

IRON DEFICIENCY AND DEPRESSIVE MOOD IN HISPANIC WOMEN

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Iron deficiency and depression occur predominantly in women, in both developed and developing countries. Our aim was to improve understanding of the association between iron deficiency and depressive mood. We explored the role of fatigue in the relationship between iron deficiency and depressive mood, the effect of confounding factors, and whether stress was a modifier of the association of iron deficiency and depressive mood. Two cross-sectional data sets were used to implement our research objectives. One was the 1982-1984 Hispanic Health and Nutrition Examination Survey (HHANES). The other was an observational study with Spanish-speaking Mexican factory workers that was conducted in 2001. In the HHANES, iron status was represented by the continuous variable body iron stores. Severe depressive mood and a fatigue index were assessed with a modified Center of Epidemiologic Studies Depression Scale. Stress was operationalized by variables representing social and biological stressors. In the Mexican study, the variables were hemoglobin, the Beck Depression Inventory, the Fatigue Severity Scale, and cortisol response over two work days. In both studies, only women who were premenopausal and not pregnant were included.

Results were consistent between the two studies. With improving iron status, the risk of severe depressive mood decreased significantly. Fatigue had no or a minor impact on the effect of iron status. No confounders were identified for the main effect model. Stress was a significant, synergistic modifier of the association between iron

status and severe depressive mood. Women exposed to both stress and iron deficiency were at significantly higher risk of severe depressive mood compared to women who experienced none or one of the risk factors. In the Mexican sample, socioeconomic status was a negative confounder of this interaction. This research indicates that studies that explore the biological association between iron deficiency and depressive mood should focus on women who experience high stress levels. In a population with limited access to medical services, improving iron status in women of reproductive age might be a good alternative to drug therapy in order to alleviate severe depressive mood.

BIOGRAPHICAL SKETCH

Maïke Rahn was born in Berlin, Germany, on January 9, 1969. After graduating from a German high school in 1988, she started her studies in biology and anthropology in Göttingen, Germany. In her search for a suitable research topic for her diploma, she encountered Jere Haas at Cornell University, who advised and mentored her throughout her cross-national research project. During the same period, she also met her future husband, Gary Siegel. After completing her German diploma in 1996, Maïke moved to New York City, where she and Gary were married. In New York City, Maïke working in Public Health research for four years; in 2000, she returned to Cornell University (and her advisor Jere Haas) in order to pursue a Ph.D. in nutrition and epidemiology. Maïke was privileged to conduct domestic and international research for her doctoral dissertation topic. During the course of her studies, Maïke and Gary became the fortunate parents to their children, Emil and Aila.

To my family, and their love, spiritedness, and support

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LIST OF ABBREVIATIONS

ACTH	Adrenocorticotropin
AUC	Area under the cortisol curve
AUC3E	Area under the cortisol curve, for 3 time points over a day, with actual time when reported, and estimated time, when not reported
BDI	Beck Depression Inventory
BMI	Body mass index
CESD	Center of Epidemiologic Studies- Depression Scale
CESD-17	CESD with 17 statements (minus fatigue-related statements)
CIDI	Composite International Diagnostic Interview
CRF	Corticotrophine releasing factor
CRP	C-reactive protein
CRH	Corticotrophine releasing hormone
DALYs	Disability-adjusted-life-years; health gap measure; incorporates years of life lost due to mortality, and years of healthy life lost to disability
DIS	Diagnostic Interview Schedule
FSS	Fatigue Severity Scale
GHQ	General Health Questionnaire
HHANES	Hispanic Health and Nutrition Examination Survey
HPA axis	Hypothalamus-pituitary gland-adrenal gland axis
ID	All iron deficiency; iron deficient
IDA	Iron deficiency anemia Hemoglobin < 120 (123) g/L; and two additional indicators beyond cut-off (MCV<80 fL, ferritin <12 µg/L, transferrin saturation < 16%, protoporphyrin > 70 µg/dL)

IDeA	Iron depletion with anemia Hemoglobin < 120 (123) g/L; and one additional indicators beyond cut-off (MCV < 80 fL, protoporphyrin > 70 µg/dL)
IDeNA	Iron depletion without anemia Hemoglobin ≥ 120 (123) g/L; and one additional indicators beyond cut-off (MCV < 80 fL, protoporphyrin > 70 µg/dL)
IDNA	Iron deficiency without anemia Hemoglobin ≥ 120 (123) g/L; and two additional indicators beyond cut-off (MCV < 80 fL, ferritin <12 µg/L, transferrin saturation < 16%, protoporphyrin > 70 µg/dL)
INSP	Mexican National Institute of Public Health (Instituto Nacional de Salud Publica).
MDD	Major depressive disorder
MCV	Mean corpuscular volume
NHANES	National Health and Nutrition Examination Survey
PMS	Premenstrual syndrome
PMDD	Premenstrual dysphoric disorder

Chapter 1: INTRODUCTION

Public Health Relevance

In adults, iron deficiency (ID) and depression are disorders found predominantly in women of reproductive age [1-10]. Both represent a substantial public health burden worldwide; prevalence of iron deficiency anemia (IDA) in reproductive age women is 48% [1]. Likewise, prevalence of lifetime major depressive disorder (MDD) in women is between 1.8 and 23.1% [2]; rates of dysthymia range from 5.4 to 12.2% [2]. In the developing world, IDA was the 3rd leading cause of increased Disability-Adjusted-Life-Years (DALYs) in women aged 15 to 45 (4.1% of all causes of DALYs) [3]. MDD worldwide was the leading cause of DALYs, with 13.7% of total DALYs in women, whereas in developed countries it accounted for 19.8%.

In the US, 5% of reproductive age women suffer from IDA; iron deficiency without anemia (IDNA) occurs in 11% [4]. Hispanic American women of reproductive age experience a wide range of ID prevalence: 4% of Mexican Americans have IDA [4], while between 6.5 and 20% of Hispanic American women experience IDNA [4, 5]. In Mexico, the prevalence of anemia (this definition includes all types of anemia, not only IDA) was 20.8% in non-pregnant women 12–49 years of age in 1999 [6]; however, this level dropped to 16% in 2006 [7]. Low transferrin saturation¹ (a measure of IDNA) was observed in 40.5% of Mexican women in 1999 [9].

Prevalence of lifetime MDD of women in the US has been estimated to be between 6 and 21.3% [10-12]; MDD within the previous 12 months was 12.9% [10]. Prevalence of lifetime MDD in Hispanic Americans was 12.3% [13]. Important to note is that lack of acculturation appears to have a protective effect in Hispanic

¹ Transferrin saturation quantifies the amount of transport iron in blood plasma. A value below 16% is considered an indicator of ID [8].

Americans; thus, recent immigrants have lower rates of depression than those who were born in the US or are more acculturated [13-19]. For women in Mexico, the lifetime prevalence of MDD is 15.9% [20]. MDD in the previous 12 months occurred in 5.8 to 7.6% of the Mexican women [20, 21], and MDD within 6 months in 5.7% [20]. Note that only the study of Bello et al. [21] was nationally representative, while the other two studies represent urban centers [20].

ID in the Brain: Effect on Neurotransmitters and Depression

Serum ID has been demonstrated to translate directly into brain ID [22-24]. Moreover, iron is a cofactor in the synthesis of serotonin, dopamine, and norepinephrine [25-27]. It stands to reason that ID should result in lower concentrations of these three neurotransmitters in the brain. The actual association between ID and the neurotransmitters serotonin and norepinephrine, though, is inconsistent. One study with ID animals showed the expected decrease in serotonin concentrations [28]; another found no change [29]. Activity of tryptophan hydroxylase, the iron-dependent enzyme that is part of the pathway that converts tryptophan to serotonin, appears to be unchanged [28, 30]. An increase [30] or decrease [30] in serotonin metabolites with ID was observed. The neurotransmitter norepinephrine has rarely been studied. It appears to remain unchanged [30] or is higher [31, 32] in ID rats. Better information is available on dopamine: ID appears to decrease the density of dopamine receptor D2 [33, 34]; dopamine transporters are downregulated as well, resulting in a slower reuptake of dopamine in the presynaptic cell [35, 36]. In conjunction, dopamine levels have been found to be higher in ID rats [29, 32]; dopamine metabolites are lower [29]. Last, monoamine oxidase, which breaks down all three neurotransmitters, has been shown to decrease in ID animals [28, 30].

In humans, observational studies generally confirm that ID is associated with depression [37-40], albeit one study found no relationship [41]. A total of 5 supplementation trials (4 with placebo controls, and 1 with a high-iron-diet control) were undertaken to explore the same association. Four of the 5 trials found no association [42-45]; all of these trials were undertaken with female participants from developed countries (USA, Australia, and Switzerland). Only one study with poor, postpartum South African women reported an effect of the supplementation compared to placebo [46].

ID, Fatigue, and Depression

ID has been associated with a decreased ability to perform physical labor [47-51], and with perceived fatigue [39, 43-45, 52]. In addition, fatigue is associated with depression, to the extent that it is a diagnostic criterion for all mood disorders related to depression [53]. Thus, correlations between fatigue and depression are generally between $r=0.3$ and 0.6 [54-59]. The associations between ID, fatigue, and depression have not been explored, even though all three have been assessed together in a number of studies [38, 39, 41, 43, 45]. This is of importance, since the lack of evaluation of the effect of fatigue on the relationship between ID and depression might result in misclassification. For example, participants who experience higher fatigue (but not depression) due to ID would nevertheless be rated as more depressed in a depression assessment instrument; this would be a consequence of their increased scores in the fatigue section. Thus, ID individuals would be at a statistically higher risk of depression, even though they are in fact not more depressed, just more fatigued. This potential for misclassification was recognized by Pollitt [60].

ID, Confounders, and Depression

ID and depression in women share several underlying causes. These include low socioeconomic status (SES) [4, 12, 18-21, 61-86]; age [4, 10, 18, 20, 21, 74, 82, 86, 87]; ethnic group [4, 12, 71, 83, 84, 88, 89]; perceived lack of control over one's environment or health [17, 63, 78, 90]; social isolation [81, 91]; disrupted marital status including divorce, widowhood, and separation [18, 20, 65, 68, 74, 92, 93]; and postpartum status or higher parity [4, 83-86, 94]. However, the majority of articles investigating the association between ID and depression in humans do not indicate control of confounders [37, 40, 41, 43, 45, 46], thus increasing the likelihood of spurious relationships.

ID, Stressor Exposure, and Depression: Interaction and Biological Mechanisms

It is intriguing that the 1 of 5 iron supplementation trials that showed significant effects of supplementation on depressive mood took place in a developing country with postpartum women [46]. In contrast, the remaining 4 trials all had negative results [42-45]; participants were women in developed countries who were not in the postpartum stage of their life. It stands to reason that these different results might be due to the interaction of stressor exposure (poverty and postpartum status) and ID. If both exposures act independently on neurotransmitter availability to postsynaptic neurons, then a synergistic increase of the risk of depression appears possible.

Stressor exposure is a well-established risk factor of depression. Chronic exposure to stressors overuses the stress system of the hypothalamo-pituitary-adrenal (HPA) axis [95]. The HPA axis is ultimately a system that evolved in order to respond to short-term (acute) stress [95, 96]. The overuse of this response system is called allostatic overload [95, 97]. A large number of stressors have been detected in previous research: low SES [64, 98], job strain [99-101], number of hours worked

[102], sleep deprivation [103], low social support at work [104] or in one's personal life [102], home strain (number of children) [102, 105], daily perceived stressors [106], and negative affect [106]. Buffers of stressors are good coping ability, high self-efficacy, and good social support [107-110]. In the literature, identified stressors that are associated with an increased risk for depression include low SES [64-66], food insecurity [111], not being married [65, 93], low social support [91], lack of control at home or at work [90], job strain [100] or longer-term unemployment [65]. Low mood has also been reported as a result of a trial inducing sleep deprivation [112].

In recent years, the biological mechanisms that associate stressor exposure or perceived stress and depression are better understood. The interlacing of three complex systems – stress, immunity, and neurotransmitters – appears to explain the association.

Stressor exposure initiates the discharge of corticotrophine releasing hormone (CRH) from the hypothalamus [96]. CRH is taken up by the pituitary gland, which in turn produces adrenocorticotropin (ACTH). ACTH initiates the release of cortisol from the adrenal gland. Cortisol increases glucose availability, effectively providing energy for a potentially life-saving “fight and flight” response [113]. Downregulation of cortisol output occurs via a negative feedback loop with two types of cortisol receptors in the brain. The mineralocorticoid receptors prevent a disturbance of the cortisol homeostatis [114]. The glucocorticoid receptors, which have a low affinity for cortisol, downregulate cortisol output back to normal levels after a stress response [114]. Binding of larger amounts of cortisol on the glucocorticoid receptors, therefore, decreases the output of the HPA cascade [115].

The key hormones of the HPA axis, which are involved in feedback loops with the immune system and the brain, are CRH and cortisol [96].

Two phenomena have been observed in stressed individuals: hypercortisolism and hypocortisolism. Hypercortisolism is usually considered a sign of acute stress [96, 113], and it is associated with depression [115, 116]. The causal mechanism is probably an impairment of the negative feedback-loop of cortisol to HPA cascade [115]; specifically, glucocorticoid receptors might not be as sensitive to cortisol as in healthy individuals [117, 118].

Hypocortisolism is thought to be a consequence of chronic stress [119] or previous traumatic experience [113, 115, 120, 121]. It is characterized by cortisol levels that are lower than normal and/or a blunted stress response [120-122]. Hypocortisolism might be caused by one or several of the following mechanisms: a) adrenal exhaustion (either primary or after prolonged periods of high cortisol output), b) downregulation of CRF after a period of hypersecretion, c) CRF hypersecretion and pituitary CRF receptor downregulation, d) increased sensitivity of glucocorticoid receptors, or e) morphological changes in tissues involved in the HPA axis [115, 120, 121]. Hypocortisolism is associated with depression, but also with a large number of other disorders such as chronic pain, arthritis, asthma, posttraumatic stress disorder, obesity, or fatigue (chronic fatigue syndrome), to name a few [96, 113, 115, 119, 120, 122, 123].

All types of stressors (acute or chronic) are associated with activation of the immune system and the release of proinflammatory cytokines such as tumor necrosis factor, interleukin-1, and interleukin-6 [115]. When negative feedback of cortisol is inhibited such as occurs in MDD, acute phase proteins and plasma and central nervous system proinflammatory cytokines are upregulated [115].

Originally, depression was considered solely a consequence of low concentrations of neurotransmitters in the synaptic cleft [124]. Therefore, signal transmission from one nerve cell to the next was impaired. Evidently serotonin,

norepinephrine, and dopamine were the primarily involved neurotransmitters [114, 122, 124-129]. In addition, evidence attributes depression to insufficient sensitivity of dopamine receptors (called D2-like receptors) [126, 127].

Recently, depression is seen as a consequence of an inflammatory immune response [115, 130-134], which in turn can be activated by stress [115]. The accumulated evidence is substantial.

In the psychoneuroendocrinological literature the phenomenon is described as “cytokine-induced sickness behavior” [132, 135, 136]. Elevated cytokine levels induce symptoms such as loss of appetite, sleepiness, withdrawal, fear, and fatigue [130, 133, 135, 136], probably as a consequence of the modulation of the neurotransmitters serotonin, norepinephrine, and dopamine in brain areas that are associated with emotional regulation and psychomotor function and reward [137]. It is striking how much sickness behavior mirrors the symptoms of depression. Thus it is thought that depression and sickness behavior may be due to the same or to a similar mechanism, inflammation in the brain [132, 135-137].

It has been shown that high levels of proinflammatory cytokine in plasma correspond to high cytokine levels in the brain [115, 130, 136]. Therapies involving the administration of the cytokine interferon- α frequently induce severe depression as a side effect [132, 133, 137].² Animals that exhibit cytokine-induced sickness-behavior recovered when treated with cytokine antagonists or anti-inflammatory cytokines [137]. In humans, cytokines (interleukin-6) appear to be upregulated in melancholic MDD compared to minor depression or non-depressed subjects [96]. Antidepressant treatment appears to reverse this dysfunction: patients who exhibit depression as a side effect of cytokine treatment can go into remission [132, 137]. In fact, antidepressant treatment reestablishes the normal inhibitory control of cortisol on

² Interferon- α activates other proinflammatory cytokines such as interleukin-6, interleukin-1, and tumor necrosis factor [138].

immune cells [115]. It has also been found to decrease proinflammatory cytokines and to increase anti-inflammatory cytokines [137, 139].

The two states of abnormal cortisol levels, hypo- and hypercortisolism, have both been linked to increased circulating cytokine concentration.

The association between high cortisol concentrations and/or stressor exposure, high proinflammatory cytokine levels, and depression is well established. Apparently, moderate stressor exposure results in a subsequent upregulation of proinflammatory cytokines [115, 120, 137, 140]. High circulating cortisol concentrations are received by cortisol receptors on lymphocytes and normally downregulate their proinflammatory cytokine output [96]. Thus, glucocorticoids act as anti-inflammatory agents [96, 115, 120, 121]. Similarly, while acute stressor exposure initially shows no reactivity of the immune system, a subsequent upregulation of immune response can be observed [120, 130, 137]. In general, healthy subjects without MDD show immune suppression when exposed to high cortisol levels, while subjects with MDD show reduced responsiveness to this cortisol challenge [115]. This is thought to be due to decreased sensitivity of lymphocytes to cortisol stimulus [141]. Evolutionary theory has been employed to expand on this evidence: individuals with a ready-to-act immune system are at an advantage compared to their less responsive peers since they have a better chance of survival (and subsequent reproduction) when faced with physical threat and potential injury [115, 122].

Depression has also been associated with hypocortisolism and/or reduced responsivity to stressors [122, 142, 143]. A second hypothesis of the association between stress and depression has therefore been developed. Since high circulating cortisol levels act as immune system suppressants, inhibiting the secretion of proinflammatory cytokine [96, 115, 120, 121], low cortisol levels might be responsible for a disinhibition of the immune system and for excessive release of cytokines [119,

140], thereby increasing the risk for depression [115, 120, 121]. Studies have shown that participants exposed to chronic stress and/or with downregulated cortisol show an upregulated immune system [119, 120, 140]. It is thought that downregulation of cortisol during chronic stress might have a protective effect, since a chronically suppressed immune system does not allow for optimal defense against antigens during acute physical trauma [115, 121, 122].

MDD has been brought into context with both hyper- and hypocortisolism. Melancholic MDD, which defines a state of hyperarousal in depressive disorder [53, 144], seems to be associated with upregulation of CRH and cortisol [96, 113, 122]. Twenty to 30 percent of all patients with MDD suffer from melancholic MDD [122].

Hypocortisolism, characterized by low levels of CRH, low or normal levels of cortisol, or reduced responsiveness of cortisol during acute stress, appears to cause atypical depression³ and seasonal affective disorder [96, 113, 122, 144]; atypical depression is defined by increased fatigue and weight gain. It should be noted that the term atypical depression may be misleading since it indeed is a common diagnosis in MDD: 15 to 30% of MDD cases experience atypical depression [122].

Both subtypes of depression are associated with depressive mood and lack of pleasure in life [122].

Studies not differentiating between melancholic and atypical depression usually find a glucocorticoid hypersecretion [115].

Hyper- and hypocortisolism are both associated with changes in neurotransmitter levels. Hypercortisolemia and melancholic MDD occur together with upregulated norepinephrine [122], albeit studies are not entirely consistent. It has also been shown that exposure to acute stress increases the amount of norepinephrine in the brain [145]. However, patients who have symptoms concordant with hypocortisolism

³ Atypical depression is characterized by hypersomnia, hyperphagia, lethargy, fatigue, and apathy [122].

show also an upregulation of the neurotransmitter norepinephrine [121]. It has been suggested that norepinephrine is centrally stimulated by cortisol or CRH [122]; in light of the previously mentioned evidence it seems most likely that the upregulation is due to CRH or cytokines. In terms of serotonin, high cortisol levels appear to increase the expression of its transporter gene, thus decreasing available serotonin at the synaptic cleft, and increasing the likelihood of depression [146, 147]. Last, low community SES and low personal SES – both chronic stressors – have been shown to decrease serotonergic responsivity [66]. Dopamine has been investigated in context with cortisol as well: hypercortisolemia is associated with suppression of the mesolimbic dopaminergic system. Lack of dopamine might be the cause for the anhedonia of depression [113].

Cytokine exposure and neurotransmitter availability are associated as well: the availability of serotonin appears to decrease during cytokine exposure as a result of degradation of tryptophane [125, 133, 137]; thus, tryptophane cannot be synthesized into serotonin. It appears as well that serotonin transporters are activated by proinflammatory cytokines, thus reducing serotonin even further [125]. Other studies, though, have found an increase in serotonin and norepinephrine upon release of proinflammatory cytokines [130, 132].

Finally, a note of caution is in order concerning the stated biological mechanisms. The interactions between the stress, immune, and neurotransmitter systems are complex. Numerous positive and negative feedback mechanisms exist. Thus, directionality is at times in question. For instance, proinflammatory cytokine exposure decreases the number and affinity of glucocorticoid receptors [120], thus creating a feedback from cytokine concentrations to the negative cortisol feedback loop. Other studies have found that cytokines stimulate the HPA axis [132, 133, 137].

Catecholaminergic fibers project into the hypothalamus, thus probably affecting CRH release [122].

In summary, ID and stress both affect neurotransmitter availability. As a consequence, they could synergistically increase the risk of depression.

The theoretical framework of this dissertation is presented in Figure 1.1. While ID and depression are associated, we find two pathways. One is the association between ID, decreased neurotransmitter concentrations in the brain, and increased risk of depression. The second pathway is the association between ID, fatigue, and depression. This would imply a misclassification of fatigued persons as depressed. Confounders might account partially or fully for the relationship between ID and depression. Last, stress might modify the association between ID and depression, resulting in a higher risk of depression in participants exposed to both ID and stress.

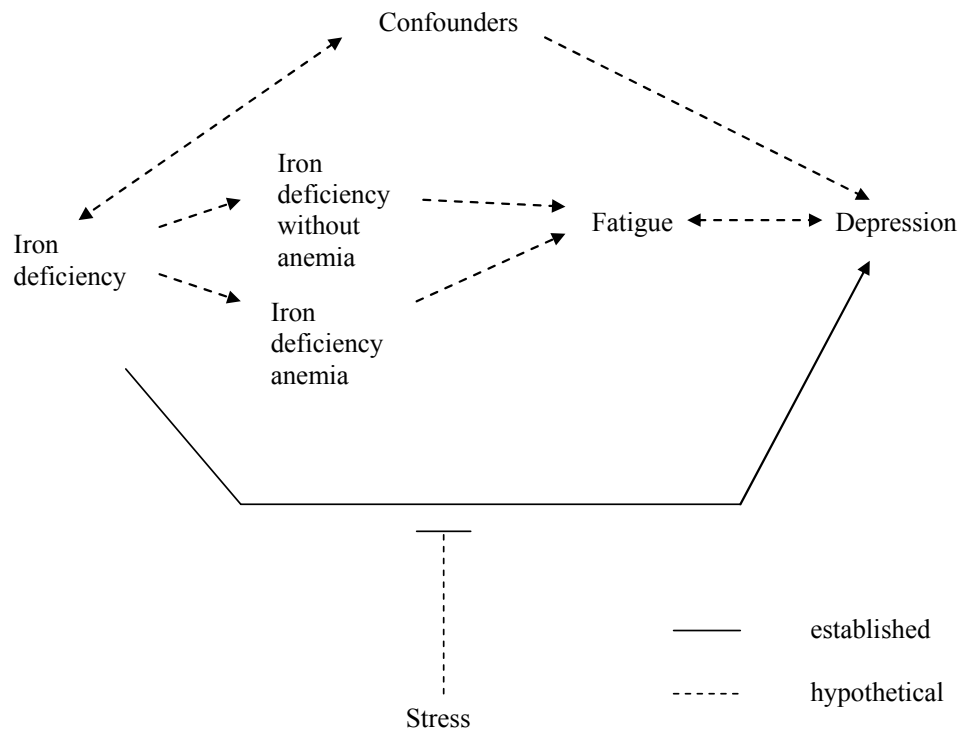


Figure 1.1: Relationship between iron deficiency and depression.

