Loneliness in healthy young adults predicts inflammatory responsiveness to a mild immune challenge in vivo

Balter, Leonie J. T.; Raymond, Jane E.; Aldred, Sarah; Drayson, Mark T.; Veldhuijzen van Zanten, Jet J. C. S.; Higgs, Suzanne; Bosch, Jos A.

DOI:
10.1016/j.bbi.2019.08.196

Loneliness in Healthy Young Adults Predicts Inflammatory Responsiveness to a Mild Immune Challenge in Vivo

Leonie JT Balter[A,D], Jane E Raymond[A], Sarah Aldred[B], Mark T Drayson[C], Jet JCS Veldhuijzen van Zanten[B], Suzanne Higgs[A] & Jos A Bosch[D]

[A]School of Psychology, University of Birmingham, Birmingham, B15 2TT, UK

[B]School of Sport, Exercise, and Rehabilitation Sciences, University of Birmingham, Birmingham, B15 2TT, UK

[C]Institute of Immunity and Immunotherapy, University of Birmingham, Birmingham, B15 2TT UK

[D]Psychology Department, Clinical Psychology, University of Amsterdam, Amsterdam, 1018 WT, NL

*__Corresponding author__: email L.J.T.balter@uva.nl

Nieuwe Achtergracht 129B

1018 WT, Amsterdam

Keywords: loneliness; mild inflammation; immune dysregulation; typhoid vaccination

ABSTRACT

The established link between loneliness and poor health outcomes may stem from aberrant inflammatory regulation. The present study tested whether loneliness predicted the inflammatory response to a standardised in vivo immune challenge. Using a within-subjects double blind placebo-controlled design, 40 healthy men (mean age = 25, SD = 5) received a Salmonella Typhi vaccination (0.025 mg; Typhim Vi, Sanofi Pasteur, UK) and placebo (saline) on two separate occasions. Loneliness was assessed using the R-UCLA loneliness scale. Regression analyses showed that those that reported feeling more lonely exhibited an elevated interleukin-6 response (β = .564, 95% confidence interval [.003, .042], $p < .05$). This association withstood adjustment for potentially confounding variables, including age, sleep quality, socio-emotional factors, and health factors. The present findings are in line with evidence that loneliness may shift immune system responsivity, suggesting a potential biobehavioural pathway linking loneliness to impaired health.

INTRODUCTION

Feeling lonely is surprisingly prevalent in today's society, with estimates stating that over 15% of British and nearly 40% of US adults report feeling lonely (Office for National Statistics, 2018; Wilson & Moulton, 2010), Loneliness is increasingly recognised as a significant social problem, whereby the British government recently appointed a Minister of Loneliness. One of the several disruptive effects of loneliness is on physical health. For example, meta-analyses show a 30% increased risk of stroke, myocardial infarction, and mortality in lonelier individuals (Holt-Lunstad, Smith, Baker, Harris, & Stephenson, 2015; Steptoe, Shankar, Demakakos, & Wardle, 2013; Valtorta, Kanaan, Gilbody, Ronzi, & Hanratty, 2016).

Immune dysregulation, in the form of enhanced inflammatory responsivity, has been proposed as a mechanism underlying the link between loneliness and health risk (Hawkley, Bosch, England, Marucha, & Cacioppo, 2007). This idea has been supported, amongst others, by evidence that inflammatory gene transcription and epigenetics are altered in lonely individuals, together with studies showing increased immune reactivity to psychological stress in lonelier individuals (Brown, Gallagher, & Creaven, 2018; Cole et al., 2007; Hackett, Hamer, Endrighi, Brydon, & Steptoe, 2012; Jaremka et al., 2013). Likewise, the inflammatory response to an immune challenge (bacterial endotoxin) is elevated in individuals who report feeling socially disconnected (Moieni, Irwin, Jevtic, Breen, & Eisenberger, 2015), which is predictive of loneliness (Cacioppo et al., 2006). However, whether loneliness itself is associated with inflammatory responsivity has yet to be determined. This proposed hypothesis was tested using an existing data-set. The current study addressed the relationship between individual variation in subjective loneliness and immune reactivity in response to a mild immune-mediated inflammatory stimulus. Analyses were adjusted for potential confounders such as age, sleep quality, socio-emotional factors (i.e., depression, anxiety, social skills, negative mood), and health factors (i.e., body weight, alcohol intake).

