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Seasonality of blood neopterin levels in the Old Order Amish

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Abstract

Seasonal changes in non-human animals and seasonal affective disorder (SAD) in humans are associated with immune activation in winter relative to summer. We intended to measure seasonal variation in neopterin, a marker of cellular immunity, and its interactions with gender and seasonality of mood. We studied 320 Amish from Lancaster, PA, USA (men = 128; 40%) with an average age [Standard deviation (SD)] of 56.7 (13.9) years. Blood neopterin level was measured with enzyme-linked immunosorbent assay (ELISA). Seasonality was measured with Seasonal Pattern Assessment Questionnaire (SPAQ). Statistical analysis included analysis of covariance (ANCOVAs) and multivariate linear regression. We also investigated interactions of seasonal differences in neopterin with gender, seasonality scores and estimation of SAD diagnosis. We found a significantly higher neopterin level in winter than in summer ($p = 0.006$). There were no significant gender or seasonality interactions. Our study confirmed the hypothesized higher neopterin level in winter. A cross sectional design was our major limitation. If this finding will be replicated by longitudinal studies in multiple groups, neopterin could be used to monitor immune status across seasons in demographically diverse samples, even if heterogeneous in gender distribution, and degree of seasonality of mood.

Keywords

Amish; immune; neopterin; SAD; seasonality

Introduction

Influence of elements of the natural environment on health have been studied extensively since ancient times [1–3]. Hippocrates postulated that “Whoever wishes to investigate medicine properly, should proceed in this way: in the first place to consider the seasons of the year and what effects each of them produces (for they are not all alike – but differ much from themselves in regard to their changes). Then the winds, the hot and the cold, especially such as are common to all countries, and then such as are peculiar to each locality” [1].

Natural environmental conditions include physical, chemical, and biological factors – some having a major geographic variation, some predominantly a temporal variation – and a sizable majority (e.g. light, temperature, precipitation, flora, microbiota) have both a temporal and geographic variation. Some of these factors manifest highly predictable temporal fluctuations, such as seasonal, diurnal, lunar and tidal variations. Animals have adapted to not only react to these changes, but to anticipate them, by aligning physiological and behavioral rhythms that are evolutionarily advantageous to specific species, by maximizing feeding and reproductive ability, and by minimizing risk of exposure to adverse environmental conditions [4]. For instance, circadian rhythms have a period of approximately 24 h, and consist of periodic fluctuations between biological day and biological night, which in a majority of individuals is most often aligned to the exposure to environmental day and night. These fluctuations are not primarily generated in response to environmental variations, not disappearing in constant conditions such as dim light. To the contrary, these rhythms are internally generated, persist in constant conditions (even in the absence of light signals) and use external signals to “entrain” to external environments and to align themselves to the external conditions, using simple yet sophisticated shifts according to predictable phase-response curves [5].

Photoperiod and seasonality

As the earth orbits the sun, and because its axis is tilted, the planet experiences periodic fluctuations in day-length leading to changes in seasons. Seasonal changes in light lead to lag changes in temperature and other climatic variables such as humidity, precipitation and wind. These changes are followed by changes in behavioral, physiological and reproductive function in microbiota, plants, animals, and ultimately, occupational, recreational and academic rhythms of human society. To anticipate the seasonal energetic bottlenecks of high thermoregulatory demands and low availability of food, and to physiologically prepare for them, many species have developed capabilities to detect and track changes in day-length, converting them into accurate biological signals, and responding to these behaviorally and physiologically [6, 7]. An early response to changes in day-length is more beneficial than a reactive response to temperature as it is beneficial to be ready, rather than getting ready to respond to thermoregulatory challenges. Seasonal variation in duration of the nocturnal melatonin secretion is the signal translating seasonal variation in the duration of the environmental night [6, 7].

Similar to many seasonal species, humans also show seasonal variations, such as those in self-perception of energy level, sleepiness, sleep, mood and appetite [8–11]. These changes are generally of a lower degree than those encountered in species living in the evolutionary macroclimate, considering our increasing capability to isolate ourselves in the microclimates of our home and work environment. Humans have developed technologies to significantly reduce exposure to seasonal photoperiodic variations. This buffering of environmental elements extends the winter day-length into late evening and early morning hours, and maintains artificial temperatures via heating in the winter and cooling in the summer. It would be expected that due to decreased exposure to seasonal environmental changes, modern humans would experience minimal, if any, seasonal variations in mood and

behavior. However, many studies have identified a sizable proportion of humans who experience seasonal variations in mood and behavior [12].

