An Experimental Treatment Approach for Inflammation-Induced Depression

Short Title: Leucine for Depression (L-DEP) Study

ClinicalTrials.gov ID: NCT03557684

Document Date: 4/30/2019

Joshua Hyong-Jin Cho, MD, PhD, Michael R. Irwin, MD, Richard Olmstead, PhD, Robert Dantzer, DVM, PhD

ABSTRACT

Depression is a major public health burden, and is even more prevalent and disabling in individuals with heightened inflammatory states due to certain diseases or treatments. Compelling evidence suggests that inflammation plays a causal role in the onset or perpetuation of some forms of depression including those in the context of interferon treatment, chronic diseases, aging, and obesity. Antidepressant medications—the mainstay of the current depression treatments, with limited efficacy, substantial adverse effects, and poor adherence—have consistently shown an even more limited efficacy in this inflammatory subtype of depression. Consequently, anti-inflammatory drugs (nonsteroidal anti-inflammatory drugs, cytokine antagonists) have been tested for the treatment of inflammation-associated depression. However, these drugs have shown limited efficacy, substantial risk profile, and prohibitive cost. Thus, novel and safe pharmacologic strategies that target mood-specific mechanisms of inflammation-associated depression are needed. One of the potential mechanisms by which inflammation causes depression is via kynurenine metabolism. Inflammation activates the tryptophan-kynurenine metabolic pathway, converting tryptophan to kynurenine. Kynurenine produced in excess at the periphery can be transported into the brain, where it is metabolized into neurotoxic kynurenine metabolites responsible for the development of depressive symptoms. As this transport system across the brain-blood barrier makes use of the large amino acid transporter LAT1, it is theoretically possible to block kynurenine entry into the brain by administering competing amino acids such as leucine. Preclinical experiments confirmed the inhibitory effects of leucine on inflammation-induced depression as a result of the blockade of excess kynurenine from entering the brain. The overarching objective of this innovative proof-of-concept trial is to test whether leucine pretreatment abrogates acute depressive responses to an inflammatory challenge (i.e., endotoxin) in humans, relative to placebo pretreatment. This study hypothesizes that, compared to placebo pretreatment, leucine pretreatment will attenuate depressive responses to endotoxin. In this double-blind randomized placebo-controlled study of healthy volunteers (N=90; 18-65 y), we aim to examine the effects of leucine vs. placebo pretreatment on: 1) endotoxin-induced depressive symptoms (primary outcome); 2) negative and positive affect responses to endotoxin (respectively, feelings of social rejection, negative bias in facial emotion recognition; and feelings of social acceptance, reward learning); and 3) cognitive responses to endotoxin (verbal and non-verbal memory, executive function, attention). If the hypotheses are confirmed, the involvement of the kynurenine pathway in the induction of depressive symptoms will be demonstrated for the first time in a human experimental study. This study will also provide instrumental information in designing future clinical trials of leucine to treat or prevent inflammation-associated depression.

RELEVANCE TO PUBLIC HEALTH

Depression is highly prevalent (~20%) and poses a huge disease burden. Among individuals with heightened inflammatory states due to certain diseases or treatments, depression is even more prevalent and disabling, and antidepressant and anti-inflammatory medications show limited efficacy and substantial adverse effects. This study—the premise of which is supported by the rigor of our preliminary data in animals—is significant because it will examine whether a novel treatment, leucine, will mitigate depression in response to inflammatory exposure, with a potential for a safe and efficacious application in clinical practice.

LAY SUMMARY

Depression is very common and poses a huge disease burden. About 20% of the US population suffers from depression at least once in their lifetime. Inflammations that are hidden inside our body as a result of aging, obesity, chronic diseases, or certain treatments (e.g., interferon for hepatitis C) appear to cause depressive symptoms and even clinical depression. Individuals with such inflammations are more likely to suffer from depression and are less likely to respond to currently available antidepressant medications. This study will test leucine, an amino acid, as a new way to mitigate depressive symptoms in response to such inflammations. This study begins with a 90-minute screening session to determine whether participants are eligible to join the main study. Those who meet the eligibility criteria will then join the main study, which will consist of taking leucine or maltodextrin (i.e., oral placebo) for 2 weeks at home and an 8-hour session at the UCLA Medical Center. A brief telephone follow-up every 3 months for 2 years with questions on mood is also planned. Approximately 90 healthy adults will be recruited for participation in the study. During the course of the study, participants will take leucine or maltodextrin for 2 weeks at home and then will be injected either lipopolysaccharide (LPS) or saline (i.e., intravenous placebo) at the UCLA Medical Center. LPS is a bacterial substance that can initiate chemical reactions that are similar to those seen in individuals with mild sickness symptoms, such as a slight increase in body temperature, muscle aches, or tiredness. It is a safe way of investigating the body's response to inflammation and how these changes may alter cognitive, emotional, or neural function. It has been given thousands of times to healthy volunteers – both younger and older adults – without any serious side effects.

SPECIFIC AIMS

Depression is highly prevalent (~20%[1]) and is a leading cause of worldwide disease burden.[2] However, antidepressants—the mainstay of the current depression treatments—have a limited efficacy typically achieving remission rates of 30% or less[3]; burden of adverse effects is substantial and adherence is poor.[4-6] Among individuals with heightened inflammatory states due to certain diseases or treatments, depression is even more prevalent and disabling.[7-9] Likewise, the efficacy of antidepressants is even more limited.[10-12]

Compelling evidence suggests that inflammation plays a causal role in the onset or perpetuation of some forms of depression including those in the context of chronic diseases (cardiovascular, infectious, rheumatic, autoimmune), aging, and obesity.[13-15] Elevated biomarkers of inflammation predict depression risk,[16,17] and inflammatory challenge (e.g., interferon, endotoxin) leads to the development of depressive symptoms.[18-23] Given the poor treatment response to conventional antidepressants in the inflammatory subtype of depression, recent interest has focused on the use of drugs that are anti-inflammatory (i.e., nonsteroidal anti-inflammatory drugs [NSAIDs], cytokine antagonists).[24-26] However, these drugs have shown limited efficacy, substantial risk profile, and prohibitive cost. Thus, novel and safe pharmacologic strategies that target mood-specific mechanisms of inflammation-associated depression are needed.

One of the potential mechanisms by which inflammation causes depression is via kynurenine metabolism.[27,28] Inflammation activates indoleamine 2,3 dioxygenase (IDO), which converts tryptophan to kynurenine (Fig. 1). Kynurenine produced in excess at the periphery can be transported into the brain, where it is metabolized into neurotoxic kynurenine metabolites responsible for the development of depressive symptoms. As this transport system across the brain-blood barrier (BBB) makes use of the large amino acid transporter LAT1, it is theoretically possible to block kynurenine entry into brain by administering competing amino acids such as leucine. Preclinical experiments confirmed the inhibitory effects of leucine on inflammation-induced depression as a result of the blockade of excess kynurenine from entering the brain (Preliminary Data). This effect appears clinically meaningful as the effect of leucine was 60% larger than that of ketamine, which is known to have potent antidepressant effects in humans.[29] Leucine, with few adverse effects, has been studied for muscle mass preservation, weight loss, and glucose homeostasis.[30-32]

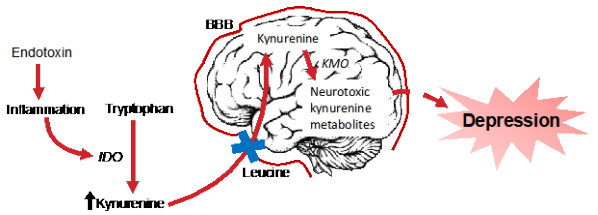

Fig. 1 Conceptual Model. Endotoxin elicits systemic inflammation, which activates indoleamine 2,3 dioxygenase (IDO). IDO converts tryptophan to kynurenine, which is transported into the brain across the brain-blood barrier (BBB). Kynurenine is metabolized by kynurenine monooxygenase (KMO) into neurotoxic kynurenine metabolites responsible for the development of depressive symptoms. Leucine blocks kynurenine entry into brain, attenuating endotoxin-induced depression.

The overarching objective of this innovative proof-of-concept trial is to test whether leucine pretreatment abrogates acute depressive responses to an inflammatory challenge (i.e., endotoxin) in humans, relative to placebo pretreatment. Endotoxin administration has been used as a novel experimental model of depression.[19] We have demonstrated that low-dose endotoxin induces depressive symptoms in humans,[21-23] which correlate with activation of brain regions implicated in depression.[33-35] Low-dose endotoxin also induces transient cognitive impairment,[20] a common symptom of depression that is often overlooked and associated with poor response to treatment.[36] Furthermore, we have found that endotoxin increases tryptophan-kynurenine conversion rate, which correlates with depressed mood (Preliminary Data).

This study hypothesizes that, compared to placebo pretreatment, leucine pretreatment will attenuate depressive responses to endotoxin. In this double-blind randomized placebo-controlled four-arm study of 90 healthy volunteers aged 18-65 years (n_1=35: oral [PO] leucine for 2 weeks followed by single intravenous [IV] endotoxin; n_2=35: PO placebo [i.e., maltodextrin] + IV endotoxin; n_3=10: PO leucine + IV placebo [i.e., saline]; n_4=10: PO placebo + IV placebo), we aim to:

1. Examine the effects of leucine vs. placebo pretreatment on endotoxin-induced depressive symptoms (primary outcome).
2. Examine the effects of leucine vs. placebo pretreatment on negative and positive affect responses to endotoxin (respectively, feelings of social rejection, negative bias in facial emotion recognition; and feelings of social acceptance, reward learning).
3. Examine the effects of leucine vs. placebo pretreatment on cognitive responses to endotoxin (verbal and non-verbal memory, executive function, attention).

