | −1.04 (.33)** | −3.17 | .002 | −.18 (.06) | [-.30, -.05] | .04 | .18 |
| Poly I:C (72 hrs) | −1.16 (.40)** | −2.90 | .004 | −.20 (.07) | [-.33, -.06] | .05 | .19 |
| Poly I:C (Slope) | −.13 (.14) | −.89 | .37 | −.12 (.12) | [-.39, .10] | .02 | .38 |
| *Natural Killer Cell Cytotoxicity* | | | | | | | |
| 100:1 Ratio | −171.36 (51.35)*** | −3.34 | .001 | −.20 (.07) | [-.34, -.05] | .07 | .20 |
| 50:1 Ratio | −136.70 (46.70)** | −2.93 | .003 | −.19 (.07) | [-.33, -.03] | .06 | .19 |
| 25:1 Ratio | −120.37 (45.61)** | −2.62 | .009 | −.17 (.07) | [-.32, -.02] | .06 | .18 |
| 12.5:1 Ratio | −102.04 (45.43)* | −2.25 | .025 | −.16 (.07) | [-.31, -.02] | .08 | .18 |
| Slope | −8.66 (3.25)** | −2.67 | .008 | −.22 (.09) | [-.39, -.04] | .05 | .31 |
| *S. aureus Growth Assay* | | | | | | | |
| 1 hr | .32 (.12)** | 2.69 | .007 | .19 (.08) | [.02, .32] | .11 | .20 |
| 2 hrs | .31 (.12)** | 2.58 | .01 | .18 (.08) | [.02, .31] | .01 | .20 |
| 3 hrs | .29 (.12)** | 2.46 | .01 | .17 (.08) | [.01, .31] | .01 | .20 |
| 4 hrs | .28 (.12)* | 2.28 | .02 | .18 (.08) | [.03, .32] | .01 | .18 |
| 5 hrs | .26 (.12)* | 2.16 | .03 | .17 (.08) | [.01, .32] | .01 | .19 |
| 6 hrs | .25 (.12)* | 2.04 | .04 | .16 (.08) | [.01, .46] | .01 | .14 |
| 7 hrs | .23 (.12)† | 1.91 | .06 | .13 (.07) | [.02, .28] | .02 | .21 |
| 8 hrs | .16 (.09) | 1.66 | .10 | .10 (.07) | [-.03, .25] | .01 | .16 |
| 12 hrs | .16 (.13) | 1.24 | .21 | .10 (.08) | [-.05, .25] | .01 | .21 |
| 24 hrs | −.01 (.17) | −.08 | .94 | −.01 (.08) | [-.16, .17] | .01 | .30 |
| Slope | −.01 (.01)* | −2.38 | .02 | −.07 (.10) | [-.24, .12] | .02 | .17 |

*Note.* Results of all models examining impact of day length on immunological and hormonal outcomes controlling for covariates (see 'Alternative Explanations' for list of covariates). TNF-α = tumor necrosis factor-alpha; IL-6 = interleukin-6; PBMC = peripheral blood mononuclear cells; media = proliferation of cells plated in media only; LPS = lipopolysaccharide; PHA = phytohaemagglutinin; poly (I:C) = polyinosinic:polycytidylic acid; NK = natural killer; *S. aureus* = *Staphylococcus aureus*; slope = change across dilution factors (NK cell assay) or time (proliferation and *S. aureus* assays). See Materials and Methods for assay details. ***$p \leq .001$; **$p \leq .01$; *$p \leq .05$; †$p \leq .07$.

trade-off was not observed for women, however, whose levels of testosterone increased (although not to a significant level) as days got shorter. Although the reason for this physiological sexual dimorphism is currently unclear, the apparent discrepancy may actually reflect a general effect in female participants that is quite similar to their male counterparts. The apparent short-day-facilitated upregulation of testosterone in females may instead potentially reflect diminished aromatization of this hormone to estradiol. Indeed, the results of prior studies lend credence to this idea (Bjørnerem et al., 2006; Nagata et al., 1997; Trainor et al., 2006), though further work is needed to explore

**Table 3**
Bivariate Correlations between Immunological Outcomes.

| Variable | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 |
|---|---|---|---|---|---|---|---|---|
| 1. Plasma IL-6 | – | | | | | | | |
| 2. Plasma TNF-α | .33*** | – | | | | | | |
| 3. Phagocytosis | .19* | .15 | – | | | | | |
| 4. NK Cell Cytotoxicity | −.04 | −.12 | .004 | – | | | | |
| 5. Proliferation – Media Only | .27*** | .09 | .01 | .22* | – | | | |
| 6. Proliferation – LPS | .28*** | .17* | .34*** | .13 | .35*** | – | | |
| 7. Proliferation – PHA | .36*** | .14 | .29*** | .05 | .36*** | .90*** | – | |
| 8. Proliferation – Poly (I:C) | .40*** | .18* | .26*** | .15 | .58*** | .78*** | .78*** | – |
| 9. *S. aureus* Growth | −.002 | .13 | .07 | −.12 | −.11 | −.21** | −.22** | −.23** |