Environmental exposure to daylight may differ due to occupation [13] and latitude and has been associated with various degrees of seasonal fluctuations in mood, weight, sleep duration and behavior [2, 14, 15]. A relationship between photoperiodic changes and incidence of exacerbation of psychiatric disorders has also been reported. The hospitalization rate in Taiwan, as a result of mania, varied significantly between seasons. Indeed, this has been attributed to an increased sensitivity to light in bipolar patients [16]. Although the majority of research reports showed significant associations between photoperiod and changes in mood and behavior [7, 14, 17, 18], there are a few studies that have not found this relationship [19, 20].

Seasonal affective disorder

Individuals who experience amplified symptoms due to negative effects of seasonality may exhibit seasonal affective disorder (SAD), defined by recurrent episodes of major depression during either the winter or summer months each year [21]. Winter SAD presents as major depression during the fall and winter months with a reversal of symptoms during the spring and summer months [21]. Summer SAD is characterized by the opposite pattern: major depression during the spring and summer months with a reversal of symptoms during the fall and winter months [2]. The Diagnostic and Statistical Manual of Mental Disorders-5 (DSM-5) criterion for major depressive disorder with seasonal pattern requires a consistent association between the season and major depression onset. Diagnosis of major depressive disorder with seasonal pattern also requires two major depressive episodes during the specific time period within the last 2 years and complete remissions during the other months [22]. Kasper et al. [23] named the less severe manifestation of this disorder as subsyndromal SAD (sSAD), which presents with minimal symptoms of depression and relative predominance of neurovegetative symptoms, such as sleepiness and increased appetite. Carbohydrate craving is a common manifestation of appetite changes across the SAD spectrum, and likely, when combined with reduced exercise, contributes to sizable weight gain in winter [21, 24].

Several mechanisms have been proposed to explain the symptoms of SAD. For instance, the dual vulnerability hypothesis suggests that individuals with SAD possess heightened susceptibility to both time-dependent physiological changes and impaired emotional regulation [25]. Wehr et al. [17] demonstrated that seasonal variation in the duration of nocturnal melatonin secretion was positively correlated with seasonal variation in the duration of night (scotoperiod) [26]. This relationship is similar to the scotoperiod variation translating into the variation of the duration of nocturnal melatonin secretion, which has been demonstrated as the key mechanism triggering seasonal changes in non-human animals [27].

Melatonin is a hormone secreted by the pineal gland and regulated by the suprachiasmatic nucleus (SCN). The SCN is also termed the “circadian pacemaker” because it generates daily endogenous circadian rhythms that are ultimately reaching all organs and tissues of the body via hormonal and neural signals. Melatonin secretion rhythmicity in terms of arousal,

neuroendocrine activity and internal body temperature, shifts between two physiological states (in terms of arousal, neuroendocrine activity and internal body temperature), an internal “biological night” and “biological day” [28]. The shift between states occurs rapidly at dawn and dusk [29]. The SCN “allows” the secretion and synthesis of melatonin during the internal biological night, with a sharp decrease in firing at dusk, and inhibits the secretion and synthesis of melatonin during the internal biological day [17, 30–32].

This endogenous rhythm allows the SCN to detect timing of dusk and dawn, and modify intensity and timing of firing accordingly [17]. Daylight is a critical zeitgeber (“time giver”) for the SCN. According to the photoperiodic hypothesis, during a shorter day-length of winter, the reduction of exposure to “morning” light (exposure which occurs earlier during summer) results in the delay of the end of internal biological night (including, but not limited to the termination of the nocturnal melatonin secretion). Similarly, the reduction of exposure to late afternoon-evening light, results in an advanced beginning of internal biological night. Thus, short photoperiods are characterized by longer durations of melatonin secretion [26]. Research in pinealectomized rodents has demonstrated that the duration of melatonin secretion is necessary and sufficient for inducing the majority of seasonal, physiological and behavioral changes [33, 34].

The duration of the melatonin secretion hypothesis, discussed above, is supported by a study from Wehr et al. [17] in SAD patients, where SAD patients exhibited longer nocturnal melatonin secretion duration in winter than in summer compared to non-SAD controls. The phase-shift hypothesis suggests that patients with SAD experience this syndrome due to a “phase delay” (shift in circadian rhythms) to offset the melatonin secretion during winter [35]. Lewy et al. [36] provided support for this hypothesis by reporting that a shorter photoperiod in the fall and winter months resulted in a delay in markers of circadian phase.

During the shorter photoperiods of the fall and winter months, many animals experience an increase in appetite and weight, in parallel with a decrease in libido and energy [7, 37]. Because the relationship between photoperiod and seasonality/SAD is attributed to a shorter photoperiod inducing major depressive episodes for SAD-winter type (i.e. in fall and winter months), the earliest effective treatment prescribed for SAD was light therapy [21]. The purpose of light therapy, according to the photoperiodic hypothesis, is to artificially extend the external and internal day-length and thus reduce the effects of shortened day-length in the fall and winter months [21, 38, 39]. According to the phase-shift hypothesis, the main positive effect of morning bright light is to phase advance the melatonin rhythms from their delayed position in the winter [35].

