Supplementary Materials

Title: The effects of acute psychological stress on circulating and stimulated inflammatory markers: A systematic review and meta-analysis

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Study Selection

Figure S1: Flow diagram of circulating study search and inclusion.

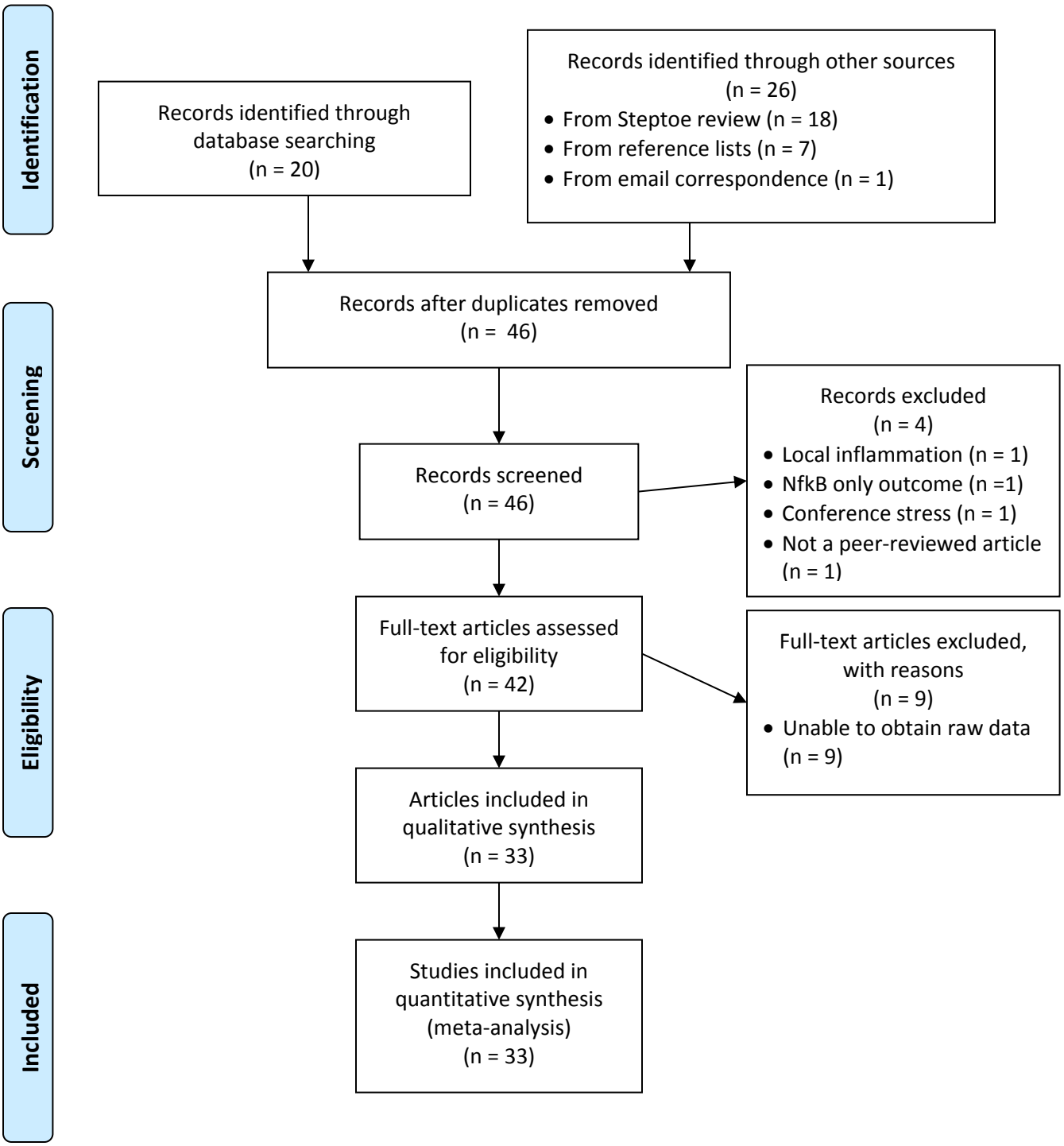
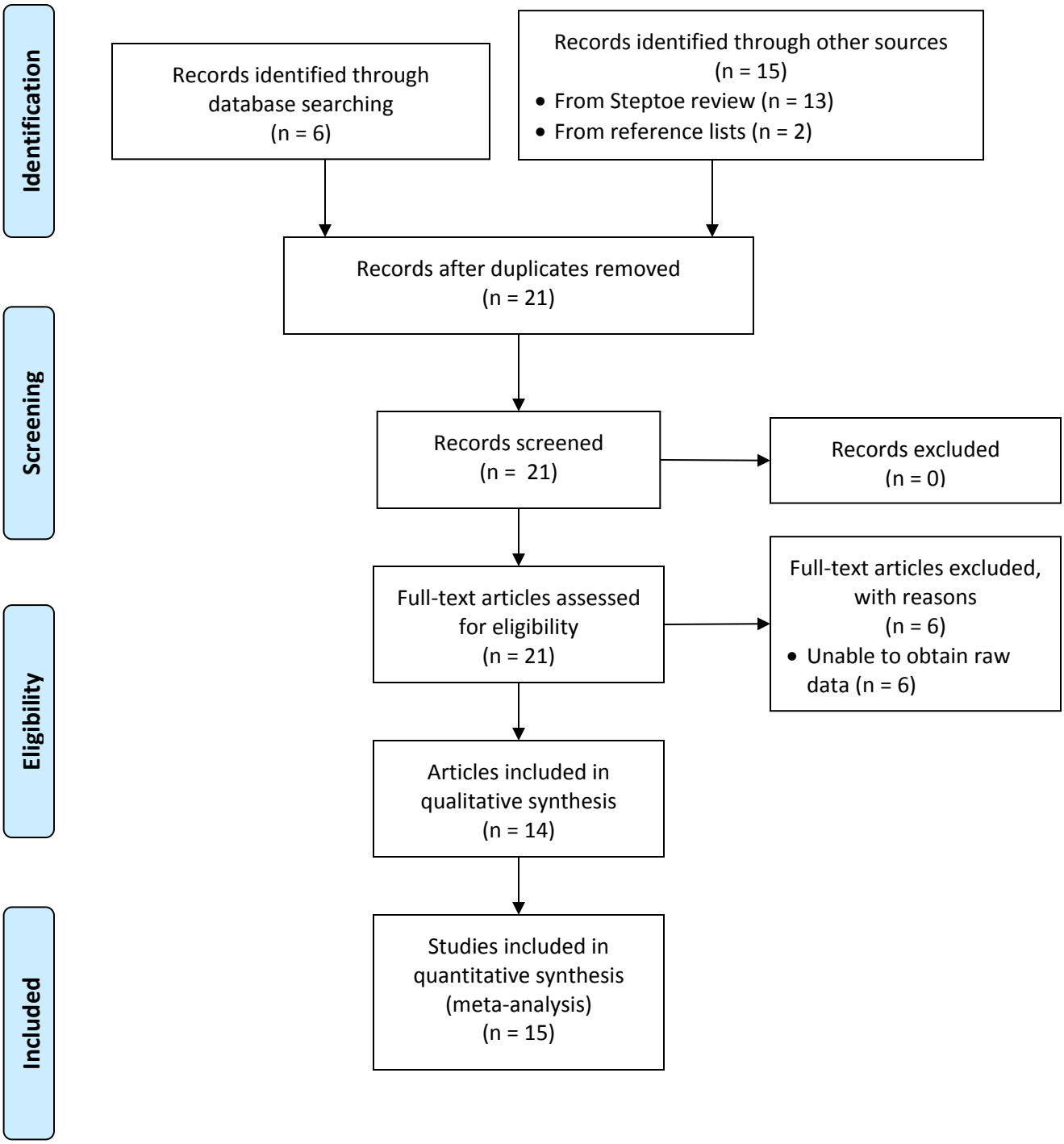


