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Social regulation of leukocyte homeostasis: The role of glucocorticoid sensitivity

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ABSTRACT

Recent small-scale genomics analyses suggest that physiologic regulation of pro-inflammatory gene expression by endogenous glucocorticoids may be compromised in individuals who experience chronic social isolation. The present study assessed the relationship between leukocyte distributional sensitivity to glucocorticoid regulation and subjective social isolation in a large population-based sample of older adults. Initial analyses confirmed that circulating neutrophil percentages were elevated, and circulating lymphocyte and monocyte percentages were suppressed, in direct proportion to circulating cortisol levels. However, leukocyte distributional sensitivity to endogenous glucocorticoids was abrogated in individuals reporting either occasional or frequent experiences of subjective social isolation. This finding held in both non-parametric univariate analyses and in multivariate linear models controlling for a variety of biological, social, behavioral, and psychological confounders. The present results suggest that social factors may alter immune cell sensitivity to physiologic regulation by the hypothalamic–pituitary–adrenal axis in ways that could ultimately contribute to the increased physical health risks associated with social isolation.

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1. Introduction

Social isolation is a risk factor for several adverse health outcomes including all-cause mortality (Berkman, 1977; House et al., 1988; Seeman, 1996; Berkman and Kawachi 2000; Cacioppo and Hawkley, 2003), and specific infectious, neoplastic, and cardiovascular diseases (Reynolds and Kaplan, 1990; Krongrad et al., 1996; Cohen et al., 1997; Eng et al., 2002; Cole et al., 2003; Soler-Villa et al., 2003; Caspi et al., 2006; Kroenke et al., 2006). A recent functional genomics analysis suggests that alterations in immune system regulation by the hypothalamic–pituitary–adrenal (HPA) axis might contribute to these effects (Cole et al., 2007). Endogenous glucocorticoids from the HPA axis play a central role in physiologic control of inflammation by inhibiting the pro-inflammatory transcription factor NF-κB (Munck and Guyre, 1986; Ruzek et al., 1999; Rhen and Cidlowski, 2005; Onard et al., 2007; Pace et al., 2007). However, the HPA axis also mediates physiologic stress responses to a variety of non-inflammatory challenges (e.g., psychological or social stressors) (Weiner, 1992; Sapolsky, 1994; Dickerson and Kemeny, 2004), and those competing functional demands can potentially undermine the optimal control of inflammatory gene expression. Studies in animal models have shown that repeated social disruption can induce a state of glucocorticoid resistance in which the glucocorticoid receptor (GR) becomes less efficient in transducing endogenous glucocorticoid signals (Quan et al., 2003; Pace et al., 2007). This dynamic is believed to stem from GR phosphorylation by the p38 mitogen-activated protein kinase signaling pathway, which results in decreased receptor affinity for glucocorticoids (Wang et al., 2004), and subsequent derepression of pro-inflammatory gene expression (Stark et al., 2001; Avitsur et al., 2005).

Studies of chronically stressed human beings have shown evidence of glucocorticoid resistance in *ex vivo* assays of leukocyte cytokine response to lipopolysaccharide (LPS) (Miller et al., 2002, 2005). Based on those findings, we hypothesized that links between social isolation and inflammation-related disease might stem from underlying alterations in leukocyte sensitivity to regulation by endogenous glucocorticoids. A recent functional genomics analysis supported this hypothesis in showing decreased transcription of GR target genes (e.g., *HIST1H2BG*, *STAT1*, *TNFRSF17*, and *TCN1*) and increased transcription of NF-κB target genes (e.g., *EGR3*, *FOSB*, *IL8*, and *PTGS2*) in leukocytes from people experiencing chronically high levels of subjective social isolation (loneliness) (Cole et al., 2007). However, it is not clear whether altered glucocorticoid regulation is a common correlate of subjective social isolation, or whether such effects occur only in individuals who show high levels of loneliness over long periods of time (e.g., consistently over 4 years in the extreme groups design of the genomics-based analysis) (Cole et al., 2007). It also remains unclear whether the effects previously observed in a small sample of older Americans from the Chicago metropolitan area might generalize to other socio-cultural contexts. To address these questions in population-based epidemiologic studies or biosocial surveys (Weinstein

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et al., 2007), it would be desirable to identify a measure of GR sensitivity that is less technically intensive than genome-wide transcriptional analysis or *ex vivo* cell culture assays.