Bright light therapy is an effective treatment for winter depressive syndrome [40–43]. Considering the “dual vulnerability” hypothesis, it is important to mention that independent of its chronobiological effects, light also has a direct antidepressant effect, thus acting on both vulnerabilities. Specifically, light is also effective in non-seasonal depression as well as in seasonal depression [21, 44, 45]. Until recently, SAD studies were exclusively conducted in modern populations [12]. However, there are populations that are more exposed to seasonal changes in the environment than the modern population, such as the pre-industrial

populations studied by Yetish et al. [46]. These populations manifested much higher differences in sleep duration between winter and summer as compared to other populations.

A population that is also more exposed to the environment than the modern population is the Old Order Amish of Lancaster, Pennsylvania. The Old Order Amish are traditionally prohibited, through self-imposed religious norms, from the use of network electric light [47]. Instead they utilize propane-powered light sources, which do not possess the same intensity or wavelength as network electric lighting [48]. Therefore, we thought that the Old Order Amish might represent an ideal population to study the prevalence of SAD because their light sources may not decrease the effects of natural seasonal variations in day-length. Raheja et al. [11] evaluated the prevalence of SAD and sSAD in the Old Order Amish of Lancaster, Pennsylvania. Contrary to their expectations, they found that the Old Order Amish have a very low prevalence of SAD [11], lower than the prevalence of SAD in population studies at comparable latitudes in predominantly Caucasian populations [2, 10]. Thus, other factors of resilience may exist that limit the response of the Amish to seasonal changes. In addition, the possible reduced artificial light exposure may be beneficial for mood dysregulation in the Old Order Amish [49]. In the Amish, while SAD prevalence is very low, there is a sizable proportion of individuals who report actual changes in appetite, sleep, weight gain, socializing and seasonal patterns in “feeling best” or “feeling worst” [6], while generally not considering these changes as problematic.

Seasonality of immune function

Photoperiodic changes have been implicated in seasonality of immune function in animals and humans. Specifically, short photoperiods have been associated with increased immune activation in primates and other animals (especially, increased lymphocyte proliferation), considered to be an adaptive anticipatory physiological change to anticipate the combination of thermoregulatory challenges and reduced availability of food [50–52]. Vulnerability to infections may also demonstrate seasonal variation [53].

Immune function and SAD

Significant immunological differences between patients with SAD and controls have been reported. Patients with winter SAD exhibited significantly higher fall/winter levels of interleukin (IL)-6 levels when compared to controls [54]. However, even if subsequent light therapy improved depressive symptoms in SAD patients, IL-6 concentrations were not significantly altered [54]. Another study used separation of lymphocytes and monocytes/macrophages in the whole blood, unstimulated and stimulated with mitogens, and demonstrated an overall immune activation in SAD patients in the winter, partially normalized after light therapy [55]. Specifically, macrophage activity (e.g. phagocytosis) was higher in SAD patients in both stimulated and unstimulated conditions. In contrast, the lymphocyte proliferation was lower at baseline, but higher after stimulation in SAD patients. Higher concentrations of T helper-1 cell (Th1) proinflammatory cytokines IL-1 β , tumor necrosis factor (TNF) and interferon gamma (IFN- γ) were produced in cultures from SAD patients, while the protective IL-2 as well as lymphocyte proliferation, were lower in SAD patients. Of interest, light treatment reversed all immune differences between SAD patients and controls [55].

Gender differences in seasonal changes in affective symptoms

A number of studies have observed seasonal variations in mood, behavior, sleep-wake cycle and physiology with definite gender effects [56]. Generally, women report a higher rate of seasonality or SAD than men in the modern population [8, 57–59]. There are a few studies that did not find significant correlations between gender and SAD prevalence [11, 60].

Gender differences in infections and immune function

In animals, a number of studies have reported sex differences in seasonal variation in immune function with generally higher variation in females in comparison to males, across species [61–63]. Males exhibit increased vulnerability to disease and heightened disease severity when compared to females exposed to the same pathogens [64–66]. There may be a hormonal component to this difference, as Huber et al. [65] reported evidence supportive of a possible modulatory role of testosterone and estrogen in mice. Testosterone facilitates the development of symptoms associated with Cocksackie B-3 virus infection, whereas estrogen ameliorates or prevents disease progression in males [65].