Figure S2: Flow of diagram of stimulated study search and inclusion.



Included Studies

Table S1: Summary of studies measuring circulating inflammatory markers

Study	N	Health Status	Age (*M*)	% Male	Stressor	Marker	Time-points
Altemus et al., 2001*	46	Healthy		0	TSST	IL-1β, IL-10, TNF-α	10, 40
Aschbacher et al., 2012	35	Healthy	61	0	TSST	IL-1β, IL-6	50
Bennett et al., 2013	89	Breast cancer survivors	51	0	TSST	IL-6	45, 120
Breines et al., 2014	41	Healthy	21	56	TSST	IL-6	30, 120
Brydon et al., 2008	67	Healthy	21	0	Stroop, Speech	IL-1RA, IL-6	0, 45
Brydon et al., 2005*	153	Healthy	52	56	Stroop, Mirror Trace	IL-6, fibrinogen	30, 75, 120
Carroll et al., 2011	102	Healthy	50	40	TSST	IL-6	30
Christian et al., 2013	39	Healthy	24	0	TSST	IL-6	45
de Brouwer et al., 2013	71	Rheumatoid arthritis	57	43	TSST	IL-1β, IL-2, IL-4, IL-5, IL-6, IL-7, IL-8, IL-10, IFNγ, TNF-α	20, 40, 80
de Brouwer et al., 2014	80	Rheumatoid arthritis (32), Psoriasis (23), Healthy (25)	58	60	TSST	IL-1β, IL-2, IL-4, IL-5, IL-6, IL-7, IL-8, IL-10, IFNγ, TNF-α	20, 40, 80
Derry et al. 2013	138	Healthy	51	33	TSST	IL-6	45