One of the longest recognized effects of glucocorticoids involves the regulation of leukocyte subset composition in circulating blood (Fauci et al., 1976). High glucocorticoid levels simultaneously increase the circulating number of neutrophils (neutrophilia) and decrease the circulating number of lymphocytes (lymphopenia) and monocytes (monocytopenia) (Dale et al., 1975; Fauci et al., 1976; Miller et al., 1994; Dhabhar et al., 1996). These effects lag glucorticoid levels by 4–6 h (Dale et al., 1975) and are driven in large part by altered trafficking of leukocyte subsets between blood and extravascular compartments (e.g., bone marrow) (Fauci and Dale, 1975; Fauci et al., 1976). Because these effects are mediated specifically by GR signaling (Miller et al., 1994; Dhabhar et al., 1996), glucocorticoid regulation of the circulating neutrophil/lymphocyte ratio or neutrophil/monocyte ratio should provide an efficient biological readout for GR functional activity in leukocytes. To the extent that leukocyte GRs show normal physiologic sensitivity to endogenous glucocorticoid levels, we would expect to observe the well-established positive correlation between cortisol levels and the neutrophil/lymphocyte ratio or the neutrophil/monoctye ratio. To the extent that leukocyte GRs are functionally desensitized, those positive correlations would be attenuated. Thus, the strength of association between endogenous glucocorticoid levels and circulating lymphocyte subset distributions may provide an efficient hematologic biomarker of leukocyte sensitivity to physiologic regulation by the HPA axis.

In the present study, we utilized this hematologic biomarker to quantify the relationship between subjective social isolation and immune system sensitivity to glucocorticoid regulation in data from SEBAS 2000—a large population-based survey of older Taiwanese adults (Chang et al., 2007). This sample has previously been studied for relationships between psychosocial characteristics and hormone levels (Seeman et al., 2004; Goldman et al., 2005), but leukocyte sensitivity to hormonal regulation has not been examined. The present analyses sought to (1) determine whether loneliness-related impairments in leukocyte sensitivity to glucocorticoid regulation occur in a large population-based sample of older adults and (2) identify the minimal extent of subjective social isolation required for the emergence of such effects.

2. Methods

2.1. Study sample

Data come from the Social Environment and Biomarkers of Aging Study in Taiwan 2000 (SEBAS) (Seeman et al., 2004; Goldman et al., 2005; Chang et al., 2007). SEBAS is a multi-site study of 1497 Taiwanese adults aged 54 and older drawn from a nationally representative sample and assessed for social, economic, psychological, and health parameters through an in-home interview in the year 2000. Participants (1023) provided overnight urine specimens in the context of a hospital-based clinical examination. Institutionalized individuals (including hospitalized) and those with serious illness were excluded from this examination. Following initial publication of study results, de-identified data were archived for secondary analysis through the Inter-University Consortium for Political and Social Research (http://www.icpsr.umich.edu/, ICPSR 3792) (Chang et al., 2007; Weinstein and Goldman, 2007). Data were collected under the supervision of Institutional Review Boards at Princeton University, The RAND Corporation, Georgetown University, the University of California at Los Angeles, and the Bureau of Health Promotion, Taiwan.

2.2. Biological parameters

Twelve-hour overnight urine collections were obtained at 7 AM and assayed for cortisol, epinephrine, and norepinephrine by Union Clinical Laboratories (Taipei, Taiwan) using high performance liquid chromatography with ultraviolet absorbance detection according to established protocols (Krstulovic, 1982; Samaan et al., 1993). Assay lower limits of detection were 2 pg/ml for catecholamines and 4 pg/ml for cortisol (Seeman et al., 2004). To control for individual differences in body size, urinary hormone values were normalized to creatinine concentrations as measured by alkaline picrate assay on a Beckman CX7 instrument (sensitivity = 10 mg/dl) (Goldman et al., 2005). White blood cell (WBC) counts, percent neutrophils, percent lymphocytes, and percent monocytes were determined in peripheral blood samples drawn at 9 AM, maintained at room temperature, and assayed within 2 h by complete blood count with five-part differential using a Coulter Gen-S automated hematology analyzer (Union Clinical Laboratories) according to the manufacturer's protocol (Coulter, Fullerton CA) (Seeman et al., 2004; Goldman et al., 2005). Leukocyte distributions at 9 AM are functionally contemporaneous with cortisol levels accumulated over the previous several hours because hematological effects lag glucocorticoid fluctuations by 4–6 h (Dale et al., 1975). Leukocyte sensitivity to glucocorticoid regulation was quantified by the strength of relationship between measured cortisol levels and measures of leukocyte subset distribution ($\log_{10}$-transformed neutrophil/lymphocyte ratios and neutrophil/monocyte ratios, and individual percentages of neutrophils, lymphocytes, and monocytes).