Similarly, robust gender differences in immune function and inflammation have been identified in humans. Autoimmune diseases, such as multiple sclerosis, celiac disease and systemic lupus erythematosus (SLE), have a significantly higher prevalence in women compared with men [67, 68]. Men have been reported to develop infections at a higher prevalence than women resulting from surgery [69]. In addition, men are more likely to develop pneumonia following an injury than women [70]. Sex steroids may mediate these differences. For instance, testosterone decreases and estrogen increases certain components of the immune response, in particular humoral immunity [67]. Elevated estrogen levels may initiate development of SLE in women with a pre-existing likelihood of developing the disease [71]. It appears that a stronger female immune response may serve a protective function from infectious agents, but is deleterious to health when an inflammation-mediated disease develops.

Neopterin

Neopterin is a marker for cellular immunity [72] that has applications in multiple branches of medicine. Neopterin is the result of interactions between Th1 lymphocytes and phagocytic cells, including monocytes, macrophages, granulocytes and dendritic cells [73, 74]. These cellular interactions lead to the formation of neopterin by incorporating guanosine triphosphate-cyclohydrolase I (GTP-CHI) in the guanosine triphosphate (GTP) pathway. Neopterin precursors are formed when IFN- γ , endotoxins, TNF and IFN- α activate GTP-CH-I within macrophages. In addition, microglial cells produce neopterin in the central nervous system [75, 76]. In order to measure neopterin levels, and by extension inflammation levels, neopterin samples can be obtained from fluids and tissues within the body including: cerebrospinal fluid, blood, urine [77], tumors [78–82], the nervous system [76], respiratory system [83], cardiovascular system [84–86], and musculoskeletal system [87].

Elevated neopterin levels were previously reported in patients with neuropsychiatric disorders such as depression [88–91], schizophrenia [75, 92, 93], attention-deficit/

hyperactivity disorder [94] and autism [95–97]. The brain produces neopterin constitutively, and its production increases during oxidative stress [72].

We have previously reported a significant but low heritability of neopterin levels in the Old Order Amish of Lancaster, Pennsylvania, as a very small environmental shared household effect [98]. Our results suggested that non-household, non-genetic effects appeared to have a more pronounced impact on neopterin variation, relative to household or genetic effects.

One such factor is occupational exposure, which is markedly different in Amish women and men. Though it is common for both men and women to work, the Old Order Amish traditionally hold jobs that are stratified by gender. That is, women generally work in indoor environments such as stores and Amish businesses, whereas men work as day laborers, and in masonry, carpentry and construction [47]. The Amish “environment”, in particular exposure to Amish agricultural techniques, could have immunoregulatory effects that may reduce the incidence and severity of conditions mediated by immune dysregulation, such as asthma and allergy [99].

As discussed above, neopterin is formed via activation of Th1 cells and is a component of cell-mediated immune responses. T helper-2 cells (Th2) cells, when activated, induce production of antibodies via ILs-4, 5, 6, 9, 13 and 10, which are components of the humoral immune response [100]. It has been reported that there is an opposing relationship between the products of Th1 and Th2 cellular immune responses. Specifically, neopterin (formed via Th1 cell activation) and immunoglobulin E (formed via Th2 cell activation via IL-4, IL-5 and IL-13 and plays a major role in allergy) concentrations exhibit an inverse relationship, such that when one is high, the other is low [101]. This may be the result of reciprocal inhibitory regulation of Th1 and Th2 cells.

A significant seasonal variation in neopterin has been described in individuals with seasonal allergic rhinitis, with lower blood levels in spring, during the high pollen season, rather than the “out of” pollen season [102]. A plausible explanation is that atopy is associated with Th2 immune response [103] known to suppress products of Th1 cell activation, such as neopterin that are formed at lower concentrations in those who suffer from allergic diseases during allergen exposure. Another study reporting seasonal variation in neopterin was in a West African population, with a peak corresponding to the months of August and September, during which transmission of malaria occurs [104].

Neopterin levels measured in the fall/winter were significantly higher in SAD patients than in controls, both before and after light therapy [105]. Light therapy did not induce a significant change in neopterin levels, although the time interval when neopterin was measured after the initiation of light therapy may have been too short for observing meaningful changes [105]. Furthermore, another potential confounding variable is timing; patients were measured in winter while controls were measured in the fall. Thus, the difference in neopterin level between patients with SAD and controls may have been entirely spurious.

The current study intended to address the: (a) limited number of studies on seasonal variation of neopterin, (b) absent measures of gender differences in neopterin seasonal

changes, and (c) minimum and potentially confounded information on the links between neopterin levels and seasonality of mood and behavior in humans. We hypothesized the following:

1. A significant seasonal variation in blood neopterin levels will be identified, with higher concentrations in winter relative to summer.
2. A gender difference in seasonal variation in blood neopterin levels will be identified.
3. The level of blood neopterin will be positively associated with Global Seasonality Score (GSS), and with total SAD (subsyndromal + syndromal).