METHOD

Participants

The study involved a within-subjects double blind placebo-controlled design, presented in detail elsewhere (Balter et al., 2018). Forty healthy young male students from the University of Birmingham were enrolled (*M* age = 24.7, SD = 5.2 years). Individuals were excluded if they self-reported a history of or suspected vaccine- or food-related allergy, inflammatory, cardiovascular, neurological, mental health, visual, or immune-related disorder, being a current smoker, and those on any medication 7 days prior to the test days. Participants received research credits or were paid £40. The study was conducted according to the guidelines laid down in the Declaration of Helsinki and all procedures were approved by the local Research Ethics Committee of the National Health Service.

Procedures

Participants visited the behavioural immunology laboratory on three separate occasions (one practice session and two test days): questionnaires were completed once during the first visit, except for negative mood, which was rated on each test day (see Materials). This was followed by two test days scheduled at least one week apart. On each test day, participants arrived at the laboratory between 8:00 and 9:00 am. A certified nurse administered Salmonella typhi capsular polysaccharide vaccine (25 μg in 0.5 mL, Typhim Vi, Sanofi Pasteur, UK) or saline placebo (0.5 mL) via intra-muscular injection in the deltoid muscle of the non-dominant arm; the injection order was counterbalanced across participants. Blood samples were taken before injection, and at 5h30min and 8h post-injection. The time points for the collection of the blood samples was based on the time course and magnitude of a variety of inflammatory markers published previously by our group (see Paine, Ring, Bosch, Drayson, & Veldhuijzen van Zanten, 2013).

The current analysis is based on the same sample as in Balter et al., (2018) and stem from secondary analysis of a larger study.

MATERIALS

Questionnaires

Questionnaires were completed in the order as presented below. Higher scores reflect worse functioning.

Alcohol intake. Average alcohol units per week (0 = 0 units, 1 = 1-5 units, 2 = 7-15 units, 3 = >15 units). One unit equals 10ml or 8g of pure alcohol and is equivalent to 1/2 pint of average-strength beer. A standard glass of wine is 2 units of alcohol. The definition of a unit of alcohol was explained to the participant.

Sleep quality. The total score of the 19-item Pittsburgh Sleep Quality Index was used to assess quality of sleep over a 1-month interval. Internal consistency (Cronbach's alpha; α) is 0.80 for the total score (Carpenter & Andrykowski, 1998).

Anxiety. The 21-item Beck Anxiety Inventory was used to assess anxiety. The Cronbach's α for non-psychiatric individuals is 0.81 (Beck, Epstein, Brown, & Steer, 1988).

Depression. The 21-item Beck Depression Inventory (BDI)-II was used to assess depressive feelings (Beck, Steer, & Brown, 1996). The BDI-II boasts high internal consistency among college students (Cronbach's α = 0.93; Dozois, Dobson, & Ahnberg, 1998).

Loneliness. Loneliness was measured via the 20-item revised UCLA Loneliness Scale (R-UCLA). The Cronbach's α reliability coefficient for the R-UCLA is 0.96 (Russell, Peplau, & Cutrona, 1980).

Social skills. The social skills subscale of the Autism Quotient was used to measure the degree of social skills a person possesses (Baron-Cohen, Wheelwright, Skinner, Martin, & Clubley, 2001). The Cronbach's α for the social skills subscale is 0.75 (Stevenson & Hart, 2017).

Mood. Negative mood on the day of testing was computed by summing five negative subscale scores (anger, confusion, depression, fatigue, and tension) and subtracting the

vigour subscale score of the Profile of Mood States Short Form. The Cronbach's α for total negative mood in a healthy sample is 0.88 (Curran, Andrykowski, & Studts, 1995).

Anthropomorphic measures

A stadiometer was used to measure height and a body composition measurement was taken using a TANITA BC-545N body composition analyser (Tanita Europe, Amsterdam, The Netherlands).