The hypotheses in this dissertation will explore the plausibility of the association between ID and depressive mood. They are the following:

- 1) ID increases the risk of depressive mood.
- 2) The association between ID and risk of depressive mood remains significant despite the statistical control for fatigue, thus indicating an effect of brain ID on depressive mood. Nevertheless, fatigue might account for some of the association of ID and risk of depressive mood, which would indicate a second pathway via the mediator fatigue.
- 3) The association between ID and risk of depressive mood is significant despite the statistical control of confounders. Confounder control might affect the strength of the association between ID and risk of depressive mood.
- 4) Inclusion of stressor exposure or stress as a modifier of the association between ID and risk of depressive mood will show a higher risk of depressive mood for individuals with ID and stress, compared to all the other possible combinations (ID and no stress exposure, no ID and no stress exposure, and no ID and stress exposure).

Chapter 2: IRON DEFICIENCY AND DEPRESSIVE MOOD IN HISPANIC AMERICAN WOMEN⁴

Abstract

Background: Both iron deficiency and depression are most likely to occur in premenopausal women. Although an association between iron deficiency and depression has been shown in a number of studies, the role of fatigue, confounding, factors and stressor exposure either was not or was only infrequently considered.

Objectives: To explore a) the mediating effect of fatigue in the relationship between iron deficiency and depression, b) the role of confounders, and c) the synergistic effect of stressor exposure.

Methods: Data from 1375 Hispanic American women of Cuban, Puerto Rican, and Mexican descent were retrieved from the Hispanic Health and Nutrition Examination Survey (HHANES), 1982–1984. The women were between 20 and 55 years of age and had not reached menopause. Exclusion criteria were menopause, pregnancy, abuse of cocaine or inhalants, macrocytic anemia, and ferritin levels > 200 µg/L. Previous week's depressive mood was assessed with the 20-statement Center of Epidemiological Studies – Depression scale (CESD-20). A continuous body iron store variable was calculated that included the iron status indicators hemoglobin, ferritin, protoporphyrin, and transferrin saturation. Adding the scores of three fatigue-related questions of the CESD-20 created a previous-week's fatigue index. Consequently, a shortened version of the CESD-20 was introduced since the CESD-17 excluded the statements that composed the fatigue scale. Binary logistic regression analysis with the dichotomized CESD-17 contrasted cases with a score above the 90th percentile against cases with a score below the 90th percentile. Body iron stores and previous-week's fatigue were the independent variables of principal interest, while other confounders

⁴ Maïke Rahn, Jere D. Hass, Division of Nutritional Sciences, Cornell University, Ithaca, NY, 14853.

were considered. Interactions between iron stores and potential stressors assessed the synergistic effect of stressor exposure on the relationship between iron stores and depressive mood. All models were adjusted for complex survey sampling methods.

Results: Of the sample, 6% were iron deficient anemic (hemoglobin < 120 g/L and two additional iron status indicators beyond cutoff⁵), and 12% were iron deficient without anemia (hemoglobin \geq 120g/L, but two additional indicators beyond cutoff⁵). Body iron stores had a mean of 295 mg and ranged from -1140 mg to 1117 mg. A total of 18% were assessed as depressed with a CESD-20 score greater than 16 points; while 7.9% were above the 90th percentile of the CESD-17. Fifty percent reported some previous-week's fatigue, and 3% reported high previous-week's fatigue. The logistic regression model revealed that iron stores showed a significant negative relationship (odds ratio 0.89, confidence interval 0.82 to 0.97) with depressive mood after controlling for previous-week's fatigue, socioeconomic status (SES), and ethnic group. Modifiers of the relationship between iron deficiency and depressive mood were the stressors post-pregnant and postpartum status, low SES, and low control over one's health. Women with lower iron stores and higher stressor exposure were at greater risk of experiencing depressive mood.

Conclusions: Iron deficiency and risk of depressive mood had an inverse association despite the inclusion of previous-week's fatigue and potential confounders. Every 100 mg decrease of iron stores increased the odds ratio of severe depressive mood by 11%. The relationship was modified by post-pregnant status, socioeconomic status, and perceived control over one's health. Exposure to both a stressor and low iron stores increased the risk of severe depressive mood significantly, while exposure to only one or no risk factor did not.

⁵ MCV<80 fL, ferritin <12 μ g/L, transferrin saturation < 16%, protoporphyrin > 70 μ g/dL

Keywords: depression, mood, iron, fatigue, stressor, SES, postpartum, Hispanic women

Introduction

Iron deficiency (ID) and depression both occur commonly in young and middle-aged women. In the US, five percent of women between 20 and 49 years of age suffer from IDA [4], while iron deficiency without anemia (IDNA) occurs in 11 percent. The prevalence of major depression over 12 months for women in the US was shown to be 12.9 percent [10].

The relationship between ID and depression has been described frequently in the research literature. Observational studies generally find an association between the two disorders [37-40]. An exception were Hunt and Penland [41], who were not able to find a connection. Recently, four randomized placebo-controlled iron supplementation trials with non-clinical populations with mixed results have been added to the literature. One study included 143 female US college students with iron status ranging from IDA to iron sufficient. The authors were not able to establish a significant improvement of depression scores with iron supplementation [42, 148]. The second study included 52 IDNA or iron-sufficient women, and did likewise not find an improvement in depression scores [43]. The third trial, with 366 IDNA women, was also not able to establish an association between iron supplementation and depression [44]. The authors had excluded all women with psychiatric disorders. The fourth study took place with 95 women in a community near Cape Town in South Africa: depression scores were alleviated in IDA women after an iron supplementation from 6 weeks to 9 months postpartum [46]. A fifth supplementation trial with 44 IDNA Australian women did not use a placebo group but instead used a high iron diet as the control condition [45]. While the iron supplemented group showed a significant

improvement in ferritin levels, no differences in mental health scores could be found between the two groups at the end of the trial.

It is generally thought that ID results in an alteration of neurotransmitter concentrations in the brain, thus causing depression. Indeed, iron is a cofactor in the synthesis of serotonin, dopamine, and norepinephrine [25-27]. Consequently, lower brain iron concentration should result in lower concentrations of at least one of the three neurotransmitters. However, the results for serotonin and norepinephrine are not consistent [28-32]. The neurotransmitter dopamine gives the best indication of iron-deficiency-induced altered nerve-signal transmission due to lower D2 dopamine receptor density and transport downregulation [29, 32-36]. In addition, the activity of monoamine oxidase, which is involved in the breakdown of all three neurotransmitters, seems to be reduced [28, 30]. Alteration of concentrations of serotonin, dopamine, and/or norepinephrine in the brain are primarily implicated as causes of depression [124, 149].

Challenging the results of the relationship between ID and depression in human studies, though, are a number of limitations that – to our knowledge – have rarely or never been addressed in the published literature. First, no study has included fatigue when considering the relationship between ID and depression. A number of studies measured all three variables [38, 39, 41, 43, 45] but did not analyze the interrelationships. This is of importance since fatigue is both a result of ID [39, 43-45, 52] and a diagnostic criterion for several types of depression [53]. Hence, fatigue and depression are associated [54-59]. Failure to account for fatigue might result in misclassification of solely fatigued cases as depressed, because depression scale scores are likely to be higher in fatigued than in non-fatigued subjects. Pollitt [60] mentioned this possibility in a discussion of ID and behavior change in children.

Second, the majority of studies investigating the association between ID and depression are observational in nature, but few employed sufficient confounder control. Thus, the observed relationship might be caused by an unknown, underlying factor [60, 150]. In reality, a number of common risk factors have been identified for depressive mood, and lower intake of iron absorption enhancers or ID in women, such as lower socioeconomic status (SES) [4, 12, 18-21, 61-86], age [4, 10, 18, 20, 21, 74, 82, 86, 87], social isolation [81, 91], disrupted marital status or being unmarried [18, 20, 65, 68, 74, 92, 93], perceived lack of control over one's environment or health [17, 63, 78, 90], ethnic group [4, 12, 71, 83, 84, 88, 89], and postpartum status or parity [4, 74, 82-86, 94, 151, 152]. In addition, food insufficiency by itself has been identified as a risk factor of depressive symptoms [111].

It is of additional interest whether specific subpopulations might be at higher risk for depression: individuals experiencing stressors such as low SES might have a greater likelihood of depression when ID than subgroups who are exposed only to one or none of the risk factors. Exposure to chronic stress appears to reduce serotonin availability [121, 122, 145] and to increase norepinephrine concentrations [6, 7, 9, 130, 132] in the brain via an upregulation of the non-specific plasma immune response and proinflammatory cytokines [115, 119, 120, 130, 136, 140]. Consequently, individuals exposed to chronic stress are at higher risk of atypical major depressive disorder (MDD) [96, 113, 122].

Our observational study conducted in Mexico in 2001 indicates that an inverse association between hemoglobin and risk of severe depressive mood is indeed maintained when controlling for fatigue and SES covariates (Chapter 3). In addition, area under the cortisol curve over time of day was a modifier of this association. Women who were below the median of the area under the cortisol curve showed a significantly increasing risk of severe depressive mood with decreasing hemoglobin

values, while women equal to or above the median did not show a significant association (Chapter 4).

In summary, three primary research questions will be explored: 1) Does fatigue account partially or fully for the relationship between ID and depression? 2) Is the relationship between ID and depression partially or fully due to confounding? and 3) Does exposure to stressors result in an amplification of the relationship between iron status and depressive mood?

Methods

Sample selection

The relationship between ID and depression in women was explored with the Hispanic Health and Nutrition Examination Survey (HHANES), a public-use data set provided by the National Center of Health Statistics (<http://www.cdc.gov/nchs/nhanes.htm>). Target populations of the 1982 to 1984 survey were Puerto Ricans in the New York City (NYC) region, Cubans in Miami, and Mexicans in the Southwest of the United States. The sampling procedures of the HHANES were complex [153]: primary sampling units (PSUs) were identified when 1980 census data indicated a county with a sizeable percentage of Hispanic population. A total of 210 PSUs were included in the sampling procedure. Criteria for stratification of the PSUs differed by region: in the NYC region it was the number of Puerto Ricans, in Miami no stratification was utilized, and in the Southwest the number of Hispanics, degree of urbanization, and median income were used. Selection of a particular household required that at least one household member claimed to be of Mexican, Puerto Rican, or Cuban origin. Last, the probability of selection of participants was based on age. Children and older adults were oversampled, with different weights assigned depending on ethnic group. Interviews were conducted in either Spanish or English.

Inclusion criteria for the present data set required that respondents had, at a minimum, completed the assessment of their iron status and depressive mood (Figure 2.1). The adult data set of the HHANES, from which most of the variables for our analysis were derived, contained women age 20 and older. Exclusion criteria were the use of inhalants or cocaine, a current pregnancy, and menopause. Since the HHANES data contained no information on acute phase response indicators, ferritin values of all women with ferritin levels greater than 200 µg/L were recoded to missing, which effectively prevented their inclusion in the calculation of the body iron stores indicator (see below). Ferritin values greater than 200 µg/L in reproductive age women are considered to be artificially high as the result of an acute infection [154]. Use of antidepressants would have been an important exclusion criterion, but this information is not available in the public use data set, even though it was collected in the original HHANES.

Variables

A continuous body iron store variable was created based on an algorithm developed by Cook et al. [155]. The iron stores (mg) variable allowed for the inclusion of the iron status indicators hemoglobin, ferritin, transferrin saturation, and erythrocyte protoporphyrin, yielding a continuous score for all levels of iron status from sufficiency to depletion. Depending on the level of hemoglobin and ferritin, a total of 3 different formulas were used to calculate iron stores. A value of iron stores of less than -300 mg indicated IDA (hemoglobin < 12 g/l, and ferritin < 12 µg/L), while a value between -300 mg and 0 mg indicated IDNA (hemoglobin ≥ 12 g/l and ferritin < 12 µg/L). A value equal to or above 0 mg implied iron sufficiency. A 100 mg increase in iron stores corresponded to an 6.6 g/L increase of hemoglobin in IDA participants.

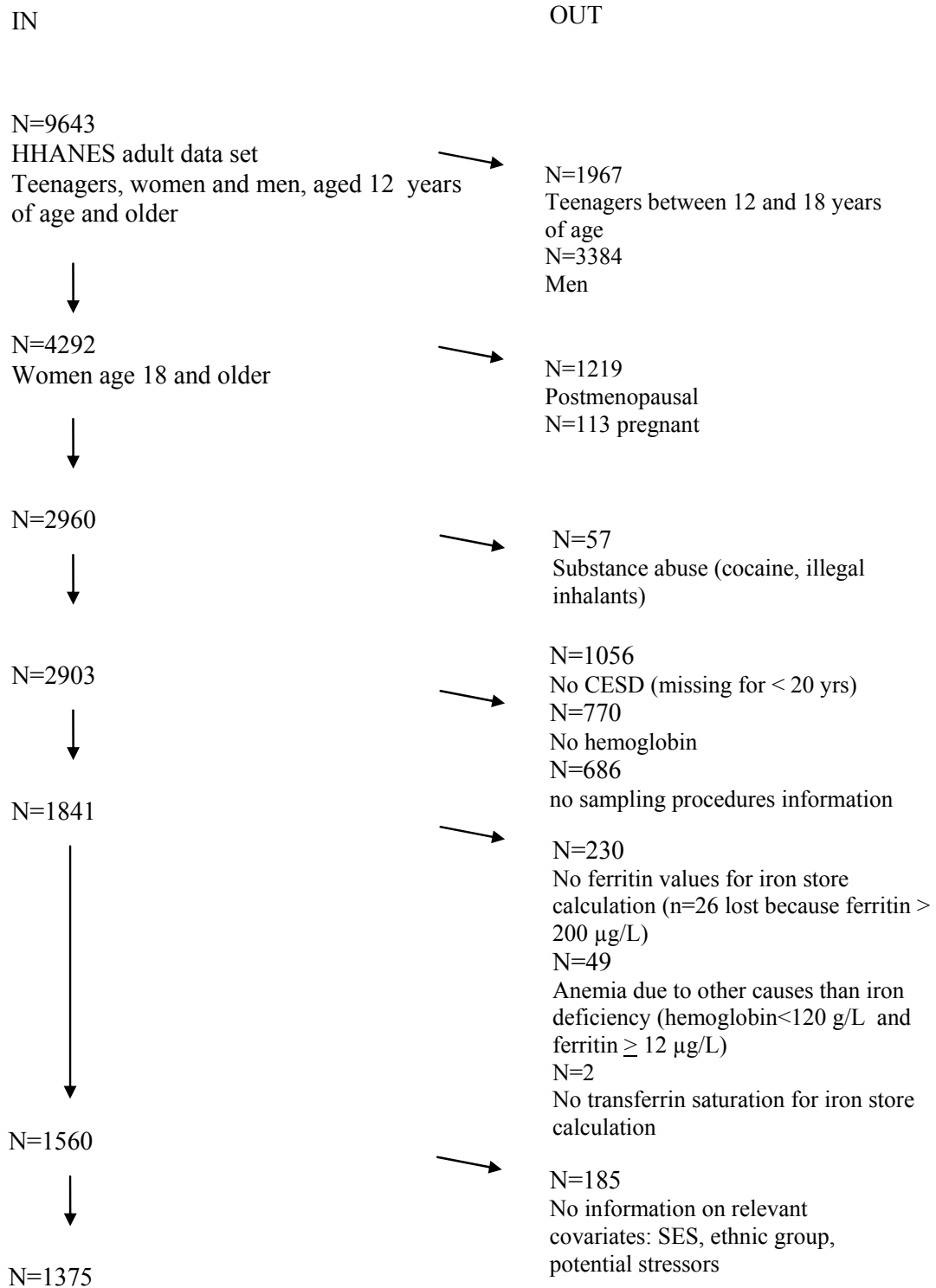


Figure 2.1: Derivation of sample size.

Depression in this population was assessed with the Center of Epidemiological Studies – Depression scale (CESD) [156]. The 20 statements of the CESD assess symptoms of depression over the week previous to the date of interview. The general domains of the scale address depressed affect, positive affect, somatic and retarded activity, and interpersonal issues. Answers are coded from 0 for “rarely or some of the time (less than 1 day)” to 3 for “most of the time (5–7 days).” The final additive score of a respondent can range between 0 and 60. A second questionnaire, the Diagnostic Interview Schedule (DIS), was used in the HHANES to assess lifetime major depression, minor depression, and dysthymia. The coding of the DIS questionnaire allowed only for differentiation between respondents with past major depressive episode(s) and with current major depressive episodes.

Two fatigue variables were created for the current analysis. Previous-week’s fatigue was constructed out of fatigue-related statements of the CESD (Appendix E, CESD). Statements relating to fatigue were “I could not get going,” “My sleep was restless,” and “I felt that everything I did was an effort.” All three statements showed sufficiently high factor loadings only in the somatic and retarded activity factor [156, 157], but not in the other factors. The scores of the three questions were added to create the previous-week’s fatigue index. The second fatigue variable, lifetime fatigue, utilized questions from the DIS: “Has there ever been a period lasting two weeks or more when you felt tired out all the time?”, “Have you ever had a period of two weeks or longer when you were sleeping too much?”, and “Has there ever been a period of two weeks or more when you talked or moved more slowly than is normal for you?” An affirmative answer was coded as 1, a negative answer as 0. The sum of the scores constituted the lifetime fatigue index.

The HHANES provides a number of potential confounders of the relationship between ID and depression (Table 2.1). Ethnicity of the participants was coded as

Puerto Rican if the respondent claimed full or partial Boricuan or Puerto Rican descent; as Cuban if she stated full or partial Cuban or Cuban American descent; and as Mexican if she claimed full or partial Mexican, Mexican American, or Chicano descent. A number of respondents who did not rate themselves according to any of the three ethnic groups were summarized into a fourth category, Undetermined (Hispano, Latin American, American, Spanish, Spanish American, Central or South American, Anglo-American, or Other).

A number of nutrients have been associated with depression and/or anemia. Vitamin B12 and folate deficiency appear to be correlated with mood changes [158-163] and to cause macrocytic anemia [8, 154]. The calculation of iron stores automatically excludes cases with macrocytic anemia, since hemoglobin levels below the cutoff of 12 g/dl have to be accompanied by ferritin levels below normal; whereas macrocytic anemia is characterized by a hemoglobin level less than 12 g/dl and a ferritin level greater than or equal to 12 $\mu\text{g/L}$. To further examine the impact of nutrient deficiencies, a variable indicating folate deficiency was created for values of red blood cell folate below 140 nmol/L [8]. Treatment with zinc appears to alleviate symptoms of depression [164-166]. Therefore, a variable signifying inadequate zinc intake was calculated based on Food Frequency Records provided in the HHANES questionnaire. Inadequate zinc intake was defined as an intake of less than 6.8 mg per day, which is the estimated average requirement for women between 18 and 50 years of age [167]. It has been suggested that increased consumption of omega-3 fatty acids (specifically docohexaenoic acid) suppresses the production of proinflammatory cytokines, which in turn could reduce the risk of depression [168]. Nutrient intake data for the HHANES have been generated only for polyunsaturated fatty acid in general, which is not specific enough for our purposes since the category includes omega-6 and omega-3 fatty acids.

Table 2.1: Independent variables considered in logistic regression analysis.

Independent variable	Explanation
Iron stores	Continuous score of iron status (mg/100)
Previous-week's fatigue	Additive score of 3 fatigue-related statements of the CESD
Lifetime fatigue	Additive score of 3 fatigue-related statements of the DIS
Ethnic group	Mexican, Puerto Rican, Cuban, Undetermined
Acculturation	Language of interview (English/Spanish)
Area of residence	in central city, not in central city, or not in standard statistical metropolitan area
Marital status	married with spouse living in the household, married with spouse not living in the household, separated, divorced, widowed, and never married
Health insurance status	health insurance (yes/no), or reason why no health insurance
Poverty index ratio	< 130%, between 130% and 300%, and > 300% smaller than 130% (yes/no)
Space available	number of rooms in household minus number of household members
Kitchen	Complete kitchen available (yes/no)
Education of respondent	Number of completed years; no high school, high school, college, graduate
Occupation of respondent	Private employee, federal government, state government, local government, self employed in incorporated business or practice, self-employed in unincorporated business, practice or farm
Education of head of household	Number of completed years; no high school, high school, college, graduate
Occupation of head of household	Private employee, federal government, state government, local government, self employed in incorporated business or practice, self-employed in unincorporated business, practice or farm
Food stamps, now	Family is presently receiving food stamps (yes/no)
Food stamps, previous month	Family received food stamps last months (yes/no)

Table 2.1 (Continued)

Independent variable	Explanation
Food stamps, not sufficient	Food stamp value was not sufficient to cover costs, and additional money had to be spend for food; food stamps received, monetary value was sufficient; no food stamps received
Past major episodes of depression	Past episode ended between 0 to 2 weeks, 2 weeks to 1 month, 1 month to 6 month, 6 months to 1 year, or more than one year; generally coded as: past major depression (yes/no)
Red blood cell folate status	< 140 nmol/L, ≥ 140 nmol/L
24-hour zinc intakes	below the EAR of 6.8 mg/day for women between 18 and 50 years of age, ≥ 6.8 mg/day
Sleep deprivation	Hours slept over last 24 hours; less than 6 (yes/no)
Post-pregnancy	Within 12 months post-pregnancy (yes/no)
Postpartum	Within 12 months postpartum, includes post-pregnant women who never had anything but life births, or who are currently breastfeeding; excludes all cases that are post-pregnant but not postpartum (yes/no)
Control over health	Control over one’s future health; a great deal, or some, very little or none

The questions determining whether a woman has been pregnant during the last 12 months were “Have you been pregnant during the last 12 months?” and “How many months ago did that pregnancy end?” (with answer categories “less than 4 months ago,” “4–7 months ago,” “7–10 months ago,” and “10–12 months ago”). The HHANES questionnaire did not determine whether a live birth had taken place within the 12 months prior to the interview. Hence, it did not differentiate between women who were postpartum (defined as delivering a live infant), and those who were post-pregnancy (which includes life birth, miscarriage, stillbirth, and abortion). In order to create a subsample with postpartum women, only those women were added who

reported a pregnancy over the 12 months prior to interview and a) whose number of live births was equal to the number of pregnancies or b) who reported that they were currently breastfeeding.