Materials and methods

Neopterin

We analyzed blood samples for neopterin levels in 2018 Old Order Amish participants [women = 1166 (57.8%), men = 852 (42.2%)], aged 18–90 years old with a mean age (SD) of 44 [17] years, taking part in the Amish Wellness Study. The participants were read the informed consent form with an Amish liaison present. The purpose of the Amish Wellness Study was to recruit willing participants within the Amish community in order to study general health and morbidity/mortality risk factors and to also provide screenings for this community. The participants for this study were all adults (greater than 18 years of age). Participants' medical and familial histories were provided to the research team and fasting blood was drawn and stored in heparinized test tubes for centrifugation following the initial visit at one of three locations: the participant's home, the mobile clinic or the Amish Research Clinic. Following centrifugation and plasma segregation, the plasma was stored at -80°C for assay. ELISA (enzyme-linked-immunosorbant assay) was utilized to measure neopterin concentrations per manufacturer's instructions (BRAHMS GmbH, Hennigsdorf, Germany) as described previously by us [106]. The assay had a sensitivity of 2 nmol/L for neopterin.

Seasonality measure

Prior to the neopterin sample collection, a subset of this population responded to a mailed Seasonal Pattern Assessment Questionnaire (SPAQ) for a study of mood and behavior [6, 11]. The SPAQ, though not a valid diagnostic method in isolation, is an effective tool for identifying individuals with seasonality and SAD. The SPAQ has been used in numerous studies of seasonality and SAD since its inception [6, 10–12]. The SPAQ screens for and evaluates the presence and relative severity of seasonality according to three domains: (1) whether the participant experiences seasonal changes in mood and behavior, (2) the amount by which seasonal changes impair daily functionality (termed “problem”), and if both conditions are satisfied, (3) the seasonal pattern (either summer type or winter type). The SPAQ also assigns a GSS, as determined by the values recorded by participants to rate their seasonality (with a range of 0 [“no change”] to 4 [“extremely marked change”] as the seasons change) along six criteria: energy level, sleep duration, mood, social activity, weight and appetite [10].

We mailed the SPAQ to 2260 Old Order Amish in Lancaster, Pennsylvania, in addition to instructions regarding how to complete the SPAQ, and a return envelope pre-stamped and addressed. A one-dollar bill was included to express our gratitude for completing the survey. The individuals contacted for this study had previously participated in various studies conducted by the University of Maryland. All participants contacted were aged 18 or older. The letter contained directions for the SPAQ and stated that completion and mailing of the SPAQ to the University of Maryland School of Medicine constituted consent to participate in the study. There were two mailings of the SPAQ: the first in May, and the second in September of 2010 to those who did not respond to the first mailing. All responses received before December 31, 2011 were included in the database. The number of responses totaled 1265, which is a response rate of 56.0%.

Responses given on the SPAQ by 1265 Amish participants (728 women, 537 men), with an average age (SD) of 55.7 (14.8) years, were obtained. SPAQ-derived variables included the GSS and “total” winter SAD (either syndromal or subsyndromal SAD). On the SPAQ, the responses for time of the year for “feeling worst” were analyzed in order to determine the pattern of seasonality [6].

For estimation of SAD, sSAD and total SAD (tSAD) we used the criteria of Magnusson [12]. Participants qualified for a SAD diagnosis if their GSS score was greater than or equal to 11. The “problem” was ranked as moderate or higher, and the seasonal pattern corresponded to fall-winter mood changes (this was determined by recording “feeling worst” on the SPAQ for at least 1 month between September and February). In addition, sSAD was assigned for participants with a GSS score of 11 or higher and with either a “mild” or “none” problem, or for participants with a GSS score of 9 or 10 with at least “mild” problems from seasonal changes. For participants who recorded months outside of the fall-winter months (September–February) in addition to these months, seasonal pattern was determined by assigning a fall-winter pattern. If there were months in both the fall-winter and the spring-summer time frame in which the participant recorded “feeling worst”, then a fall-winter pattern was assigned, if there were at least two more months that were within the fall-winter time frame than the spring-summer months (March–August). If the participant listed several months consecutively that spanned both the spring-summer and fall-winter months, a fall-winter pattern was assigned if two or more months were in the fall-winter months and only 1 month was marked for the spring-summer months.

The subsample of individuals having data for both neopterin, as a marker of inflammation and seasonality of mood included 320 participants [women = 192 (60%), men = 128 (40%)] with an average age (SD) of 56.7 (13.9) years.