Hypothesis: Compared to placebo pretreatment, leucine pretreatment for 2 weeks will attenuate endotoxin-induced depression outcomes including depressive symptoms, negative affect, and positive affect.
Impact Statement: If the hypotheses are confirmed, this proof-of-concept study will provide instrumental information in designing future clinical trials of leucine to treat or prevent inflammation-associated depression.

RESEARCH STRATEGY

A. SIGNIFICANCE

A.1. Burden of Depression and Limitation of Current Treatments: Depression is a major public health burden: highly prevalent (~20% in US[1]) and projected to become the leading cause of disease burden.[2] However, antidepressants, the mainstay of the current depression treatments, have a limited efficacy typically achieving remission rates of 30% or less when used as a monotherapy.[3] They are also associated with poor adherence and substantial adverse effects.[4-6] Depression is even more prevalent and disabling in individuals with inflammatory conditions such as chronic diseases (cardiovascular, infectious, rheumatic, autoimmune), aging, obesity, and interferon treatment.[7-9] Moreover, poor treatment response to antidepressants has been consistently shown in those with inflammatory disorders, inflammation-related gene variants, and elevated levels of inflammatory markers.[10-12] *This study is significant as it will examine novel inflammation-related mechanisms with translational potential for treatment or prevention of this major public health burden.*

A.2. Inflammatory Mechanisms of Depression: Compelling evidence suggests that inflammation plays a causal role in the onset or perpetuation of some forms of depression.[13-15] Elevated biomarkers of inflammation, including C-reactive protein (CRP) and proinflammatory cytokines, predict depression risk,[16,17] and inflammatory challenge (interferon, endotoxin) leads to the development of depressive symptoms.[18-23] Given the poor treatment response to conventional antidepressants in the inflammatory subtype of depression, recent research has focused on the use of drugs that are anti-inflammatory (e.g., NSAIDs, cytokine antagonists).[24-26] However, these drugs show limited efficacy and also have a broad range of actions that can adversely affect other than mood domain with serious side effects (e.g., gastrointestinal bleeding, severe infection). Also, the cost of cytokine antagonists is prohibitive (~$3K per dose). *Thus, novel and safe pharmacologic strategies that target mood-specific mechanisms of inflammation-associated depression are needed, as proposed here.*

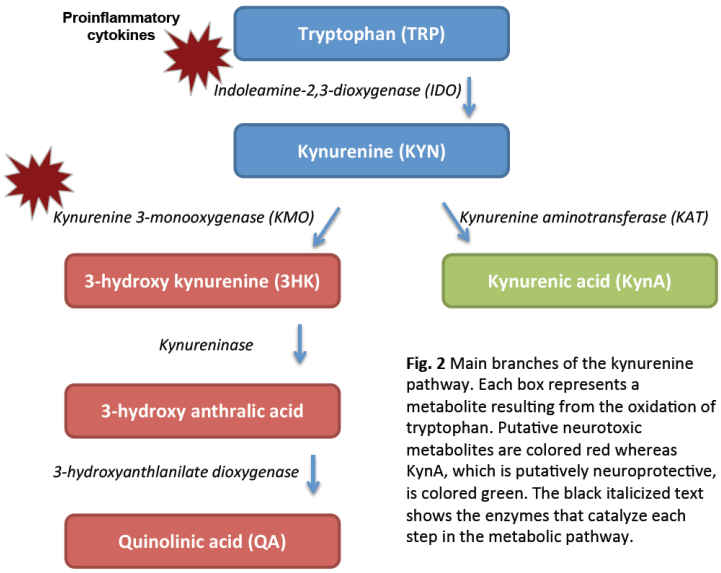

Fig. 2 Main branches of the kynurenine pathway. Each box represents a metabolite resulting from the oxidation of tryptophan. Putative neurotoxic metabolites are colored red whereas KynA, which is putatively neuroprotective, is colored green. The black italicized text shows the enzymes that catalyze each step in the metabolic pathway.

A.3. Kynurenine Metabolism and Leucine: Kynurenine metabolism is increasingly recognized as a key neurochemical pathway in the link between inflammation and depression.[27] Inflammatory mediators activate IDO, an enzyme that metabolizes tryptophan (TRP) to kynurenine (KYN); hence KYN/TRP ratio represents a measure of IDO activity (Fig. 2). Circulating KYN is transported into brain by the large amino acid transporter LAT1, which is expressed in endothelial cells of the BBB (Fig. 3).[28] KYN is further metabolized into 3-hydroxykynurenine (3HK) by the enzyme kynurenine monooxygenase (KMO), leading to an increase in quinolinic acid (QA) on the putatively neurotoxic branch of the kynurenine pathway. Of note, besides IDO, KMO is also upregulated under inflammatory conditions (Fig. 2),[29,37] and endotoxin has been shown to almost exclusively increase neurotoxic metabolites including 3HK and QA but not kynurenic acid (KynA) on the putatively neuroprotective branch.[29] 3HK is a free radical generator, and QA, *an N-methyl-D-aspartate (NMDA) receptor agonist*, has been associated with a plethora of neurotoxic effects.[38,39] KynA is an *NMDA receptor antagonist* and is protective against the excitotoxic action of QA.[40,41] Hence, a shift in the balance of these neurotoxic and neuroprotective kynurenine metabolites is believed to be responsible for the development of depressive symptoms.[42-48]

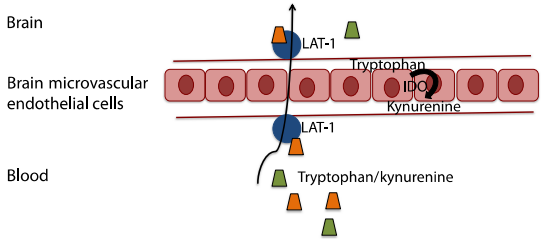

Fig. 3 Transportation of tryptophan and kynurenine across the blood–brain barrier by the large neutral amino acid transporter (LAT-1). Tryptophan and kynurenine enter the brain via a sodium-independent large neutral amino acid transporter known as System L which sits on either side of the blood–brain barrier (BBB). This allows peripheral tryptophan and kynurenine to be transported through the microvascular endothelial cells of the BBB as well as the transportation of kynurenine endogenously produced in these endothelial cells of the brain when indoleamine 2,3 dioxygenase (IDO) is activated

One way to block the shift in the balance of kynurenine metabolites is to block the entry of KYN into brain. KYN is transported into brain via LAT1. It is therefore theoretically possible to decrease the amount of

KYN transported into brain by using competing amino acids at the level of LAT1. Leucine is the amino acid that has the highest affinity for LAT1. Preclinical experiments in a murine model of inflammation-induced depression show that leucine competitively inhibits LAT1 transport of excess KYN into brain in inflamed mice (Preliminary Data). Through this competitive inhibition of KYN transport, leucine is capable of abrogating the development of depressive symptoms in response to inflammatory challenge, but without affecting neuroinflammation or inflammation-induced sickness. Endotoxin is known to induce or increase the secretion of cytokines from the cells which comprise the BBB and to activate IDO at this level.[49] However, as leucine does not affect peripheral inflammatory responses or neuroinflammation (Preliminary Data), its specific effects on mood may be unaltered by the endotoxin-induced immune activation of the BBB itself. Furthermore, given the observation that endotoxin upregulates the neurotoxic branch of the kynurenine pathway without altering the neuroprotective branch,[29] the blockade of KYN transport by leucine appears to affect primarily the neurotoxic branch. Notably, the effect of leucine was about 60% larger than that of ketamine.[29] Ketamine, an *NMDA receptor antagonist*, has a rapid and robust antidepressant effect in humans.[50] *This study is significant by evaluating the potential antidepressant effects of leucine for the first time in humans.*

A.4. Complexity of Kynurenine System Balance and Inflammatory Depression: As in neurodegenerative diseases, an increased influx of KYN from the periphery to CNS can increase the neurotoxic metabolites (3HK and QA) and decrease the neuroprotective KynA, augmenting neuronal vulnerability.[38] Thus, this neurotoxic shift is believed to contribute to the development of depressive symptoms. In line with this hypothesis, a recent meta-analysis has reported decreased serum and CSF levels of KynA in depressed patients compared to controls.[51] KynA is an NMDA receptor antagonist like ketamine, which has a rapid and robust antidepressant effect in humans. On the other hand, the observation that ketamine produces schizophrenia-like symptoms in normal individuals and exacerbates psychotic features in schizophrenic patients has led to the idea that the excessive KynA might have a role in schizophrenia pathology. Increased CNS levels of KynA have been found in schizophrenic patients, and further studies in animals and humans indicate that increased KynA levels may be especially relevant for the cognitive deficits present in schizophrenia.[38] As cognitive deficit is also seen in depression, a more complex kynurenine system balance in depression is indeed possible; e.g., the key for mental health might be an optimal balance between 3HK+QA and KynA rather than a simple predominance of KynA. However, the existing data largely support that a neurotoxic shift of the kynurenine pathway is involved at least in depression pathophysiology[51]; and most importantly, the key premise of our project focuses on the blockade of the BBB transport of KYN, a step upstream to the aforementioned balance mechanism between 3HK+QA and KynA. Thus, the premise of our project overcomes the controversy regarding whether a deficient or an excessive presence of KynA leads to depression. Furthermore, the increase of KYN relative to TRP in the periphery, indicating an increased IDO activity, has been consistently associated with inflammatory depression.[52] Importantly, our previous human endotoxin study also showed a robust increase in the ratio KYN/TRP after endotoxin administration and a significant correlation between this ratio and depressive symptoms.[53] Thus, although there is indeed a complexity of kynurenine system balance in relation to psychopathology, our proposed intervention targets an upstream level that has been consistently associated with inflammatory depression.