Dugue et al., 1993*	7	Healthy	18	100	Stroop	CRP, IL-1β, IL-6, TNF-α	0
Edwards et al., 2006*	40	Healthy	21	50	Mental Arithmetic	IL-6	1, 30, 60
Fagundes et al., 2013	138	Healthy	51	33	TSST	IL-6	45, 120
Hackett et al., 2012	524	Healthy	63	54	Stroop, Mirror Trace	IL-1RA, IL-6	1, 45
Hamer & Steptoe 2007	207	Healthy	52	53.6	Stroop, Mirror Trace	IL-1RA, IL-6, TNF-α	45
Hamer et al., 2006*	92	Healthy	33	100	Speech, Mirror Trace	CRP	10
Heesen et al., 2002*	50	Multiple Sclerosis (35), Healthy (15)	35	40	Mental Arithmetic Stroop, Speech	IL-6, sIL-6r, TNF-α	1, 60
Heffner et al., 2012	83	Healthy	61	46	Auditory Challenge	IL-6	60
Kop et al., 2008	64	Coronary artery disease (36), Healthy (28)	57	66	Anger Recall, Mental Arithmetic	CRP, IL-6	1, 30
Kuebler et al., 2015	25	Healthy	36	100	TSST	IL-1β, IL-6, IL-10	10, 20, 60, 90, 120
Lutgendorf et al., 2004*	14	Healthy	34	50	Stroop	IL-6	10, 15, 30, 60
Miller et al., 2005*	72	Depressed (36), Healthy (36)	27	0	Speech, Puzzle	CRP, IL-6, TNF-α	1, 30
Muscatell et al., 2015	31	Healthy	19	0	Social Rejection	IL-6, TNF-α	30, 60, 90
Owen & Steptoe, 2003*	211	Healthy	52	56	Stroop, Mirror Trace	IL-6, TNF-α	45
Pace et al., 2006	28	Depressed (14), Healthy (14)	29	100	TSST	IL-6	30, 60, 75, 90

Pace et al., 2009	61	Healthy	19	50	TSST	IL-6	30, 60, 75, 91
Puterman et al., 2013	48	Healthy	63	0	TSST	IL-6	30, 50, 90
Rohleder et al., 2006*	12	Healthy	27	100	TSST	IL-1RA	1, 20, 45, 90
Steptoe et al., 2001*	12	Healthy	41	46	Stroop, Mirror Trace	CRP, IL-1RA, IL-6, TNF-α	45, 120
Weick et al., 2014	28	Bipolar (13), Healthy (15)	47	0	TSST	IL-2, IL-6, TNF-α	40
Weinstein et al., 2010	28	Depressed (14), Healthy (14)	40	50	Frustration Recall, Mental Arithmetic	CRP, IL-6, TNF-α	1, 30
Yamakawa et al., 2009	16	Healthy	21	100	TSST	IL-2, IL-6, IL-12, IFNγ, TNF-α	1, 30, 60, 90

Notes: All times refer to minutes post-stress task; TSST = Trier Social Stress Test; IL = interleukin; CRP = C-reactive protein; TNF = tumor necrosis factor; IFN = interferon; ra = receptor antagonist

* study included in Steptoe et al. (2007) review

Table S2: Summary of studies measuring stimulated inflammatory markers.

Study	N	Health Status	Age (*M*)	% Male	Stressor	Marker	Time-points	Stimulant (Concentration, Incubation Time)
Ackerman et al. 1998*	50	Multiple Sclerosis (25), Healthy (25)	37	28	TSST	IL-1β, IL-4, IFNγ, TNF-α	1, 5, 20, 60	LPS (5ug/mL, 48hr)[a] PHA (5ug/mL, 48hr)[a]
Bellingrath et al., 2010	55	Healthy	50	38	TSST	IL-2, IL-4, IL-6, IL-10, IFNγ,TNF-α	1	PHA (.5mg/mL, 22hr)[b]
Bower et al., 2007*	25	Breast Cancer Survivors	58	0	TSST	IL-1β, IL-6, TNF-α	30	LPS (100pg/mL, 20hr)[a]
Buske-Kirschbaum, 2007*	48	Psoriasis (23), Healthy (25)	30	48	TSST	IL-2, IL-4, IL-10, IFNγ,	1, 10, 60	PHA (5ug/mL, 48hr)[b]
Dickerson et al., 2009	39	Healthy	21	0	TSST	TNF-α	1, 40	LPS (100pg/mL, 20hr)[a]
Gaab et al., 2005*	41	Chronic Fatigue (21), Healthy (20)	36	52	TSST	IL-6, TNF-α	10, 60	LPS (30ng/mL, 18hr)[a]
Jacobs et al., 2001*	31	Rheumatoid Arthritis (9), Lupus (7), Healthy (15)	41	0	Speech	IL-2, IL-6, IL-10	60	LPS (not specified, 36hr)[a] PHA (not specified, 24hr)[a]
Jaremka et al., 2013a	134	Healthy	51	31	TSST	IL-6, TNF-α	45, 120	LPS (1ug/mL, 24hr)[b]
Jaremka et al., 2013b	144	Breast Cancer Survivors	51	0	TSST	IL-1β, IL-6, TNF-α	45, 121	LPS (1ug/mL, 24hr)[b]
Miller et al., 2005*	72	Depressed (36), Healthy (36)	27	0	Speech, Puzzle	IL-6, TNF-α	1, 30	LPS (100ng/mL, 6hr)[a]
Peters et al., 1999*	96	Healthy	22	100	Mental Arithmetic	IL-6	1, 15, 30	LPS (10, 1, .1, .001ng/mL, 18hr)[a]
Prather et al., 2009	61	Healthy	50	46	TSST	IL-1β, IL-6, TNF-α	1, 30	LPS (2.5ug/mL, 24hr)[a]
Rohleder et al., 2003*	25	Healthy	24	0	TSST	IL-6	10, 60	LPS (18hr, 30ng/mL)[a]