The analysis of the modifying effect of stressors on the relationship between ID and depression tested for interactions between potential stressors and iron stores. Stressors that are known to increase the risk for depression are available in the HHANES. Food insufficiency (food stamps not sufficient for food purchases), crowding (number of rooms available per household member), disrupted marital status (divorced, widowed, separated), postpartum status, lack of sleep, and lack of control over one's future health were considered stressors. In the HHANES survey, Puerto Ricans have consistently been shown to have higher rates of depression even after controlling for potential explanatory variables [67]. Therefore, the ethnic category of Puerto Rican was considered to code for an unknown stressor, and was tested as such in interactions. While SES is not necessarily to be equated with stressors, low SES is one of the fundamental causes of negative health outcomes [169]. Therefore, a higher exposure to stressors (which are more proximal to the health outcome) is associated with lower SES. Thus, measures of low SES (poverty index ratio, years of education, health insurance, availability of a full kitchen, and food stamp recipient) were also investigated as modifiers.

Statistics

Since the independent variable previous-week's fatigue was created from three CESD statements, the depression score of the CESD was summed from the 17 remaining statements that had not been used for the fatigue variable (named here depressive mood or CESD-17). In this fashion, a spurious correlation between the same statements in outcome and predictor variable was avoided. The established cutoff for the CESD-20 of a score of 16 and greater has been developed by Radloff

[156] with a white and African American study population. The necessity of a higher cutoff for the CESD-20 in groups other than the original study population has been suggested [170-175]; with cutoffs ranging from 17 to 23, depending on ethnic and income group. Hence, two dichotomous variables of the CESD-20 for a cutoff score greater than 16 and greater than 23 were created. In order to transfer the cutoffs of the CESD-20 to the CESD-17, the approximate percentile equivalents in this data set were assessed. The 80th percentile of the CESD-20 score distribution equaled a score of 16, the 90th percentile a score of 25. Thus, cases in the upper 80th and the upper 90th percentile of the CESD-17 were defined as suffering from moderate and severe depressive mood, respectively. Internal consistency of all newly created scales or indices was assessed with Chronbach's alphas.

Descriptive statistics such as means and frequencies were calculated with survey statistics provided by SAS Statistical Software, version 9.1 [176]. In the same fashion, significance testing with Chi-square or Fisher's-Exact test was performed with the *surveyfrequency* procedure provided by SAS. Associations between a categorical and a continuous variable were tested by using *proc surveyregression* or *proc surveylogistic*.

Since the right-tailed distribution of the scores of the CESD-17 violated assumptions for residual distribution of simple linear regression, the outcome was dichotomized and logistic regression was performed. Logistic regression analysis was conducted with SAS Statistical Software [176]. The outcome variable was CESD-17, with a cutoff above the 90th percentile. The determining variables of main interest were previous-week's fatigue and iron stores. The iron stores variable (mg) was divided by 100 in order to allow for a more meaningful interpretation of the magnitude of the effect. In addition, a dichotomized version of the variable with a cutoff of -200 mg was used; this cutoff was suggested by Cook [155] after analyzing the distribution

of iron stores of women of reproductive age in the National Health and Nutrition Examination Survey III (NHANES III). Other variables of interest are listed in Table 2.1.

All logistic regression models were adjusted for the complex design of the survey, specifically weighing, stratification, and clustering PSUs in the SAS Statistical Software survey analysis module. The expansion weights provided in the HHANES were transformed to relative weights = $n/N \times \text{weight}$ [177]. This allowed for comparison between weighted and unweighted models since it preserved the original sample size. Design effects were calculated as the square root of the ratio of the standard errors adjusted for all survey sampling techniques (weighing, stratification, and clustering) to the standard errors adjusted for simple random sampling without survey sampling adjustment [177].

Final logistic regression models were determined on the basis of theoretical assumptions, model significance, and variable significance. Model diagnostics were performed with deviance residuals, assessing the overall fit of the model, looking for outliers, and outlying relative weights. In addition, the associations of relative weight with key variables were investigated. Covariates considered in the logistic regression models were confirmed with factor analysis (see Appendix A, section 1.2.1, Factor analysis). Interactions were considered to be significant, with an alpha below 0.2.

Results

Descriptive statistics

A total of 1375 participants matched the inclusion criteria; 314 women had iron stores below 0 mg (Table 2.2). The weighted mean body iron stores of the whole sample was 295 mg, while unadjusted mean iron stores was 305 mg. The minimum iron stores value was -1140 mg, and the maximum value was 1117 mg.

Table 2.2: Participant characteristics. Weighted and unweighted means and unadjusted standard deviations (SD). Statistical testing with Chi-square or t- test. Significance based on comparison with iron sufficient group.

Variable	Iron stores < -200 mg			Iron stores -200 to 0 mg			Iron stores ≥ 0 mg		
	Weighted mean	Unweighted mean	Unadjusted SD	Weighted mean	Unweighted mean	Unadjusted SD	Weighted mean	Unweighted mean	Unadjusted SD
N (unweighted)	137			177			1061		
N (weighted)	136			193			1046		
Age (years)	33.3	35.1*	9.3	31.8	32.9	8.9	30.9	32.5	8.5
Iron Stores (mg)	-403.6*	-400.5*	160.8	-96.7*	-93.1*	53.3	458.6	462.3	277.4
Hemoglobin g/L	115.1*	115.3*	12.7	132.4*	133.2*	7.4	136.5	136.4	8.5
Ferritin µg/L	3.3*	3.4*	2.6	6.7*	7.0*	3.0	48.2	48.6	36.6
Protoporphyrin µg/dL	128.4*	121.9*	140.0	57.2*	56.9*	15.2	49.7	50.2	15.5
Transferrin Saturation %	10.7*	11.1*	5.4	20.8*	20.9*	7.6	27.3	27.1	10.5

*p<0.05 between ID and iron sufficient group

Table 2.3 presents frequencies of relevant variables by iron status. Seventy-seven women were iron deficient anemic (IDA) with hemoglobin levels below 120 g/L and two other indicators of iron deficiency beyond cutoff.⁶ A total of 170 women matched the criteria of iron deficiency without anemia (IDNA), which was defined as hemoglobin levels above or equal to 120 g/L, but with two additional iron status indicators beyond cutoff.⁶ Hemoglobin levels ranged from 64 g/l to 167 g/L; the minimum ferritin was 1 µg/L, and the maximum was 196 µg/L.

The overall number of cases with a weighted poverty index ratio below 130% was 161 (40.8%). In terms of ethnic group, the majority of the sample was of Mexican descent (69%), followed by 12.1% Puerto Rican, and 4.8% Cuban; a total of 13.6% had declared Hispanic ethnicity but undetermined geographic origin.

In terms of the variables assessing depressive mood, a total of 272 women (18%) were above the cutoff of 16 points of the CESD-20 recommended by Radloff [156]. However, it has been argued that disadvantaged populations and/or ethnicities other than the original study population might require cutoffs higher than 16, in order to assess depression accurately [11, 170-175]. At the highest recommended cutoff of 23 [11, 174], 145 women (9.3%) showed depressive symptomatology. Two hundred and sixty-seven women (17.4%) were above the 80th percentile of the CESD-17. A total of 131 women (7.9%) were in the upper 90th percentile of the CESD-17. Scores of the CESD-20 ranged from 0 to 60, scores of the CESD-17 ranged from 0 to 51. A total of 40 women (2.4%) were identified by the DIS as suffering from a current major depressive episode. Of these, 29 were above the cutoff of 16 of the CESD-20, 27 were above the cutoff of 23 of the CESD-20, and 26 were above the 90th percentile of the CESD-17. A total of 63 women had suffered from at least one episode of major depression in the past.

⁶ Mean corpuscular volume (MCV) < 80 fL, ferritin <12 µg/L, transferrin saturation < 16%, protoporphyrin > 70 µg/dL

The index of previous-week's fatigue ranged from 0 to 9 points, with an overall weighted mean score of 1.5. The lifetime fatigue index (DIS) ranged from 0 to 2.

Table 2.3: Participant characteristics. Frequencies in percent. Adjusted for stratification, clustering, and probability of selection. Statistical testing with Chi-square or Fisher's-Exact test. Significance based on comparison with iron sufficient group.

Variable	Iron stores < -200 mg	Iron stores -200 to 0 mg	Iron stores \geq 0 mg
N (unweighted)	137	177	1061
N (weighted)	136	193	1046
% Ethnic group			
Puerto Rican	8.0 ^a	9.7 ^a	82.3
Cuban	8.5 ^a	10.9 ^a	80.7
Mexican	11.2 ^{ab}	15.8 ^a	73.1
Undetermined	5.5 ^{ab}	10.3 ^a	84.1
% IDA	56 ^a	0	0
% Ferritin < 12 $\mu\text{g/L}$	100 ^a	8.7 ^a	4.4
% Poverty index ratio < 130	12.6 ^a	13.2 ^a	74.1
% Poverty index ratio \geq 130–300	8.6 ^{ab}	14.1 ^a	77.3
% Poverty index ratio \geq 300	6.9 ^{ab}	15.6 ^a	77.4
% Living with spouse	64.8	67.9	64.1
% Less rooms than people in household	38.7 ^a	27.1	23.0

^a $p < 0.05$ between ID and iron sufficient group

^b $p < 0.05$ between ID groups

^c Red blood cell folate only assessed in women 18 to 44 years of age

Table 2.3 (continued)

Variable	Iron stores < -200 mg	Iron stores -200 to 0 mg	Iron stores \geq 0 mg
% High lifetime fatigue	1.3	0.9	1.6
% High previous-week's fatigue	1.2	4.1	2.8
% CESD-20 > 16	14.9	18.5	16.2
% CESD-20 > 23	8.7	10.7	8.5
% CESD-17 90th percentile	8.7	8	7.7
% Zinc (24-hour) intake < 6.8 mg/day	0	0	0.2
n RBC folate ^c (unweighted)	109	131	839
N RBC folate (weighted)	111	146	866
% RBC folate < 140 nmol/L	3.9	5.9	4.4

Internal consistency

The CESD-20 had a standardized Cronbach's alpha of 0.91. The newly created CESD-17 scale showed good internal consistency, with a standardized Cronbach's alpha of 0.89. The index of previous-week's fatigue had a standardized Chronbach's alpha of 0.65, while the lifetime fatigue index had a poor internal consistency, with a standardized Chronbach's alpha of 0.50. It was therefore decided to use previous-week's fatigue in the following analysis.

Logistic regression analysis

The logistic regression analysis with CESD-17 (90th percentile cutoff) as the outcome variable showed a significant inverse correlation: a 100 mg increase in iron stores resulted in an 11% decrease in odds of depressive mood after controlling for all covariates (Table 2.4, Model 4). All models presented in Table 2.4 were significantly different with $p < 0.001$ (Chi-square test). No relationship between iron stores and CESD-17 with the 80th percentile cutoff could be established (not shown). Iron status as a categorical variable, instead of the continuous variable iron stores, was tested as well; neither IDA (hemoglobin < 120 g/L and ferritin $< 12\mu\text{g/L}$) nor IDNA (ferritin $< 12\mu\text{g/L}$) was statistically significant (not shown).

Role of fatigue

In models without previous-week's fatigue, the iron stores variable showed a marginally significant or significant inverse relationship with depressive mood (Table 2.4, Models 1 and 3). The inclusion of previous-week's fatigue strengthened the effect of iron stores slightly (Table 2.4, Models 2 and 4). Previous-week's fatigue was strongly correlated with the outcome: a unit increase in the score of previous-week's fatigue increased the odds of depressive mood by 2.14.

Confounders and covariates

The inclusion of covariates that might be potential confounders made no difference in the odds ratios of iron stores (Appendix Table A.1). The three variables that were finally added as covariates were ethnic group, living with spouse, and space in the household (Table 2.4). In addition to model building, factor analysis was used to confirm confounding (Appendix Table A.1). The three covariates reflected three identifiable factors coding for ethnic status, SES based on marital status, and SES based on personal achievements. The inclusion of ethnic group, living with spouse,

and space in the household increased the explanatory power of the model substantially: from an area under the receiver operator curve of 0.7 to 0.9 (Table 2.4, Models 2 and 4). The poverty index ratio was not significant in models adjusted for relative weight.

Interactions

Interactions were included in the final model to explore the modifying effect of stressor exposure on the relationship between iron stores and depressive mood. They were judged to be significant at a p-value less than 0.2. While not all interactions showed a difference of alpha less than 0.05 between the group with the highest risk and the reference, the general pattern that emerged was the same: it was found that ID increased the likelihood of depressive mood (CESD-17>90th percentile) when exposure to stressors was present as well.

Women who were between 0 and 12 months post-pregnancy with iron stores below -200 mg had an almost 5 times greater odds ratio of depressive mood than women who were not post-pregnant and had iron stores greater or equal to -200 mg (Figure 2.2). This relationship was replicated in an interaction between the variables postpartum status and iron stores as well: postpartum women with iron stores smaller than -200 mg had an odds ratio of 4.8 compared to women who were not postpartum and had iron stores greater or equal to -200 mg ($p=0.02$; not shown). The odds of depressive mood for women with a poverty index ratio below 130% and iron stores below -200 mg was almost 3 times greater than for women with a poverty index ratio greater or equal to 130% and iron stores greater or equal to -200 mg (Figure 2.3). The finding with poverty index ratio was replicated with other indicators of SES (Appendix Figure A.1, Appendix Figure A.2, Appendix Figure A.3). Finally, expected moderate to low control over one's future health was identified as a risk factor for severe depressive mood in ID women (Figure 2.4): women who were exposed

experienced a 1.6 greater odds ratio of depressive mood when ID than women with high expected control and iron stores greater than -200 mg.

Discussion

In summary, risk of depressive mood was significantly decreased with increasing iron status in the premenopausal Hispanic American women of this sample. A 100 mg increase in iron stores resulted in an 11% decrease in the odds of depressive mood measured by the CESD-17 (Table 2.4, Model 4). This relationship was significant while controlling for previous-week's fatigue, SES, and ethnic group. Previous-week's fatigue did not act as a mediator as hypothesized. Likewise, SES and ethnic group did not confound the relationship between iron stores and depressive mood. However, their inclusion reduced the confidence interval of the coefficient of iron stores, making iron stores significant. Thus, either previous-week's fatigue, or SES and ethnic group, or both, are necessary in the model. Stressor exposure modified the relationship between ID and depressive mood, thus increasing the probability of depressive mood in ID women.

Limitations and plausibility

Causality

As a result of the observational study design of the HHANES, a causal relationship between ID and depressive mood could not be established. Associations might have been due to a common underlying factor that had not been controlled in the statistical analysis. In addition, directionality cannot be assessed with the current study design since all measures were taken concurrently. Likewise, the identified modifying relationships between iron stores and stressors could not establish which variable is the modifier and which the modified. It is important to keep in mind, though, that the purpose of the present analysis was not to establish a causal link

between ID and depressive mood. Instead, the HHANES data set presented a time- and cost-effective way to conduct a plausibility analysis while addressing challenges to previously published research.

Past depression

The correlation between iron stores and depressive mood might be a result of previous impairments in psychological functioning. Severe depression in the past increases the likelihood of a recurrence of depression [178]. Past depression might also result in dietary habits that predispose a person to ID, although to our knowledge no study has explored this relationship. Including past major depression⁷ in the full model (Table 2.4, Model 4) did not change the odds ratio (OR) of iron stores (OR=0.89) or previous-week's fatigue (OR=2.1), which indicates that past major depression is not a confounder. Unexpectedly, the variable *past major depression* showed a lower probability of current depressive mood in the full model with an OR of 0.14 (confidence interval [CI] 0.05, 0.41) compared to cases with no history of major depression. This result is counter to established relationships [93], and calls into question the validity of the past major depression variable. Further investigation of the variable revealed that the effect is due to one group of 17 cases who reported that their last bout of major depression ended sometime between 1 and 6 months prior to the survey (OR=0.098, CI 0.04,0.26). Two of these cases ranked in the upper 90th percentile of CESD-17. Scrutiny of potential explanatory variables revealed no pattern in terms of health insurance, ethnicity, residence, marital status, reproductive status, or measures of SES (not shown). A number of possible explanations for this finding can be suggested. This group might contain a larger number of users of antidepressant medication whose likelihood of depression is lower than expected based on their history. Unfortunately, no information on medication usage is available in this data

⁷ For definition see Appendix E, 1. Diagnostic Interview Schedule, Depression Module.

set. Another explanation might be that this group constitutes persons who are most recently in partial or full remission, full remission of a major depressive episode being defined as being symptom-free for at least 2 months [179]. They therefore have a lower likelihood than cases in longer time intervals of remission to currently experience depressive mood. Finally, it should be cautioned that the recall of the timing of previous depressive episodes in the DIS has been reported to be inaccurate [180].

Validity of the subsample of the HHANES

The final sample used for analysis represents only a subset of the full HHANES sample. While cases were excluded on the basis of distinct criteria that might have affected the investigated relationship, or because of missing variables, a bias could have been introduced that reduced the validity of the final conclusions for a general female Hispanic sample. A comparison was undertaken between the final sample of 1375 cases and the 1528 women who were excluded based on missing information on depression, sampling procedures, or hemoglobin values (Figure 2.1, n=2903). No significant differences between the two groups were found in terms of depression scores, ethnic group composition, or poverty index ratio (Appendix Table A.3). The subsample of 1375 women was significantly younger than the excluded group (33 years of age versus 35 years).

Validity of Hispanic sample

The external validity of the present analysis is by nature limited, since the population sampled in the HHANES were US Hispanics in the early 1980s. Risk factors of depression might differ today or in other ethnic groups.

Table 2.4: Logistic regression model with CESD-17 as dependent variable (event defined as being in the upper 90th percentile of the distribution), n=1375. Odds ratios (OR), lower and upper limit of confidence intervals (CI) of odds ratio, and design effects (DEF). The models are adjusted for stratification, clustering (PSUs), and relative weights. Ref PR = reference Puerto Rican, c = area under the ROC curve.

Variable	Model 1			Model 2			Model 3			Model 4		
	OR	CI	DEF	OR	CI	DEF	OR	CI	DEF	OR	CI	DEF
Intercept	0.10	0.09, 0.12	0.73	0.01	0.009, 0.015	0.31	0.38	0.29, 0.50	0.51	0.38	0.02, 0.7	0.67
Iron stores (mg)/100	0.95	0.90,1.006	1.33	0.89	0.82, 0.97	1.24	0.94	0.89,0.99	1.37	0.89	0.82,0.97	1.38
Previous-week's fatigue				2.15	1.95,2.36	0.80				2.14	1.95,2.35	0.72
Living space							0.84	0.77, 0.92	1.24	0.82	0.73,0.92	1.07
Spouse in house (Ref not)							0.45	0.37, 0.56	0.19	0.52	0.33,0.81	0.54
Ethnic group (ref PR)	Cuban Mexican Undeter.						0.43	0.21, 0.87	1.05	0.87	0.35, 2.17	0.96
							0.35	0.25, 0.47	0.47	0.40	0.22, 0.74	0.79
							0.40	0.20, 0.78	0.84	0.31	0.12, 0.82	0.88
-2LL	753			436			705			412		
c	0.514			0.923			0.708			.936		

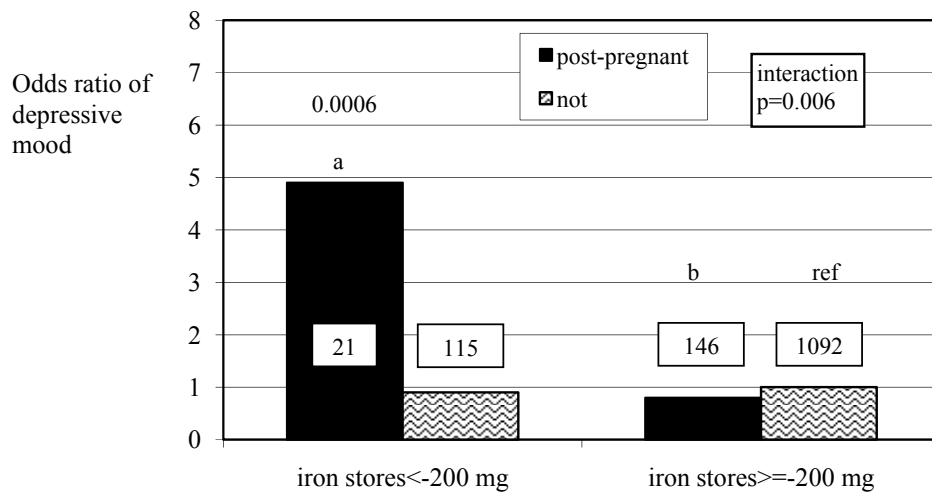


Figure 2.2: Odds ratios of depressive mood with iron stores < -200 or ≥ -200 mg, grouped by post-pregnant status. Logistic regression model with CESD-17 as dependent variable (event defined as being in the upper 90th percentile of the distribution). Covariates were previous-week’s fatigue, space available, living with spouse, and ethnic group. The model excluded cases that were post-pregnant but not postpartum. The model is adjusted for stratification, clustering (PSUs), and relative weights. Group size in rectangles above bars. P-value of difference from reference group (ref), and differences with significance below 0.05 shown by different letters.

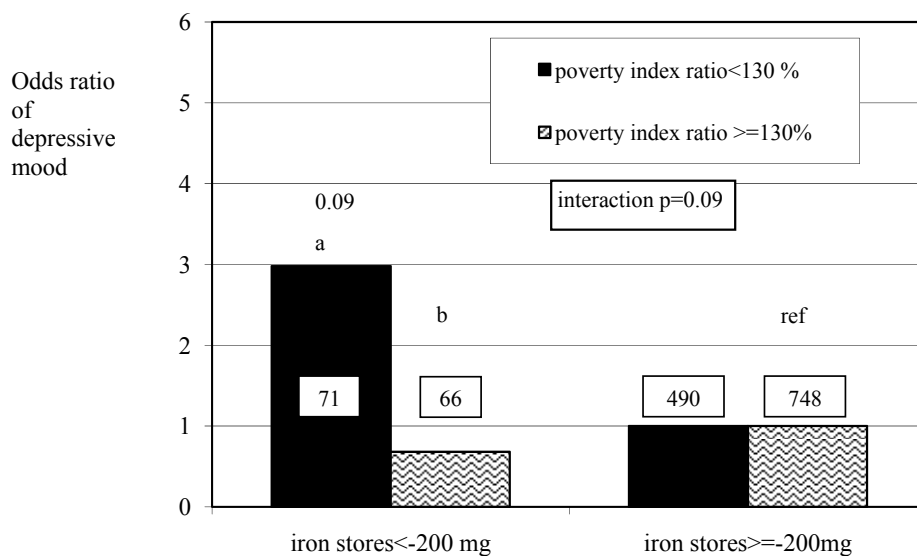


Figure 2.3: Odds ratios of depressive mood with iron stores < -200 or ≥ -200 mg/100, grouped by poverty index ratio $< 130\%$, or $\geq 130\%$. Logistic regression model with CESD-17 as dependent variable (event defined as being in the upper 90th percentile of the distribution). Covariates were previous-week's fatigue, and ethnic group. The model is adjusted for stratification, clustering (PSUs), and relative weights. Group size in above bars. P-value of difference from reference group (ref), and differences with significance below 0.05 shown by different letters.