A.5. Human Experimental Studies of Endotoxin-Induced Depression: Our and other groups have demonstrated that low-dose endotoxin induces depressive symptoms in humans as well[19-23], which correlate with activation of brain regions implicated in depression.[33-35] We have also found that endotoxin increases TRP-KYN conversion rate, which correlates with depressed mood (Preliminary Data). Increases of inflammation in response to low-dose endotoxin are comparable with the magnitude of increases in acute inflammation (e.g., infection) and chronic inflammation (e.g. cardiovascular disease, cancer, aging processes, sleep disturbance, or obesity). Chronic inflammation predicts depression risk.[54,55] Chronic inflammation primes inflammatory and depressive responses to challenges that acutely activate inflammation (e.g., infection, interpersonal stress).[56-64] Moreover, acute inflammatory reactivity predicts acute increases in depressive symptoms,[58,65] as well as increases in depression over the following year.[65] Although this model is specific to inflammation-associated depression, it should be noted the burden of inflammation-associated depression itself is huge for two reasons. First, a high proportion of depressed individuals identified in community present with elevated inflammation. In the National Health and Nutrition Examination Survey (NHANES), 47% of individuals with the Patient Health Questionnaire (PHQ-9) scores indicative of significant depression (≥ 10) had CRP levels ≥ 3 mg/L and 29% had CRP levels ≥ 5 mg/L.[66] Second, poor treatment response to antidepressants has been consistently shown in those with inflammatory disorders, inflammation-related gene variants, and elevated levels of inflammatory markers [10-12]. Furthermore, the findings of this study may provide

some justification to test leucine in a broader context of clinical depression. *This study is significant as a unique opportunity to advance translational depression research using a novel human experimental model and examining a potential biological mechanism of depression identified by rigorous pre-clinical research.*

A.6. Depressive vs. Sickness Symptoms in Endotoxin Model: Low-dose endotoxin can induce not only depressive symptoms but also some sickness symptoms (i.e., lethargy, fatigue, muscle pain, headache, shivering, and nausea). Although in clinical practice depressed individuals experience both emotional and somatic symptoms of depression in varying degrees, a possible critique on the endotoxin model would be that depressed mood might be majorly influenced by sickness symptoms. To minimize this possibility, we have previously used a depression scale that only assesses emotional symptoms such as "unhappy", "sad," and "hopeless" (Profile of Mood Scale [POMS][67]).[21-23] Furthermore, we have used analytical procedures to ensure the effects of endotoxin on depressed mood are independent of sickness symptoms (Preliminary Data). In this study, beyond these two measures, we will use experimental tasks of negative and positive affect to assess mood-specific processes (see C.2.6. Experimental Tasks). This innovative approach is particularly important for this study as we hypothesize that leucine pretreatment—which interferes with the transport of excess KYN into brain without modifying inflammatory responses to endotoxin—will specifically abrogate depressed mood but not sickness symptoms induced by endotoxin. *This study is significant because it will examine depressive vs. sickness symptoms in a human endotoxin model of depression by employing mood-specific assessments and especially by testing a treatment that mat act on a mood-specific mechanism without modifying inflammatory responses to endotoxin.*

A.7. Transient Cognitive Impairment in Endotoxin Model of Depression: Cognitive impairment is a common symptom of depression, experienced by two-thirds of depressed patients and associated with poor treatment response, but often overlooked by clinicians.[36] Low-dose endotoxin transiently impairs verbal and nonverbal memory functions in humans.[20] Independent preclinical data demonstrate that peripherally administered endotoxin induces memory deficit through IDO-dependent neurotoxic kynurenine metabolism; furthermore, a single peripheral injection of KYN is sufficient to induce memory deficit.[68] Notably, human studies have shown that the supplementation with branched-chain amino acids (leucine, isoleucine, and valine) has protective effects against cognitive deficits during strenuous exercises.[69-72] Based on the evidence from animal and human research, it is expected that the blockade of the BBB transport of KYN by leucine pretreatment will attenuate endotoxin-induced cognitive impairment.

A.8. Potential Impact of the Current Study and Future Directions: If the hypotheses are confirmed, the involvement of the kynurenine pathway in the induction of depressive symptoms, including cognitive impairment, will be demonstrated for the first time in a human experimental study. Furthermore, this proof-of-concept study will provide instrumental information in designing future trials of leucine to treat or prevent inflammation-associated depression. Given the minimal adverse effects and the additional benefits such as muscle mass preservation, leucine will be a prime candidate for a pharmacologic strategy to treat or prevent inflammation-associated depression.

B. INNOVATION

B.1. A highly controlled experimental model of depression to examine a novel mood mechanism
Low-dose endotoxin administration is a highly innovative experimental model to understand the causal role of inflammation in the induction of depressive symptoms; yet, it is a procedure that has only been implemented by a few laboratories in the world with only a small number of studies conducted to date (n=12).[19,73] Using this experimental model, in addition to a randomized controlled trial design, we will evaluate the kynurenine pathway, beyond the inflammatory cascade, as a potential mechanism of inflammation-associated depression. To our knowledge, this will be the first human experimental study to evaluate the involvement of the kynurenine pathway in the induction of depressive symptoms.

B.2. A novel pharmacologic agent shown to act on a novel mood mechanism in animal model
Leucine has been shown to abrogate endotoxin-induced depression-like behavior in mice by specifically acting on blood-to-brain transport of excessively produced KYN without affecting inflammatory responses (Preliminary Data). Although leucine has been studied in humans for other purposes, it has never been tested for depression. This study will employ this novel paradigm for the first time in humans.

B.3. Task based multi-dimensional methods to evaluate affective processes
No prior experimental research on inflammation and depression has used innovative objective measures to assess negative and positive affective processes. Given that social disconnection plays a critical role in the

onset and perpetuation of depression,[58,74] we will examine distress following an experimental episode of social rejection as a function of pretreatment conditions. Further, we will examine the impact of pretreatments on accurate judgment of facial emotions with implications for perceived threat (i.e., angry face) or reward value (i.e., happy face).[75] Facial emotion recognition task is used to identify negatively biased processing of emotional faces and is associated with depression and risk of relapse in depressed patients.[76,77] This study is novel also by evaluating positive affective responses, using reward-based tasks.[78-80] Use of these experimental affect tasks is strategic in distinguishing the effects of endotoxin on depressive symptoms from its effects on sickness symptoms. Furthermore, understanding how leucine pretreatment influences negative and positive affect in response to endotoxin has the potential to refine pharmacologic strategies of depression treatment and to inform the design of neuroimaging studies aimed at identifying the neural sensitivities related to inflammation. For example, findings related to reward deficits could direct attention toward pharmacologically targeting neurotransmitters (i.e., dopamine) and toward scanning reward-related brain regions.

C. APPROACH

C.1. Preliminary Studies

C.1.1. Leucine abrogates inflammation-induced depression-like behavior

Aim: We examined whether L-leucine (hereafter referred to as leucine), which has a high affinity for LAT1, competitively inhibits KYN binding to LAT1, reduces blood-to-brain KYN transport, and blocks endotoxin-induced depression-like behavior in mice. *Methods:* CD-1 mice (N=20; n=5 per group) were injected lipopolysaccharide (LPS = endotoxin, 0.83 mg/kg, intraperitoneal [IP]) or saline and treated with leucine (50 mg/kg, IP) or vehicle administered before and 6 h after LPS (Fig. 4). Depression-like behavior was measured at 24 h by increased duration of immobility in the forced swim test (FST) and decreased sucrose preference. *Results:* Leucine blocked LPS-induced depression-like behaviors (p's<0.05) (Fig. 5). Leucine had no effect on sickness behavior and neuroinflammation. Consistent with our hypothesis that leucine would competitively inhibit blood-to-brain KYN transport, leucine had no effect on LPS-induced increases in plasma KYN/TRP ratio (i.e., IDO activity) or brain TRP levels but significantly reduced brain KYN levels. Using an in vitro model of BBB transport based on primary cultures of brain microvasculature endothelial cells, we confirmed the ability of leucine to block KYN influx via LAT1 and demonstrated it did not interfere with KYN efflux. *Conclusion:* Leucine abrogated LPS-induced depression-like behavior without affecting LPS-induced sickness, inflammation, and IDO activation. Leucine reduced blood-to-brain transport of KYN and decreased brain KYN levels. *These data suggest that targeting KYN transport mechanisms at the BBB level using leucine is a viable approach to attenuating inflammation-induced depression in humans.*