2.3. Psychological, social, behavioral, and health parameters

SEBAS measures of psychosocial, behavioral, and health parameters have previously been described (Seeman et al., 2004; Goldman et al., 2005; Chang et al., 2007; Weinstein and Goldman, 2007). For the present analysis, subjective social isolation was assessed by SEBAS item B14-5, which asks respondents how often they felt lonely during the past week (not at all, rarely = 1 day during the past week, sometimes = 2–3 days during the past week, often = 4 or more days during the past week). This one-item indicator has been shown to be a valid measure of subjective social isolation (Hughes et al., 2004). Objective social isolation was measured by SEBAS items A1 (not married), A3-A (lives alone), and B9-A–H (does not participate in a neighborhood, religious, political, social service, village/lineal, elderly club, or elderly education organization). Depressive symptoms were assessed by a nine-item variant of the Center for Epidemiologic Studies Depression scale (CESD) that has been validated for Taiwanese adults (Cheng and Chan, 2005) and omits one item directly tapping social isolation. Socioeconomic status (SES) was measured as a composite of (*z*-transformed) measures of male/husband's education (MANED00), perceived SES relative to other Taiwanese (D1), and low personal economic stress (C3-B). Physical health was represented as a composite of (*z*-transformed) subjective physical health (B1) and absence of medically significant chronic illness (including diabetes, cancer, renal disease, cardiovascular disease, respiratory disease, and cerebrovascular disease). Alcohol consumption and cigarette smoking were assessed by items ALC and SMK.

2.4. Statistical analyses

Relationships between hematologic parameters and hormone levels were analyzed using Spearman rank correlations estimated by SAS PROC CORR and general linear models fit using SAS PROC GLM (SAS Institute, Cary NC) (McCullagh and Nelder, 1991). Linear models analyzed variation in the distribution of hematological parameters ($\log_{10}$-transformed neutrophil/lymphocyte and neutrophil/monocyte ratios, and individual percentages of neutrophils, lymphocytes, and monocytes) as a function of measured cortisol levels while controlling for any potential confounding between loneliness and other biological, social, psychological, behavioral, or health parameters. Glucocorticoid sensitivity was quantified by the linear model parameter relating leukocyte subset distributions to cortisol levels, and differences in the strength of that relationship for high- vs. low-lonely participants were quantified by a Cortisol × Loneliness interaction term (in a model including a main effect of Loneliness). Residual plots verified that results met the distributional assumptions of general linear models (Miller, 1986). The lowest level of subjective social isolation at which altered leukocyte sensitivity to glucocorticoid regulation could be detected was identified using profile contrasts. Among the total 1023 SEBAS participants, data on loneliness were missing for 20 individuals and hematologic measures were unavailable for 8, leaving a final analyzed sample size of 995. All available data were analyzed, and all reported *p*-values represent two-sided significance levels.

3. Results

Table 1 presents demographic, behavioral, psychosocial, and endocrine characteristics of the SEBAS 2000 sample. As previously detailed (Seeman et al., 2004; Goldman et al., 2005; Chang et al., 2007), this sample represents a relatively healthy cohort of older, community-dwelling Taiwanese adults. Socioeconomic status varied widely, but was generally satisfactory to respondents. Rates of depressive symptoms were low, and endocrine and hematologic parameters fell within established reference ranges (Seeman et al., 2004; Weinstein and Goldman, 2007).