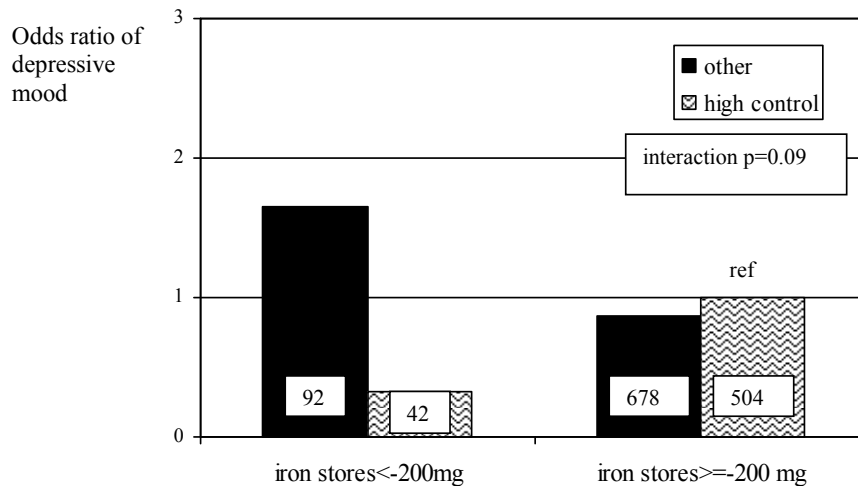


Figure 2.4: Odds ratio of depressive mood with iron stores < -200 or ≥ -200 mg, grouped by expect high control over one’s future health or other. Logistic regression model with CESD-17 as dependent variable (event defined as being in the upper 90th percentile of the distribution), $n=1298$. Covariates were previous-week’s fatigue, space available, and ethnic group. The model is adjusted for stratification, clustering (PSUs), and relative weights. Group size in bars. P-value of difference from reference group (ref), and differences with significance below 0.05 shown by different letters.

Validity of the CESD-20

General

The CESD-20 was created specifically to assess the level of depressive symptomatology in a population, not to be used as a clinical screening tool [156]. Cross-validations of the CESD-20 with more extensive mental health screening instruments assessing a range of depressive disorders have found sensitivities between 40 and 95%, specificities between 61 and 95%, and positive predictive values (PPV) between 6 and 48% [170, 173, 175, 181, 182]. Consequently, the CESD-20 at times does not perform well as a clinical assessment tool of depression. Comparison to the DIS has also suggested that CESD-20 assesses anxiety as well [181, 182]. It has therefore been suggested to use the CESD-20 as a tool for assessing emotional distress [172, 183]. It should be kept in mind, though, that anxiety disorder is also a common

comorbidity of depressive mood [53], which might account for the higher rates of anxiety in cases identified as depressed by the CESD.

Hispanic culture

The CESD-20 was developed with an English-speaking sample of White and African American respondents [156]. A large number of articles have investigated the cultural definition of depression in the Hispanic culture. Specific cultural terms for mood disorders are not addressed in the CESD-20 translations. For example, in Mexican culture, the term “nervios” (loosely translated as “nerves”) includes somatic disorders (such as gastrointestinal problems, vomiting, lump in throat, and headaches), as well as dysthymia, major depression, and anxiety [184]. In Puerto Rican culture the term “ataques de nervios” can include a number of behavioral and somatic symptoms, all indicative of depression or other mental health issues [185]. No information was found on special expressions for depression in Cuban culture.

Somatization of dysthymia or major depression is more frequent in Hispanic populations; this is especially true in conjunction with being above 40 years of age and having a low level of acculturation [157, 186, 187]. Low levels of acculturation have been shown to be a protective factor against depression in Mexican Americans and Puerto Ricans. In the HHANES it was found that Mexican Americans and Puerto Ricans have lower risk of depression when less acculturated [18, 19].

In summary, the CESD-20 might not be as accurate as more extensive depression assessment instruments. Moreover, in Hispanics, depression appears to be experienced differently than in White comparison groups. More physical symptoms are associated with depressive mood, and culturally specific language terms are employed that at times describe a variety of emotional disorders. None of this is accounted for in the CESD-20. In addition, the CESD-20 appears to also identify cases with high anxiety. In the present analysis, misclassification of cases by the CESD-17

should result in a weakening of the association between ID and depressive symptomatology since a high rate of false negatives and false positives occurs. Nevertheless, we were able to demonstrate a relationship between ID and depressive mood, which indicates the strength of the relationship in this data set.

Scale construction of CESD-17 and previous-week's fatigue

The variables of previous-week's fatigue and depressive mood were both created from the CESD-20. Thus, neither variable is an established measure, which calls into question their validity. However, the internal validity of the two constructed scales was good, and the inverse association between iron status and depressive mood despite the control for fatigue has been confirmed in our study in Mexican women (Chapters 3 and 4). It is important to recognize that the current analysis solved the important problem of correlating a fatigue scale with a depression scale, while both instruments contain questions assessing fatigue. Thus, the relationship might have been considered spurious, if not for the removal of the fatigue statements from the CESD-20. Finally, it should be mentioned that no useful alternative measures of fatigue or depression were available in the HHANES data set. Lifetime fatigue from the DIS had poor internal consistency, and the depression measure of the DIS could only be coded concurrently for major depression.

Antidepressant use

A significant limitation of this data set is that that no information on antidepressant medication use was available. Although use of antidepressant medication had been collected during the HHANES survey, the data are in poor condition, and have therefore not been made available to the public (E.A. Frongillo, personal communication; also consult the NCHS website <http://www.cdc.gov/nchs/nhanes.htm>) [188]. Since antidepressant use could not be

controlled for statistically, women with low iron stores might show low levels of depression solely as a result of the alleviating effects of antidepressant medication. On the other hand, it might be safe to assume that the number of women who are using antidepressants in this sample is small. The data were collected in the early 1980s, when antidepressant use was not yet widespread. In addition, the sample population includes a significant percentage of the poor and/or immigrants, who might not have access to medical services or, consequently, to antidepressants. It should also be noted that women who use antidepressants should experience less depression, therefore weakening a potential relationship between iron stores and depressive mood. Nevertheless, we were able to demonstrate an effect.

Substance abuse

Users of illegal substances were excluded from the sample, as long as they reported having used drugs within the last month (n=57, see compare Figure 2.1), since they represent a subpopulation that might have reduced the external validity of the results to the general population. The HHANES questionnaire assessed abuse of illegal inhalants and cocaine, but no information on heroin use was collected. Additional data on current prescription drug and alcohol use were gathered. Prescription drug use was self-rated; participants answering questions about the use of “pills just to enjoy the feeling they give” or for “nonmedical reasons” within the past month. Alcohol use was self-reported as well, with answers ranging from “abstainer” to “heavy drinker” (“Do you now consider yourself a...?”). Data on 1349 cases were available for prescription drug use, and for the full data set on alcohol use. In the current sample, only 1 participant indicated abuse of prescription drugs, 85 rated themselves as medium to heavy drinkers, and 6 as heavy drinkers. The inclusion of alcohol abuse as a possible confounder in the final model (Table 2.4, Model 4) showed that the variable was not significant (not shown).

It is important to keep in mind that all substance abuse was self-reported. Consequently, prevalence of substance abuse might have been underestimated, and not all substance abusers might have been excluded or controlled for. Substance abuse can be associated with low consumption of enhancers of iron absorption (such as vitamin-C-containing fruits and vegetables) [189, 190], although two studies found no difference in iron status between drug abusers and non-abusers [191, 192]. Substance abuse is also associated with depression [53, 193-197]. In summary, unmeasured substance abuse might still have contributed as a positive confounder to the significant relationship between iron stores and depressive mood.

Premenstrual syndrome (PMS) and perimenopause

While postmenopausal women were excluded from the current sample, no information was available on perimenopause and premenstrual status. Perimenopause is associated with a higher risk of depression [198-200]. In addition, perimenopausal women might have a lower prevalence of ID than the younger age groups, because of decreased menstrual blood loss. This is supported by studies assessing iron status in menopausal or late middle-age women [155, 201-203]. Thus perimenopausal status fulfills the criteria of a confounder of our analysis, albeit a negative one. Even without adjusting for perimenopausal status, the relationship between iron stores and depressive mood in this analysis is significant. Older chronological age was used as an indicator for the role of perimenopause in the relationship between iron status and depressive mood; it was not significant. PMS and premenstrual dysphoric disorder (PMDD) have been shown to be associated with depressive mood [179, 198, 204-206]. No information on either disorder or on the exact menstruation cycle was gathered in the HHANES. However, iron status is not affected by menstruation cycle [207]. Therefore, a systematic strengthening of the association between iron stores and depressive mood in premenopausal women is unlikely.

Other nutrients that may cause depression

A number of nutrients have been associated with depression and/or anemia, including zinc, folate, vitamin B12, and ω -3 polyunsaturated fatty acids (specifically docosahexanoic acid) [158-163, 168]. For Hispanics, only levels of ω -3 polyunsaturated fatty acids in breast milk, not in the diet, have been reported [208]. In the analysis, all nutrients except ω -3 polyunsaturated fatty acids were accounted for; no information for ω -3 polyunsaturated fatty acids was provided in the data set [209].

In summary, if cases with low iron stores were also deficient in ω -3 polyunsaturated fatty acids, the nutrient might have acted as a positive confounder. However, it is also important to keep in mind that the evidence of the depression-lowering properties of ω -3 polyunsaturated fatty acids is inconsistent and controversial [210-212].

Post-pregnancy variable

While the HHANES contains an extensive section on reproductive history, it was not documented whether the last pregnancy ended in a live birth, abortion, miscarriage, or stillbirth. Thus the variable that connotes post-pregnancy (n=167) probably contains a mix of cases. Therefore, a more stringent postpartum variable was constructed. Criteria for inclusion were that the woman reported that 12 months or less passed since her last pregnancy, and that she is either breastfeeding now, or only had live births. It is possible, although highly unlikely, that a woman was currently breastfeeding a child from a previous pregnancy even though her most recent pregnancy did not end in a live birth. More probable is that a number of valid cases of postpartum women with recent live births were screened out of the postpartum variable; thus the true number of postpartum women might have been higher than 74 cases. Despite the small number of cases, the interaction between iron stores and postpartum status was significant (p-value of 0.02); postpartum women with ID had an

odds ratio of depressive mood that was 4.8 times greater than the reference group (women who were not post-pregnant and had iron stores > 200 mg; p-value of comparison 0.07). Thus, the calculated odds ratio of depressive mood of the postpartum group with ID was almost the same as the one of the post-pregnant group (4.9 for post-pregnancy, Figure 2.2). On the other hand, a miscarriage or a stillbirth might present a risk factor for depression in itself [213], and – once iron stores are low – a contributing factor to the higher odds ratio.

Internal validity

Body iron stores was derived from hemoglobin, ferritin, protoporphyrin, and transferrin saturation. Point estimate and distribution of iron stores is accordingly mirrored by all four of the iron status measures (Table 2.2).

Validity of depressive mood (CESD-17) was assessed by comparison with other variables available in the data set. The cutoff of the 90th percentile of the CESD-17 (a score ≥ 21) was set to correspond with the 90th percentile of the CESD-20 (score ≥ 26). In order to validate the cutoff of the CESD-17, the second depression scale used in the HHANES survey, the DIS, was employed. The DIS is used to assess lifetime depression, but it can be coded to reflect current major depression by combining the diagnosis of major depression with a question inquiring about the end of the last episode of low mood (see Appendix E, Diagnostic Interview Schedule, Depression Module). In the current sample, the concordance between current major depression (DIS) and depressive symptomatology (CESD-17) was as follows (Appendix Table A.4): sensitivity = 80%, specificity = 92.6%, and PPV = 24%. The equivalent results for survey-adjusted frequencies (not shown) would be a sensitivity of 79.3%, a specificity of 93.9%, and a PPV of 24.4%. Other studies have cross-validated the CESD with the diagnosis of major depression of the DIS. For Caucasians they show a sensitivity between 45 and 95%, a specificity of 64 to 95%, and a PPV of 15 to 28.4%

[170, 173, 181, 182]. Comparison of the CESD with the diagnosis of major depression of the Schedule for Affective Disorders and Schizophrenia (SADS) yielded a sensitivity of 60 to 63%, a specificity of 83.4 to 93.9%, and a PPV of 6.7 to 33% [173, 175]. When the CESD was compared with the SADS while focusing on any depressive illness, the sensitivity was 40.8 to 59.4%, the specificity was 85.9 to 94.9%, and the PPV was 24.7 to 47.6% [173, 175]. A comparison of the CESD with the DIS diagnosis of major depression in US Hispanics yielded the following: in English-speaking participants, sensitivity of 81.2% and specificity of 60.8%; in Spanish-speaking participants, sensitivity of 84.2% and specificity of 64.5% [182].

One hundred and twenty-eight cases are above the cutoff of 23 points in the CESD-20. However, of these, only 108 women are above the 90th percentile of the CESD-17 (Appendix Table A.4). Cases that were at or below the 90th percentile but above 23 points in the CESD-20 were nevertheless reclassified as not severely depressed. The majority of these cases (91%) had between 23 and 25 points (mean 24). When comparing the cases above the 90th percentile of the CESD-20 and the CESD-17, 1016 cases were classified as not experiencing depressive mood, and 105 cases were classified as depressed by both scales (Appendix Table A.5). Only 3 cases were below or equal to the 90th percentile of the CESD-20 and above the 90th percentile of the CESD-17. Conversely, 6 cases were above the 90th percentile of the CESD-20 and below or equal to the 90th percentile of the CESD-17. Thus, differential classification of the different scales was minimal.

In summary, in the present sample the CESD performed well when compared to the DIS diagnosis of major depression, especially when compared to results of other studies. Sensitivity and specificity were high; and although the PPV appears low, it depends on prevalence and lies within the range of what has been previously observed in the literature. It should be noted that the CESD has been created with the intention

of capturing cases in the general population that are suffering from depressive symptomatology [156]. Thus, it was developed as a screening tool, and a range of types of depression might be identified as cases. It is therefore safe to assume that the women considered depressed according to the CESD-17 probably suffer from less severe forms of depression as well, such as dysthymia or minor depression (compare Appendix D). It is important to keep in mind as well that the CESD-20 appears to assess only not depressive mood, but also anxiety.

Fatigue in the previous week had only a minor effect on the relationship between iron stores and depressive mood (Table 2.4). In fact, its inclusion in the model appeared not to be necessary when covariates concerning ethnic group and SES were also present. Previous-week's fatigue showed good internal consistency, with a Chronbach's alpha of 0.65. It correlated significantly ($r=0.23$, $p<0.0001$, weighted correlation) with the lifetime fatigue measure constructed from the DIS (Appendix E, Lifetime Fatigue Score, DIS). Likewise, of the 18 cases with high lifetime fatigue (a DIS fatigue score of 3), 22% rated high in previous-week's fatigue as well (score greater than 5); while only 7% of the cases with low to moderate DIS lifetime fatigue rated high in previous-week's fatigue (Chi-square $p=0.005$; not shown). Previous-week's fatigue was also correlated with the CESD-17 with a coefficient of 0.69 ($p<0.0001$, weighted correlation). A correlation of this magnitude is to be expected based on the published literature [54-57]. When evaluating frequency distributions, only 3% of the respondents without depressive mood (90th percentile of the CESD-17) complained about high previous-week's fatigue – compared to 53% of the respondents with depressive mood (Chi-square $p<0.001$; not shown). This bivariate distribution between high fatigue and depressive mood has previously been observed [58, 59]. In order to further validate previous-week's fatigue, its relationship with iron stores was explored. It was assumed that lower iron stores would be associated with

higher fatigue. However, the opposite was true: a 100 mg increase in iron stores was associated with a 6% increase in the odds of high previous-week's fatigue (Appendix Table A.6). Since the previous-week's fatigue variable behaved as expected overall, characteristics of this sample could well be implicated with the positive correlation. Therefore, the relationship between previous-week's fatigue and iron stores was examined for confounding and influential subgroups. Depressive mood turned out to be an important negative confounder of the relationship between fatigue and iron stores. However, the inclusion of other potential confounders such as poverty index < 130%, ethnic group, postpartum status, parity, age, body mass index (BMI), oral contraceptive use, or physical activity did not alter the positive relationship between the two variables of interest. It was found that only younger participants showed a positive relationship between the odds of high previous-week's fatigue and iron stores (interaction, $p=0.007$; Appendix Figure A.5). No other variables available in the data set were considered helpful in elucidating the unexpected finding. One might speculate that the younger subgroup, because of youthful vigor, may not have experienced the debilitating effects of lower iron stores to the same degree as an older group. Therefore, the younger age group would not rate as high in the previous-week's fatigue variable.

In light of these findings, we examined the association of depressive mood and iron stores with the CESD-20 with a cutoff of 23, and without previous-week's fatigue in the model; marital status, ethnic group, and crowding were covariates. We found that the results are comparable to our models presented in Table 2.4. With every 100 mg increase in body iron stores, the odds ratio of depressive mood decreased significantly by 5% (Appendix Table A.8).

In order to further validate our constructed depressive mood and fatigue variables, a comparison with data from our Mexican sample (Chapter 3) was

undertaken. The CESD-17 ranged from 0 to 48 points in the Mexican data set of $n=100$, with a mean of 17 points (not shown). The CESD-20 had a mean of 20 and ranged from 0 to 55 points. Previous-week's fatigue had a mean of 2.8 and ranged from 0 to 9 points. The correlation coefficient between the CESD-17 and previous-week's fatigue was 0.63 ($p<0.0001$). The correlation coefficients of the Fatigue Severity Scale (FSS, description in Chapter 3) with the CESD-17 and previous-week's fatigue were 0.47 and 0.46 ($p<0.001$), respectively. The CESD-20 was associated with the CESD-17 ($r=0.99$, $p<0.001$). Since the 90th percentile of the CESD-17 was much higher in the Mexican sample (31 points) than in the HHANES, no categorical comparisons between the two CESD-17 versions were undertaken. When comparing the CESD-17 with the Beck Depression Inventory (BDI, Chapter 3), the correlation coefficient was 0.59 ($p<0.0001$), which was comparable to the correlation between the BDI and the CESD-20 ($r=0.63$, $P<0.0001$; not shown). The BDI and previous-week's fatigue were significantly ($P<0.001$) correlated as well ($r=0.58$), whereas the BDI and the FSS had a correlation coefficient of 0.50 ($p<0.0001$).

We also replicated the logistic regression models that we employed in the HHANES analysis. Sensitivity/specificity analysis in the Mexican data set indicated that the CESD-20 cutoff of 21 performed best when compared to the severe depressive mood of the BDI: sensitivity was 78%, specificity 92% (not shown). With the cutoff of 21, 40% of the cases were labeled as severely depressed. When reanalyzing the association between the CESD-20 (with a cutoff of 21) and hemoglobin in the Mexican data set, severe depressive mood decreased significantly ($p=0.004$) by 4% with every g/L increase of hemoglobin (not shown). Covariates were a factor indicating SES and crowding, and $MCV>94$ fL (compare Chapter 3). When including the FSS as an additional covariate in this model, the effect of hemoglobin on severe depressive mood (CESD-20 with cutoff of 21 points) increased to a 5% rise with every

additional change in g/l of hemoglobin ($p=0.004$). Alternatively, when assessing the association between the CESD-17 with hemoglobin in the Mexican data set, the 90th percentile of the CESD-17 yielded only 10 cases. Thus, the cutoff for the CESD-17 of the HHANES was used as an outcome (score of 21). When assessing the effect of hemoglobin in this model, it had an OR of 0.98, but the p-value ($p=0.18$) indicated only a trend (not shown). This model controlled for previous-week's fatigue, factor SES, and crowding, and $MCV>94$ fL (compare Chapter 3).

We concluded that the results of the analysis of the associations between indicators of fatigue, depressive mood, and iron status with the Mexican data set implied that the CESD-17 and previous-week's fatigue correlated in the expected direction with fatigue and depression instruments, and with iron status in our Mexican data set.

Mechanisms

ID in this study was associated with a higher likelihood of depressive mood. The explanation for this finding might be that ID affects concentrations of neurotransmitters in the synaptic cleft between nerve cells; alterations in serotonin, epinephrine, or dopamine levels are generally considered a cause of depression [124]. The relationship between ID and neurotransmitter levels in the brain has been explored in animal studies. Iron is a cofactor for the synthesis of all three aforementioned neurotransmitters [26]. Serotonin concentration, or serotonin synthesis or breakdown, appears to be affected by ID, although not all studies show consistent results: ID rats have exhibited reduced concentrations of serotonin in the brain [28]. The concentration of the serotonin metabolite 5-hydroxyindole-3-acetic acid was found to be lower (while serotonin concentrations were not changed) [29], or higher [31] with ID. The synthesis of serotonin by tryptophane hydroxylase was found to be unchanged [28], or increased [214] with ID. The neurotransmitter norepinephrine has

rarely been investigated; it appears to remain unchanged [30] or is higher [31, 32] in ID rats. More consistent information is available on dopamine. ID appears to decrease the density of dopamine receptor D2 [33, 34]. In addition, dopamine transporters are downregulated, resulting in a slower reuptake of dopamine in the presynaptic cell [35, 36]. Consequently, dopamine levels have been found to be higher in ID rats [29, 32]; and dopamine metabolites to be lower [29]. The activity of monoamine oxidase, which is involved in the breakdown of serotonin, seems to be reduced during ID [28, 30].

In this analysis we have also shown that the effect of ID on depressive mood is modified by SES, post-pregnancy status, and perceived control over one's health. The potential mechanism for this potentiating effect might be that lack of iron in the brain and exposure to social stressors both act on the neurotransmitter systems of the brain. Consequently, the probability of depressive mood is higher with exposure to both risk factors. This is, to our knowledge, the first analysis to explore this interaction. In the social epidemiological literature, the relationship between stressor exposure and risk of depression has been well documented. The suggested mechanism is that chronic stressor exposure creates an overuse of one of the stress systems (the hypothalamo-pituitary-adrenal [HPA] axis) that is generally responsible for a short-term response to stressors; the overuse of this response system is called allostatic overload [95, 97]. Factors that increase stress have been identified, such as low SES [64, 98], sleep deprivation [103], job strain [99-101], low social support at work [104], low social support in private life [102], home strain (number of children) [102, 105], number of hours worked [102], daily perceived stressors [106], and negative affect [106]. Factors that might buffer the effect of stressors appear to be good coping ability, high self-efficacy, and good social support [107-110]. In addition, factors that are associated with an increased risk for depression have been documented; they include low SES [64-66], low social support [91], not being married [65, 93], low control at home or at

work [90], job strain [100], or longer-term unemployment [65]. Low mood has been reported as a result of a trial inducing sleep deprivation [112].