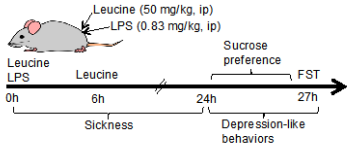

Fig. 4 Experimental time course for the prevention of LPS-induced depression-like behavior by leucine

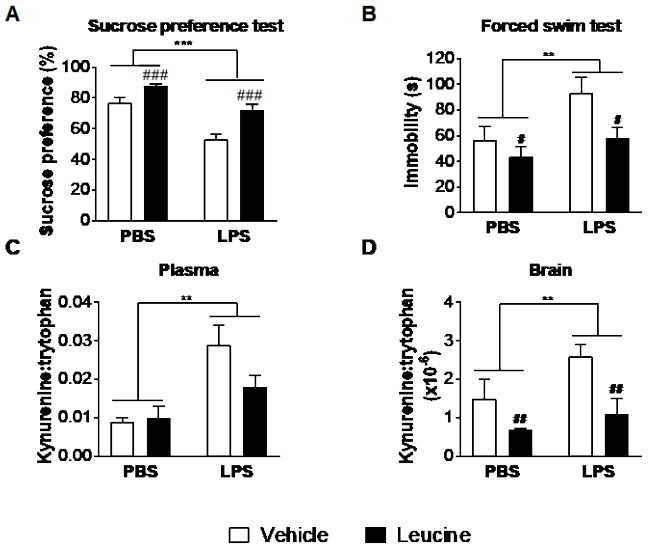

Fig. 5 Leucine reduces brain KYN:TRP ratios and blocks depression-like behavior. **A,B** Mean sucrose preference (%) and time spent immobile in the forced swim test (s) (± SEM) of mice treated with leucine or vehicle immediately before and 6 h after LPS or phosphate-buffered saline (PBS) (n > 14 per group). **C,D** Mean KYN:TRP ratios in plasma and brains (± 442 SEM) of mice treated with 50 mg/kg leucine or vehicle immediately before and 6 h after LPS or PBS (n = 5 per group). **p < 0.01, ***p < 0.0001 for LPS vs. PBS main effects. #p < 0.05, ##p < 0.01, ###p < 0.0001 for leucine vs. vehicle main effects.

C.1.2. Inflammatory challenge (i.e., endotoxin) induces increases in depressed mood in humans

In a randomized double-blind placebo-controlled design, 115 participants (69 female, mean age: 24.1± 6.3 years) received either low-dose endotoxin (0.8 ng/kg of body weight, n=61) or placebo (saline, n=54). Circulating interleukin-6 (IL-6) and tumor necrosis factor-α (TNF-α) were measured hourly post-injection for the next six hours (T1-T6), along with assessment of depressed mood using the POMS. Endotoxin (vs. placebo) led to significant increases in IL-6 (p<0.001) and TNF-α levels (p<0.001). Endotoxin (vs. placebo) also led to significantly greater increases in depressed mood (p<0.001), and these effects did not change when controlling for sickness symptoms (p<0.05).[22] Endotoxin induced increases in depressed mood, which were not due to sickness symptoms. *These data show the feasibility and safety of the human endotoxin model.*

C.1.3. Endotoxin induces changes in KYN metabolism, which correlates with depressed mood

Aim: In the aforementioned study in C.1.2. (N=115), we examined whether endotoxin induces changes in kynurenine metabolism and whether these changes correlate with inflammation-induced depressed mood. *Methods:* Circulating kynurenine metabolites (TRP, KYN, QA, KynA) were measured at T_0, T_2, and T_6. The primary outcome was IDO activity assessed using KYN/TRP. *Results:* Participants exposed to endotoxin (vs. placebo) showed greater increases in IDO activity ($p<0.0001$). Among endotoxin-exposed participants (N=61; 38 females), the correlation between IDO activity and depressed mood was highly significant ($p=0.004$). *Conclusion:* Inflammatory challenge increased IDO activity, which in turn was significantly correlated with inflammation-induced depressed mood. *These data confirm endotoxin-induced increases in peripheral conversion of TRP to KYN as well as increases in depressed mood, and thus show the feasibility of inhibiting blood-to-brain transport of KYN as well as inflammation-induced depressed mood.*

C.2. Research Design and Methods

C.2.1. Overview of Study Design (Fig. 6): In this double-blind randomized placebo-controlled four-arm study, a total of 90 healthy volunteers (ages 18-65) will be randomly assigned to a two-week pretreatment of L-leucine (hereafter referred to as leucine) or PO placebo (i.e., maltodextrin). After the pre-treatment period, they will receive low-dose endotoxin or IV placebo (i.e. saline): n_1=35 leucine + endotoxin; n_2=35 PO placebo + endotoxin; n_3=10 leucine + IV placebo; n_4=10 PO placebo + IV placebo. (see Justification of Sample Size for the rationale for smaller IV placebo arms)

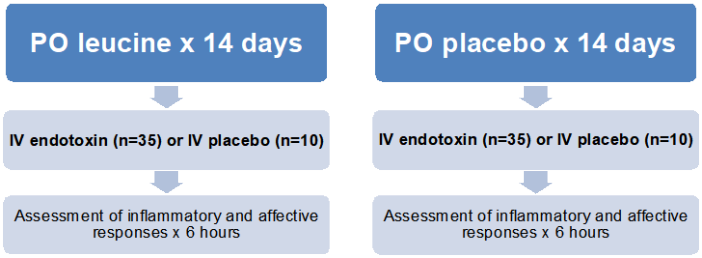

Fig. 6 Overview of study design

C.2.2. Subjects: Participants will be recruited from: 1) flyers that will be posted at the UCLA campus including the UCLA Medical Center; 2) advertisements posted in local university and community newspapers and websites (Daily Bruin, Brentwood News, Santa Monica Daily Press, www.bruinwalk.com and www.dailybruin.com); and 3) lists of telephone numbers and mailing addresses purchased from GENESYS Sampling Systems (Fort Washington, PA), a company that maintains a bimonthly updated database of all available listed telephone households in the US, following our previously successful recruitment strategies. A list of households with at least one adult aged 18 to 65 years within a 10-mile radius of UCLA Westwood Campus will be purchased every other month. Age information for each household is based on either known age-related data or a statistical estimate of age, predicted on individual household characteristics and Census demographic information. We have used these lists for our previous telephone survey of community-dwelling older adults (R01AG034588). A letter will be sent to the individuals listed by GENESYS Sampling Systems, briefly describing the study and inviting them to participate in the study. Participants interested in the study will contact the project coordinator by email or phone. All interested participants will then go through a two-step screening procedure, consisting of a structured telephone interview and an in-person screening session. The initial screening process will be completed over the phone by the project coordinator. In addition to the telephone screening, we will use a web survey (https://tinyurl.com/UCLA-LDEP). When potential participants type this link on their Internet browser or click the advertisement banner on one of the websites, they will be directed to the web survey. Lastly, we will include a QR code on the flyers to facilitate the enrollment. By scanning the QR code, the prospective participants will be directed to the web survey as a screening procedure. The second screening will be done in person by one of the study physicians (Dr. Joshua Hyong-Jin Cho, Dr. Michael Irwin, or Dr. Ariya Mahbod). Most of the assessment will be conducted by the study physician, especially for medical and psychiatric comorbidities. The other interview components including C-SSRS and SCID will be conducted by the project coordinator and reviewed by the study physician. Participants' eligibility will ultimately be determined by the study physician, based on the results from the blood and urine tests as well as the medical and psychiatric evaluation obtained during the in person screening processes. To maximize the participant retention, they will be regularly contacted by phone throughout the study, especially during the 2-week pretreatment period.

Inclusion Criteria: Participants will be required to be in good general health (as evaluated during the phone and in-person screening sessions) and aged 18-65 years.

Exclusion Criteria: Individuals with the following conditions will be excluded: history of maple syrup urine disease (a contraindication to leucine treatment), chronic diseases; current use of prescription medications such as steroids, NSAIDs, immune modifying drugs, opioid analgesics, and psychotropics; Axis I psychiatric disorders including current major depressive disorder; current depressive symptoms assessed by the Patient

Health Questionnaire (PHQ-9 ≥ 5); heavy smoking (1 pack or more per day) or excessive caffeine use (>600 mg/day); BMI > 35 due to the effects of obesity on cytokine activity; evidence of recreational drug use or pregnancy from urine test; evidence of clinically significant rhythm abnormality on a resting electrocardiogram (ECG); clinically significant abnormalities on screening laboratory tests. (see Protections of Human Subjects section for the full criteria)

C.2.3. Procedures

Pretreatment: Leucine is commercially available in US as dietary supplements. For this study, we will use oral preparation of the pharmaceutical grade leucine (12 g/day in 2 divided doses for 2 weeks; Ajinomoto, Raleigh, NC) mixed with effervescent powder. PO placebo will resemble the compounded mixture of leucine and effervescent powder. Clinical studies of leucine have used doses ranging from 2 to 18 g/day.[81-87] According to a meta-analysis, the long-term supplementation dose (i.e., 10 days to 6 months) ranged from 2.8 to 16.1 g/day with a weighted mean of 8.5 g/day.[30] Casperson et al. found that leucine 12 g/day for 2 weeks improved muscle protein synthesis with a concomitant increase in phosphorylation of mechanistic target of rapamycin (mTOR) following the stimulus of a meal.[87] As this above-average dose given for 2 weeks caused increases in post-prandial protein synthesis and mTOR activation, both mechanisms also observed in preclinical studies of leucine after the stimulus of endotoxin,[88] we will use 12 g/day for 2 weeks. Participants will be given leucine or PO placebo on the day of in-person screening and will take it for 2 weeks including the morning of endotoxin administration, hence a total of 28 doses. At the end of pretreatment, subjects will indicate the presence or absence of a number of possible adverse effects in a questionnaire using a 4-point scale from 'not at all' to 'very often': symptoms include not feeling well, headache, weakness, fatigue, nausea, vomiting, burping, stomachache, bloating, flatulence, constipation, diarrhea, dry mouth, change of taste, cough, skin complaints, muscle pain, weight gain, weight loss, dizziness, sweating, and shaking. The adherence will be assessed using the Patient Adherence Questionnaire (PAQ)[89] and the count-back method (i.e., counting doses remaining in returned packets)[90] at the end of the pretreatment (i.e., immediately before endotoxin).