Wirtz et al., 2007	44	Healthy	43	100	TSST	IL-6, TNF-α	1, 20, 60	LPS (15ng/mL, 18hr)[a]
Wolf et al., 2009	29	Healthy	36	41	TSST	IL-6	10, 60	LPS (30ng/mL, 18hr)[a]

Notes: All time-points refer to minutes post-stress task. Methods used to stimulate immune markers varied considerably. TSST = Trier Social Stress Test; IL = interleukin; CRP = C-reactive protein; TNF = tumor necrosis factor; IFN = interferon; ra = receptor antagonist; LPS = lipopolysaccharide; PHA = phytohaemaglutinin
[a] whole blood incubation; [b] peripheral blood mononuclear cell incubation
[*] study included in Steptoe et al. (2007) review

Table S3.

Assays used to assess circulating levels of interleukin-6

Study	Quantification method	Company	Sensitivity	Intra-Assay CV (%)	Inter-assay CV (%)
Aschbacher et al., 2013	ELISA	Meso Scale Discovery	.07	3	4
Bennett et al., 2013	ELISA	R&D Systems (HS)	.039	6.9-7.8	6.5-9.6
Breines et al., 2014	ELISA	R&D Systems (HS)	.09	5.3	9.2
Brydon et al., 2008	ELISA	R&D Systems (HS)	.09	5.3	9.2
Brydon et al., 2005	ELISA	R&D systems (HS)			
Carroll et al., 2011	ELISA	R&D Systems (HS)	.09	5	7
Christian et al., 2013	Multiplex	Meso Scale Discovery	.025	4.3	5.2
de Brouwer et al., 2013	Cytokine multiple kits	Invitrogen Corporation	<5		
de Brouwer et al., 2014	Cytokine multiple kits	Invitrogen Corporation			
Derry et al. 2013	Multispot cytokine kits	Mesoscale Discovery	.26	5.64-6.7	5.16-10.2
Dugue et al., 1993	Radio-immun assay				

Edwards et al., 2006	ELISA	Diaclone Research (HS)		5.5	1.4
Fagundes et al., 2013	Multispot cytokine kits	Mesoscale Discovery	.26	5.64-6.7	5.16-10.2
Hackett et al., 2012	ELISA	R&D Systems (HS)	.016-.110	7.3	7.7
Hamer & Steptoe 2007	ELISA	R&D Systems (HS)	.09	5.3	9.2
Heesen et al., 2002	ELISA	R&D Systems			
Heffner et al., 2012	ELISA	R&D Systems (HS)		<10	<10
Kop et al., 2008	ELISA	R&D Systems		5.0-8.6	5.0-8.6
Kuebler et al., 2015	ELISA	Mesoscale discovery (HS)	.117	4.5	7.3
Lutgendorf et al., 2004	ELISA	R&D Systems (HS)	.10		7
Miller et al., 2005	ELISA	R&D Systems (HS)	.7	4.6	
Muscatell et al., 2015	ELISA	R&D Systems (HS)		<9	<9
Owen & Steptoe, 2003	ELISA	R&D Systems (HS)	.09	5.3	9.2
Pace et al., 2006	ELISA	R&D Systems			
Pace et al., 2009	ELISA	R&D Systems		10	6
Puterman et al., 2013	immunoassay	Mesoscale Discovery	.46	4	6

Steptoe et al., 2001	ELISA	R&D Systems(HS)	.09	5.3	9.2
Weick et al., 2014	Cytokine bead array	BD biosciences			
Weinstein et al., 2010	ELISA	R&D Systems (HS)	.156-10.0	6.3	
Yamakawa et al., 2009	Flowcytomix Assay	Bender Medsystems	1.2	4.5-12	3.3-16.3

Results of Meta-Regression

Figure S3: Meta-regression with age regressed onto effect size for acute stress on IL-6