Hypocortisolism has been associated with chronic stress [102, 119, 123, 215, 216] and a higher risk of atypical major depressive disorder (MDD) [96, 113, 122]. The mediating mechanism might be an upregulation of the immune system due to chronically low cortisol levels [115, 130-134], which is reflected in high concentrations of plasma and brain proinflammatory cytokines, namely tumor necrosis factor, interleukin 1, and interleukin 6 [115, 119, 120, 125, 130, 133, 136, 137, 140]. Elevated cytokine concentrations in the brain reduce the availability of serotonin [121, 122, 145], whereas norepinephrine concentrations appear to increase [6, 7, 9, 130, 132].

External validity

Prevalence of IDA in the present sample was 6% in 182, which is higher than the US average of 5% in 1988 [4]; IDNA in this sample was 12%, which is 1% higher than the more current US average. Thus a total of 18% of the present sample suffers from ID.

The iron stores variable in the present analysis was calculated from a formula developed by Cook et al. with the NHANES III (1988–1994) [155]. The mean unadjusted iron store of 305 mg of the HHANES sample was comparable to the authors' mean iron store of 309 mg for women 18 to 44 years of age; while women 45 to 64 years of age had a much higher mean value with 608 mg. The variation in iron stores in the present analysis was, with an unadjusted standard deviation of 398 mg, similar to Cook et al.'s study, which reported standard deviations in the younger women of 346 mg and of 372 mg in the older. The wider variation of the HHANES sample was due to more extreme negative values. A newer formula by Cook et al. that

utilizes the ratio of serum transferrin receptors to serum ferritin [217] could not be used because serum transferrin receptors were not determined in the HHANES.

The prevalence of depressive mood in the present sample was as follows: percent lifetime prevalence of major depression (definition in Appendix D, Major Depressive Disorder) as assessed by the DIS was 7.0%, prevalence of depressive symptomatology over the last week based on the cutoff of 16 of the CESD-20 was 18.1%, prevalence of depressive symptomatology based on the CESD-20 cutoff of 23 was 9.3%, and prevalence of depressive mood based on the 90th percentile of the CESD-17 was 7.9%. This compares to a lifetime prevalence of major depression for women in the US of between 6 and 21.3% [10-12]. Vega et al. [13] showed a prevalence of lifetime major depression of between 10 and 14% for Californian Mexican Americans. The rate of depressive symptomatology assessed by the CESD in women was found to be 33% in Hispanic Americans [218] and 37% in the general US population [170].

The significant inverse association between iron stores and depressive mood that was found in the present analysis (Table 2.4) is confirmed by a large host of studies. The majority are cross-sectional [37-40]; only one is a randomized trial with placebo group [46]. In contrast, negative results were found by one cross-sectional study [41], three randomized placebo-controlled iron supplementation trials [43, 44, 148, 219], and one supplementation trial that used a high iron diet as the control condition [45]. It is important to note that the randomized trial that had a significant relationship between iron supplementation and lowered depression scores took place with IDA postpartum women in South Africa [46], while the four trials with negative results all included partially or exclusively non-IDA participants; the participants were not postpartum, and came from developed countries (Switzerland, Australia, and USA) [42-45]. Our observational study with female Mexican factory workers

indicated that the inverse association between iron status and risk of severe depressive mood remains after controlling for fatigue and SES (Chapter 3).

One can speculate that the difference between the South African trial and the four others might be due to the fact that a) the South African trial supplemented only IDA women, allowing for maximum effect size of iron supplementation on alleviation of depression scores; and b) iron supplementation had a stronger effect in women who were postpartum and from South Africa because it disrupted the synergistic effect of higher exposure to stressors and ID on depression.

No study has been identified in the literature that explores the more complex relationship between ID, fatigue, and depression, even though a number of studies assessed all three [38, 39, 41, 43-45]. Moreover, to our knowledge, this is the first study exploring the modifying effect of stressors on the relationship between ID and depressive mood. The only references that might allude to our hypothesized interaction between stressors and ID could be the results of the recent randomized iron supplementation trials [43-46, 54-57, 148, 219]: South African women who are, in addition, postpartum, simply might have higher stress levels than the women in the other four trials and therefore show a greater remission of depression with iron supplementation.

In the present study, previous-week's fatigue had a positive correlation with depressive mood. A strong association between the two has also been shown in a number of other studies: correlation coefficients were between 0.35 and 0.62 [58]. Fuhrer et al. [59] found that fatigued participants had a higher depression score of 23.5 compared to peers reporting no fatigue (score 17.6; $p=0.0001$); Hickie et al. [53] showed that 21% of their fatigued participants were also distressed, versus 7% of the non-fatigued subjects. This is logical, given that fatigue is one of the diagnostic criteria of depression [39, 43-45].

Fatigue and ID are related; in our analysis we found a positive correlation of previous-week's fatigue and iron stores, which is contrary to the published literature [38, 41]. Two studies found no relationship between fatigue and iron status [220]. Since our variable, previous-week's fatigue, appears to be a valid measure of fatigue, the remaining conclusion would be that confounding or a subgroup influenced the expected results. We were not able to identify such a confounding variable. However, previous-week's fatigue in our analysis does not appear to have the originally hypothesized major influence on the relationship between ID and depressive mood.

We are not aware of any published study that explores the effect of stress as a modifier of the association between iron status and depressive mood. The results of our Mexican study indicate that women with low salivary cortisol levels throughout the workday have an increasing risk of severe depressive mood with decreasing hemoglobin values, whereas this association is not significant in women with cortisol values equal to or above the median (Chapter 4).

Clinical significance of association

The magnitude of the effect of ID on depressive mood should determine whether iron supplementation is considered an important therapy for depression. The accepted current therapy for depression is administration of antidepressants such as selective serotonin reuptake inhibitors. To our knowledge, only one randomized controlled trial assessing the efficacy of antidepressant therapy has been published that can be compared with our type of data; this study used a different depression scale (Hamilton Rating Scale for Depression) to assess their results, and chose as one of their outcomes the probability of remission after therapy. Detke et al. [153] tested the antidepressants Duloxetine and Paroxetine on a total of 367 patients with major depressive disorder. Depending on the dosage, Duloxetine reduced the probability of remission by 21 to 28% compared to a placebo, while Paroxetine reduced it by 17%.

From our cross-sectional analysis we can estimate the potential effect of improving body iron status. This simulation is based on a logistic regression model adjusted for survey methods that uses as the dependent variable non-depressive mood, which is defined as all cases equal to or below the 90th percentile of the CESD-17. The covariates of this model were previous-week's fatigue, space available, spouse in house, and ethnic group, the same as in Model 4, Table 2.4. The odds ratio of the iron stores variable was 1.12 (CI 1.03, 1.22). In our study, a change from iron stores of –600 mg (IDA equivalent to a hemoglobin value of 10 g/dL) to 0 mg (iron sufficient) would increase the probability of remission of depressive mood by 3.24%. For an IDNA woman with an iron store of –200 mg, the probability of remission when reaching iron sufficiency would increase by 1%. An iron-sufficient person with an iron store of 0 mg who changes to a positive iron store of 600 mg would have an increased probability of remission of depressive mood of 1.8%. When assessing the effect of improving from an iron store of –600 mg to iron sufficient (0 mg) in women with stressor exposure (a poverty index ratio of < 130%), the probability of remission increased 3.21%. Thus, the effect of an improvement of iron status is, at best, approximately one fifth of the effect of an antidepressant; iron supplementation when depressive mood and ID are present has therefore only a substantial effect in women who present with severe to moderate IDA. It should be noted that the definition of remission in Detke et al.'s study (score of ≤ 7 points on a depression scale after 8 weeks of treatment) differs substantially from the present research, which impairs the comparison of outcomes.

Conclusions

In conclusion, a significant increase of risk of depressive mood was shown with decreasing iron stores in US Hispanic premenopausal women; this was true even after statistically controlling for fatigue and potential confounders. A synergistic

relationship between iron stores and a variety of stressors was found: depressive mood was highest in women with low iron stores and exposure to a stressor.

Comparison to the published literature invites speculation about the reasons why the present analysis showed support for the main study hypotheses:

- Using a continuous measure of ID (iron stores) versus categorical threshold variables (IDA or IDNA) might have increased the likelihood to detect the relationship. This sample might otherwise not have had a large enough portion of IDA or IDNA women to detect a difference in depressive mood. Second, it appears as though the relationship between ID and depressive mood in our sample might span the full spectrum of iron status; testing only IDA could have missed a large and significantly affected group.
- The HHANES might have been a good sample for testing the hypothesized relationship since a large percentage of women are below 130% of the poverty index ratio. Having a higher stressor exposure might have made participants more susceptible to the effect of ID on depressive mood.
- It should be noted that the effect of alleviation of ID on remission of depressive mood is at best one fifth of the effect of antidepressants.

Based on the results of the present analysis, it is recommended that future research

- include mid- and high-level depression as an outcome
- analyze the relationship in IDA and severely IDNA women to maximize the effect of ID on depressive mood
- use a continuous measure of iron status in addition to the traditional categorical cutoffs
- target the analysis to young and early middle-aged women
- focus on groups that are exposed to stressors

- assess interactions between stressors and iron status.

Future exploration of this topic should continue by means of a randomized placebo-controlled iron supplementation trial in order to allow for more definitive answers about causal relationships. In addition to adhering to the aforementioned improvements, the plausibility would be increased if

- the response of fatigue and depression scores to supplementation could be observed in two separate groups, one fatigued and not depressed, and one depressed. The accuracy of the diagnosis should be assured by conducting clinical interviews at baseline.
- better measurements of fatigue and depressive mood were to be used
- a measure of internal stress were to be included instead of measuring only external stressors. A possible biological marker of perceived stress is cortisol.

Chapter 3: IRON DEFICIENCY AND DEPRESSIVE MOOD IN FEMALE MEXICAN FACTORY WORKERS⁸

Abstract

Background: Iron deficiency and depression are both disorders found primarily in premenopausal women. Results of studies that explore the association between the two are inconsistent.

Objectives: To investigate the magnitude of association of iron deficiency and depressive mood in poor female Mexicans. To assess the effect of a) fatigue as a mediator and of b) potential confounders.

Methods: Iron status, fatigue, depressive mood, and socioeconomic and behavioral factors were assessed in 100 female Mexican garment-factory workers. Iron status was assessed with hemoglobin, ferritin, transferrin saturation, mean corpuscular volume, and protoporphyrin. Severity of depressive mood was defined with the Beck Depression Inventory cutoffs for severe (≥ 29) and moderate (20–28) depressive mood. Fatigue was assessed with the Fatigue Severity Scale. The magnitude of the association between depressive mood and iron deficiency was investigated with logistic regression analysis. Outcomes were the risk of moderate or severe depressive mood. Iron status indicators were used as either continuous or dichotomized variables, or as composites. Covariates were fatigue and potential confounders. Socioeconomic and lifestyle covariates were further summarized with factor analysis.

Results: The participants were on average 27 years old, and 51% were above a BMI of 25 kg/m². Ninety-two percent had a household income below the national Mexican poverty line for household income. Average hemoglobin value was 131 g/L

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± 16 ; the 25th percentile was at 123 g/l. Anemia was therefore present in 24% of the sample, whereas 21% had iron depletion (hemoglobin < 123 g/L, and at least one other indicator of iron deficiency beyond cutoff). The percentage of moderate and severe depressive mood was 25 and 26, respectively. Mean fatigue score was 3.8, ranging from a score of 1 to 7. Only hemoglobin level was significantly related with risk of severe depressive mood. For every g/L increase in hemoglobin, the odds ratio of severe depressive mood decreased by 6%. Including fatigue and a factor score for socioeconomic status and crowding into the statistical model did not substantially change this association. Risk of moderate depressive mood was not associated with iron status.

Conclusion: In female Mexican factory workers, lower hemoglobin levels are strongly associated with increased risk of severe depressive mood.

Keywords: Mexican, hemoglobin, iron, depression, fatigue, confounder

Introduction

Iron deficiency (ID) and depression are both prominent public health problems in premenopausal women. Forty-eight percent of women in developing countries have iron deficiency anemia (IDA) [1]. Prevalence of major depressive disorder (MDD) in developing countries is between 2 and 23%. In Mexico, prevalence of anemia in women is between 16% (in 2006) and 21.5% (in 1999) [6, 7]; whereas 41% are ID [9]. Prevalence of lifetime MDD is 7.8 to 15.9% in Mexican women [13, 20], and prevalence within the previous year is 5.8 to 7.6% [20, 21].

ID and depressive mood are positively associated in the majority of observational studies [37-40]. Iron supplementation trials have inconsistent results, with 4 trials in industrialized nations finding no association [42-45], while 1 trial with South African postpartum women shows a significant improvement in depression scores with iron supplementation compared to placebo [46]. This study is the only one

addressing the association in a developing nation. It appears that an efficacious supplementation with iron might be more probable in women living in poverty beyond the levels found in developed countries.

The role of fatigue in this relationship has not been investigated in the published literature. However, fatigue is not only associated with iron status [39, 43-45, 52], but is also a part of the clinical diagnosis of depression [53]. Indeed, it is significantly associated with depressive mood [54-59]. Thus, misclassification of ID and fatigued participants as depressed could occur when depressive mood is measured with depression assessment instruments that include fatigue as part of the assessment.

An additional challenge to the validity of the findings is that the majority of the studies have not controlled for confounders [37, 40, 41, 43, 45, 46]. Several factors such as low socioeconomic status (SES) [4, 12, 18-21, 61-86], ethnic group [4, 12, 71, 83, 84, 88, 89], marital status [18, 20, 65, 68, 74, 92, 93], social isolation [81, 91], and locus of control [17, 63, 78, 90] are associated with both risk of depression and ID. Therefore, without confounder control, the association could be spurious.

Our work with the Hispanic Health and Nutrition Examination Survey (HHANES) explored these research questions as well. It shows that fatigue and other covariates affect the inverse association between iron status and risk of depressive mood only minimally or not at all (Chapter 2).

In order to elucidate the nature of the relationship between iron status and depressive mood, this chapter investigates the following hypotheses:

- 1) ID and risk of depressive mood have a significant inverse association in a sample of poor female Mexicans.
- 2) The association between the ID and depressive mood will remain significant, despite the control for fatigue in the model. Fatigue might be a mediator of the relationship between ID and depressive mood.

- 3) ID and depressive mood will continue to be associated, even with the inclusion of confounders in the statistical model.

Methods

Location

In the summer of 2001, an observational study with female garment-factory workers was undertaken in the Mexican state of Morelos. Morelos is located in the central mountainous region of Mexico, 50 miles south of Mexico City, at a mean elevation of 1548 m (4856 ft). The factories were located in a garment-factory complex (Ciudad de la Confección) south of the city of Cuernavaca. The study assessed the effect of ID on two outcomes: emotional health and work productivity. This chapter will report on the emotional health study; the results of the productivity study can be found in Hernandez-Cordero et al. [221]. Logistic and technical support was provided by the National Institute of Public Health of Mexico (INSP).

Screening and matching

Managers of the Ciudad de la Confección were asked in the summer of 2000 whether they would be interested in participating in the study. A total of 4 managers of the 6 factories agreed. In those factories, all women were invited to participate in the first stage of the study in the summer of 2001. This screening stage measured a number of characteristics necessary for the selection of the sample. Hemoglobin status was initially assessed by HemoCue (HemoCue AB, Aengelholm, Sweden) from a sample of capillary blood. Calibration of the Hemocue with a reference cuvette provided by the manufacturer was performed daily before the start of measurements. A two-page questionnaire was administered to obtain information about the type of work, number and ages of children, age of the worker, and reproductive status.

Women who were pregnant, were younger than 18 years of age, or had a child younger than 1 year of age were not eligible for participation in the study.

Since the initial prevalence of anemia of 11% during screening turned out to be much lower than the originally anticipated 20 to 23% [6], a block design for sampling was used. Women with anemia (defined as hemoglobin levels < 120 g/L) were given priority. Women without anemia (hemoglobin ≥ 130 g/L) were matched to the anemic women. Matching criteria deemed influential on the measured outcomes were physical intensity of the work (productivity part), and family situation (emotional health study). Family situation grouped women into 1) younger than 40 years of age and having no children under 10 years, 2) younger than 40 years of age and having children under 10, and 3) older than 40 years. Clusters consisted of one anemic participant and two to three non-anemics who were randomly chosen from the pool of eligible non-anemic women. Women who did not fit any of the inclusion criteria were excluded from further participation. After 3 weeks in the sampling pool, non-anemic cases were dropped. All women who were excluded received information on their weight, height, body mass index (BMI), and hemoglobin status the next day. Women who had screening hemoglobin levels below 120 g/L were counseled to see a physician and referred to the free medical clinical on site. They also received a month's supply of iron supplement capsules with instructions on how to take them. Women whose hemoglobin levels were less than 80 g/L or greater than 169 g/L were immediately counseled to see the physician on site. They were not included in the study. Once all interested women were identified and matched, the next factory was approached. Anemics who had no match in their factory were matched to non-anemics from a different factory. Thus, matched pairs might not have necessarily been nested within the same factory.

Data collection

The second stage of the study included a venous blood draw, which was collected into vacutainer tubes and refrigerated until arrival at the INSP, where all blood samples were analyzed. Hemoglobin [g/L] and mean corpuscular volume (MCV) [fL] were assessed in an automated system, Cell Dyn (Abott, Santa Clara, California, USA); protoporphyrin [$\mu\text{g}/\text{dL}$] concentrations were measured with a ZPP Hematofluorometer (AVIV Biomedical, Lakewood, New Jersey, USA). Sampling clusters with complete information were selected randomly into a subsample for a more extensive iron status evaluation (compare Appendix B, section 1.2.1, Methods for subsample: Sample selection). Subsequent analysis revealed that hemoglobin was the only iron status indicator in this sample affecting risk of depressive mood. Therefore, in order to maximize sample size, only hemoglobin, MCV, and protoporphyrin were used in the analysis. For methods of the more extensive iron status evaluation in the subsample, see Appendix B, section 1.2.1, Methods for subsample: Iron status.

All iron status indicators were evaluated as continuous and categorical variables. Cutoffs were the following: hemoglobin $< 123 \text{ g/L}$,⁹ protoporphyrin $> 70 \mu\text{g}/\text{dL}$, and MCV $< 80 \text{ fL}$. In addition, composite measures were developed. Iron depletion without anemia (IDeNA) was defined as hemoglobin $\geq 123 \text{ g/L}$, and MCV and/or protoporphyrin beyond cutoff. Iron depletion with anemia (IDeA) was hemoglobin $< 123 \text{ g/L}$ and MCV or protoporphyrin beyond cutoff. All other cases with hemoglobin $\geq 123 \text{ g/L}$ and no IDeNA were defined as iron sufficient.

In addition, stage 2 included depression assessment with the Spanish version of the Beck Depression Inventory-II (BDI; The Psychological Corporation, Harcourt Brace & Company: San Antonio) [223] (Appendix E, Beck Depression Inventory).

⁹ As a result of the higher altitude of Cuernavaca, hemoglobin values are generally higher than at sea levels. Hence, the suggested cutoff for anemia is 123 g/L instead of the usual 120 g/L [222].

The BDI is a clinical screening tool that assesses the presence and severity of depression in adults and adolescents [223, 224]. The instrument contains 21 statements with four answer choices ranging in scoring from 0 to 3. A total additive score ranges from 0 to 63 points. The BDI provides cutoffs for minimal (score of 0–13), mild (score of 14–19), moderate (score of 20–28), and severe (≥ 29) depression. Women who answered questions on the BDI about suicide in the affirmative were referred to the free medical service in the Ciudad de la Confección. They also received travel fare to a social service center that addressed mental health needs at no cost to their clients. For validation purposes of the BDI, the Spanish version of the Center of Epidemiologic Studies Depression Scale (CESD) [225] was included as well (Appendix E, CESD). The CESD is a depression screening tool originally developed by Radloff et al. [156] for an English-speaking population. It consists of 20 items. It has been validated and used in Mexican populations [218, 225, 226]. Order of administration of the two depression scales was alternated for every new participant entering stage 2 of the study.

Fatigue was assessed with the Fatigue Severity Scale (FSS) [227] (Appendix E, Fatigue Severity Scale). The FSS was chosen because it is a short fatigue-severity assessment tool and has shown good differentiation between fatigue in non-depressed and fatigue in depressed subjects [227]. It was originally developed from a 29-item fatigue assessment instrument [228]. Participants estimate their range of fatigue from 1 to 7; a visual aid was provided that allowed participants to point to the level of fatigue they had experienced in the past 2 weeks. The final additive score of the 9-statement scale is divided by 9. The FSS had not been translated into Spanish previously; therefore, translation was undertaken with the help of a professional translator. The scale was subsequently back-translated into English by a bilingual

speaker other than the translator. Discrepancies were resolved by discussion with native speakers. This method was recommended by Brislin et al. [229].

Additional questionnaires included a health history; a short food-intake record; an SES evaluation that was originally used by the Mexican National Nutrition Survey of 1999 [230]; information on the living situation such as marital status, household composition, time spent with second jobs, or child care support (Appendix E, Mexico 2001). Since only the SES evaluation was developed in Spanish at the INSP, all other questionnaires were translated with the aforementioned method [229].

All questionnaires were tested with volunteers in order to explore comprehension; they were then modified if necessary. Interviewers were trained for several days in the administration of the questionnaires; the training included mock interviews with the coordinators and volunteers from the INSP staff. All parts of the questionnaires were read to the respondent; thus it was ensured that semiliterate and illiterate women had a good understanding of the questions and the answer options.

After completion of the interview, participants received information on their weight, height, BMI, and iron status. Upon completion of the study within a factory, an item considered useful by the factory workers was donated (e.g., a microwave or a first aid kit). The project had been approved by both the Human Research Review Boards at Cornell University and the INSP.

Statistical analysis

Statistical analysis was carried out with SAS 9.1 [176]. Two main outcome variables were created from the BDI: severe depressive mood (≥ 29 points), with all other categories (none to moderate; < 29 points) as a reference; and moderate and severe depressive mood (≥ 20 points), with all others (none to mild; < 20 points) as a reference. In addition, two outcome variables contrasted severe depressive mood (≥ 29

points) with none to mild (0–19 points), and moderate depressive mood (20–28 points) with none to mild (0–19 points).

Because of the study design, modeling was done using several procedures. Adjustment for the higher level of factory was done with a mixed-model procedure for logistic regression (proc glimmix). Matching was adjusted for by inserting the matching criteria as control variables into the model. In addition, proc logistic was used to estimate factory effect as a modifier of the relationship between iron status and depressive mood. The magnitude of the effect of the factory variable and the matching criteria were assessed. Models were adjusted according to the statistical importance of the variables and their impact on the effect of iron status.

Variables indicating iron status were the independent variables of main interest. All iron status indicators were tested as both continuous and categorical variables. Composite indicators were tested as well. Curvilinear relationships were assessed. Hypotheses 2 and 3 were tested by inserting fatigue and potential confounders into the established models with the iron status indicators. Covariates were initially entered individually; subsequently, all variables with a $p < 0.2$ were tested in various combination, in order to explore their importance.