Table 1
SEBAS 2000 sample characteristics

Characteristic	Distribution
Age	68.3 (8.5) years[a]
Sex	57.7% Male 42.3% Female
SES	73.8% no economic stress 26.2% economic stress 6.8 (4.5) years education[a] 9.2% college or post-graduate 11.6% high school 56.1% primary school 15.3% illiterate 7.8% information unavailable
Physical health	75.2% no medical condition 24.8% medical condition 96.5% good health 3.5% poor health
Loneliness (subjective social isolation)	81.5% none 7.1% rare (1 day/week) 6.9% occasional (2–3 days/week) 4.5% frequent ($\geqslant$4 days/week)
Objective social isolation	96.0% integrated 4.0% isolated
Depression (CESD10)	7.9 (5.5) U (0–30 scale)[a] 20.7% score depressed
Smoking	76.4% none 1.4% occasionally with friends 0.2% only after meals 22.0% every day
Alcohol consumption	77.0% none 16.4% sometimes 1.6% frequently 5.1% every day
Cortisol	28.3 (51.9) μg/g creatinine[a]
Epinephrine	21.9 (9.9) μg/g creatinine[a]
Norepinephrine	2.6 (2.6) μg/g creatinine[a]
WBC count	6120 (1597) cells/μL[a]
Leukocyte subset distributions	32.6 (8.8) % lymphocytes[a] 7.3 (2.0) % monocytes[a] 56.7 (9.5) % neutrophils[a]

[a] Mean (SD).

The vast majority of respondents reported no subjective experience of social isolation, with fewer than 10% reporting either rare loneliness (1 day per week), occasional loneliness (2–3 days per week), or frequent loneliness (4 or more days per week). Subjective social isolation was weakly correlated with objectively measured social isolation (Spearman $r_S = .106$, $p = .0008$), age ($r_S = .134$, $p = .0001$), and poor physical health ($r_S = .189$, $p < .0001$), and more strongly correlated with low SES ($r_S = .228$, $p < .0001$), and depressive symptoms ($r_S = .415$, $p < .0001$). Females reported marginally higher rates of loneliness (biserial $r = .061$, $p = .054$). Each of these potential confounders was controlled for in subsequent multivariate analyses relating loneliness to glucocorticoid sensitivity. Loneliness was not significantly correlated with any of the other risk factors listed in Table 1 (including smoking and alcohol consumption).

3.1. Cortisol regulation of leukocyte distribution

Table 2 reports results from multivariate analyses modeling circulating leukocyte subset distributions as a function of cortisol, epinephrine, and norepineprhine levels. Consistent with the established effects of glucocorticoids on leukocyte subset trafficking (Dale et al., 1975; Fauci and Dale, 1975; Fauci et al., 1976; Miller et al., 1994), results show the expected positive relationship between cortisol levels and circulating neutrophil percentages, and the expected negative relationships between cortisol levels and the percentages of circulating lymphocytes and monocytes. The net effect was a highly significant positive correlation between cortisol levels and the neutrophil/lymphocyte ratio and the neutrophil/monocyte ratio. Those relationships were specific to cortisol, as no significant relationships emerged for norepinephrine or epinephrine levels. Thus, circulating leukocyte subset distributions provide a specific biomarker of immune cell regulation by endogenous glucocorticoids.

3.2. Effects of loneliness on glucocorticoid regulation of leukocyte distributions

To determine whether subjective social isolation was associated with reduced leukocyte sensitivity to glucocorticoid regulation, we carried out two types of analyses assessing the sensitivity of circulating leukocyte subset distributions to cortisol levels in lonely vs. non-lonely individuals. Initial non-parametric analyses stratified data from lonely vs. non-lonely individuals and quantified the association between cortisol level and leukocyte distributional parameters within each stratum (Table 3). Results from the 812 non-lonely individuals showed the generally observed positive relationship between cortisol levels and circulating neutrophil/lymphocyte ratios and neutrophil/monocyte ratios. However, consistent with the hypothesis of reduced leukocyte sensitivity to glucocorticoid regulation, data from the 183 lonely individuals showed no statistically significant relationship between cortisol and either hematologic ratio. The difference in the magnitude of sensitivity correlations was statistically significant for both neutrophil/lymphocyte ratios ($p = .0359$ by z-test for independent correlations) and neutrophil/monocyte ratios ($p = .0370$). Similar effects emerged in analyses distinguishing those who reported

Table 2
Neuroendocrine correlates of circulating leukocyte subset distributions

	WBC count (10^3 cells/μL)	Lymphocytes (% WBC)	Monocytes (% WBC)	Neutrophils (% WBC)	Neutrophil/ Lymphocyte ratio[a]	Neutrophil/ Monocyte ratio[a]
Cortisol[b]	1.0 (0.9)[c] $p = .3008$	−16.1 (5.3) $p = .0024$	−2.9 (1.2) $p = .0203$	21.5 (5.7) $p = .0002$	0.42 (0.12) $p = .0005$	0.34 (0.09) $p = .0004$
Epinephrine[b]	−30.7 (20.2) $p = .1286$	−59.0 (110.3) $p = .5929$	−31.3 (25.5) $p = .2200$	120.9 (119.4) $p = .3111$	1.45 (2.50) $p = .5364$	3.17 (2.00) $p = .1122$
Norepinephrine[b]	9.8 (5.4) $p = .0697$	−45.3 (29.4) $p = .1238$	0.9 (6.8) $p = .8949$	41.3 (31.8) $p = .1943$	1.13 (0.67) $p = .0895$	0.29 (0.53) $p = .5913$