Endotoxin Administration (Main Study Session): The main study session including endotoxin administration and experimental tasks will be conducted at the UCLA Clinical & Translational Research Center (CTRC). Participants will arrive at the CTRC at 07:00. Upon arrival, a urine sample will be collected and tested for drug use and pregnancy in order to ensure continued eligibility for study participation. A CTRC nurse will assess vital signs (blood pressure, pulse, temperature). An indwelling venous catheter with a heparin lock will be inserted into the participant's dominant forearm for hourly blood draws and one into the non-dominant forearm for endotoxin administration. Oral hydration with water or juice will be offered hourly in order to minimize the negative side effects of endotoxin (hydration reduces risk of diastolic blood pressure decreases). Following these procedures, participants will eat a light breakfast before undergoing experimental tasks and taking the last dose of study drug at 08:30. At 09:00 (T_0), participants will receive endotoxin (0.8 ng/kg of body weight) or IV placebo as an intravenous bolus over 30-60 seconds. Endotoxin to be used in this study is derived from *Escherichia coli* (*E. coli* group O:113) and will be provided by the NIH Clinical Center.[91]

Safety Monitoring and Discharge from Study: Participants will be monitored as per the protocol (see Protections of Human Subjects section) and discharged following the last blood draw upon approval from the study physician. Briefly, in addition to the continuous observation, scheduled nursing assessments including vital sign check will be performed every 30 minutes for the first 2 hours after LPS administration and then hourly. If any potential adverse reactions are noted as detailed in the protocol, the study physician will be contacted and the pre-determined actions will be taken as appropriate such as ECG, verbal or manual stimulation, relieving medications, or discontinuation of the study procedures.

C.2.4. Baseline Measures (Prior to Pretreatment): During the in-person screening session, the following measures will be assessed: sociodemographic information, sleep (DSM-5 insomnia using the SCID-5[92] and sleep disturbance using the Pittsburgh Sleep Quality Index[93]), depressive/anxiety symptoms (Inventory of Depressive Symptomatology[94], Beck Depression Inventory[95], Beck Anxiety Inventory[96], POMS[67], and the Montgomery-Asberg Depression Rating Scale [MADRS][97]), cognitive functions (estimated IQ using Test of Premorbid Functioning from Advanced Clinical Solutions™; verbal memory, visual memory, executive function, and attention using computerized tests from CNS Vital Signs™ including Verbal Memory Test, Visual Memory Test, Stroop Test, Shifting Attention Test, and Continuous Performance Test), systemic inflammation (CRP), and kynurenine metabolism (TRP, KYN, 3HK, QA, and KynA). Baseline measures such as sex, sleep disturbance, systemic inflammation, and KYN metabolism will be tested as potential moderators of the pretreatment effects on endotoxin-induced depression outcomes. In particular**,** we expect stronger effects of leucine pretreatment in females compared to males because our previous studies indicate that: 1) whereas

endotoxin increases circulating proinflammatory cytokines and IDO activity (i.e., KYN/TRP ratio) equally in males and females,[22,53] it increases depressed mood much more robustly in females[22]; 2) the correlations between cytokines and depressed mood are stronger in females[22]; and 3) the correlations between IDO activity and depressed mood are stronger in females[53]. Furthermore, before and after pretreatment, blood samples will be drawn for the assays of intracytoplasmic cytokine expression (IL-1, IL-6 and TNF-α) and nuclear signaling of inflammation (nuclear factor [NF]-κB) using ex-vivo stimulation with endotoxin (also known as lipopolysaccharide [LPS] stimulation). These assays will measure the cellular production of cytokines in response to LPS stimulation and thus the sensitivity of peripheral blood mononuclear cells (PBMC) to an inflammatory challenge before and after pretreatment.

C.2.5. Repeated Measures (Main Study Session)

Behavioral Measures: Depressed mood, a core symptom of depression, will be assessed immediately before endotoxin administration (T_0) and then hourly for the next 6 hours (T_1-T_6) using the POMS[67] (primary outcome). We will also use the MADRS[97] hourly (T_0-T_6) as it is sensitive to acute changes in depressed mood and anhedonia following endotoxin[19] and is less oriented towards somatic symptoms than the Hamilton Depression Rating Scale. Because feelings of social disconnection and loneliness predict depression and co-occur with depressed mood following inflammatory challenge, feelings of social disconnection (Feelings of Social Disconnection Scale[21]) and loneliness (adapted UCLA Loneliness Scale[98]) will also be assessed. Participants will also complete self-report measures of sickness symptoms (e.g., muscle pain, fatigue).
Cognitive Measures: Three items from the POMS-Confusion subscale (unable to concentrate, forgetful, confused) will be assessed at T0 and then hourly (T1-T6).[67] Verbal memory, visual memory, executive function, and attention be assessed at T0 and then between T2 and T3 using computerized tests from CNS Vital Signs™: Verbal Memory Test, Visual Memory Test, Stroop Test, Shifting Attention Test, and Continuous Performance Test. Reichenberg et al. assessed cognitive impairment at 1, 3, and 9 hours after endotoxin and found it to be worst at 3 hours.[20] The order of tests will be counterbalanced across subjects to avoid any non-random version-dependent bias.[99]
Cytokines, Kynurenine Metabolites & Gene Expression Analysis: Blood samples will be collected immediately before endotoxin administration (T_0) and then hourly (T_1-T_6). Samples will then be appropriately processed and stored for later assessment of proinflammatory cytokines (IL-1 receptor antagonist, IL-6, TNF-α, soluble TNF receptor) and KYN metabolites (TRP, KYN, 3HK, QA, KynA). Depressed mood and biomarkers will be hourly assessed even after the time of peak responses (T_2) because depressed mood can linger and has been shown to correlate with cytokines and kynurenine metabolites.[16,42] At baseline prior to pretreatment, immediately before endotoxin injection (T_0), and 30 minutes post-injection ($T_{0.5}$), blood samples will be drawn in PaxGene RNA tubes, which preserve RNA integrity. Genome-wide transcriptional profiling will be conducted using Illumina HT-12 BeadArrays. Gene expression analysis will provide information on the underlying biological mechanisms of inflammatory and affective responses, and in particular, will be used to independently replicate the findings of our prior endotoxin studies.

C.2.6. Experimental Tasks (Main Study Session)

For Negative Affect: *Subjective Sensitivity to Social Rejection (Cyberball Social Exclusion Task):* Feelings of 'social disconnection' play a critical role in the onset and perpetuation of (non-inflammatory forms of) depression.[100] Inflammatory processes can trigger social withdrawal[101] and increase feelings of distress and related neural sensitivity (i.e., activation of the dorsal anterior cingulate cortex).[21] Using the Cyberball Social Exclusion Task,[102] we will examine feelings of social distress. Participants will be told that they will play a virtual ball-tossing game with two other players over the Internet. In reality, they will play with a preset computer program. In the first round (inclusion), participants will play with the two other players for the entire period. In the second round (exclusion), participants will receive the ball for 7 throws and then will be excluded for the rest of the round when the two players will stop throwing the ball to the participant. Following this task, they will complete a measure of social distress in response to social exclusion.[103]
Negative Bias in Facial Emotion Recognition (Emotional Face Recognition Task): Depressed subjects have a negative bias in the perception of emotions expressed in faces as they tend to interpret neutral faces as sad and happy faces as neutral.[76] Furthermore, such a bias appears to play a role in the etiology and maintenance of depression as it predicts relapse in currently depressed and remitted patients.[77] Emotional Face Recognition Task[104] consists in showing participants a series of black and white photographs (Ekman Pictures of facial affect), in which the facial expression is morphed from neutral to either Sad, Angry, or Happy.[105] For each image, participants will be asked to make a forced choice about the emotion expressed, and rate their certainty on a 0-9 scale. Earlier recognition of a Sad face with certainty would be indicative of negative bias.