Covariates of interest included physical characteristics, lifestyle and family situation, and SES. More specifically, they were a) physical characteristics: BMI; age; reproductive status; medications taken; oral contraceptive use; alcohol use; b) family situation: marital status; number of children under 5, 10, or 18 years of age; whether the father helps with child care; whether the woman lives with family, friends, and if yes, with whom; or alone, c) work: physical strenuousness of the work at the factory; whether the woman worked in a second job, and if yes, how many days of the week; d) SES: quality of the roof, walls, and floor; water source; sanitation; household income; number of possessions; crowding (number of rooms – number of persons

living in home); and whether the kitchen was separate or not, or no kitchen existed. For a more specific description of the variables, see Appendix E, Mexico 2001.

Folate and vitamin B12 deficiencies have both been shown to be associated with depression [160-163, 231]. Likewise, folate and vitamin B12 deficiencies are associated with macrocytic anemia [8, 154]. Thus, cases with MCV>94 fL [154] were identified as macrocytic and potentially folate- or vitamin-B12-deficient. Cases with anemia (hemoglobin < 123 g/dL) and macrocytosis were considered to be folate-deficient anemic or vitamin-B12-deficient anemic, and were excluded. Models assessing the effect of MCV>94 fL were treated as follows: cases with high MCVs were initially excluded from the statistical analysis; subsequently, MCV>94 fL was included as a control variable.

Residual plots were investigated for outliers. In order to explore the relative importance of specific cases, observations were excluded and models rerun; odds ratios of the reduced data set were compared with those of the full data set. Exclusion was based on extreme values of depression scores or iron status indicators, as well as high residual values. Best models were chosen based on theoretical considerations, significance of the variables, significance of the models, and parsimoniousness.

In addition, factor analysis (SAS 9.1, Promax procedure) was performed in order to assess interrelationships between social, economic, and demographic variables, and to summarize variables that expressed a similar domain in the preliminary regression analysis. Factors were set to be correlated (oblique rotation). Variables included were type of wastewater removal; quality of floor, walls, and roof; quartiles of number of possessions; water source; availability of separate kitchen for cooking; household income (<4377 pesos,¹⁰ ≥4377 pesos), quartiles of crowding (number of rooms – number of persons living in house); household composition

¹⁰ equivalent of US \$157/month in summer of 2001; national household poverty line in Mexico in 2001 [232]

(living alone, with parents, with brothers or sisters, with a friend, or other); marital status (married or living with boyfriend, single, or divorced, separated, widowed, single mother); father helping with child care (no kids, yes, no); participant's number of work days in a second job; children under 10 (yes, no; quartiles), under 5 (yes, no; quartiles), and under 18 (yes, no; quartiles). All variable levels were re-coded according to their risk of being a cause for depression based on the literature review. For instance, women reporting higher poverty, crowding, a second job, children under 10, or a disrupted marital status would be considered at higher risk. Factor models were evaluated based on scree plots, underlying expectations, interpretability, and amount of variance explained. Resulting factor scores were tested as independent effects in the logistic regression model.

Sample selection

A total of 337 women were screened. The overall response rate to the invitation to the initial screening stage was 69%. The breakdown by factory was as follows: Unger 85%, Phantom 73%, CIM 65%, and Avi 32%. Of the screened individuals, 134 were invited to participate in the 2nd stage of the study. Of those, 4 cases refused, 2 women could not be located, 4 reduced their hours of work to 6, and for 6 women no match could be found. A final total of 118 women was included based on inclusion criteria, blocking, and matching characteristics. Figure 3.1 shows the creation of the final sample size of 100 cases. For selection of the subsample with complete iron status data, refer to Appendix B, section 1.2.1, Methods. A comparison of characteristics between women participating in the screening stage only and the final study participants showed no significant differences ($p < 0.05$) in terms of marital status, height, weight, and age (Appendix Table B.1).

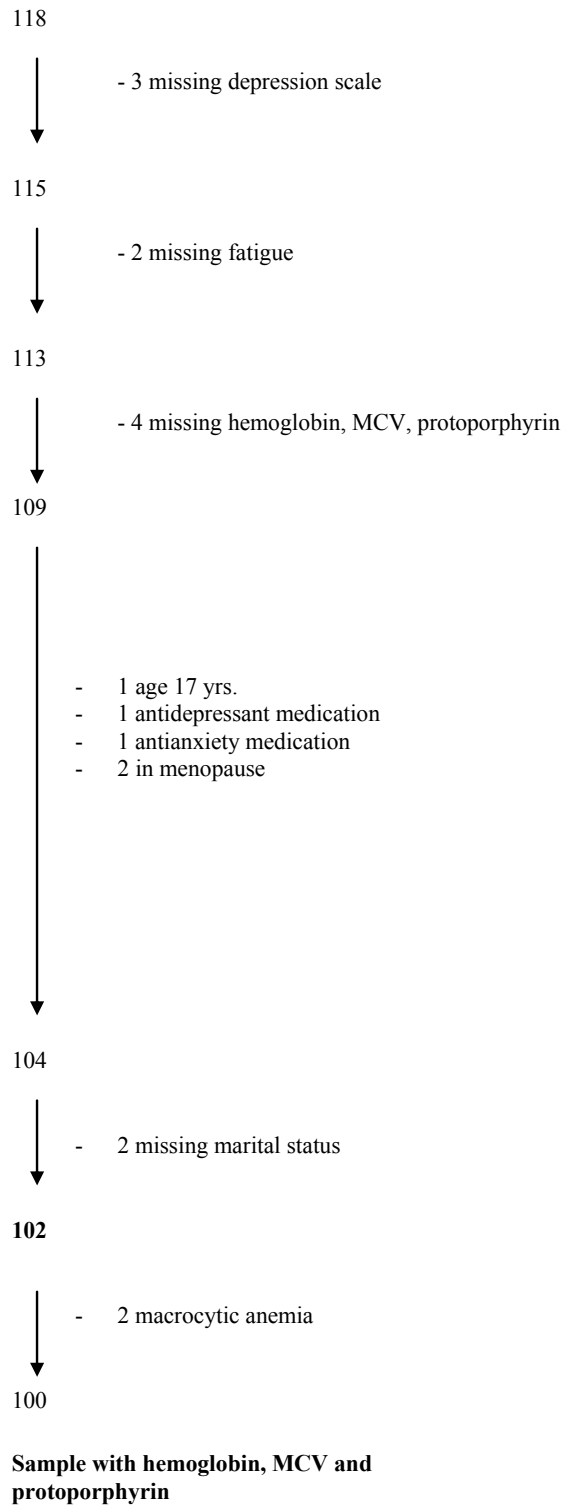


Figure 3.1: Sample selection.

Results

Sample description

A prominent feature of this sample was the high percentage of depression (Table 3.1). Twenty-five percent suffered from moderate depressive mood, whereas 26% had severe depressive mood. With the recommended cutoff of the CESD of 16 points [156], 59% of the sample can be considered depressed. BDI scores ranged from 0 to 61 points. Twenty-four percent were anemic, 21% had IDeA, and 15% suffered from IDeNA (Table 3.2). Hemoglobin ranged from 87 to 161 g/L. The percentage of participants who had MCVs greater than 94 fL was 23. This sample might, therefore, have a high percentage of folate or vitamin B12 deficiency that has not yet resulted in anemia. In addition, 51% of the participants were overweight or obese (Table 3.3). Only 5% of the sample used oral contraceptives; none of the women reported drinking more than 1.5 glass of alcohol on a daily basis (not shown). The proportions of women from different factories were as follows: Unger were 55% of the sample, Phantom 24%, CIM 15%, and Avi 6% (not shown). For a description of the subsample with complete iron status indicators, consult Appendix B, section 1.2.2, Results for subsample.

Socioeconomic covariates: Factor analysis

Analysis of potential confounders showed that variables indicating crowding in the home were associated with severe depression. Since several of the categorical crowding indicators had small cell sizes, a factor analysis was performed, creating the continuous factor “crowding” (factor 2; Table 3.4). A higher quality of the roof and the walls in this factor was associated with having a kitchen exclusively for cooking (instead of no kitchen, or one for cooking and sleeping), more rooms for the number of people in the house, and no children under 10 years of age. Factor 2 showed that crowding is also a socioeconomic phenomenon, since it includes the quality of the

house. Other factors identified in the analysis were a wealth factor (factor 1) and a child care/work hours factor (factor 3). Factor 1 showed that better wastewater removal, better water source in the house, higher quality of house construction, being married, and a higher number of possessions were associated. Factor 3 indicated that women with children under 10 years of age were less likely to work in a second job. When women were working in a second job, and had children under 10, fathers were more likely to be involved in child care. It should be noted that only 13% of the women had a second job, and 26% had children under 10 years of age (not shown). Factors correlations were between $|0.057|$ and $|0.28|$ (not shown). Household income and family composition did not load high enough (loading $|0.4|$) in any of the factors to be included in the final analysis.

Main effects model: Influence of fatigue and socioeconomic covariate

Factory as a random factor in the glimmix procedure demonstrated a lack of effect on hemoglobin, even though factory accounted for some of the variation (Table 3.5, Model 5). Moreover, all matching criteria included in the model were not significant and did not affect the coefficient of iron status (not shown). Thus, the most parsimonious model, a linear logistic regression without factory as a random factor, and without adjustment for matching criteria, was chosen as the appropriate model (Table 3.5, Model 4). Initial logistic regression models excluded all cases with macrocytosis ($MCV > 94$ fL). Despite the smaller sample size, the effect of hemoglobin on severe depressive mood was significant (not shown). Also, a model having $MCV > 94$ as a control variable worked just as well, while maintaining the full sample size. Hence it was the preferred model.

Table 3.1: Subject characteristics, mood; mean (SD), median, 25th and 75th percentile, or %; n=100.

Variable	Mean (SD)	Median	25th p.	75th p.	% of whole sample
BDI	22.1 (14.0)	20	13.5	29.5	
Fatigue	3.8 (1.5)	3.7	2.7	4.9	
CESD-20 ^a	20.1 (10.6)	18.5	13.0	26.0	
% BDI moderate score 20–28					25%
% BDI severe score ≥ 29					26%
% CESD-20 > 16					59%

^a The Spanish version of the Center of Epidemiologic Studies Scale (CESD) [156] was used as a validation tool in this study; a score above 16 is the recommended cutoff for depressive disorders.

Table 3.2: Subject characteristics, iron status; mean (SD), median, 25th and 75th percentile, or %; n=100; iron depletion is defined as hemoglobin < 12.3 g/dl, and mean corpuscular volume (MCV) or protoporphyrin beyond cutoff.

Variable	Mean (SD)	Median	25th p.	75th p.	% of whole sample
Hemoglobin g/L	131 (16)	134	123	141	
MCV fL/l	88.7 (8.5)	91.3	85.4	93.6	
Protoporphyrin µg/dL	79.0 (67.3)	55.0	36.5	94.7	
%Hemoglobin < 12.3 g/dL					24%
% MCV < 80 fL					15%
% Protoporphyrin > 70 µg/dL					37%
% Iron depletion with anemia					21%
% MCV or protoporphyrin beyond cutoff; but hemoglobin ≥ 12.3 g/dL					15%
% MCV>94 ^a					23%

^a indicator of macrocytosis, therefore potential folate and/or vitamin B12 deficiency

Table 3.3: Subject characteristics, general; mean (SD), median, 25th and 75th percentile, or %; n=100.

Variable	Mean (SD)	Median	25th p.	75th p.	% of whole sample
Age (yrs)	27.1 (7.8)	24.5	20	33	
BMI kg/m ²	25.6 (4.2)	25.1	22.6	28.6	
Factor 2 (Table 2) (crowding, SES)	0.0 (1.0)	0.2	-0.5	0.6	
% BMI>25 kg/m ²					51%
% BMI>30 kg/m ²					14%
% Household Income<4377 pesos ^a					92%
% Dirt floor					10%
% dirt floor, walls and roof plastic, asbestos or metal					4%

^a equivalent of US \$157/month in summer of 2001; poverty line level 1 for households in 2000 [232]

Table 3.4: Factor structure for socioeconomic variables. Bold numbers reflect factor loadings $> |4|$.

Variable	Factor 1 House/SES	Factor 2 Crowding, SES	Factor 3 Life stage
Wastewater removal	0.61	-0.07	-0.07
Quality of floor	0.75	0.38	-0.02
Number of possessions (quartiles)	0.61	0.20	0.15
Water source	0.61	0.34	-0.16
Marital status	0.47	-0.1	-0.15
Quality of walls	0.59	0.41	0.17
Quality of roof	0.58	0.48	0.10
Availability of kitchen	0.09	0.72	0.02
Crowding (quartiles)	0.27	0.68	-0.02
Father's involvement in child care	-0.01	0.01	0.82
Mother works 2nd job	-0.07	-0.25	0.53
Children under 10	-0.01	-0.47	-0.70
Variance explained	2.6	1.99	1.6

Table 3.5: Logistic regression models; outcome Beck Depression Inventory (BDI) severe depressive mood; OR (95% CI); n=100.

Variables	Fixed factor models; OR (95% Confidence Intervals)				Mixed model
	Model 1	Model 2	Model 3	Model 4	Model 5
Intercept	0.21 (0.11, 0.40)****	0.006 (0.00, 0.05)****	0.20 (0.10, 0.39)****	0.003 (0.00, 0.03)****	0.002 (0.00, 0.03)**
Centered hemoglobin g/L	0.95 (0.92, 0.98)**	0.94 (0.90, 0.97)**	0.95 (0.92, 0.98)**	0.94 (0.90, 0.98)**	0.95 (0.91, 0.98)**
Fatigue		2.26 (1.50,3.42)****		2.52 (1.61, 3.94)****	2.94 (1.75, 4.92)****
Factor 2 (crowding, SES)			0.72 (0.45, 1.15)	0.52 (0.28, 0.96)**	0.43 (0.22, 0.85)**
MCV>94 fL	3.07 (0.99, 9.54)*	3.74 (1.004, 13.91)**	2.90 (0.92, 9.12)*	3.44 (0.87, 13.55)*	3.97 (0.88, 17.87)
N	100	100	100	100	100
Area under the ROC	0.703	0.848	0.725	0.858	n/a
-2LL	102.3	82.4	100.4	77.5	531.6 ¹
Random factor factory: Cov parameter estimate (std error)	n/a	n/a	n/a	n/a	0.357 (0.735)

¹ Pseudo-log Likelihood for Proc glimmix

* p-value ≥ 0.05 and < 0.1 ; ** p-value < 0.05 ; *** p-value < 0.001 ; **** p-value < 0.0001

After analyzing the effect of all indicators of iron status (not shown), only hemoglobin was related to depressive mood. Assessment of curvilinearity yielded no significant findings. Similarly, only severe depressive mood, defined as BDI ≥ 29 points, performed well in the models; while moderate depressive mood (BDI > 20 points) did not (Appendix Table B.2). Likewise, reducing the potential outcome to no and mild depression versus severe depressive mood (excluding moderate depressive mood); or no and mild depression versus moderate depressive mood (excluding severe

depressive mood) did not add additional information (Appendix Table B.3).

Hypotheses 2 and 3 postulated an effect of fatigue and potential confounders. Neither one affected the coefficient of hemoglobin in a substantial manner, nor in the expected direction (Table 3.5, Models 1–4). Interactions of iron status with MVC>94, factory, and oral contraceptive use were not significant (not shown).

In summary, the preferred model (Table 3.5, Model 4) showed that each unit increase in hemoglobin (g/L) was associated with a 6% decrease in the odds ratio of severe depressive mood. When using IDeA (hemoglobin < 12.3 g/dL, and MCV and/or protoporphyrin beyond cutoff), the odds ratio of severe depressive mood was 3.44 (CI 0.87, 13.63, p-value 0.07) compared to all other cases (Appendix Table B.4). Residual plots showed no outliers (not shown). All fixed models in Table 3.5 are significantly ($p < 0.05$) different from each other, assuming a difference of degrees of freedom of at least 1.

For a description of the results of the logistic regression analysis for the subsample with complete data, consult Appendix B, section 1.2.2, Results of subsample.

Discussion

In summary, in the present sample of female Mexican factory workers, hemoglobin was inversely associated with severe depressive mood. For every unit increase of hemoglobin (g/L), the odds ratio of severe depressive mood decreased 6%. Controlling for fatigue and potential confounders as covariates did not affect the coefficient of hemoglobin.

Limitations and plausibility

Causality

Since the present study is observational, we are limited to plausibility statements. In addition, no causal inferences can be drawn, since directionality cannot be established. A randomized, controlled trial would allow one to draw causal inferences, but only 1 out of 5 supplementation trials has shown that iron supplementation results in a reduction of depression scores [42-46]. Moreover, depressive disorders can be associated with weight gain or weight loss [53], which implies an alteration in diet and/or nutrient composition of the diet (e.g., a shift away from iron-rich foods, or vitamin C-rich foods that increase absorption of iron). The effect of depression on healthy eating or supplement use has been explored by Bowen et al. [233]; the authors have not found an association between depression and fruit and vegetable consumption. Moreover, observational studies are vulnerable to confounding; thus, association might be spurious if confounder control was inadequate.

Past depression

Past depression was not assessed in the present sample. Severe past mood disorder increases the likelihood of recurrence of depression [178]; past depression might affect eating behavior and thus iron status, even though, to our knowledge, no published study has investigated this relationship. Mean age of first onset of MDD is between 25 and 31 years of age in international samples [2]. In the US, lifetime prevalence of major depressive disorder (MDD) at between 18 and 29 years of age is 15% [62]. Since the present sample was relatively young (mean age 27 years), the majority of participants who will experience MDD in their lifetime might therefore currently be experiencing their first episode or have yet to experience it. Thus, confounding by past depression might not be as severe as in an older sample.

Antidepressant intake due to past and/or current depression might have influenced the results, but the only woman reporting it was excluded.

Validity of sample selection

The final sample is only a subset of the women working in the factory and of the screened women. Thus, selection bias could be a problem. First, 69% of all women in the four factories participated in the screening stage. While this average between the four factories is acceptable, the individual percentages indicate that the factory Avi had only 32%, which is probably too low to be representative of a broader population. Differences between factories in the effect of hemoglobin on depressive mood were assessed during the analysis. Factory was controlled for in several ways, with no significant effect. Thus, even though the group from Avi may not be representative of the factory population, it did not differ from the other three factories with respect to the variables of interest.

Women were selected for the final sample according to anemia status and matching criteria. While every anemic woman was included, non-anemic women were selected randomly in order to ensure a representative sample. Women in the final sample did not differ from women in the screening stage in select characteristics. It is therefore likely that the screening sample and the study sample are comparable.

Validity of Mexican garment-worker sample

The present sample of Mexican female garment-factory workers might only be representative of poor Mexican women in a semiurban environment. Albeit, in light of the scarcity of information on the relationship between iron status and depressive mood in developing countries, this study adds valuable information.

Validity of the Beck Depression Inventory (BDI)

General

The BDI was originally developed with a non-Hispanic White and African American sample [223, 224]. The BDI-II is a relatively new scale; therefore, the number of studies evaluating it is limited. In English-speaking samples that did not have a prior diagnosis of current depression, the validity of the BDI is high when assessing Chronbach's alphas; effect of ethnicity, age, or gender; factor structure; level of depression; and discriminant validity from anxiety [172, 223, 234-237]. For more information on the validity of the BDI, please consult the internal validity section of this chapter.

Hispanic

A drawback of the Spanish version of the BDI-II is that cultural terms for mood disorders are not addressed in the translation. In Mexican culture the term "nervios" (loosely translated as "nerves") indicates somatic disorders (such as gastrointestinal problems, vomiting, lump in throat, and headaches), dysthymia and major depression, and anxiety [184]. Somatization of dysthymia or major depression is frequent in Hispanic populations [157, 186, 187].

A number of BDI validation studies have been undertaken in Hispanic populations, including Mexicans. In general, the validity of the Spanish BDI-II appears to be as high as that of the English-language version. Moreover, language of interview does not appear to affect test scores [238-242]. It is also noteworthy that exclusion of somatic questions did not improve the performance of the Spanish BDI-II [241].

For associations between the BDI-II and the CESD in our data set, please consult the internal validity section of this chapter.

In summary, the BDI-II appears to be a good measure to assess depression in Spanish-speaking populations. Challenges to accurately assessing depression severity with the BDI-II in a Mexican population with low educational level might be the high item difficulty, which reduces understanding; lack of culturally specific terms for depression; and lack of discrimination between depression and anxiety. However, a misclassification of cases by the BDI-II would result in a weaker association between hemoglobin and severe depressive mood. We were, on the other hand, able to show a significant relationship between hemoglobin and depressive mood.

Validity of the Fatigue Severity Scale (FSS)

The FSS has been an established measure of fatigue but was developed for English-speaking patients and healthy control subjects [227]. The FSS is usually evaluated in comparison to clinical diagnosis. To our knowledge, in 2001, when this study was conducted, no fatigue scale existed in Spanish. The English version of the FSS has good validity in terms of Chronbach's alphas; discrimination between fatigued and non-fatigued, and fatigued and depressed; test-retest reliability; and correlations with other fatigue scales [227, 243-246] (compare also the internal validity section of this chapter). However, our Spanish translation has not been validated in a broader population. Likewise, the concept of fatigue has to our knowledge not been explored in the Mexican population. Thus, the present study might not measure fatigue accurately. It should be kept in mind that the internal validity of the Spanish FSS appeared to be good (see the internal validity section of this chapter). Moreover, this study aimed at producing a short measure of fatigue severity, for which the FSS was deemed to be appropriate.

Substance abuse

Substance abuse was not specifically assessed, although participants were asked about their alcohol and medication use. Substance abuse is associated with malnutrition and depression [53, 189, 190, 193-198, 204-206]; thus it is a potential confounder. While no women reported drinking more than 1.5 glasses of alcohol per day, abuse of substances other than alcohol might have increased the strength of the relationship between hemoglobin and depression. In addition, abuse of alcohol or medication might have been underreported. A review of the literature indicates that substance abuse in semirural settings close to Mexico City occurs predominantly in young Mexican males [247-249]; in young women, prevalence of abuse of substances other than medications is less than 1% to 2.81% [247, 249]. Substance abuse for both genders was 1.5% in metropolitan areas, and not significantly different or lower for the region that would include the state of Morelos, where the present study took place [247, 249]. Moreover, during the analysis phase, cases with high depression scores had been removed to test influential data points; results did not differ substantially. In summary, in this sample of rural, semiurban, and urban Mexican women, prevalence of substance abuse other than alcohol and medications might have affected the results, even though the likelihood is probably low, as a result of low reported prevalences.

Premenstrual syndrome (PMS) and perimenopause

No information was gathered relating to perimenopause. Menopausal women were excluded from the present sample, and the sample is relatively young (mean age of 27 years; 75th percentile 33 years), which makes perimenopause an unlikely contender for a confounder.

Premenstrual syndrome and premenstrual dysphoric disorder (PMDD) have been shown to be associated with depressive mood [223, 234-236]. No information on either disorder or on the exact menstruation cycle was gathered. A review of the

literature did not yield information about the association between iron status and the likelihood of PMS. Iron indicator values are not affected specifically during the time of menstruation [207]. In conclusion, women who had PMDD and were assessed at that time of their cycle could have had higher depressive mood, but the hemoglobin values were probably not affected.