[a] Units = $\log_{10}$(ratio).
[b] Units = g hormone/g creatinine.
[c] Partial regression coefficient point estimate (standard error) from multivariate analysis of each hematologic parameter as a function of cortisol, epinephrine, and norepinephrine levels.

Table 3
Loneliness-related differences in hematologic biomarkers of glucocorticoid sensitivity: non-parametric univariate analyses

	WBC count (10^3 cells/μL)	Lymphocytes (% WBC)	Monocytes (% WBC)	Neutrophils (% WBC)	Neutrophil/ Lymphocyte ratio	Neutrophil/ Monocyte ratio
Non-lonely	$r = -.069$[a] $p = .0509$[b]	$r = -.136$ $p = .0001$	$r = -.195$ $p < .0001$	$r = .196$ $p < .0001$	$r = .159$ $p < .0001$	$r = .236$ $p < .0001$
Lonely	$r = -.101$ $p = .1740$	$r = -.002$ $p = .9733$	$r = -.103$ $p = .1645$	$r = .025$ $p = .7380$	$r = .012$ $p = .8675$	$r = .093$ $p = .2098$

[a] r = Spearman rank correlation between cortisol concentration (g/g creatinine) and the indicated hematologic parameter.
[b] p = two-tailed significance level.

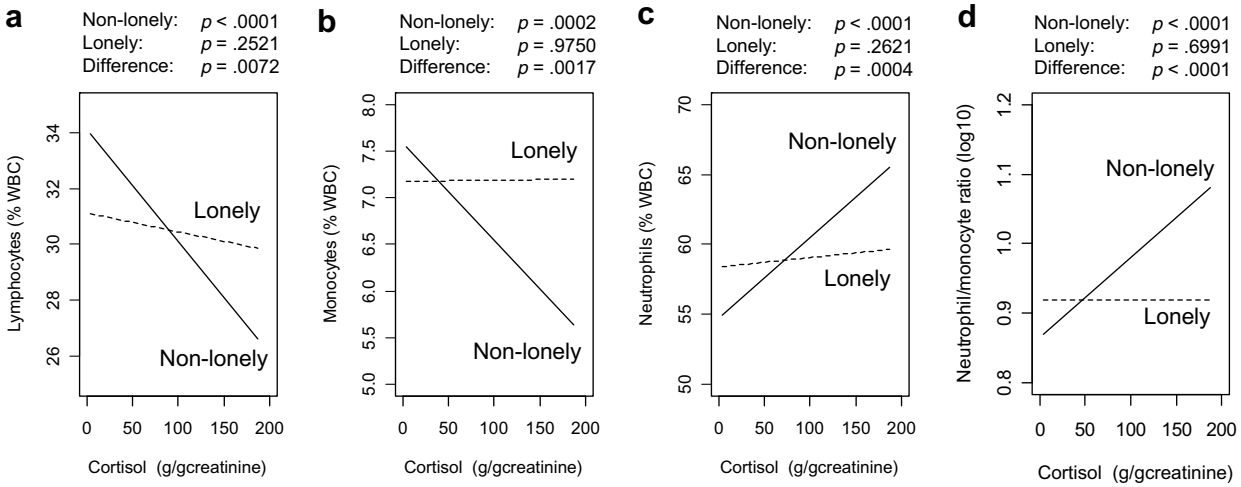

Fig. 1. Sensitivity of circulating leukocyte subset distributions to cortisol level. Regression lines indicate the variation in hematologic paramters as a function of cortisol level in 812 non-lonely participants (solid line) and 183 lonely participants (dashed line) for (a) percent lymphocytes, (b) percent monocytes, (c) percent neutrophils, and (d) neutrophil/monocyte ratios. p-values give statistical significance of the slope of regression lines for non-lonely individuals, lonely individuals, and the difference between groups (Cortisol × Loneliness interaction) in analyses controlling for BMI, Age, Sex, Age × Sex interaction, smoking, alcohol consumption, SES, physical health status, depression, and objective social isolation.

the highest level of loneliness (frequent) from others. High-lonely individuals showed non-significant correlations between leukocyte distributional parameters and cortisol levels (all $p > .25$), whereas leukocyte subset distributions showed highly significant correlations with cortisol levels in the remainder of the sample (all $p < .0001$). The use of rank-based non-parametric measures ensures that these results are robust to assumptions regarding statistical distribution (Miller, 1986).