For Positive Affect: *Subjective Sensitivity to Social Acceptance (Positive Social Feedback Task):* Decreased sensitivity to positive social experiences, like social acceptance, is another mechanism that might account for cytokine-induced feelings of social disconnection and depressed mood. The Positive Social Feedback Task was developed based on Izuma et al.'s study involving monetary and social rewards, which demonstrated that positive social feedback robustly activates reward-related brain areas, notably the striatum, and these overlap with the areas activated by monetary rewards.[106] Prior to the experimental session, participants will be asked to complete a survey that contains several personality questionnaires (NEO Five Factor Inventory),[107] as well as several open-ended questions ("What do you do in your free time?" "Briefly describe your personality." "What is your main goal in the future?"). Then, participants will be video-recorded for 2-5 minutes as they discuss what they like about themselves. Participants will be told that 8 people (4 male, 4 female) will form impressions of them—based on their answers to the personality questionnaires and the video-taped self-introduction—by selecting personality traits to describe them. Participants will then see a photograph of themselves along with a descriptive word underneath (supposedly provided by the evaluators), which will be pre-rated based on desirability, and will be asked to rate subjective happiness when each of the feedback items is presented, using a 7-point scale.

Reward (Reward Learning Task): Anhedonia is a core feature of major depressive disorder and includes a reduction in experienced pleasure (liking reward) and dysfunction in reward anticipation reward learning.[78-80] We have found that inflammatory challenge reduces neural sensitivity to reward anticipation.[108] We will use a laboratory based probabilistic reward task that objectively measures participants' ability to modulate behavior as a function of reward, because this task identifies reduced reward learning in depressed patients which is state-dependent and also predicts the persistent diagnosis of depression in the midst of treatment.[78-80] This computerized reward-learning task is extensively described in Pizzagalli et al.[109]

Order and Timing of Experimental Tasks: The order of the tasks will be first a randomized sequence of Reward Learning and Emotional Face Recognition, then followed by a randomized sequence of Positive Social Feedback and Cyberball Social Exclusion. Because the latter two tasks involve deception, they will be completed only once after the former two tasks, at T_2, time of peak cytokine responses. The former two tasks will be completed at T_0 and T_2.

C.2.7. Interpretation of Results and Potential Pitfalls

As leucine may not inhibit endotoxin-induced inflammation, unopposed sickness symptoms could mask the antidepressant effects of leucine assessed using the POMS. Thus, the results will be statistically adjusted for sickness symptoms. Furthermore, we will use experimental tasks of negative and positive affect to assess mood-specific processes. Although the proposed leucine dose is above average,[30,87] the adequate antidepressant dose is unknown. Thus, if an interim data analysis for the first 30 participants using 12 g/day shows no signal of an antidepressant effect, we will consider increasing the dose to 16 g/day for the same period of 14 days. Of note, 16 g/day was the maximum dose of leucine used in long-term supplementation studies according to a meta-analysis.[30]

C.2.8. Statistical Analysis

The basic design is a 2 group (leucine vs. PO placebo) by 2 condition (endotoxin vs. IV placebo) independent analysis of variance (ANOVA) with one or more repeated outcome measures analyzed with linear mixed models (LMM) with primary focus upon the two endotoxin-exposed groups. The number of repeated measures varies by assessment. The key results are the main effects of pretreatment (i.e., group) while the main effects of group, condition, and their interaction will be tested. For analyses with repeated measures, the two main effects and interaction will further interact with the time variable. POMS will be the primary outcome measure. Simes correction for multiple testing will be applied for the secondary outcome measures as they are expected to be correlated.[110]

C.2.9. Justification of Sample Size

The effect sizes for the reduction of endotoxin-induced depressive symptoms by leucine within the animal data are very large (d=.90), but we have assumed some attenuation in humans (d=.70). Assuming an effect of this size, with n=35 per group, there is >80% power to detect differences between the leucine and PO placebo pretreatments with endotoxin exposure for the single time point outcomes (e.g., Cyberball Social Exclusion); comparisons for repeated measures assessments will have more power (>90%) and able to detect more subtle effects at >80% power. Both 'leucine + IV placebo' and 'PO placebo + IV placebo' arms are expected to show minimal depression with means over time to be very near zero but exist as reference points to the endotoxin condition, which our previous work suggests to have large effects. With repeated measures, n=10 can detect

medium sized effects (d=.50) with power >80%. Thus, there is more than adequate power with 35 vs. 10 to show the effect of endotoxin exposure within pretreatment groups.

REFERENCES

1. Bromet E, Andrade LH, Hwang I, et al. Cross-national epidemiology of DSM-IV major depressive episode. *BMC Med.* 2011;9:90.
2. Mathers CD, Loncar D. Projections of global mortality and burden of disease from 2002 to 2030. *PLoS Med.* 2006;3(11):e442.
3. Depression Guideline Panel: Clinical Practice Guideline 5: Depression in Primary Care, vol 2: Treatment of Major Depression. In: US Department of Health and Human Services PHS, Agency for Health Care Policy and Research, ed. Rockville, MD.1993.
4. Ashton AK, Jamerson BD, W LW, Wagoner C. Antidepressant-related adverse effects impacting treatment compliance: Results of a patient survey. *Curr Ther Res Clin Exp.* 2005;66(2):96-106.
5. Lee SH, Paz-Filho G, Mastronardi C, Licinio J, Wong ML. Is increased antidepressant exposure a contributory factor to the obesity pandemic? *Transl Psychiatry.* 2016;6:e759.
6. Bockting CLH, ten Doesschate MC, Spijker J, Spinhoven P, Koeter MWJ, Schene AH. Continuation and Maintenance Use of Antidepressants in Recurrent Depression. *Psychotherapy and Psychosomatics.* 2008;77(1):17-26.
7. Maes M, Kubera M, Obuchowiczwa E, Goehler L, Brzeszcz J. Depression's multiple comorbidities explained by (neuro) inflammatory and oxidative & nitrosative stress pathways. *Neuroendocrinol Lett.* 2011;32(1):7-24.
8. Moussavi S, Chatterji S, Verdes E, Tandon A, Patel V, Ustun B. Depression, chronic diseases, and decrements in health: results from the World Health Surveys. *The Lancet.* 2007;370(9590):851-858.
9. Yirmiya R, Pollak Y, Morag M, et al. Illness, cytokines, and depression. *Annals of the New York Academy of Sciences.* 2000;917(1):478-487.
10. Vogelzangs N, Beekman AT, van Reedt Dortland AK, et al. Inflammatory and metabolic dysregulation and the 2-year course of depressive disorders in antidepressant users. *Neuropsychopharmacology.* 2014;39(7):1624-1634.
11. Strawbridge R, Arnone D, Danese A, Papadopoulos A, Herane Vives A, Cleare AJ. Inflammation and clinical response to treatment in depression: A meta-analysis. *Eur Neuropsychopharmacol.* 2015;25(10):1532-1543.
12. Barnes J, Mondelli V, Pariante CM. Genetic contributions of inflammation to depression. *Neuropsychopharmacology.* 2017;42(1):81-98.
13. Dantzer R, O'Connor JC, Freund GG, Johnson RW, Kelley KW. From inflammation to sickness and depression: when the immune system subjugates the brain. *Nat Rev Neurosci.* 2008;9(1):46-56.
14. Lotrich FE. Inflammatory cytokine-associated depression. *Brain Res.* 2015;1617:113-125.
15. Kunugi H, Hori H, Ogawa S. Biochemical markers subtyping major depressive disorder. *Psychiatry Clin Neurosci.* 2015;69(10):597-608.
16. Gimeno D, Kivimaki M, Brunner EJ, et al. Associations of C-reactive protein and interleukin-6 with cognitive symptoms of depression: 12-year follow-up of the Whitehall II study. *Psychol Med.* 2008:1-11.
17. Pasco JA, Nicholson GC, Williams LJ, et al. Association of high-sensitivity C-reactive protein with de novo major depression. *Br J Psychiatry.* 2010;197(5):372-377.
18. Udina M, Castellvi P, Moreno-Espana J, et al. Interferon-induced depression in chronic hepatitis C: a systematic review and meta-analysis. *J Clin Psychiatry.* 2012;73(8):1128-1138.
19. DellaGioia N, Hannestad J. A critical review of human endotoxin administration as an experimental paradigm of depression. *Neurosci Biobehav Rev.* 2010;34(1):130-143.
20. Reichenberg A, Yirmiya R, Schuld A, et al. Cytokine-associated emotional and cognitive disturbances in humans. *Arch Gen Psychiatry.* 2001;58(5):445-452.
21. Eisenberger NI, Inagaki TK, Mashal NM, Irwin MR. Inflammation and social experience: an inflammatory challenge induces feelings of social disconnection in addition to depressed mood. *Brain Behav Immun.* 2010;24(4):558-563.
22. Moieni M, Irwin MR, Jevtic I, Olmstead R, Breen EC, Eisenberger NI. Sex differences in depressive and socioemotional responses to an inflammatory challenge: implications for sex differences in depression. *Neuropsychopharmacology.* 2015;40(7):1709-1716.
23. Cho HJ, Eisenberger NI, Olmstead R, Breen EC, Irwin MR. Preexisting mild sleep disturbance as a vulnerability factor for inflammation-induced depressed mood: a human experimental study. *Transl Psychiatry.* 2016;6(3):e750.