Statistical analysis

We analyzed the data with ANCOVAs and linear and logistic regressions. All tests were two-tailed and the criterion alpha was set at 0.05. SYSTAT Version 13 (San Jose, CA, USA) and SAS 9.4 (Cary, NC, USA) were the programs used for statistical analysis. All data were adjusted for age and, when not stratified for gender, also adjusted for gender and interactions. Overall significance in multivariable tests was followed by pairwise

comparisons using the Tukey statistic. Neopterin levels required a preanalytic logarithmic transformation to reach a normal distribution (with resulting variable called log-neopterin).

Results

The demographics are presented in Tables 1 and 2. Table 1 presents the average GSS scores and seasonal pattern in the sample with neopterin and seasonality data. Table 2 shows neopterin values by season of draw. The hypothesis driven results of the study are presented below.

There was a significant effect of season of blood collection on plasma neopterin levels in ANCOVA model adjusted for age and gender $F_{(3,2005)} = 4.43$, $p = 0.004$, with higher log-neopterin in winter than in summer ($p = 0.006$), as hypothesized. There was no significant gender X season interaction on log-neopterin levels $F_{(3,2005)} = 1.07$, $p = 0.361$ (Figure 1).

There was no significant association between GSS and log-neopterin in a multivariable linear model that included age and gender. The overall model was significant $F_{(3,307)} = 22.80$, $p < 0.001$, but not significant for the effects of GSS ($\beta = -0.008$, $p = 0.24$) and gender ($\beta = -0.005$, $p = 0.90$).

Similarly, in an ANCOVA model there were no differences in log-neopterin between those with versus those without tSAD ($p = 0.90$). Finally, there was no significant difference in log-neopterin levels between those with fall-winter pattern of “feeling worst” and those with other patterns or showing no pattern.

A multivariable model with age and gender showed a non-significant effect of seasonal pattern $F_{(2,309)} = 0.889$, $p = 0.41$, and no significant interaction between gender and seasonal pattern $F_{(2,309)} = 1.397$, $p = 0.25$ on plasma log-neopterin levels.

Discussion

We confirmed the hypothesized seasonal variation in neopterin levels, with a peak in winter when compared to summer, but we could not establish a significant gender difference in neopterin levels or an effect of seasonality or SAD. To our knowledge, two other studies have identified seasonal variation in neopterin and related it to environmental exposure to immune triggers, specifically (a) to allergens in atopic individuals, hypothesized to reduce neopterin levels via a Th2 shift [101], and (b) to seasonal variation in parasitic infection, specifically malaria transmission [104], hypothesized to trigger Th1 responses and elevate neopterin levels.

Finding elevated winter neopterin levels in our sample is consistent with Nelson’s theory of an enhanced immune function in the winter [52]. Nelson [52] went even further and speculated that photoperiodism, with the accompanying reduction or even suppression in many behaviors and physiological processes (such as reproductive behaviors), is a consequence rather than the cause of immune activation in the winter, since the immune activation is energetically costly and requires energy conservation via reduction of vitally unnecessary activity, such as play and reproduction [52].

Melatonin duration, the main molecular mediator of seasonal changes, has also been associated with bolstering of immune function during short photoperiods [50, 107]. For example, melatonin was reported to restore an immune system weakened by viral disease [50]. In addition, melatonin has been implicated in enhancing Th1 [108], and suppressing Th2 [109] lymphocyte activity. It has been hypothesized that a longer duration of nocturnal melatonin secretion in the winter, as uncovered in SAD patients by Wehr et al. [17], may stimulate T-lymphocytes and macrophages and shift equilibrium between Th1 and Th2 towards Th1 responses, with production of proinflammatory cytokines and a suppression of anti-inflammatory cytokines (Th2).

Our SAD and seasonal pattern-related analysis of neopterin levels found no associations with GSS or SAD estimation, in contrast to a previous report of higher neopterin levels in SAD patients than in controls [105]. However, in that study, blood samples in patients were collected in the fall and winter, while controls had their blood samples drawn in the fall only. It is thus possible that the difference between SAD and non-SAD individuals in the previous study was spurious. Our negative results for associations between SAD and seasonal pattern-related analysis of neopterin levels were also inconsistent with other previous articles suggesting that non-specific immune differences between SAD patients and healthy controls were unaffected [54] or affected by light treatment [55]. Those studies used clinical populations and clinical tools as opposed to our study, which was based on the SPAQ. This could be responsible, at least partly, for the different results. Another possibility for no association between SAD and seasonal pattern-related analysis of neopterin levels could be due to the Amish being culturally stoic [47], potentially underreporting their symptoms of depression. Nevertheless, in another study performed by our group in the Old Order Amish, we identified that dysphoria/hopelessness, a symptom of depression, was significantly associated with *Toxoplasma gondii* IgG sero-intensity [110].