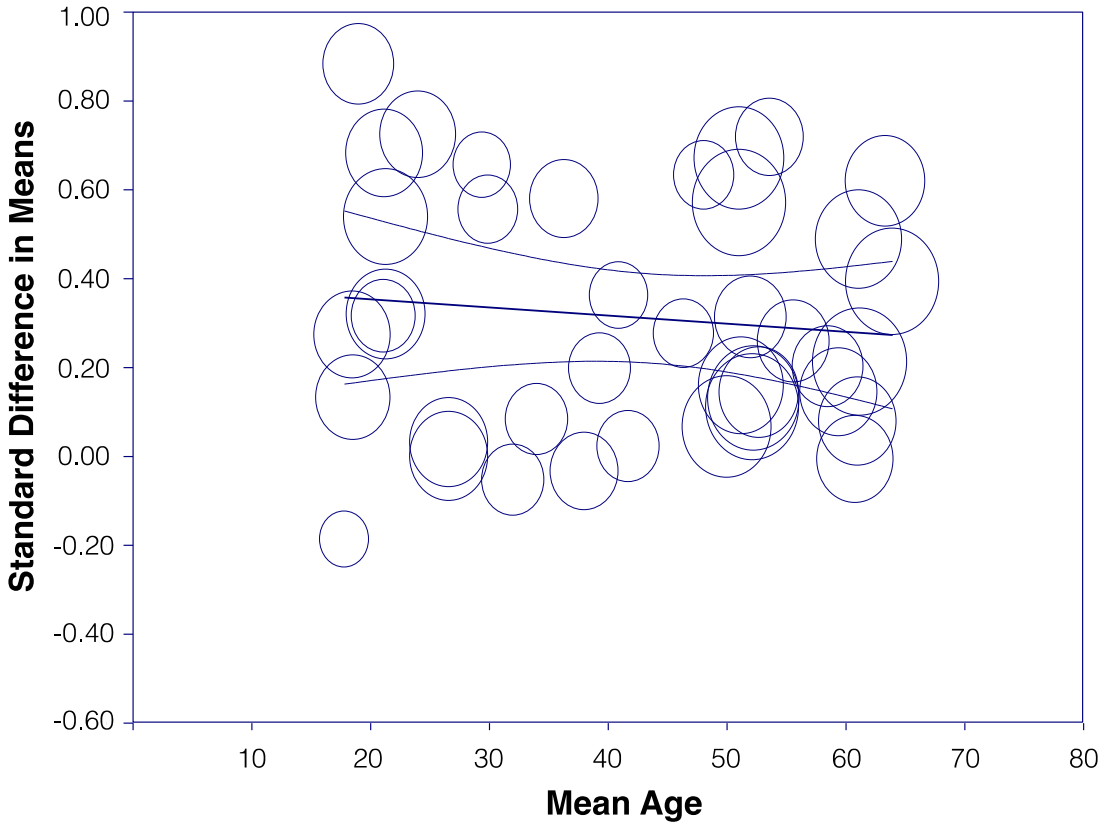
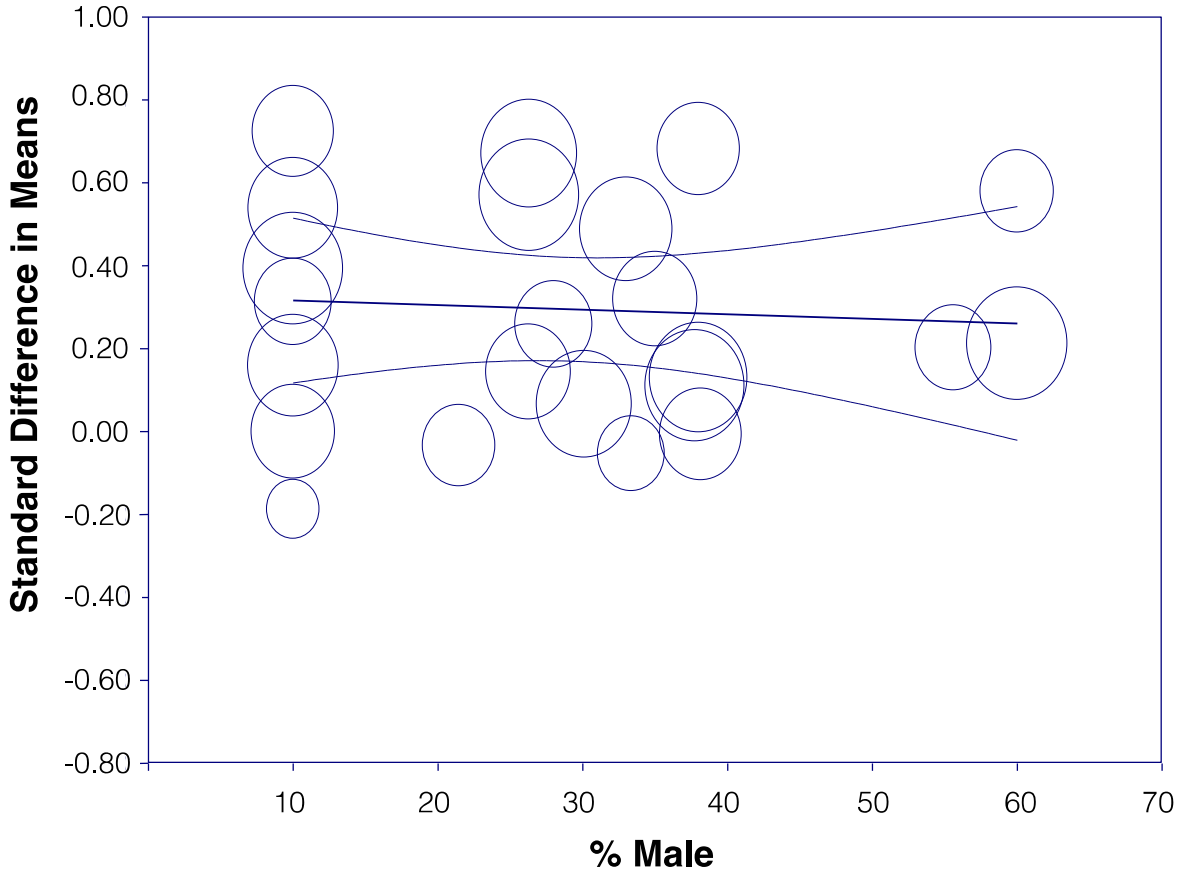