24. Raison CL, Rutherford RE, Woolwine BJ, et al. A randomized controlled trial of the tumor necrosis factor antagonist infliximab for treatment-resistant depression: the role of baseline inflammatory biomarkers. *JAMA Psychiatry.* 2013;70(1):31-41.
25. Fields C, Drye L, Vaidya V, Lyketsos C. Celecoxib or naproxen treatment does not benefit depressive symptoms in persons age 70 and older: findings from a randomized controlled trial. *Am J Geriatr Psychiatry.* 2012;20(6):505-513.
26. Kohler O, Benros ME, Nordentoft M, et al. Effect of anti-inflammatory treatment on depression, depressive symptoms, and adverse effects: a systematic review and meta-analysis of randomized clinical trials. *JAMA Psychiatry.* 2014;71(12):1381-1391.
27. Reus GZ, Jansen K, Titus S, Carvalho AF, Gabbay V, Quevedo J. Kynurenine pathway dysfunction in the pathophysiology and treatment of depression: Evidences from animal and human studies. *J Psychiatr Res.* 2015;68:316-328.
28. Dantzer R. Role of the Kynurenine Metabolism Pathway in Inflammation-Induced Depression: Preclinical Approaches. *Current topics in behavioral neurosciences.* 2016.
29. Walker AK, Budac DP, Bisulco S, et al. NMDA receptor blockade by ketamine abrogates lipopolysaccharide-induced depressive-like behavior in C57BL/6J mice. *Neuropsychopharmacology.* 2013;38(9):1609-1616.
30. Xu ZR, Tan ZJ, Zhang Q, Gui QF, Yang YM. The effectiveness of leucine on muscle protein synthesis, lean body mass and leg lean mass accretion in older people: a systematic review and meta-analysis. *Br J Nutr.* 2015;113(1):25-34.
31. Pedroso JA, Zampieri TT, Donato J, Jr. Reviewing the Effects of L-Leucine Supplementation in the Regulation of Food Intake, Energy Balance, and Glucose Homeostasis. *Nutrients.* 2015;7(5):3914-3937.
32. Layman DK. The Role of Leucine in Weight Loss Diets and Glucose Homeostasis. *The Journal of Nutrition.* 2003;133(1):261S-267S.
33. Inagaki TK, Muscatell KA, Irwin MR, et al. The role of the ventral striatum in inflammatory-induced approach toward support figures. *Brain Behav Immun.* 2015;44:247-252.
34. Eisenberger NI, Berkman ET, Inagaki TK, Rameson LT, Mashal NM, Irwin MR. Inflammation-induced anhedonia: endotoxin reduces ventral striatum responses to reward. *Biological Psychiatry.* 2010;68(8):748-754.
35. Eisenberger NI, Inagaki TK, Rameson LT, Mashal NM, Irwin MR. An fMRI study of cytokine-induced depressed mood and social pain: the role of sex differences. *Neuroimage.* 2009;47(3):881-890.
36. Rock PL, Roiser JP, Riedel WJ, Blackwell AD. Cognitive impairment in depression: a systematic review and meta-analysis. *Psychological Medicine.* 2013;44(10):2029-2040.
37. Saito K, Markey SP, Heyes MP. Effects of immune activation on quinolinic acid and neuroactive kynurenines in the mouse. *Neuroscience.* 1992;51(1):25-39.
38. Schwarcz R, Bruno JP, Muchowski PJ, Wu HQ. Kynurenines in the mammalian brain: when physiology meets pathology. *Nat Rev Neurosci.* 2012;13(7):465-477.
39. Guillemin GJ. Quinolinic acid, the inescapable neurotoxin. *FEBS J.* 2012;279(8):1356-1365.
40. Stone TW, Darlington LG. Endogenous kynurenines as targets for drug discovery and development. *Nat Rev Drug Discov.* 2002;1(8):609-620.
41. Kim JP, Choi DW. Quinolinate neurotoxicity in cortical cell culture. *Neuroscience.* 1987;23(2):423-432.
42. Myint AM, Kim YK, Verkerk R, Scharpe S, Steinbusch H, Leonard B. Kynurenine pathway in major depression: Evidence of impaired neuroprotection. *J Affect Disord.* 2007;98(1-2):143-151.
43. Savitz J, Dantzer R, Wurfel BE, et al. Neuroprotective kynurenine metabolite indices are abnormally reduced and positively associated with hippocampal and amygdalar volume in bipolar disorder. *Psychoneuroendocrinology.* 2015;52:200-211.
44. Savitz J, Drevets WC, Smith CM, et al. Putative neuroprotective and neurotoxic kynurenine pathway metabolites are associated with hippocampal and amygdalar volumes in subjects with major depressive disorder. *Neuropsychopharmacology.* 2015;40(2):463-471.
45. Singh R, Savitz J, Teague TK, et al. Mood symptoms correlate with kynurenine pathway metabolites following sports-related concussion. *J Neurol Neurosurg Psychiatry.* 2015.
46. Schwieler L, Samuelsson M, Frye MA, et al. Electroconvulsive therapy suppresses the neurotoxic branch of the kynurenine pathway in treatment-resistant depressed patients. *J Neuroinflammation.* 2016;13(1):51.

47. Bay-Richter C, Linderholm KR, Lim CK, et al. A role for inflammatory metabolites as modulators of the glutamate N-methyl-D-aspartate receptor in depression and suicidality. *Brain Behav Immun.* 2015;43:110-117.
48. Erhardt S, Lim CK, Linderholm KR, et al. Connecting inflammation with glutamate agonism in suicidality. *Neuropsychopharmacology.* 2013;38(5):743-752.
49. Banks WA, Erickson MA. The blood–brain barrier and immune function and dysfunction. *Neurobiology of disease.* 2010;37(1):26-32.
50. Monteggia LM, Zarate C. Antidepressant actions of ketamine: from molecular mechanisms to clinical practice. *Current opinion in neurobiology.* 2015;30:139-143.
51. Nakajima SL, Ogyu K, Kubo K, et al. Kynurenic acid levels in major depressive disorder: a systematic review and meta-analysis. American College of Neuropsychopharmacology Annual Meeting; 2017; Palm Springs, CA.
52. Muller N, Myint AM, Schwarz MJ. Inflammatory biomarkers and depression. *Neurotox Res.* 2011;19(2):308-318.
53. Cho HJ, Kruse J, Eisenberger NI, Irwin MR. Inflammation, kynurenine metabolism, and depressed mood: a human experimental study. *Neuropsychopharmacology.* 2016;41:S516-S517.
54. Gimeno D, Kivimaki M, Brunner EJ, et al. Associations of C-reactive protein and interleukin-6 with cognitive symptoms of depression: 12-year follow-up of the Whitehall II study. *Psychol Med.* 2009;39(3):413-423.
55. Raison CL, Miller AH. Role of inflammation in depression: implications for phenomenology, pathophysiology and treatment. *Mod Trends Pharmacopsychiatri.* 2013;28:33-48.
56. Chung HY, Cesari M, Anton S, et al. Molecular inflammation: underpinnings of aging and age-related diseases. *Ageing Res Rev.* 2009;8(1):18-30.
57. Franceschi C, Campisi J. Chronic inflammation (inflammaging) and its potential contribution to age-associated diseases. *J Gerontol A Biol Sci Med Sci.* 2014;69 Suppl 1:S4-9.
58. Slavich GM, Irwin MR. From stress to inflammation and major depressive disorder: a social signal transduction theory of depression. *Psychol Bull.* 2014;140(3):774-815.
59. Irwin MR, Olmstead R, Carrillo C, et al. Sleep loss exacerbates fatigue, depression, and pain in rheumatoid arthritis. *Sleep.* 2012;35(4):537-543.
60. Pace TW, Negi LT, Sivilli TI, et al. Innate immune, neuroendocrine and behavioral responses to psychosocial stress do not predict subsequent compassion meditation practice time. *Psychoneuroendocrinology.* 2010;35(2):310-315.
61. Pace TW, Mletzko TC, Alagbe O, et al. Increased stress-induced inflammatory responses in male patients with major depression and increased early life stress. *Am J Psychiatry.* 2006;163(9):1630-1633.
62. Weinstein AA, Deuster PA, Francis JL, Bonsall RW, Tracy RP, Kop WJ. Neurohormonal and inflammatory hyper-responsiveness to acute mental stress in depression. *Biol Psychol.* 2010;84(2):228-234.
63. Dassonville C, Bonfils P, Momas I, Seta N. Nasal inflammation induced by a common cold: comparison between controls and patients with nasal polyposis under topical steroid therapy. *Acta Otorhinolaryngol Ital.* 2007;27(2):78-82.
64. McIntire CR, Yeretssian G, Saleh M. Inflammasomes in infection and inflammation. *Apoptosis.* 2009;14(4):522-535.
65. Aschbacher K, Epel E, Wolkowitz OM, Prather AA, Puterman E, Dhabhar FS. Maintenance of a positive outlook during acute stress protects against pro-inflammatory reactivity and future depressive symptoms. *Brain Behav Immun.* 2012;26(2):346-352.
66. Rethorst CD, Bernstein I, Trivedi MH. Inflammation, obesity, and metabolic syndrome in depression: analysis of the 2009-2010 National Health and Nutrition Examination Survey (NHANES). *J Clin Psychiatry.* 2014;75(12):e1428-1432.
67. McNair DM, Lorr M, Droppleman LF. *Profile of Mood States, POMS.* Educational and industrial testing service; 1981.
68. Heisler JM, O'Connor JC. Indoleamine 2,3-dioxygenase-dependent neurotoxic kynurenine metabolism mediates inflammation-induced deficit in recognition memory. *Brain Behav Immun.* 2015;50:115-124.
69. Hassmen P, Blomstrand E, Ekblom B, Newsholme EA. Branched-chain amino acid supplementation during 30-km competitive run: mood and cognitive performance. *Nutrition.* 1994;10(5):405-410.