Interleukin-6 analysis

Blood (6 ml) was collected from an antecubital vein in the forearm into a vacutainer containing ethylenediaminetetraacetic acid (EDTA) as anticoagulant (Becton Dickinson Diagnostics, Oxford, United Kingdom). Samples were immediately centrifuged at 1500g for 10 min at 4 °C and plasma was aliquoted and stored at -80 °C for later cytokine assessment of plasma interleukin-6 (IL-6) using high-sensitivity ELISA (Quantikine HS Human IL-6 ELISA, R&D Systems, UK) in accordance with the manufacturer's instructions. The limit of detection of this assay was 0.11 pg/mL, with an intra-assay coefficient of variation (CV) of 4.2%. All samples were well above the detection limit (the sample values ranged between 0.33 and 9.62 pg/mL). To minimize assay variation, all samples from the same participants were assayed in the same run.~~.~~

STATISTICAL ANALYSIS

Data were analysed using SPSS v.24.0 (IBM-SPSS Inc., Chicago, IL, USA). IL-6 data of three participants were ~~excluded~~ removed because IL-6 data of three participants were removed because for two participants the inflammation induction did not induce an inflammatory response of two of these three participants showed a high baseline balue (> 2.5 SD above mean) that was indicative of a possible immune activation. ~~of high baseline values indicative of a possible infection~~ Additionally, ~~and~~ 5% of IL-6 data was missing due to occasional failure to take a blood draw. Data were analysed using bivariate correlation

analysis and linear regression analysis with log transformed IL-6 response (difference from baseline to peak IL-6 at either 5h30 or 8h post-injection) in the vaccine condition. Model 1 included loneliness, model 2 and 3 additionally included variables previously shown to be associated with inflammation or loneliness: depression, anxiety, negative mood, sleep quality, social skills, alcohol intake (model 2), age, and body mass index (BMI) (model 3).

RESULTS

Loneliness scores ranged between 22-64 (*M* = 39, SD = 10) and typhoid vaccination increases in IL-6 ranged from 1.1-8.8 pg/mL (*M* = 3.8, SD = 1.6) (see also Balter et al., 2018). At baseline, IL-6 was not significantly correlated with loneliness scores ($r(36) = -.123$, $p = .487$). However, as shown in Figure 1, loneliness positively correlated with the IL-6 response (difference from baseline to peak IL-6 at either 5h30 or 8h post-injection) to typhoid vaccination ($r(34) = .383$, $p = .026$). In 92% of the cases the peak IL-6 occurred at 5h30. Analyses using IL-6 responses at a single time point (5h30 or 8h) yielded essentially similar results. None of the other socio-emotional variables significantly correlated with the IL-6 response (Table 1). No significant correlations emerged in the placebo condition (*p's* > .60). Regression analysis showed that individual variation in loneliness was associated with the IL-6 response (model 1), independent of depression, anxiety, negative mood, quality of sleep, social skills, alcohol intake (model 2), age and BMI (model 3) (Table 2).

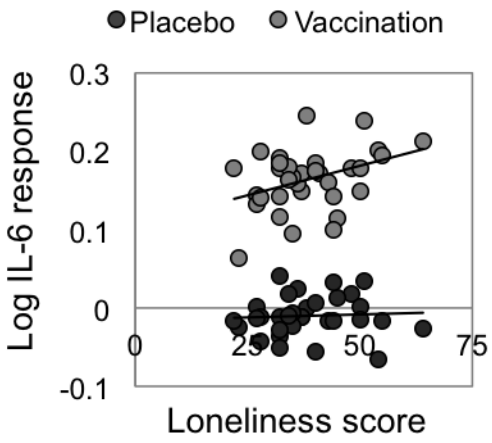

Figure 1. Correlations between log IL-6 response (difference from baseline to peak IL-6 at either 5h30 or 8h post-injection) and loneliness separately for the placebo and vaccination condition.