*Note.* Values for natural killer cell cytoxicity were collapsed across effector:target ratios for this analysis. Proliferation and *Staphylococcus* aureus growth values were collapsed across time-points for this analysis.TNF-α = tumor necrosis factor-alpha; IL-6 = interleukin-6; PBMC = peripheral blood mononuclear cells; proliferation = peripheral blood mononuclear cell proliferation; LPS = lipopolysaccharide; PHA = phytohaemagglutinin; poly (I:C) = polyinosinic:polycytidylic acid; NK = natural killer; *S. aureus* = *Staphylococcus aureus*. ***$p \leq .001$; **$p \leq .01$; *$p \leq .05$; †$p \leq .07$.

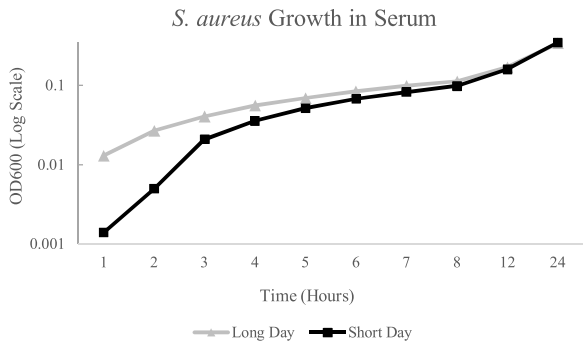


**Fig. 1.** Growth of *Staphylococcus aureus (S. aureus)* in participant serum. Shown here are the slopes of *S. aureus* growth in participant serum across time (1–24 h). Separate slopes shown for one standard deviation above the mean of day length (Long Day) and one standard deviation below the mean of day length (Short Day). Optical Density (OD) values at each time-point shown here on a logscale.

this possibility in human females. Moreover, in addition to shortened-photoperiod-induced reductions in testosterone production and aromatization, earlier rodent work argues that shorter photoperiods may also blunt the behavioral effects of estrogens (Mangels et al., 1998). The results of these studies, in addition to those of the current research, together suggest that seasonal factors may influence aspects of female endocrine function that have implications for fertility and reproductive health.

There are several limitations of the current research that must be noted. First, the data collected for this study were cross-sectional in nature. Although experimentally manipulating ambient photoperiod in humans presents various methodological and ethical issues, future studies might extend the current findings by making use of longitudinal designs. In capturing within-individual shifts in immune function across seasons, longitudinal studies would help assess the magnitude of the effect of day length on immunological outcomes across time. Such studies might also help rule out alternative explanations for seasonal shifts in immune function, such as seasonal variation in exposure to allergens or specific classes of pathogens (e.g., viral vs. bacterial illnesses). Additionally, we cannot rule out the possibility that the effects of day length on immune function are due to other environmental, social, or behavioral factors not measured in the current research. Future work examining relationships between day length and immune function in humans may further explore this possibility by collecting data on a broader range of psychological and environmental variables.

Further, although a number of immune measures were examined in the current study, together they represent only a limited snapshot of the tremendous complexity and wide-ranging diversity of immune function. Future research would therefore also benefit from collecting a broader range of immune measures. Finally, the current research did not experimentally address the mechanisms driving the observed immunological effects, also an important area for future research. One promising possibility for such inquiry is examining the role played by pineal melatonin, which is inversely proportional to day length (Underwood and Hyde, 1989; Wehr, 1997, 1991) and is known to exert complex effects on the HPA axis (Konakchieva et al., 1997), the HPG axis (Tamarkin et al., 1985), and immune function (Demas and Nelson, 1998a,b,c; Yellon et al., 1999a,b).

### 4.1. Conclusions

The current results provide some of the first evidence that multiple components of immune function in healthy human participants are sensitive to the change of the seasons. These results lay the groundwork for future research examining the impact of photoperiodic cues on the activities of the immune system, and have important implications for health, clinical treatment, and research that includes measures of immune function.

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## Data availability

The data analyzed in the current study can be found on the Open Science Framework (DOI: 10.17605/OSF.IO/R9SUF).

## Author contributions

J.G., R.P.P.L., J.D.W., J.L.P, M.L.P., M.J.E., S.M., H.K.B., E.K.C., D.J.C., and G.W.B., assisted with sample collection and processing. G.W.B. and S.E.H. designed the study and oversaw data collection and analysis. J.G. performed data analysis. S.M., J.G., H.K.B., G.W.B., and S.E.H wrote the first draft of the manuscript. All authors edited and revised later drafts of the manuscript prior to submission.

## Competing interests

The authors have no competing interests to declare.

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## Appendix A. Supplementary data

Supplementary material related to this article can be found, in the online version, at doi:https://doi.org/10.1016/j.psyneuen.2019.05.011.

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