3.3. Biological, social, behavioral, and psychological confounders

To ensure that the differential linkage of cortisol to circulating leukocyte subset distributions in lonely vs. non-lonely individuals did not stem from confounded differences in other biological, social, behavioral, or psychological characteristics, a second set of analyses used general linear models to quantify relationships between cortisol and leukocyte distributions while controlling for body mass index (BMI), Age, Sex, Age × Sex interaction (to accommodate sex differences in age trends observed for some hematologic parameters), objective social isolation, depression, SES, smoking, alcohol consumption, and physical health. As shown in Fig. 1, Cortisol × Loneliness interaction terms remained statistically significant. To confirm that effects of subjective loneliness were not attributable to the objective absence of social contact, glucocorticoid sensitivity analyses were repeated substituting measures of objective social isolation for loneliness. None of the leukocyte distributional parameters showed a significant Cortisol × Isolation interaction (all $p > .2126$).

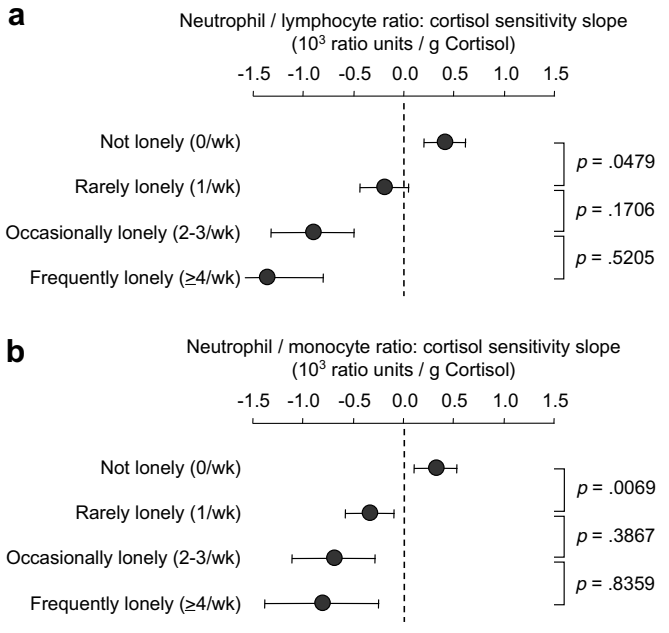

Fig. 2. Relationship between subjective social isolation and magnitude of leukocyte distributional sensitivity to cortisol level. Data represent means ± standard error of sensitivity coefficients relating variation in cortisol concentration to (a) neutrophil/lymphocyte ratios, and (b) neutrophil/monocyte ratios at varying levels of loneliness. p-values denote statistical significance of profile contrasts comparing adjacent sensitivity coefficients.

3.4. Threshold of regulatory alterations

To identify the threshold level of loneliness at which alterations in leukocyte sensitivity to glucocorticoid regulation become detectable, a set of profile contrasts compared cortisol sensitivity parameters for respondents who reported not being lonely at all vs. rarely lonely (1 day/week), rarely vs. occasionally lonely (2–3 days/week), and occasionally lonely vs. frequently lonely ($\geqslant$4 days/week). As shown in Fig. 2, this analysis showed a mildly decelerating dose–response relationship between frequency of subjective isolation and reduced leukocyte distributional sensitivity to cortisol. A statistically significant decrement occurred between "not lonely" and "rarely lonely" for both neutrophil/lymphocyte ratios and neutrophil/monocyte ratios. No other pair-wise profile contrast was statistically significant in either analysis. Thus, even minimal levels of subjective social isolation are associated with alterations in leukocyte sensitivity to glucocorticoid regulation.