	IL-6 response
Loneliness	.383*
Depression	.053
Anxiety	.060
Negative mood	.106
Social skills	.146

*Table 1. Correlation coefficients between the log IL-6 response (difference from baseline to peak IL-6 at either 5h30 or 8h post-injection) to vaccination and socio-emotional variables; * indicates statistical significance (p < .05).*

	t	β	*p*	95% CI
Model 1 (R^2 = .146)			.026*	
Loneliness	2.343*	.383*	.026*	.001 .029
Model 2 (R^2 = .281)			.302	
Loneliness	2.179*	.517*	.040*	.001 .040
Depression	-0.207	-.055	.838	-.032 .026
Anxiety	0.404	.086	.690	-.014 .021
Sleep quality	-1.558	-.321	.133	-.029 .004
Negative mood	0.421	.105	.678	-.019 .028
Social skills	-0.783	-.182	.442	-.025 .011
Alcohol intake	-1.168	-.238	.255	-.026 .007
Model 3 (R^2 = .347)			.325	
Loneliness	2.407*	.579*	.025*	.003 .042
Depression	-0.772	-.224	.449	-.044 .020
Anxiety	0.278	-.069	.784	-.024 .018
Sleep Quality	-0.802	-.182	.431	-.026 .011
Negative mood	0.755	.194	.458	-.016 .033
Social skills	-0.199	-.056	.844	-.025 .020
Alcohol intake	-1.521	-.374	.143	-.034 .005
Age	-1.031	-.325	.322	-.037 .013
BMI	-0.573	-.121	.572	-.021 .012

*Table 2. Standardised regression coefficients (β), t- and p-values, and 95% confidence intervals (95% CI) of models predicting the IL-6 response (difference from baseline to peak IL-6 at either 5h30 or 8h post-injection) to the immune challenge; * denotes statistical significance (p < .05).*

DISCUSSION

The results presented here showed that those that reported feeling more lonely exhibited an enhanced inflammatory response to a mild immune stimulus. This association was robust to adjustment of age, BMI, and socio-emotional variables. A prior study showed that feelings of social disconnection were associated with an elevated immune response to endotoxin, an inflammatory stimulus that raises IL-6 about 100-fold (Moieni, Irwin, Jevtic, Breen, Cho, et al., 2015; Moieni, Irwin, Jevtic, Olmstead, et al., 2015). The current study extends this finding to loneliness, showing that a mild inflammatory stimulus, raising IL-6 levels about 4-fold, similarly evokes an enhanced inflammatory response in more lonely individuals. Although loneliness and social disconnection tend to co-occur, there is a conceptual distinction between the two, whereby feeling lonely is considered a result of social disconnection (Cacioppo et al., 2006). However, strong genetic overlap between social isolation and loneliness as well as depression has been reported (Matthews et al., 2016). The observation that neither depression, anxiety, social skills nor negative mood were correlated with the inflammatory response, suggest that the relationship between loneliness (or social disconnection, as shown by Moieni et al., (2015)) and immune responsiveness is unlikely to be confounded by other negative socio-emotional factors.

Since we and others identified loneliness as a predictor of immune dysregulation, screening for loneliness in populations with inflammation-related complaints, and other high-risk populations such as older adults, may be warranted as a target for further study. Admittedly, a causal role of loneliness remains speculative at this point, but the present findings as well as those of others, do provide a rationale to explore if interventions that focus on reducing feelings of loneliness may simultaneously help ameliorate inflammatory dysregulation. Likewise, evidence of a possible causal role of loneliness might be strengthened by studies that manipulate subjective loneliness for example via a false feedback paradigm (see Lamster, Nittel, Rief, Mehl, & Lincoln, 2017). The current findings

are limited in terms of generalizability due to the experimental nature of the study and that only healthy young males were assessed. Despite this consideration, research could assess whether lonely individuals may also have stronger responses to more naturalistic inflammatory insults such as a cold or flu. Furthermore, although the present study was aligned with prior research and was hypothesis driven, the current results stem from secondary analysis of existing data, and replication seems therefore warranted.

In summary, the current results showed that, among healthy young adults, those feeling more lonely exhibited a higher inflammatory response to a mild immune challenge, that appeared independent of negative mood and common confounders related to social or health behaviours.