Other nutrients that may cause depression

A number of nutrients have been associated with depression and/or anemia, including zinc, folate, vitamin B12, and ω -3 polyunsaturated fatty acids (specifically docosahexanoic acid) [158-163, 168]. Vitamin B12 and folate deficiency cause macrocytic anemia [8, 154]. In the present study, macrocytic anemics were excluded, and macrocytosis without anemia was statistically controlled for in the models. Intake of zinc or ω -3 polyunsaturated fatty acids, though, was not assessed. Prevalence of zinc deficiency was 30% in rural and urban Mexican women in 1999 [9]; considering the frequency of occurrence, a sizeable proportion of women in this sample might have been zinc deficient. Note, though, that Chapter 2 of this dissertation did not find an association between iron status, zinc intake, and severe depressive mood. Information on ω -3 polyunsaturated fatty acid status for Mexicans is only available for breast milk [250]. Mean concentration in Mexico is not significantly different from industrialized countries (mean % of total fatty acids is 0.26%) [250], albeit lower than the worldwide mean of 0.32% [208]. It is also important to keep in mind that the depression-lowering properties of ω -3 polyunsaturated fatty acids are inconsistent and controversial [210-212].

In summary, if cases with low hemoglobin values were also deficient in zinc or ω -3 polyunsaturated fatty acids, either one of those two nutrients might have acted as a positive confounder.

Internal validity

Hemoglobin

Hemoglobin and all other iron status measures were assessed with established methods of laboratory analysis. The National Mexican Institute of Public Health (INSP) laboratory that analyzed the blood samples had received excellent ratings by the CDC in terms of precision of measurements [251]. Hemoglobin, MCV, and protoporphyrin correlate significantly ($p < 0.0001$), indicating that low hemoglobin values are accompanied by low MCV and high protoporphyrin values; correlations are of the magnitude of $r = |0.67|$ to $|0.70|$ (not shown). Having hemoglobin less than 123 g/L was also significantly associated with being IDeA ($p < 0.0001$, Fisher's-Exact test; $n = 100$) and IDA ($p < 0.0001$ Fisher's-Exact test; $n = 76$).

Beck Depression Inventory (BDI)

The validity of the BDI has been explored extensively in previous studies. Our literature review shows that the BDI-II's Chronbach's alphas are high (between 0.73, 0.89, and 0.95) [223, 234, 236]; scores were independent of ethnicity and age in three studies [223]; however, one study found significantly higher scores for women [223], while another identified lower scores for younger participants [223, 234-236]; the BDI-II correlates well with other depression scales (e.g., the Revised Hamilton Psychiatric Rating Scale for Depression has a correlation with the BDI-II of $r = 0.71$) [172, 237]; it consistently groups symptoms into 2 factors, affective-cognitive and somatic [172, 223, 237]; the scale has also been shown to differentiate between psychiatric and non-psychiatric populations, and can distinguish levels of depression severity [223, 237].

Review articles dealt with the older version of the BDI. Identified limitations are that the factor structure is variable depending on population (between 2 and 9 factors); that the high difficulty of the items reduces the usefulness as a simple field

tool [237]; and the discriminant validity in relation to anxiety [237]. Correlations between anxiety scales and BDI can be high in non-psychiatric populations (0.22 to 0.82) [223, 237]. It should be noted that Beck et al. [223] state that the scale discriminates well when comparing mean scores of depressed (BDI-II) and anxious participants, although correlation between the BDI-II and anxiety scales (Beck Anxiety Inventory, Revised Hamilton Anxiety Rating Scale) is between $r=0.47$ and 0.60. Moreover, the test-retest reliability of the English version of the BDI is controversial, since it is dependent on time interval [238-240].

Previous studies investigated the validity of the BDI scale in Spanish-speaking Puerto Rican, Spanish, and Argentinian populations [238-240]. All of them confirm high internal consistency (Chronbach's $\alpha = 0.87$ to 0.91) [240], independent of age, education, and income; high test-retest reliability [238]; and that the scale is highly correlated with other depression assessment instruments (Symptoms Scale Checklist-90) [238]; that likewise, in terms of criterion validity, groups at higher risk (women, young adults, and low SES) had higher scores than other groups [238-240]; and that factor analysis revealed 2 to 4 factor solutions [240-242]. The validity of the Spanish version of the BDI-II has only recently undergone an evaluation in Hispanic Americans of predominantly Mexican descent [240-242]. Chronbach's alphas are high in Spanish speakers (0.89 to 0.95) [240, 242]; scores were independent of age, gender, and SES [242], while higher acculturation resulted in lower BDI scores in one of the studies [240], but not in the other [240]; test-retest reliability was good [240, 242]; a 2-factor solution was favored [240, 242]; and language of interview made no significant difference in test scores [241]. Discrimination between anxiety and depression was assessed by Novy et al. [252]; they found a correlation of 0.60 between the Spanish versions of the Beck Anxiety Inventory and the BDI-II, although the authors interpret the correlation as low (an indicator of good discriminate validity) compared to higher

correlations between anxiety assessment tools of 0.77. Little information exists on the validity of the BDI in Mexicans in Mexico. A study from 1997 with middle-aged Mexican rheumatology patients from Mexico City shows that the agreement between clinical diagnosis of any depressive disorder and BDI was as follows: sensitivity=92%, specificity=86%, positive predictive value=0.80 [240]¹¹; interestingly, the exclusion of somatic symptoms did not improve the validity of the BDI. This is of importance, since somatization is an important feature of depression in Hispanics [157, 186, 187].

In the present study, the BDI-II had a high Chronbach's alpha (0.90). It correlated well with the CESD¹² [156]: the Pearson correlation was 0.62 ($p < 0.001$). When assessing sensitivity and specificity of the two depression scales, the CESD performed best at a cutoff of 21 (sensitivity=78%, specificity=92%) when compared to severe depressive mood of the BDI (not shown). When assessing moderate depressive mood, CESD cutoffs never rose above a sensitivity or specificity of 70% when compared to moderate depressive mood of the BDI. The expected association of the BDI-II with the FSS was found ($r = 0.50$, Pearson correlation; $p < 0.0001$).

In summary, the Spanish BDI-II appears to be a valid tool for depression severity assessment in the present Mexican sample.

Fatigue Severity Scale (FSS)

The FSS has not been used in a Latin American population previously, and at the time of the present study (2001) it had not been translated into Spanish. The English version of the FSS has been shown to have a high Chronbach's alpha (0.88 to 0.94) [227, 243, 244]; to discriminate between fatigued and healthy subjects [227, 243, 244]; to be correlated with most other fatigue scales ($r = 0.47$ to 0.81) [243, 244] except

¹¹ Sensitivity and specificity assessment with the BDI are rare, since it is usually compared to clinical diagnosis, not other scales.

¹² The CESD has experienced extensive use in Latin populations, including Mexico [218, 225, 226].

the Fatigue Impact Scale ($r=0.15$) [227, 243]; and to be unidimensional (1 factor only) [227], although 2 factors were demonstrated by Hagell et al. [243]. In addition, it showed substantially reduced scores with improvement of fatigue in clinical patients [227] and good test-retest reliability [227, 244]; furthermore, the associations with depression (CESD) were relatively weak to moderate (0.2 to 0.46), depending on population tested [227, 245]; mean scores of the FSS for patients with severe fatigue were higher [243, 245]; and scores were independent of gender, but not of age [243]. In a Spanish-speaking population, the FSS correlated significantly with the Fatigue Impact Scale ($r=0.68$) [246].

Since the FSS had previously not been validated in a Spanish-speaking population, we did a more extensive validation with our Spanish version. The FSS had a high internal consistency (Cronbach's $\alpha = 0.78$); and correlated significantly with a summary score of the three fatigue questions of the CESD ($r=0.46$; $p<0.0001$) (not shown). This strength of association was to be expected [227, 245]. As demonstrated in previously published studies that assessed the association between depression and fatigue [54-57], the FSS also showed a significant correlation with the BDI-II (Pearson correlation $r=0.50$; $p<0.001$). The FSS was created from a 29-item questionnaire [228] and represents the unidimensional fatigue severity factor. Principal component analysis with subsequent rotation (Varimax and Promax) with the criteria of a minimum Eigenvalue of 1 for factor determination revealed 2 factors. Factor 1 loaded 5 items that indicated more severe fatigue and interference with social functioning, factor 2 loaded motivational and exercise-related fatigue (Appendix Table B.5; Appendix E, Fatigue Severity Scale). The motivational item was previously reported as not fitting with the unidimensional assumption of the FSS [243]. The FSS was associated with time spent relaxing ($r=0.25$; $p=0.07$) in a subsample of 49 factory workers. FSS was not significantly associated with iron status (even when testing the

effect of matching, factory, covariates, and curvilinearity). In the best model, every unit rise in hemoglobin reduced the odds of a fatigue score above the 75th percentile of the FSS by 1.2%, $p=0.21$ (Appendix Table B.6). It is possible that fatigue in this sample of poor women is associated with factors other than iron status; these might act as confounders. Another explanation might be that this relatively young sample does not become fatigued enough to show an association between fatigue and hemoglobin. An indication might be the relatively low average score (3.8) compared to studies with fatigued subjects (4.6–6.6) [227, 243-245]. Last, the translated version might not measure the concept of severe fatigue accurately in Spanish-speaking Mexican women, consequently reducing the range of possible answers.

In summary, fatigue severity appears to be measured appropriately by the FSS in the present sample, although the FSS is not unidimensional as originally intended, and it is not associated with iron status.

Mechanisms

Lower hemoglobin levels were associated with a higher risk of severe depressive mood in the present study. Animal studies indicate a biological explanation: serum ID translates directly into brain ID [22-24]. Moreover, iron is a cofactor in the synthesis of serotonin, dopamine, and norepinephrine [25-27]. Consequently, lower brain iron concentration should result in lower concentrations of at least one of the three neurotransmitters. Alteration of concentrations of serotonin, dopamine, and/or norepinephrine in the brain are implicated as a cause of depression [114, 122, 124-129], probably because of impaired signal transmission between nerve cells. Recently, a decrease of D2-like dopamine receptors has been shown in depressed patients [126, 127]. Findings for serotonin and norepinephrine, though, are inconsistent [28-32], with dopamine giving the best indication of ID-induced impaired nerve-signal transmission due to lower D2 dopamine receptor density and transport

downregulation [29, 32-36]. In addition, the activity of monoamine oxidase, which is involved in the breakdown of serotonin, seems to be reduced [28, 30].

External validity

Anemia was assessed by Hemocue; the prevalence was 11%. Compared to Shamah-Levy et al. [6], who found a prevalence of 16 to 21% in rural and urban non-pregnant women in Mexico City and the central region with Hemocue in 1999, this value is lower. Mundo et al. [7] reported a prevalence of 14.7 to 16.8% in 2006. Iron status values of the venous blood sample analysis were not representative of the population of factory workers as a whole, since women who were identified as anemic by Hemocue were oversampled. Only 1% of the sample had transferrin saturation below the cutoff of 16% (Appendix Table B.9), which is extremely low compared to the 36 to 52% of the 1999 National Mexican Nutrition Survey [9].

Since anemic women were oversampled, and ID is associated with depressive mood in this analysis, the percentage of women suffering from severe depressive mood in the present sample (26%) is not representative of the overall population in the factories. Lifetime prevalence of MDD in unacculturated female Mexican American immigrants is 8.4%, and of dysthymia 1.6% [13]. Women in the Mexican state of Morelos, where the present study took place as well, had a prevalence of MDD in the last year of 5% [21]. In surveys assessing MDD in women in Mexico in general, the prevalence for the previous 12 months was 7.6%, and for the last 6 months was 5.7% [20, 21]; lifetime prevalence was between 7.8 and 15.9% [13, 20].

The average fatigue score in the present sample was 3.8. Compared to means from other studies, it lies between the means of non-fatigued subjects and fatigued subjects. Non-fatigued participants have an average score of 2.3 to 3.1 [227, 243]. Fatigued and/or ill subjects have a mean FSS score of 4.6 to 6.6 [227, 243-245].

Hemoglobin and depressive mood showed a significant inverse association in the present sample. Observational studies in humans generally confirm an association between ID and depressive mood [37-40], with one exception [41]. In our analysis of the HHANES we showed a significant ($p < 0.05$) decrease of the OR of depressive mood with every g/L increase in hemoglobin (Chapter 2). Five supplementation trials explored the same association, but 4 of the 5 trials had negative results [42-45]. All trials with negative results took place in industrialized countries with women who were not explicitly postpartum. Only one study with postpartum South African women showed a decrease in depression scores after iron supplementation, compared to placebo [46]. It is possible that the association between ID and depressive mood is strengthened in populations that are exposed to more, and to more severe stressors. Poverty and the postpartum period are considered such stressors, and they increase the risk of depression [53, 64-66, 86, 253-256].¹³ Biologically, chronic stressor exposure probably results in a downregulation of cortisol release [215, 216] and an upregulation of the immune system [119, 140]. Increased release of proinflammatory cytokines, which are part of the unspecific immune response of the body [138], is now thought to be one of the causes of depressive disorder [115, 120, 121]. Thus, assuming a synergistic relationship between stress and ID, the Mexican women in our study might have been more likely to show a statistically significant relationship between iron status and depressive mood.

While fatigue did not affect the relationship of hemoglobin and depressive mood in the present study, a literature review revealed no other work that allowed for comparison of the results. The significant association between fatigue and depressive

¹³ Established risk factors for postpartum depression coincide with risk factors outside the postpartum period, such as low SES. However, pregnancy-specific risk factors are unwanted pregnancy, low birth-weight babies, maternity blues, lack of social support, number of children, and a baby who cries a lot.

mood has previously been demonstrated by a number of other sources [58, 59, 179, 244].

In the present study, no association between perceived fatigue and iron status was detected. The published literature suggests that fatigue increases with iron deficiency [39, 43-45, 52]; however, two studies found no relationship between fatigue and iron status [38, 41]. Since the FSS appears to be a valid measure of fatigue severity, non-significance might be due to unaccounted confounders, lack of severe fatigue, or cultural bias.

To our knowledge, no published study has assessed the effect of fatigue and SES covariates on the relationship between iron status and risk of depressive mood. We investigated the associations with the HHANES data set (Chapter 2). Our research shows that the inverse association between iron status and depressive mood is only minimally or is not affected by the inclusion of fatigue, SES, and ethnic group as independent variables. The OR of depressive mood decreased by 11% with every 100 mg increase of body iron stores [155].

Clinical relevance

Assessing the magnitude of the effect of low hemoglobin values on severe depressive mood would help to determine the potential effectiveness of iron supplementation as a therapy for depression. A literature review revealed only one randomized controlled trial assessing the efficacy of antidepressant therapy that can be compared with our type of analysis, although this study used a different depression scale (Hamilton Rating Scale for Depression). The authors [220] use the probability of remission after therapy as their outcome, and tested the antidepressants Duloxetine and Paroxetine on a total of 367 patients with MDD. Depending on the dosage, Duloxetine increased the probability of remission by 21 to 28% compared to a placebo, while Paroxetine increased it by 17%. In the present study, when defining a

BDI score below 29 as remission (reference BDI score ≥ 29), we can estimate the potential effect of improving hemoglobin status. Hemoglobin (g/L) was the variable of interest; covariates were fatigue, factor 2, and MCV >94 . The odds ratio of remission of severe depressive mood with a unit increase in hemoglobin was in this case 1.06 (CI 1.02, 1.10). A change from a hemoglobin value of 100 g/L to 123 g/L would increase the probability of remission of severe depressive mood by 29.7 percent. This magnitude is comparable to the effect of antidepressant treatment in the aforementioned study. However, since our study was observational, it cannot conclusively answer the directionality of the association between hemoglobin values and depressive mood. Likewise, the definitions of remission in Detke et al.'s treatment trial (depression scores ≤ 7 points after 8 weeks of treatment) and in our study are very different. We compared the probability of remission in this group of poor Mexican women to the one in the HHANES, which is 3.2% (Chapter 2). The great difference in magnitude of the effect between the samples might be explained by the much higher stress exposure in Mexican factory workers (consult also the mechanisms section in this chapter), or by the greater homogeneousness of the Mexican sample that reduced extraneous variability.

Conclusion

In conclusion, a significant increase of risk of severe depressive mood with decreasing hemoglobin values was demonstrated in this sample of female Mexican garment-factory workers. The relationship persisted after controlling for fatigue and socioeconomic or lifestyle covariates.

Comparison to the published literature invites speculation about the reasons why the present analysis supported the hypothesized association between hemoglobin and severe depressive mood:

- The sample of Mexican factory workers might have shown the association more clearly than a more advantaged population, because the magnifying effect of the stressor poverty might increase vulnerability to the negative effect of ID (compare Chapter 2 and Chapter 4). It is well documented that chronic stressor exposure raises the risk of depressive mood. Thus, women who are living in poverty and are ID are exposed to two risk factors, and this double exposure might increase their risk for depressive mood substantially (consult the mechanisms section of this chapter).
- The magnitude of the probability of remission of severe depressive mood with improvement of hemoglobin levels to non-anemic levels is comparable to treatment with antidepressants. In more affluent nations, antidepressants are the best-known available treatment for depression; their administration is therefore normative. Poor developing nations might not be able to afford expensive treatment options; screening for ID of severely depressed women and administration of iron supplements might be an accepted treatment option or a preventative public health approach in financially constrained situations.

Based on the results of the present analysis, it is recommended that future research

- focus on severe depressive mood as an outcome
- use continuous measures of iron status in addition to the traditional categorical cutoffs
- target studies to poor populations, preferably in developing countries
- compare effect of iron status supplementation on improvement of depression scores to antidepressant treatment.

Chapter 4: CORTISOL, IRON STATUS, AND SEVERE DEPRESSIVE MOOD IN FEMALE MEXICAN FACTORY WORKERS¹⁴

Abstract

Background: Stressor exposure increases the risk of depressive mood in iron deficient individuals. No study has investigated whether a biological marker of perceived stress such as cortisol shows a similar modifying effect.

Objectives: To explore the modifying effect of stress on the relationship between iron deficiency and depressive mood.

Methods: In 2001, an observational study with 48 Mexican female factory workers was conducted. Iron status was assessed via venous blood sample with measures of hemoglobin, mean corpuscular volume (MCV), and protoporphyrin. Severity of depressive mood was measured with the Spanish Beck Depression Inventory-II (BDI). Fatigue severity was assessed with a Spanish translation of the Fatigue Severity Scale. Internal stress was measured with salivary cortisol, sampled 3 times a day (30 minutes, 3 and 7 hours after waking) on 2 randomly chosen workdays. For the statistical analysis, iron status was operationalized as a) the continuous variable hemoglobin (g/L) while controlling for macrocytosis, and b) the categorical variable iron depletion (hemoglobin < 123 g/L, and MCV < 80 fL and/or protoporphyrin > 70 µg/dL; vs. hemoglobin ≥ 123 g/L). Severe depressive mood was defined as BDI scores ≥ 29 points vs. the reference of < 28 points. Area under the cortisol concentration curve over the time of the day (AUC) was calculated; then the variable was dichotomized at the median. Values below the median were considered low, while variables equal to or greater than the median were considered high, reflecting hyper- or hypocortisolism at the extremes. Logistic regression was

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performed with severe depressive mood as the outcome, and iron status, fatigue, macrocytosis, AUC cortisol, the interaction between AUC cortisol and iron status, and potential confounders such as socioeconomic status.

Results: The sample of factory workers had a mean age of 29 years; mean BMI was 26 kg/m². Thirty-one percent of the women had severe depressive mood. The percentage of anemia was 29%, and iron depletion with anemia was 25%. Mean hemoglobin concentration was 131 ± 18 g/l, with a range between 8.8 and 16.1 g/l. The average Fatigue Severity Scale score was 4.1 ± 1.6, reflecting moderate fatigue severity. Mean AUC cortisol was 16 ± 15.4 nmol/L; the median was 14.1 nmol/L. The interaction between hemoglobin (continuous) and dichotomized AUC cortisol, and iron depletion (dichotomous) and dichotomized AUC cortisol had p-values of p=0.08. Cases with AUC cortisol levels below the median showed a significant 6% increase of the odds of severe depressive mood with each g/L decrease in hemoglobin (p-value of slope 0.04). No significant association was found in cases with AUC cortisol values above the median. The statistical model controlled for fatigue, crowding, and socioeconomic status. Macrocytosis was not a significant predictor of severe depressive mood in this model.

Conclusion: AUC cortisol modified the effect of hemoglobin on severe depressive mood. Individuals with low AUC cortisol show a significant rise in risk of severe depressive mood with decreasing hemoglobin levels.

Keywords: stress, cortisol, depression, hemoglobin, hypocortisolism, Mexico

Introduction

Iron is a cofactor in the synthesis of three neurotransmitters: serotonin, norepinephrine, and dopamine [25-27]; their change in concentration in the brain is associated with depression [114, 122, 124-129]. Consequently, iron deficiency (ID) is related to depressive mood in human observational studies [37-40]. Our analysis of the

association between risk of depressive mood and iron status with the Hispanic Health and Nutrition Examination Survey (HHANES) and Mexican women corroborates these findings (Chapter 2 and Chapter 3). However, supplementation trials have less consistent results. A total of 5 studies supplemented participants with iron, and compared depression scores between supplemented participants and controls at the end of the intervention [42-45]. In 4 of the studies, controls had received a placebo: in 1 the control condition was an iron-rich diet [42-45]. Only 1 of the supplementation interventions was able to find a significant decrease in depression scores in the supplemented group [46]. Interestingly, this particular study took place in South Africa, with women less than 1 year postpartum [46]. All other trials with negative results were conducted in industrialized nations without a postpartum-inclusion criterion [42-45].

In a previous study (Chapter 2), we were able to show a modifying effect of exposure to stressors on the relationship between iron stores [155] and severe depressive mood in Hispanic American women: poverty, lack of control over one's health, and post-pregnant status increase the risk of severe depressive mood in ID women, while women who are not ID and/or not exposed to a stressor are at no increased risk (Chapter 2). Based on the results in Chapter 2, we suggest that stress and ID interact, so that women exposed to both risk factors face a much higher risk of depressive mood than women with exposure to only one or none of the factors. It is well known that the stress of poverty increases the risk of depression [64-66]; postpartum status can be a risk factor as well [53, 86, 253-256].¹⁵ The likely mechanism is that chronic stressor exposure results in a habituation of the stress system, reflected in a downregulation of cortisol [215, 216], and a chronic

¹⁵ While established risk factors of depression outside of the postnatal period (such as low SES) are by far the strongest determinants of postpartum depression, pregnancy-specific risk factors have been identified as well. These include unwanted pregnancy, low birth-weight babies, maternity blues, lack of social support, number of children, or a baby who cries a lot.

upregulation of the immune system [119, 140], thereby increasing the likelihood of depressive disorders [115, 120, 121]. Neurotransmitter concentrations are altered by both hypocortisolism and high concentrations of proinflammatory cytokines [66, 121, 125, 130, 132, 133, 137]. Likewise, animal studies have shown that ID alters neurotransmitter availability in the brain [28, 29, 32-36], albeit without entirely consistent results [28-32].