4. Discussion

The present study used a novel cell trafficking-based measure to assess the relationship between subjective social isolation and leukocyte sensitivity to glucocorticoid regulation. Motivated by previous functional genomics studies indicating altered glucocorticoid regulation of genome-wide transcriptional profiles in a small sample of lonely older Americans (Cole et al., 2007), this analysis sought to verify those findings in a larger population-based study of older Taiwanese adults showing a broad range of loneliness levels. Initial biomarker analyses found the ratio of circulating neutrophils/monocytes and neutrophils/lymphocytes to be particularly sensitive to physiologic variation in the endogenous glucocorticoid, cortisol. Substantive analyses found reduced leukocyte distributional sensitivity to cortisol in people who experienced frequent loneliness, with significant effects detectable even among people who report feeling lonely as little as 1 day per week. These effects were specific to the experience of subjective social isolation, and could not be attributed to any correlated differences in physical health, SES, depression, smoking, alcohol consumption, or objective social isolation. Thus, the present findings are consistent with previous observations (Cole et al., 2007) in suggesting that subjective social isolation represents an independent social risk factor for alterations in leukocyte sensitivity to glucocorticoid regulation.

The present findings are consistent with data from experimental animal models indicating that social stress can influence immune cell sensitivity to glucocorticoid regulation (Stark et al., 2001; Bailey et al., 2004; Engler et al., 2004), and with data from human clinical studies linking social risk factors to *ex vivo* measures of leukocyte glucocorticoid resistance (Miller et al., 2002, 2005). However, it is unclear how closely the present study's *in vivo* cell trafficking-based biomarker of glucocorticoid regulatory sensitivity corresponds to the benchmark measure of "glucocorticoid resistance" based on pharmacologic inhibition of LPS-induced cytokine production *ex vivo*. No data are currently available to directly correlate these two approaches in humans, but socially stressed animals show simultaneous changes in both parameters (Engler et al., 2005). Hematologic biomarkers and *ex vivo* LPS stimulation assays are each likely to reflect some unique features in addition to whatever variance they share due to the common role of GR signaling in regulating their expression. For example, circulating leukocyte subset distributions may be affected by several other factors in addition to endogenous cortisol levels, including inflammation (subset-specific chemotaxis) (Mackay, 1993), hematopoiesis (differential production of leukocyte subsets) (Fey, 2007), and autonomic nervous system activity (catecholamine modulation of subset trafficking) (Ottaway and Husband, 1994; Carlson, 2001). Likewise, *ex vivo* cytokine production assays are sensitive to variations in LPS signal-transduction pathways and the presence of plasma glucocorticoids in cell cultures (Arbour et al., 2000; Bhattacharyya et al., 2007; Bower et al., 2007; Jaekal et al., 2007; MacRedmond et al., 2007). Despite these differences, leukocyte distribution and cytokine inhibition measures of glucocorticoid regulatory sensitivity are likely to covary substantially because both depend centrally on the ability of the GR to transduce glucocorticoid signals into functional alterations in leukocyte biology (Miller et al., 1994). Direct comparison of these two approaches is an important target for future research.

The utility of hematologic biomarkers of glucocorticoid sensitivity might be substantially improved by intra-individual assessments over time (e.g., repeated measurements of cortisol and leukocyte subset distributions over their diurnal cycle (Winkel et al., 1981)). That would hold constant other hematologic influences that likely added substantial noise to the present cross-sectional analysis (and may explain why relationships between cortisol levels and hematologic parameters rarely exceeded $r = .20$ in magnitude). The present cross-sectional measures can only identify group-level factors that influence glucocorticoid sensitivity (e.g., degree of loneliness) and cannot support individual-specific inferences about biologic function or physical health. The present data do suggest that neutrophil/monocyte ratios constitute the most sensitive hematologic biomarker of GR sensitivity, as that parameter showed the strongest quantitative relationship to endogenous cortisol levels. The biological basis for that advantage remains to be defined, but neutrophil/monocyte ratios may control for hematopoietic variation more effectively than other distributional summaries because neutrophils and monocytes differentiate from a common myeloid progenitor cell (Friedman, 2002) that is subject to regulation by glucocorticoids (Motomura et al., 1983; Suda et al., 1983; Rinehart et al., 1997). The neutrophil/monocyte ratio is also comparatively insensitive to potential biological confounders such as BMI, whereas neutrophil/lymphocyte ratios were significantly correlated with BMI (data not shown). Despite these complexities, hematologic assessment of glucocorticoid sensitivity provides substantial cost and feasibility advantages over previous measures such as *ex vivo* cytokine stimulation assays (Stark et al., 2001; Miller et al., 2002; Bailey et al., 2004; Miller et al., 2005; Pace et al., 2007) and bioinformatic analysis of genome-wide transcriptional profiles (Cole et al., 2007).