70. Blomstrand E, Hassmen P, Ek S, Ekblom B, Newsholme EA. Influence of ingesting a solution of branched-chain amino acids on perceived exertion during exercise. *Acta Physiol Scand.* 1997;159(1):41-49.
71. Blomstrand E, Hassmen P, Ekblom B, Newsholme EA. Administration of branched-chain amino acids during sustained exercise--effects on performance and on plasma concentration of some amino acids. *Eur J Appl Physiol Occup Physiol.* 1991;63(2):83-88.
72. Portier H, Chatard JC, Filaire E, Jaunet-Devienne MF, Robert A, Guezennec CY. Effects of branched-chain amino acids supplementation on physiological and psychological performance during an offshore sailing race. *Eur J Appl Physiol.* 2008;104(5):787-794.
73. Schedlowski M, Engler H, Grigoleit JS. Endotoxin-induced experimental systemic inflammation in humans: a model to disentangle immune-to-brain communication. *Brain Behav Immun.* 2014;35:1-8.
74. Eisenberger NI. Social pain and the brain: controversies, questions, and where to go from here. *Annu Rev Psychol.* 2015;66:601-629.
75. Stuhrmann A, Suslow T, Dannlowski U. Facial emotion processing in major depression: a systematic review of neuroimaging findings. *Biol Mood Anxiety Disord.* 2011;1(1):10.
76. Bourke C, Douglas K, Porter R. Processing of facial emotion expression in major depression: a review. *Aust N Z J Psychiatry.* 2010;44(8):681-696.
77. Bouhuys AL, Geerts E, Gordijn MC. Depressed patients' perceptions of facial emotions in depressed and remitted states are associated with relapse: a longitudinal study. *J Nerv Ment Dis.* 1999;187(10):595-602.
78. Whitton AE, Treadway MT, Pizzagalli DA. Reward processing dysfunction in major depression, bipolar disorder and schizophrenia. *Curr Opin Psychiatry.* 2015;28(1):7-12.
79. Vrieze E, Pizzagalli DA, Demyttenaere K, et al. Reduced reward learning predicts outcome in major depressive disorder. *Biol Psychiatry.* 2013;73(7):639-645.
80. Pizzagalli DA, Iosifescu D, Hallett LA, Ratner KG, Fava M. Reduced hedonic capacity in major depressive disorder: evidence from a probabilistic reward task. *J Psychiatr Res.* 2008;43(1):76-87.
81. Bjorkman MP, Pilvi TK, Kekkonen RA, Korpela R, Tilvis RS. Similar effects of leucine rich and regular dairy products on muscle mass and functions of older polymyalgia rheumatica patients: a randomized crossover trial. *J Nutr Health Aging.* 2011;15(6):462-467.
82. Kirby TJ, Triplett NT, Haines TL, Skinner JW, Fairbrother KR, McBride JM. Effect of leucine supplementation on indices of muscle damage following drop jumps and resistance exercise. *Amino Acids.* 2012;42(5):1987-1996.
83. Zemel MB, Bruckbauer A. Effects of a leucine and pyridoxine-containing nutraceutical on fat oxidation, and oxidative and inflammatory stress in overweight and obese subjects. *Nutrients.* 2012;4(6):529-541.
84. Walker TB, Smith J, Herrera M, Lebegue B, Pinchak A, Fischer J. The influence of 8 weeks of whey-protein and leucine supplementation on physical and cognitive performance. *Int J Sport Nutr Exerc Metab.* 2010;20(5):409-417.
85. Leenders M, Verdijk LB, van der Hoeven L, et al. Prolonged leucine supplementation does not augment muscle mass or affect glycemic control in elderly type 2 diabetic men. *J Nutr.* 2011;141(6):1070-1076.
86. Ispoglou T, King RF, Polman RC, Zanker C. Daily L-leucine supplementation in novice trainees during a 12-week weight training program. *Int J Sports Physiol Perform.* 2011;6(1):38-50.
87. Casperson SL, Sheffield-Moore M, Hewlings SJ, Paddon-Jones D. Leucine supplementation chronically improves muscle protein synthesis in older adults consuming the RDA for protein. *Clin Nutr.* 2012;31(4):512-519.
88. Walker AK, Danysh BP, Christian DL, Remus JL, Hofmann M, Dantzer R. Rapamycin inhibits antidepressant effects of leucine in lipopolysaccharide-treated mice. *Brain, Behavior, and Immunity.* 2016;57, Supplement:e12-e13.
89. Morris DW, Trivedi MH. Measurement-Based Care for Unipolar Depression. *Current Psychiatry Reports.* 2011;13(6):446-458.
90. Zhang Z, Peluso MJ, Gross CP, Viscoli CM, Kernan WN. Adherence reporting in randomized controlled trials. *Clin Trials.* 2014;11(2):195-204.
91. Suffredini AF, Hochstein HD, McMahon FG. Dose-related inflammatory effects of intravenous endotoxin in humans: evaluation of a new clinical lot of Escherichia coli O:113 endotoxin. *J Infect Dis.* 1999;179(5):1278-1282.

92. Research Version of the SCID for DSM-5 (SCID-5-RV). Structured Clinical Interview for DSM Disorders (SCID). 2018; https://www.columbiapsychiatry.org/research/research-areas/services-policy-and-law/structured-clinical-interview-dsm-disorders-scid-2. Accessed August 31, 2018.
93. Buysse DJ, Reynolds CF, 3rd, Monk TH, Berman SR, Kupfer DJ. The Pittsburgh Sleep Quality Index: a new instrument for psychiatric practice and research. *Psychiatry Res.* 1989;28(2):193-213.
94. Trivedi MH, Rush AJ, Ibrahim HM, et al. The Inventory of Depressive Symptomatology, Clinician Rating (IDS-C) and Self-Report (IDS-SR), and the Quick Inventory of Depressive Symptomatology, Clinician Rating (QIDS-C) and Self-Report (QIDS-SR) in public sector patients with mood disorders: a psychometric evaluation. *Psychol Med.* 2004;34(1):73-82.
95. Beck AT, Steer RA, Ball R, Ranieri W. Comparison of Beck Depression Inventories -IA and -II in psychiatric outpatients. *J Pers Assess.* 1996;67(3):588-597.
96. Beck AT, Epstein N, Brown G, Steer RA. An inventory for measuring clinical anxiety: psychometric properties. *J Consult Clin Psychol.* 1988;56(6):893-897.
97. Montgomery SA, Asberg M. A new depression scale designed to be sensitive to change. *Br J Psychiatry.* 1979;134(4):382-389.
98. Russell D, Peplau LA, Cutrona CE. The revised UCLA Loneliness Scale: concurrent and discriminant validity evidence. *J Pers Soc Psychol.* 1980;39(3):472-480.
99. McCaffrey RJ, Ortega A, Orsillo SM, Nelles WB, Haase RF. Practice effects in repeated neuropsychological assessments. *The Clinical Neuropsychologist.* 1992;6(1):32-42.
100. Heinrich LM, Gullone E. The clinical significance of loneliness: a literature review. *Clin Psychol Rev.* 2006;26(6):695-718.
101. Kelley KW, Bluthe RM, Dantzer R, et al. Cytokine-induced sickness behavior. *Brain Behav Immun.* 2003;17 Suppl 1:S112-118.
102. Eisenberger NI, Lieberman MD. Why rejection hurts: a common neural alarm system for physical and social pain. *Trends Cogn Sci.* 2004;8(7):294-300.
103. Williams KD, Cheung CK, Choi W. Cyberostracism: effects of being ignored over the Internet. *J Pers Soc Psychol.* 2000;79(5):748-762.
104. van der Helm E, Gujar N, Walker MP. Sleep deprivation impairs the accurate recognition of human emotions. *Sleep.* 2010;33(3):335-342.
105. Dodich A, Cerami C, Canessa N, et al. Emotion recognition from facial expressions: a normative study of the Ekman 60-Faces Test in the Italian population. *Neurol Sci.* 2014;35(7):1015-1021.
106. Izuma K, Saito DN, Sadato N. Processing of Social and Monetary Rewards in the Human Striatum. *Neuron.* 2008;58(2):284-294.
107. Costa PT, Jr., McCrae RR, Norris AH. Personal adjustment to aging: longitudinal prediction from neuroticism and extraversion. *J Gerontol.* 1981;36(1):78-85.
108. Eisenberger NI, Berkman ET, Inagaki TK, Rameson LT, Mashal NM, Irwin MR. Inflammation-induced anhedonia: endotoxin reduces ventral striatum responses to reward. *Biol Psychiatry.* 2010;68(8):748-754.
109. Weischer M, Nordestgaard BG, Cawthon RM, Freiberg JJ, Tybjaerg-Hansen A, Bojesen SE. Short telomere length, cancer survival, and cancer risk in 47102 individuals. *J Natl Cancer Inst.* 2013;105(7):459-468.
110. Simes RJ. An improved Bonferroni procedure for multiple tests of significance. *Biometrika.* 1986;73(3):751-754.