Figure S4: Meta-regression with sex regressed onto effect size for acute stress on IL-6



Identified Moderators

Table S4: Moderators of circulating inflammatory response

Moderator	Study	Sig?	Cytokine(s) affected, Time Point(s), Control Condition(s)	Statistical Controls
Cigarette smoking history	Bennett et al., 2013	y	Exaggerated IL-6 response at 2 hours in breast cancer survivors who were former smokers compared to those who had never smoked	age, BMI, time since cancer treatment, education, anti-depressant use
Abdominal obesity (waist circumference)	Brydon et al., 2008	y	Higher IL-1ra response at 45 min in individuals in highest tertile waist circumference (> 70cm) compared to individuals in the lower two tertiles (< 70cm)	age, ethnicity, smoking status, baseline levels of cytokines
African-American race	Christian et al., 2013	y	Higher IL-6 response at 2 hours in African Americans compared to Whites	baseline IL-6; groups not different in age, education status, income, nulliparity, BMI
Pregnancy	Christian et al., 2013	n	Tendency towards higher IL-6 response at 2 hours in pregnant compared to non-pregnant women	baseline IL-6; groups not different in age, education status, income, nulliparity, BMI
Physical fitness (exercise heart rate)	Hamer & Steptoe, 2007	y	Those in high fitness group (lowest exercise HR tertile) had smaller IL-6, TNF-α response at 45 min compared to those in the lowest fitness group (highest HR tertile)	age, sex, BMI, smoking, alcohol, SES, basal levels of inflammatory markers
Cortisol response	Kunz-Ebrechet et al., 2003	y	Individuals with the lowest cortisol response to stress (lowest 40% of sample = cortisol non-responders) showed greater IL-6, IL-1ra response at 45 min compared to individuals with the highest cortisol response (highest 40% of sample = cortisol responders)	IL-6: age, sex, BMI, waist/hip ratio, change in hematocrit, method of blood sampling; IL-1ra: sex, BMI, waist/hip ratio, change in hematocrit, time of day, use of hormone replacement therapy
Norepinephrine response	Kop et al, 2008	y	Highest norepinephrine responses (above median split) were related to greater IL-6, CRP response at 29 min compared to lowest norepinephrine responses (below median split)	age, BMI

Positive outlook	Aschbacher et al., 2012	y	Decreased positive outlook during the task associated with higher IL-1β response from pre- to 50 min (no comparison group)	age, BMI, caregiver status, anti-depressant use, baseline cytokine levels
Self-compassion	Breines et al., 2014	y	Higher self-compassion associated with lower IL-6 response at 120 min (no comparison group)	age, sex, ethnicity, BMI, self-esteem, depressive symptoms, post-TSST distress, TSST timing
Affective response to task	Carroll et al., 2011	y	Angry/anxious responses to stress task associated with increased IL-6 response at 30 min (no comparison group)	sex, age, race, BMI, and menopausal status
Anger, perceived social support	Puterman et al., 2013	y	Anger measured 10 min after stress task predicted increased rate of change in IL-6 through 90 min (no comparison group); effect larger for those with low social support.	age, BMI, caregiver status
Loneliness	Hackett et al., 2012	y	Greater loneliness (highest tertile) associated with greater IL-6, IL-1ra response in women at 45 min, compared to the lowest tertile of loneliness	age, grade of employment, BMI, smoking status
Effort-reward balance	Hamer et al., 2006	y	Men in the highest tertile of effort-reward imbalance showed greater CRP response to stress task at 10 min, compared to men in the lowest tertile	age, BMI, baseline levels of CRP
Sleep quality	Heffner et al., 2012	y	Poor sleep quality on the PSQI (PSQI > 5) had larger IL-6 responses at 60 min, compared to good sleepers (PSQI <=5)	age, BMI, depressive symptoms, perceived stress, loneliness
Subjective social status (SSS)	Derry et al., 2013	y	Individuals reporting lower SSS showed higher IL-6 response at 45 min and 2 hours compared to those wither higher SSS (no comparison group)	age, sex, BMI, visit, Omega-3 supplementation group
SES (employment grade)	Steptoe et al., 2002	n	No relationship between employment grade (high, intermediate, low) and change in TNF-a, IL-1ra, IL-6	SES groups did not differ in age, sex, BMI, waist/hip, time of day