The absence of gender differences in seasonal variation in neopterin was surprising considering the known gender differences in immune function [67, 68], seasonal changes in immune function [61–63], overall seasonality of physiology and behavior [8, 57–59], seasonal environmental exposures in the Old Order Amish [6, 47] major occupational differences in the Amish between genders, in particular in the summer [47], and finally, the known effects of gonadal steroids on immune function [67, 71]. The lack of replication suggests that neopterin levels were indeed robust to at least occupation and gender, and possibly to hormonal influences. This adds to our recent contribution to neopterin physiology suggesting a low heritability (in contrast to previously reported heritability of other immune parameters) [98].

In line with the findings of the studies linking obesity and inflammation, body mass index (BMI) as a measure of obesity has also been associated with higher levels of inflammatory markers [111]. Furthermore, BMI has also been reported to have seasonal variation, with higher values in winter as compared to summer seasons [112, 113]. A number of studies have found positive association between BMI and C-reactive protein (CRP) levels [114–117]. Panagiotakos et al. [118] reported that in subjects from both genders, BMI was positively associated with various indicators of inflammation, such as white blood cell (WBC) counts, TNF, IL-6, CRP and amyloid A. A study in pregnant women reported that

the levels of high-sensitivity CRP and leptin increased as the BMI of the study participants increased [119]. Inflammation has also been reported to be a key mediator of the risk for preeclampsia-risk associated with higher BMI [120]. Saito et al. [117] reported that serum albumin and fibrinogen levels were also positively associated with BMI, further providing evidence that BMI is positively associated with inflammation. Also, obesity has been previously related to markers of inflammation, including CRP, IL-6, TNF, resistin, leptin, fibrinogen, monocyte chemoattractant protein-1 (MCP-1) and plasminogen activator inhibitor-1 (PAI-1) [121–123]. IL-6, a pleiotropic cytokine that is elevated during inflammatory responses, has been shown to be produced by the subcutaneous adipose tissue in humans [124]. It has been shown that macrophages accumulate within the adipose tissue and contribute highly to the production of IL-6 and TNF [125]. Also, loss of weight in obese people has been linked to improved inflammatory profile [126] and decrease in concentrations of IL-6, TNF and CRP [127].

An alternative interpretation of our findings is that variation in immune parameters could be the consequence of seasonal variation in exposure to microorganisms, collectively referred to as the “Old Friends” [128] implicated in promoting immunoregulation. Immunoregulation refers to a balanced expression of effector T-cells (i.e. Th1, Th2 and Th17 cells) and regulatory T-cells (Treg), that produce anti-inflammatory cytokines such as IL-10 and transforming growth factor-beta (TGF- β) [129, 130]. Increased exposure to “Old Friends” (bacteria in soil, for instance) during the summer would be expected to increase Treg and decrease Th1 immunity, in line with the lower neopterin concentrations during summer. Throughout human evolution, “Old Friends” needed to be tolerated by the immune system as they were either part of host physiology (human microbiota), or were harmless but inevitably contaminating air, food and water (environmental microbiota), or caused severe tissue damage when attacked by the host immune system (helminthic parasites) [128]. Seasonal variation in exposure to “Old Friends” could result from either seasonal dietary changes or seasonal changes in activities with high exposure to environmental bacteria, such as working with the soil during seasonal farming activities. Consistent with this hypothesis, in a longitudinal study of healthy participants, expression of forkhead box protein 3 (Foxp3), a specific marker of Treg, was found to be higher during summer [131].

Also, the Amish eat a seasonally varied diet [47, 132]. Though diet has been implicated as a likely correlate to seasonal variation in gut microbiomes, most studies examining this relationship contain confounding elements [133–135]. This includes variations in diet between participants and other differences across populations, such as environmental variation, gene expression or differences, cultural factors resulting in different health practices and variation in access to health resources [136–138]. However, a recent study in a Hutterite population reported a correlation between seasonal variation in diet and annual changes in gut microbiomes [139]. This study provided an analysis of this relationship with fewer confounding variables by accounting for longevity, with measurements of gut microbiota during the summer and winter, and a generally homogenous environment via their lifestyle. This study reported seasonal variations in gut microbiota that were correlated with changes in diet between the summer and winter months. Specifically, microbiome samples contained more diverse microbiota during the winter months than summer months. An explanation postulated by the authors suggested that nutrients available during summer

months facilitate the growth and proliferation of certain microbiota at the expense of others, which are “outcompeted” [139].

Yet another possibility is that seasonal changes in food availability and preferences (e.g. green leaves, legumes, fruits and prebiotics) may contribute to seasonal changes in gut microbiota that play a crucial role in immunomodulation, resulting in increased levels of inflammatory markers in winter [130]. Seasonal changes in immune function could also be associated with potential reactivation of *T. gondii* in the Amish, which could further increase inflammation, and has also been related to dysphoria/hopelessness [110].