Several additional limitations need to be considered when interpreting the substantive results of this study. First, these data come from a correlational analysis that cannot decisively establish causal direction. Previous experimental studies show that glucocorticoid manipulation can influence circulating leukocyte distributions (Dale et al., 1975; Fauci and Dale, 1975; Fauci et al., 1976; Miller et al., 1994; Dhabhar et al., 1996). Correlations between endogenous glucocorticoid levels and hematologic parameters cannot be attributed to a reverse causal effect because experimental alteration of leukocyte subset distributions does not affect circulating cortisol levels (Rovelli et al., 1995; Schuld et al., 1999). Thus, circulating leukocyte subset distributions represent a causally valid biomarker of leukocyte sensitivity to glucocorticoid signaling. However, the relationship between glucocorticoid sensitivity and social risk factors requires careful interpretation. The present study was motivated by the hypothesis that subjective social isolation influences immune system sensitivity to regulation by endogenous GCs (Cole et al., 2007), but a reverse causal influence is conceivable in the possibility that altered glucocorticoid regulation of inflammation might affect social perception or behavior (e.g., via cytokine effects on the brain (Dantzer et al., 2008)). It is also possible that the observed correlation between re-

duced glucocorticoid sensitivity and social isolation is a spurious consequence of those variables' common relationship to a third causal variable. The present study ruled out some of the most plausible potential confounders, including demographic characteristics, SES, physical health status, smoking, alcohol consumption, depression, and objective social isolation. Nonetheless, it is conceivable that other determinants which remain unmeasured in this study could potentially affect both neuroendocrine-immune dynamics and experienced social isolation. Another limitation of this study involves its assessment of subjective social isolation using a one-item measure obtained on a single occasion. Although that one-item measure has been shown to correlate well with multi-item loneliness scales in previous studies (Hughes et al., 2004), future research would benefit from more extensive assessment of subjective social isolation across multiple occasions.

The present findings are consistent with the general theory that social factors can modulate immune system sensitivity to regulation by the nervous system (Amdam et al., 2005; Cole, 2005; Cole et al., 2007; Sloan et al., 2007; Sloan et al., 2008). The implications of such effects for immunologic effector functions or disease pathophysiology remain unclear because SEBAS lacks direct measures of those processes. However, the pattern of decreasing leukocyte functional sensitivity to HPA regulation with increasing levels of subjective social isolation is consistent with the profile of increased inflammatory disease risk observed in human epidemiologic studies (Berkman, 1977; House et al., 1988; Reynolds and Kaplan, 1990; Krongrad et al., 1996; Seeman, 1996; Cohen et al. 1997; Berkman and Kawachi, 2000; Cacioppo and Hawkley, 2003; Cole et al., 2003; Soler-Villa et al., 2003; Caspi et al., 2006; Kroenke et al., 2006) and in animal models (Sklar and Anisman, 1980; Ben-Nathan et al., 1989; Shively et al., 1989; Clausing et al., 1994; Hilakivi-Clarke and Dickson, 1995; Villano Bonamin et al., 2001; Wu et al., 2001; McClintock et al., 2005; Hermes et al., 2006; Thaker et al., 2006). A significant challenge for future research lies in understanding the teleologic basis for these relationships. Why has evolution selected for the development of an immune system that escapes its chief counter-regulatory influence when individuals are bereft of close social ties? The fact that the HPA axis has evolved to serve as both a primary regulator of endogenous inflammation (Munck and Guyre, 1986; Ruzek et al., 1999; Rhen and Cidlowski, 2005; Onard et al., 2007) and a primary regulator of physiologic responses to social threat (Dickerson and Kemeny, 2004) implies that there may be a significant selective benefit from the dynamic alteration of immune biology in response to changing social conditions (e.g., as noted for development of the nervous and endocrine systems (Hofer 1984; Panksepp, 1998; Zhang et al., 2006)). Defining the nature of individual physiologic threats and resources that are conferred by the close presence of conspecifics (e.g., as analyzed in (Cole, 2005)) could help unravel the evolutionary basis for the epidemiologic relationship between social factors and physical health (Berkman, 1977; House et al., 1988; Seeman, 1996; Berkman and Kawachi, 2000).

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