Stress management training	de Brouwer et al., 2013	y	Among patients with rheumatoid arthritis, stress management training associated with lower IL-8 response at 9-week follow-up (baseline through 80min) compared to control group, no difference in IL-6, IL-1β, IL-2, IL-4, IL-5, IL-6, IL-7, IL-10, IFN-γ, TNF-α	sex, hormonal contraceptive use, baseline anxiety, nonsteroidal anti-inflammatory drug use
Compassion meditation training	Pace et al., 2009	n	Participation in 6 weeks of compassion meditation training did not change IL-6 reactivity when compared to a health discussion group; practice time in the intervention group associated with decreased IL-6	baseline cytokine level, time of day; groups not different on age, sex, BMI, depression score

Notes: all times refer to post-stress task; BMI = body mass index; CTQ = Childhood Trauma Questionnaire; SES = Socioeconomic Status; MDD = major depressive disorder; PSQI = Pittsburgh Sleep Quality Questionnaire; IL = interleukin; TNF = tumor necrosis factor; IFN = interferon; ra = receptor antagonist

Table S5: Moderators of stimulated inflammatory response

Moderator	Study	Sig?	Cytokine(s) affected, Time Point(s), Control Condition(s)	Statistical Controls
BMI	Huang et al., 2011	y	BMI positively correlated with % change in LPS-stimulated IL-6, not TNF-α, immediately following the stressor (no comparison group)	none
Menopause	Prather et al., 2009	y	Post-menopausal women had greater IL-6 response compared to men immediately post task & at 30 min, and greater TNF-α response at post-task, but not 30 min	groups matched in age, race, BMI, or baseline levels of stimulated cytokine, cytokine values were corrected for number of WBCs
Oral contraceptive use	Rohleder et al., 2003	y	Women using oral contraception showed exaggerated IL-6 responses at 60min compared to women in the luteal phase	cytokine levels corrected for monocyte number in sample
IR (HOMA)	Suarez et al., 2006	y	Higher HOMA-IR associated with greater stress-induced increases in IL-1β, TNF-α at 15 min (no comparison group)	age, BMI, race/ethnicity

Loneliness	Jaremka et al., 2013a	y	Among healthy, sedentary, overweight individuals, loneliness associated with greater increases in production of IL-6, TNF-α at 45 min and 2 hours (no comparison group)	age, sex, abdominal diameter, baseline levels of cytokine
Loneliness	Jaremka et al., 2013b	y	Among breast cancer survivors, loneliness associated with greater increases in production of IL-6, IL-1β at 45 min and 2 hours (no comparison group)	age, abdominal diameter, baseline levels of cytokine
Negative affect	Suarez et al., 2006	y	Among men with higher HOMA-IR (above median), negative affect associated positively with stimulated secretion of TNF-α, IL-6 at 15 min (no comparison group); among men with lower HOMA-IR (below median), greater negative affect associated inversely with TNF-α, IL-6 secretion at 15 min (no comparison group)	age, BMI, race/ethnicity
Primary Appraisal Secondary Appraisal (PASA) scale score	Wirtz et al., 2007	y	Higher PASA "stress index" associated with greater stimulated TNF-α, IL-6 production (AUC through 60min); higher PASA subscale "control expectancy" associated with lower TNF-α, IL-6 production (AUC through 60min); greater PASA subscale "challenge" associated with higher IL-6 production (AUC through 60min)	age, BMI, mean arterial pressure
Effort-reward imbalance (ERI) and over commitment (OC)	Bellingrath et al., 2010	y	Higher ERI associated with greater decrease in stimulated IL-10, greater increase in stimulated TNF-α/IL-10 ratio immediately post-stress (1min) (no comparison group, ERI continuous); higher scores for OC associated with greater stimulated IL-2 production immediately post-stress (1 min) (no comparison group, OC continuous)	age, depressive symptoms, sex, group variable (men, premenopausal women in luteal phase, post-menopausal women)
Effort & controllability of task	Peters et al., 1999	y	Low control condition associated with decreased production of IL-6 at 15 and 30 min compared to the high control condition	subjects were randomized to condition

Cancer-related fatigue	Bower et al., 2007	y	Breast cancer survivors with persistent fatigue (SF-36 vitality scale < 50) showed greater stimulated production of IL-1β, IL-6 at 30 min compared to breast cancer survivors without persistent fatigue (SF-36 vitality scale > 70)	age, marital status, BMI, caffeine use

Notes: All times refer to post-stress task; BMI = body mass index; IR = insulin resistance; HOMA = homeostatic model assessment, a measure of IR; LPS = lipopolysaccharide; WBCs = white blood cells; SF-36 = 36-item short form survey from the Medical Outcomes Study; IL = interleukin; TNF = tumor necrosis factor