REFERENCES

Balter, L. J. T., Hulsken, S., Aldred, S., Drayson, M. T., Higgs, S., Veldhuijzen van Zanten, J. J. C. S., … Bosch, J. A. (2018). Low-grade inflammation decreases emotion recognition – Evidence from the vaccination model of inflammation. *Brain, Behavior, and Immunity*. https://doi.org/10.1016/j.bbi.2018.05.006

Baron-Cohen, S., Wheelwright, S., Skinner, R., Martin, J., & Clubley, E. (2001). The autism-spectrum quotient (AQ): evidence from Asperger syndrome/high-functioning autism, males and females, scientists and mathematicians. *Journal of Autism and Developmental Disorders*, *31*(1), 5–17. https://doi.org/10.1023/A:1005653411471

Beck, A. T., Epstein, N., Brown, G., & Steer, R. A. (1988). An Inventory for Measuring Clinical Anxiety: Psychometric Properties. *Journal of Consulting and Clinical Psychology*. https://doi.org/10.1037/0022-006X.56.6.893

Beck, A. T., Steer, R. A., & Brown, G. K. (1996). *Beck depression inventory-Second Edition*. *San Antonio*.

Brown, E. G., Gallagher, S., & Creaven, A. M. (2018). Loneliness and acute stress reactivity: A systematic review of psychophysiological studies. *Psychophysiology*. https://doi.org/10.1111/psyp.13031

Cacioppo, J. T., Hawkley, L. C., Ernst, J. M., Burleson, M., Berntson, G. G., Nouriani, B., & Spiegel, D. (2006). Loneliness within a nomological net: An evolutionary perspective. *Journal of Research in Personality*. https://doi.org/10.1016/j.jrp.2005.11.007

Carpenter, J. S., & Andrykowski, M. A. (1998). Psychometric evaluation of the Pittsburgh Sleep Quality Index. *Journal of Psychosomatic Research*, *45*(1), 5–13. https://doi.org/10.1016/S0022-3999(97)00298-5

Cole, S. W., Hawkley, L. C., Arevalo, J. M., Sung, C. Y., Rose, R. M., & Cacioppo, J. T. (2007). Social regulation of gene expression in human leukocytes. *Genome Biology*. https://doi.org/10.1186/gb-2007-8-9-r189

Curran, S. L., Andrykowski, M. A., & Studts, J. L. (1995). Short Form of the Profile of Mood States (POMS-SF): Psychometric information. *Psychological Assessment*, *7*(1), 80–83. https://doi.org/10.1037/1040-3590.7.1.80

Dozois, D. J. A., Dobson, K. S., & Ahnberg, J. L. (1998). A psychometric evaluation of the Beck Depression Inventory-II. *Psychological Assessment*. https://doi.org/10.1037/1040-3590.10.2.83

Hackett, R. A., Hamer, M., Endrighi, R., Brydon, L., & Steptoe, A. (2012). Loneliness and stress-related inflammatory and neuroendocrine responses in older men and women. *Psychoneuroendocrinology*. https://doi.org/10.1016/j.psyneuen.2012.03.016

Hawkley, Louise C.; Bosch, Jos, A; England, Christopher G.; Marucha, Phillip T. & Cacioppo, J. T. (2007). *Loneliness, dysphoria, stress and immunity: A role for cytokines*. Cytokines: Stress and immunity.

Holt-Lunstad, J., Smith, T. B., Baker, M., Harris, T., & Stephenson, D. (2015). Loneliness and Social Isolation as Risk Factors for Mortality: A Meta-Analytic Review. *Perspectives on Psychological Science*. https://doi.org/10.1177/1745691614568352

Jaremka, L. M., Fagundes, C. P., Peng, J., Bennett, J. M., Glaser, R., Malarkey, W. B., & Kiecolt-Glaser, J. K. (2013). Loneliness Promotes Inflammation During Acute Stress. *Psychological Science*. https://doi.org/10.1177/0956797612464059