In addition, one study reported higher rather than lower neopterin level in atopics, thus inconsistent with Th1 suppression, and no differences between atopic patients successfully and unsuccessfully treated with immunotherapy that is geared, at least partially, towards shifting the balance from Th2 to Th1 [140]. Thus, in our opinion, it is more likely that the findings by Ciprandi et al. [102] may have been driven by endogenous immune anticipatory adaptation to season (heightened Th1 response during the winter with physiological rebounding of Th2 in spring) and not seasonal differences in allergen exposure.

Limitations of our study include using a survey instrument that measures subjective seasonal depressive symptoms through self-report rather than objective diagnoses of depressive disorders with seasonal patterns in individuals directly and the cross-sectional design, which did not allow for longitudinal measurement of changes in neopterin. Thus, our seasonal estimates are not based on repeated values within subjects, but on measurements across subjects. The sample for testing interactions with self-reports of seasonality was relatively small, and for the associations between neopterin levels and SPAQ-derived SAD estimates, it was extremely small. Yet, the strengths of the study include using a sample that was moderately powered to investigate seasonal variation in neopterin, allowing testing interactions between gender, seasonality (by GSS) and season of blood collection without critically losing statistical power. Generalizability may be limited due to studying a unique population – the Old Order Amish. Yet, the advantage of studying this population includes extremely low incidence of smoking and drinking alcohol, both factors relating to depression and inflammation, and previously manifesting considerable seasonal variation [141, 142] in modern populations. Similarly, reduced social and educational disparities within the Amish community decrease in our sample heterogeneity in pathogens and allergen exposure, as well as extraneous seasonal variation in immune function, thus providing an enhanced ability to uncover associations that would have otherwise been blurred, masked or falsely claimed.

The SPAQ was distributed twice during 2010 with the first mailing in May (spring) and the second mailing in September (fall). We utilized all responses received before December 31, 2011 [6, 11, 143]. Between the two administrations, there was no significant difference in responses from the Amish participants. However, to our knowledge, the difference between winter and summer administration of the SPAQ and difference in response has not been directly studied. For this reason, though it is unlikely that the seasonal administration of the SPAQ may have affected responses, this is possible. In a study of college students during different seasons, there were no significant seasonal differences in the SPAQ-derived GSS

and winter SAD [144]. Our findings may have also been driven by summer exposure to allergens such as grass, weed and pollen, consistent with the Th2 shift theory presented in Ciprandi et al. [102]. However, it has been reported that the Amish, developmentally exposed to Amish farming, may possess a degree of protection from allergy and asthma [99].

Conclusions

In conclusion, neopterin levels appear to be different across seasons but independent from effects of gender and degree of seasonality of mood and behavior. Thus, studies on neopterin crossing temporal divides of season would have to adjust for seasonal differences in collection, and not to worry as much about gender differences and effects of seasonality scores or SAD diagnostic status of the participants. Therefore, neopterin could be used for monitoring of immune status across seasons in demographically diverse samples, even if heterogeneous in regards to gender composition and degree of seasonality of mood and behavior.

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Figure 1.
Seasonal variation in log-neopterin (mean and standard error).
Y axis, log-neopterin concentrations (SE).

Table 1

Seasonality of mood and behavior, patterns and gender differences.

Parameters	Total	Men	Women
N	320	128	192
Age (mean, SD)	54.7 (13.9)	54.2 (14.1)	55.0 (13.9)
GSS (mean, SD)	4.19 (3.06)	4.52 (2.99)	3.95 (3.10)
Winter pattern, N (%)	N = 45 (14.06)	N = 15 (11.72)	N = 30 (15.63)
Summer pattern, N (%)	N = 39 (12.19)	N = 20 (15.63)	N = 19 (9.90)

GSS, Global seasonality score; winter pattern, feeling worst in winter, on the seasonal pattern questionnaire (SPAQ); summer pattern, feeling worst in summer, on SPAQ.

Table 2

Neopterin levels (nmol/L) in winter and summer in the total sample, men and women.

Parameters	Total sample	Men	Women
Neopterin (mean $\pm$ SD)	1.75 $\pm$ 0.33	1.74 $\pm$ 0.32	1.76 $\pm$ 0.34
Summer neopterin (mean $\pm$ SD)	1.72 $\pm$ 0.33	1.73 $\pm$ 0.34	1.72 $\pm$ 0.33
Winter neopterin (mean $\pm$ SD)	1.81 $\pm$ 0.36	1.81 $\pm$ 0.37	1.82 $\pm$ 0.36

Neopterin, Average levels across the entire year; summer neopterin, neopterin levels in blood collected in summer; winter neopterin, neopterin levels in blood collected in winter.