Publication Bias Results

Figure S5. Funnel plots of standard error by standard difference in means

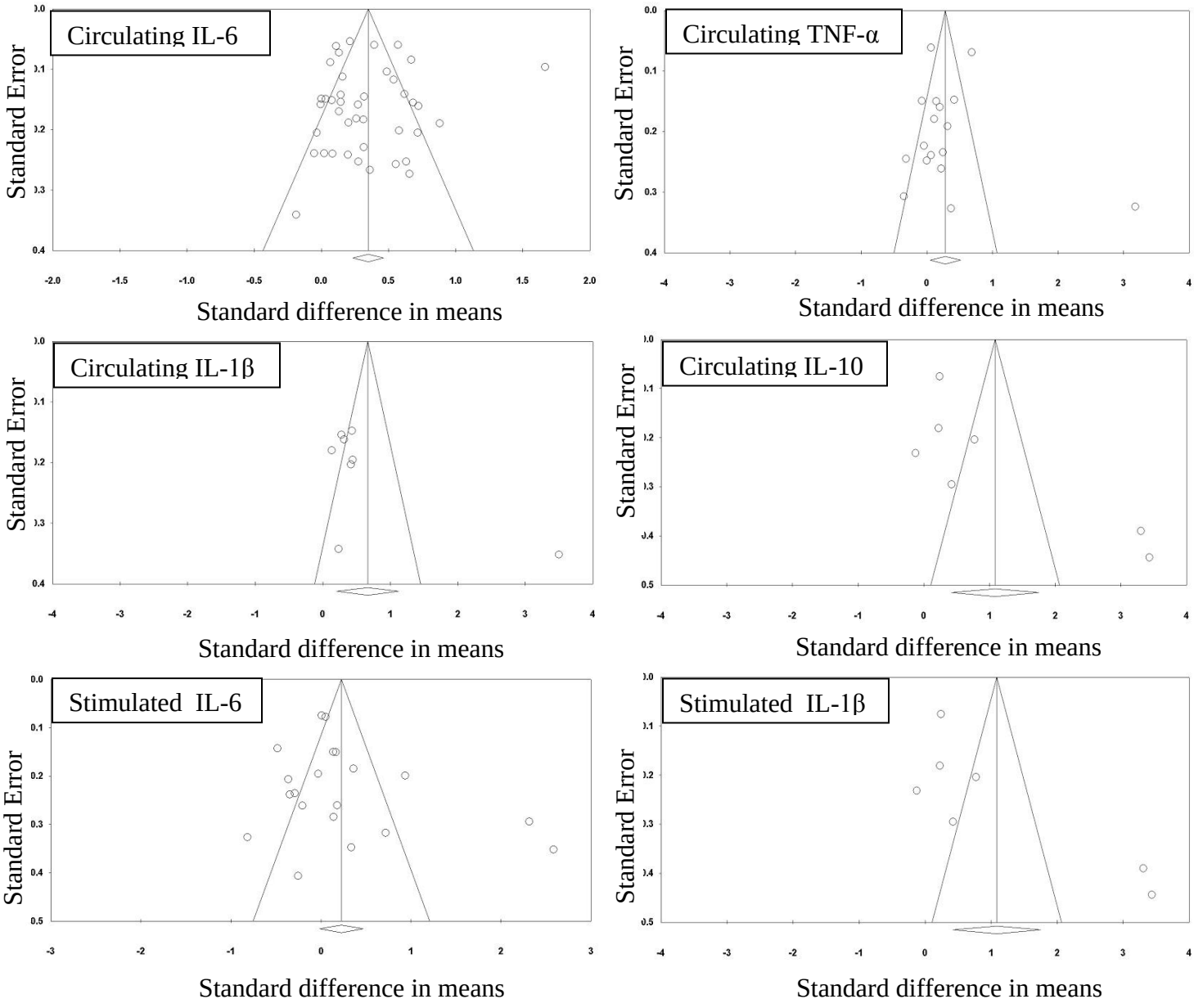


Table S6: Publication bias statistics

Cytokines (minutes post stress)	Number of samples	Fail-Safe N	Eggers Regression Intercept
Circulating IL-6 (11-120min)	40	2076	-0.03
Circulating IL-1β (20-120min)	8	123	7.40*
Circulating TNF-α (20-120min)	17	191	-0.10
Circulating IL-10 (20-120min)	6	46	14.49*
Circulating IL-2 (40-50min)	7	35	-0.38
LPS-stimulated IL-6 (11-30min)	20	57	1.67
LPS-stimulated IL-1β (20-120min)	7	160	4.93*
LPS-stimulated TNF-α (20-30min)	9	84	8.03*
PHA-stimulated IFN-γ (0-10min)	5	42	1.59
PHA-stimulaed IL-4 (0-10min)	5	25	-6.82*

Notes: All times refer to minutes post-stress task; TSST = Trier Social Stress Test; IL = interleukin; CRP = C-reactive protein; TNF = tumor necrosis factor; IFN = interferon; ra = receptor antagonist; LPS = lipopolysaccharide; PHA = phytohaemaglutinin
* $p < .10$