Lamster, F., Nittel, C., Rief, W., Mehl, S., & Lincoln, T. (2017). The impact of loneliness on paranoia: An experimental approach. *Journal of Behavior Therapy and Experimental Psychiatry*. https://doi.org/10.1016/j.jbtep.2016.06.005

Matthews, T., Danese, A., Wertz, J., Odgers, C. L., Ambler, A., Moffitt, T. E., & Arseneault, L. (2016). Social isolation, loneliness and depression in young adulthood: a behavioural genetic analysis. *Social Psychiatry and Psychiatric Epidemiology*, *51*(3), 339–348. https://doi.org/10.1007/s00127-016-1178-7

Moieni, M., Irwin, M. R., Jevtic, I., Breen, E. C., Cho, H. J., Arevalo, J. M. G., … Eisenberger, N. I. (2015). Trait sensitivity to social disconnection enhances pro-inflammatory responses to a randomized controlled trial of endotoxin. *Psychoneuroendocrinology*. https://doi.org/10.1016/j.psyneuen.2015.08.020

Moieni, M., Irwin, M. R., Jevtic, I., Breen, E. C., & Eisenberger, N. I. (2015). Inflammation impairs social cognitive processing: A randomized controlled trial of endotoxin. *Brain, Behavior, and Immunity*, *48*, 132–138. https://doi.org/10.1016/j.bbi.2015.03.002

Moieni, M., Irwin, M. R., Jevtic, I., Olmstead, R., Breen, E. C., & Eisenberger, N. I. (2015). Sex differences in depressive and socioemotional responses to an inflammatory challenge: Implications for sex differences in depression. *Neuropsychopharmacology*. https://doi.org/10.1038/npp.2015.17

Office for National Statistics. (2018). Loneliness - What characteristics and circumstances are associated with feeling lonely?, 1–19. https://doi.org/10.1177/1745691614568352

Paine, N. J., Ring, C., Bosch, J. A., Drayson, M. T., & Veldhuijzen van Zanten, J. J. C. S. (2013). The time course of the inflammatory response to the Salmonella typhi vaccination. *Brain, Behavior, and Immunity*, *30*, 73–79. https://doi.org/10.1016/j.bbi.2013.01.004

Russell, D., Peplau, L. A., & Cutrona, C. E. (1980). The revised UCLA Loneliness Scale: Concurrent and discriminant validity evidence. *Journal of Personality and Social Psychology*. https://doi.org/10.1037/0022-3514.39.3.472

Steptoe, A., Shankar, A., Demakakos, P., & Wardle, J. (2013). Social isolation, loneliness, and all-cause mortality in older men and women. *Proceedings of the National Academy of Sciences of the United States of America*, *110*(15), 5797–5801. https://doi.org/10.1073/pnas.1219686110

Stevenson, J. L., & Hart, K. R. (2017). Psychometric properties of the autism-spectrum quotient for assessing low and high levels of autistic traits in college students. *Journal of Autism and Developmental Disorders*. https://doi.org/10.1007/s10803-017-3109-1

Valtorta, N. K., Kanaan, M., Gilbody, S., Ronzi, S., & Hanratty, B. (2016). Loneliness and social isolation as risk factors for coronary heart disease and stroke: Systematic review and meta-analysis of longitudinal observational studies. *Heart*. https://doi.org/10.1136/heartjnl-2015-308790

Wilson, C., & Moulton, B. (2010). Loneliness among Older Adults : A National Survey of Adults 45 +. *Aarp*. https://doi.org/10.1016/j.geomorph.2004.07.013

ACKNOWLEDGEMENTS

The authors would like to thank Sasha Hulsken for her contribution in conceiving and executing the experiment. The authors would like to thank Farahdina Bachtiar, Anne Clemens, Jessica Maund, Alexandra Morrison, Greta Ontrup, and Marina Wissink for their valuable contribution in recruiting and testing participants and the nursing staff of the School of Nursing (University of Birmingham) for administering the injections.

FUNDING AND DISCLOSURE

This research was funded by internal funds of the University of Amsterdam and University of Birmingham. The authors report no conflicts of interest, financial or otherwise.