In the present study, we aimed to test for a synergistic relationship between iron status, stress, and depressive mood in a population from Mexico. Moreover, instead of using stressor exposure as an approximation of experienced stress, we employed a biological marker of stress, cortisol. We expect that ID women experiencing high stress levels are at higher risk for depressive mood than women who were not as stressed and/or not ID.

Methods

Location

In the summer of 2001, an observational study with female garment-factory workers was undertaken. The factories were located in the Mexican state of Morelos, in the central mountainous region of Mexico, 50 miles south of Mexico City, at high elevation (1548 m or 4856 ft). The factories were part of a garment-factory complex (Ciudad de la Confección) south of the city of Cuernavaca. Logistic and technical support for the research was provided by the Mexican National Institute of Public Health (INSP, Instituto Nacional de Salud Publica). The project had been approved by both the Human Research Review Boards at Cornell University and the INSP.

Screening and matching

A subsample of 48 women was randomly selected from a larger sample of 100 participants (see Chapter 3). Women from 4 of 6 factories in the factory complex

participated in the three stages of the larger study. The screening stage consisted of a short assessment of a number of characteristics important for participant selection. These included hemoglobin status, which was assessed by HemoCue (HemoCue AB, Aengelholm, Sweden) from a sample of capillary blood, work type, number and ages of children, age of the participant, and whether she was lactating or pregnant. Women who were pregnant, younger than 18 years of age, or less than 1 year postpartum were not eligible for participation in the study.

The initial prevalence of anemia of 11% during screening was lower than the originally anticipated 20 to 23% [6]. Therefore, a block design for sampling was used in which inclusion of women with anemia (defined as hemoglobin levels < 120 g/L) was given priority. Women without anemia (hemoglobin ≥ 130 g/L) were matched to the anemic women based on the physical intensity of their work (important for the assessment of productivity),¹⁶ and family situation (mood component of the study). Family situation grouped women into a) younger than 40 years of age and having no children under 10, b) younger than 40 years of age and having children under 10, and c) older than 40 years. The clusters consisted of one anemic participant and two to three non-anemics who were randomly chosen from the pool of eligible non-anemic women. Once all women who wanted to participate were identified and matched, the next factory was approached. Anemic women who had no match in their factory were matched to non-anemics from a different factory. Thus, matched pairs might not have necessarily been nested within the same factory. Screening and matching procedures are also described in Chapter 3.

Women who did not fit any of the inclusion criteria were excluded from further participation. After 3 weeks in the sampling pool, non-anemic cases were dropped. All

¹⁶ The study consisted of two parts. One part assessed the association between ID and productivity; the other investigated the relationship between ID and mood. This chapter will not report on the methods and results of the productivity study. For further information, please compare with Hernandez-Cordero et al. (2003) [221].

women who were excluded received information on their weight, height, body mass index (BMI), and hemoglobin status the next day. Women who had hemoglobin levels below 120 g/L, who were either dropped because of exclusion criteria or who completed the study, were counseled to see a physician and referred to the free medical clinical on site. They also received a month's supply of iron supplement capsules with instructions on how to take them. Women whose hemoglobin levels were below 80 g/L or above 169 g/L were immediately counseled to see the physician on site. They were not included in the study.

Data collection

The second stage of the study included a venous blood draw, which was collected into vacutainer tubes. All blood samples were analyzed at the INSP. Hemoglobin [g/L] and mean corpuscular volume (MCV) [fL] were assessed in an automated system, Cell Dyn (Abott, Santa Clara, California, USA); protoporphyrin [$\mu\text{g}/\text{dL}$] concentrations was measured with a ZPP Hematofluorometer (AVIV Biomedical, Lakewood, New Jersey, USA). Based on the results of the analysis in Chapter 3, non-significant iron status measures such as ferritin and transferrin saturation were used only for internal validity assessments. All iron status indicators were evaluated as continuous and categorical variables. Cutoffs were as follows: hemoglobin $< 123 \text{ g/L}$,¹⁷ protoporphyrin $> 70 \mu\text{g}/\text{dL}$, and MCV $< 80 \text{ fL}$. In addition, composite measures were developed. Iron depletion without anemia (IDeNA) was defined as hemoglobin $\geq 123 \text{ g/L}$, and MCV and/or protoporphyrin beyond cutoff. Iron depletion with anemia (IDeA) was hemoglobin $< 123 \text{ g/L}$, and MCV or protoporphyrine beyond cutoff. All other cases with hemoglobin $\geq 123 \text{ g/L}$ and no IDeNA were defined as iron sufficient.

¹⁷ Because of the higher altitude of Cuernavaca, hemoglobin values are generally higher than at sea levels. Hence, the suggested cutoff for anemia is 123 g/L instead of the usual 120 g/L [222].

Stage 2 also included an assessment of severity of depression with the Spanish version of the Beck Depression Inventory-II (BDI; The Psychological Corporation, Harcourt Brace & Company: San Antonio) [223] (Appendix E, Beck Depression Inventory). The BDI is a clinical screening tool that assesses presence and severity of depression [223, 224]. It contains 21 statements, each with 4 answer choices ranging in score from 0 to 3. The total additive score ranges from 0 to 63 points. The BDI provides cutoffs for minimal (score of 0–13), mild (score of 14–19), moderate (score of 20–28), and severe (≥ 29) depression. In order to assess the construct validity of the BDI, the Spanish version of the Center of Epidemiologic Studies Depression Scale (CESD) [225] was included as well (Appendix E, CESD). The CESD is a depression screening tool developed by Radloff et al. [156] for an English-speaking population. It consists of 20 items, and the Spanish version has been validated and used in Mexican populations [218, 225, 226]. Order of administration of depression scales was alternated for every new participant entering stage 2 of the study.

Women who answered questions on the BDI related to suicide in the affirmative were referred to the free medical service in the Ciudad de la Confeción. They also received travel fare to a social service center that addressed mental health needs at no cost to their clients.

The Fatigue Severity Scale (FSS) was used to assess fatigue [227] (Appendix E, Fatigue Severity Scale). The FSS was chosen because it is a short measure of fatigue severity; in addition, it has shown good differentiation between fatigue in non-depressed and fatigue in depressed subjects [227]. It was originally developed from a 29-item fatigue assessment instrument [228]. Participants estimate their range of fatigue from 1 to 7 in the past 2 weeks; a visual aid was provided that allowed participants to point to the level of fatigue they experienced. The final additive score of the 9-statement scale is divided by 9. However, no Spanish version of the FSS

existed in 2001; therefore, it was translated by a professional translator. The scale was subsequently back-translated into English by a bilingual speaker. Discrepancies were resolved by discussion with the translators and native speakers. This method was recommended by Brislin et al. [229].

Additional questionnaires included a health history; a short food-intake record; a socioeconomic status (SES) evaluation originally developed for the Mexican National Nutrition Survey in 1999 [230]; information on the respondent's living situation such as marital status, household composition, time spent in a second job, and paternal support with child care (Appendix E, Mexico 2001). Since only the SES evaluation was developed in Spanish at the INSP, all other questionnaires were translated with the aforementioned method [229]. All questionnaires were tested with volunteers in order to explore comprehension; they were then modified if necessary. Interviewers were trained for several days in the administration of the questionnaires; the training included mock interviews with the coordinators and volunteers of the INSP. All parts of the questionnaires were read to the respondent; thus it was ensured that semiliterate and illiterate women had a good understanding of the questions and answer options.

Stage 3 of the study addressed the level of internal stress a participant experienced. A randomly selected subset of women with complete data from the 2nd stage was invited to the 3rd stage. The 3rd stage included 2 days of cortisol sampling, and the General Health Questionnaire (GHQ) that assesses emotional health [257, 258] (Appendix E, GHQ). It was previously used successfully in conjunction with cortisol [100].

Cortisol from saliva samples was used as the biological marker of perceived stress. Saliva samples were collected with sampling tubes called Salivettes (Sarstedt Corp, Newton, NC, USA). The tubes contain a polyester swab on which participants

chewed to generate saliva; the saliva was then deposited into the tube by spitting. The sampling scheme called for 4 samples per day for 2 days. Sampling days were chosen randomly from the 5 workdays of the week. For each woman, sampling intervals were timed according to time of morning waking. The intervals were the same for each participant in order to generate comparable curves: 30 min after waking,¹⁸ 3 hours after waking, 7 hours after waking, and 10 hours after waking. Sampling was timed to exclude lunchtime and to leave at least an hour after lunch. Women were advised not to eat for half an hour before sampling, since food intake increases cortisol levels [260]. Since the first sample was to be taken at home, women had one practice session on the afternoon before the sampling day. They were then sent home with one Salivette, and filled it the next morning while recording the actual time of sampling; the interviewer collected the filled Salivette on the morning of the sampling day. Times of waking and of first saliva collection were recorded, and interviewers calculated the subsequent times of sampling for the day. The women received the projected sampling times on a piece of paper in order to assure compliance with the sampling times. Interviewers came to the women at their workplace to take the next sample. At that time women were also reminded of the next sampling time. At the end of the day, women were asked to report any events during the day that they experienced as stressful and their time of occurrence (Appendix E, Mexico 2001, 3rd stage). Cortisol samples were refrigerated in the factory and frozen upon arrival at the INSP. Cortisol is very stable and easily withstood the short transports from the participant's home to the factory, and from the factory to the INSP [261]. The frozen cortisol was analyzed with a radioimmunoassay using cortisol labeled with 125 Iodine, in a solid phase system. The kit used was Cort-CT2, purchased from Schering-CisBiorad International, Gif-Sur-Yvette Cedex, France. Variability (coefficient of

¹⁸ Saliva cortisol levels are at their highest peak 30 min after waking [259, 260].

variation) within cortisol sample assessments was 5.4%, between cortisol samples 7.3%, at a level of 52.3 nmol/L [262].

In order to further assess the validity of cortisol, a Spanish version of the GHQ was administered to the participants after completing the second cortisol sampling day [257, 258]. The GHQ is a tool for detecting a range of psychiatric disorders in the general population. It contains 28 statements that assess frequency of symptoms [258]. The GHQ incorporates the domains of somatic symptoms, anxiety and insomnia, social dysfunction, and severe depression. Answer categories vary according to question but generally range from “not at all” to “much more than usual.” The final additive score ranges from 0 to 63, and the higher the score, the worse the general health. The Spanish version was originally developed for Hispanic Americans, including Mexican Americans [257].

After completion of the interview and cortisol sampling, participants received information on their weight, height, BMI, and iron status. Upon completion of the study within a factory, an item considered useful by the factory workers themselves was donated (e.g., a microwave or a first aid kit).

Cortisol: Data cleaning and preliminary analysis

While 67% of the sample had complete information on cortisol and time of sampling, 33% had no recorded time for some or all of their cortisol values. For the missing times, the mean of the available sampling time for all subjects was inserted. Mean actual sampling times were very close to the sampling times that were projected by the interviewers (Appendix Table C.1). However, time of sampling after waking was self-reported, and misreporting of the self-reported sampling time is common (about 38%) [263]. Each participant’s individual curve of cortisol concentration over the adjusted time of sampling per day was plotted; spikes in the cortisol curves were compared with self-reports of stressful events of the day. On only one occasion could

a spike on sampling time be explained by a stressful event that occurred 2–30 minutes prior to sampling.¹⁹ In this case, the curve was smoothed between sampling times 2 and 4. All other data points remained unchanged. Undetectable levels of cortisol (<0.5 nmol/L) were set at 0.05 nmol/L in order to include data points in the analysis. Cortisol values below the 5th and above the 95th percentile were compared with the reported literature in order to confirm that they were not due to measurement error. No values were excluded on that basis.

Three methods of assessment of high- and low-cortisol groups were tested: 1) area under the timed concentration curve (AUC), 2) cortisol values 30 minutes after waking, and 3) slopes of cortisol concentration over time. The final preferred method was area under the curve for three sampling times (AUC3) (30 min after waking, 2nd and 3rd samples in factory). The reason for excluding the 4th sampling time was that 16% of the sample had an unexplained increase of cortisol concentrations between sampling time 3 and 4 that exceeded 1 unit of cortisol. Since women were paid by the quantity of completed garments, it is possible that stress increased toward the end of the workday, when women tried to meet their production goals. In order to reduce this additional external source of variability, only the first 3 sampling times were used. AUC3 was calculated with the pharmacological program *pkexamine* of the statistical Stata software, version 9.0 [265]. When 2 days of sampling were available (83% of the sample), the mean between the two curves was taken. Otherwise, the AUC3 of the one available day was used. *From here on, only the results for AUC3 with the combination of recorded and estimated time of sampling will be reported. The variable will be called AUC3E (area under the concentration curve, for 3 samples, with actual and estimated times).* For information on calculation, methods, and results of all cortisol

¹⁹ Saliva cortisol levels reflect spikes of plasma cortisol within 1 to 2 minutes; cortisol generally is highest 3 to 4 minutes after an exposure to an acute stressor. [260, 264]

measures, please see Appendix C, section 1.2, Methods and results of cortisol measures.

Statistical analysis

Statistical analysis was carried out with SAS 9.1 [176]. The two main outcome variables were severe depressive mood (BDI \geq 29 points), with no to moderate depressive mood (0–28 points) as a reference; and moderate and severe depressive mood (BDI \geq 20 points), with no to mild depressive mood (0–19 points) as reference. Moreover, two additional outcome variables contrasted severe depressive mood (\geq 29 points) with no to mild (0–19 points), and moderate depressive mood (20–28 points) with no to mild (0–19 points).

To account for the study design, statistical modeling was performed with several procedures. The higher design level of “factory” was adjusted for with a mixed model procedure for logistic regression (proc glimmix). Matching was controlled for by inserting the matching criteria as control variables into the model. The magnitude of the effect of the variable factory and of the matching criteria was assessed. Models were developed according to the statistical importance of the variables and their impact on the coefficient of the iron status variable.

Iron status measures were the independent variables of main interest. All iron status indicators were tested as both continuous and categorical variables. Composite indicators were tested as well (see above). Curvilinear relationships of the continuous iron status indicators were assessed. Covariates were initially entered individually; subsequently, all variables with a $p < 0.2$ were tested in various combination, in order to explore their importance.

Covariates of interest included personal physical characteristics, lifestyle and family situation, and SES. More specifically, they were a) physical or demographic characteristics: BMI; age; medications taken; oral contraceptive use; alcohol use; b)

family situation: marital status; number of children under 5, 10, or 18 years of age; whether the partner helps with the children; whether the woman lives with family, friends, and if yes, with whom; or alone.; c) work: physical strenuousness of the work at the factory; whether the woman worked in a second job, and if yes, how many days of the week; d) SES: quality of the roof, walls, and floor; water source; sanitation; household income; number of possessions; crowding (number of rooms – number of persons living in home); and whether the kitchen was separate or not, or no kitchen existed. In addition, factors that summarized wealth (factor 1), crowding in the household and SES (factor 2), and the presence of young children, paternal support, and working in a second job (factor 3) were included. These factors had been generated for the larger sample in Chapter 3. For a more specific description of the variables, consult Appendix E, Mexico 2001.

Folate and vitamin B12 deficiency are associated with depression [160-163, 231]. Moreover, folate and vitamin B12 deficiency cause macrocytosis [8, 154]. Therefore, cases with $MCV > 94$ fL [154] were identified as macrocytic and potentially folate or vitamin B12 deficient. Cases with anemia (hemoglobin < 123 g/dL) and macrocytosis were considered to be folate-deficient anemic or vitamin-B12-deficient anemic, and were excluded. Models assessing the effect of $MCV > 94$ fL were treated as follows: cases with high MCVs were initially excluded from the statistical analysis; subsequently, $MCV > 94$ fL was included as a control variable while maintaining sample size.

AUC3E cortisol was dichotomized with the median as the cutoff. Women with AUCE cortisol below the median were considered to have low cortisol levels, while women with values equal to or above the median had high cortisol. The variable was inserted as a modifier of the effect of iron status on depressive mood, while controlling

for fatigue, other potential covariates, and MCV>94 fl. Interactions were considered influential with a p-value<0.2. All aforementioned measures of iron status were tested.

The model's residual plots were investigated for outliers. In order to explore the relative importance of specific cases, observations were excluded and the model rerun; odds ratios of the reduced data set were compared with those of the full data set. Exclusion was based on extreme values of depression scores or iron status indicators, high residual values, and cortisol values above the 95th or below the 5th percentile. Best models were chosen based on theoretical considerations, results from Chapter 3, significance of the variables, significance of the models, and parsimoniousness.

Sample selection

A total of 100 women participated in the 2nd part of the study. Figure 4.1 describes the selection of the final sample of 48 cases of the 3rd stage.

Results

Descriptive statistics

The distribution of cases between factories was as follows: 10% from Avi, 21% from CIM, 19% from Phantom, and 50% from Unger. A comparison between the women of the subsample and those who participated in the screening stage only showed that the subsample was older (29 vs. 27 years of age, $p<0.05$) and included a lower proportion of single women and a higher proportion of women who were divorced, separated, single mothers, or widowed ($p<0.05$; Appendix Table C.2).

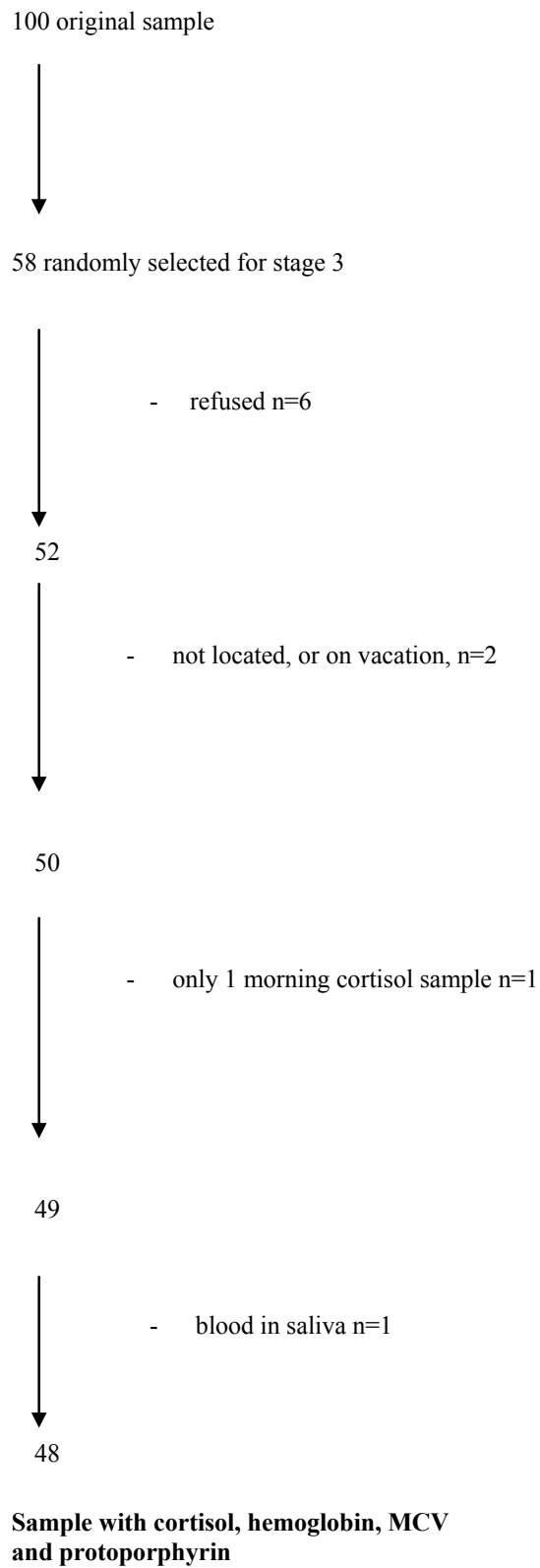


Figure 4.1: Sample selection.

A comparison between the subsample of 48 women and those excluded from the larger group (n=52) found that women in the subsample were significantly ($p<0.05$) older (29 vs. 25 years of age) and that a significantly greater proportion had CESD scores above 16 (71% vs. 48%; Appendix Table C.3). In addition, FSS scores were higher in the subsample, with a p-value of 0.06 (mean 4.1 vs. 3.5). Moreover, the subsample had a higher proportion of women who were married and a smaller proportion of women who were single ($p=0.05$).

Percentage of depressive mood was – based on the BDI – 25 for severe and 31 for moderate depressive mood (Table 4.1), which was comparable to levels in the larger sample (Chapter 3). The recommended cutoff of 16 points for the CESD [156] placed 71% of the sample in the depressive range. Actual cortisol values ranged from 0.5 to 32 nmol/L for the morning sample, and from undetectable levels to 17.9 nmol/L for the rest of the day. Means were 5.2 nmol/L for the morning sample, 2.3 nmol/L for sample 2, 1.9 nmol/L for sample 3, and 2.4 nmol/L for sample 4 (Appendix Table C.4). AUC3E cortisol ranged from 1.5 to 84.6 nmol/L. Twenty-nine percent of the sample was anemic, and 25% of the participants suffered from iron depletion with anemia (IDeA; Table 4.2). Hemoglobin ranged from 8.8 to 16.1 g/L (not shown). Twenty-three percent had $MCV>94$ fl (meaning macrocytosis, an indication for folate and/or vitamin B12 deficiency; Table 4.2) [154]. Fifty-eight percent of the participants were overweight or obese (Table 4.3). Factor 2 (which reflected crowding and SES) was below 0, but did not differ significantly from the larger sample (Appendix Table C.3). Eight percent of the sample used oral contraceptives (not shown). Alcohol consumption was low, with the highest amount consumed being 3 glasses per week (not shown).

Table 4.1: Subject characteristics, mood; mean (SD), median, 25th and 75th percentile, or %; n=48.

Variable	Mean (SD)	Median	25th p.	75th p.	% of whole sample
BDI	22.9 (13.7)	21	14	33.5	
Fatigue	4.1 (1.6)	4.1	3.0	5.3	
CESD-20 ^a	23.0 (11.7)	23.5	15	28.5	
% BDI moderate score 20–28					25%
% BDI severe ≥ 29					31%
% CESD-20 > 16					71%
Cortisol (AUC3E) nmol/L	16.0 (15.4)	14.1	6.3	17.0	

^a The Spanish version of the Center of Epidemiologic Studies Scale (CESD) [156] was used as a validation tool in this study; a score above 16 is the recommended cutoff for depressive disorders.

Table 4.2: Subject characteristics, iron status; mean (SD), median, 25th and 75th percentile, or %; n=48; iron depletion is defined as hemoglobin < 12.3 g/dl, and MCV or protoporphyrin beyond cutoff.

Variable	Mean (SD)	Median	25th p.	75th p.	% of whole sample
Hemoglobin g/L	131 (18)	134	121	143	
MCV fL/l	88.4 (9.8)	91.0	85.4	93.7	
Protoporphyrin µg/dL	87.6 (82.4)	54.2	34.2	110.7	
% Hemoglobin < 12.3 g/dL					29%
% MCV < 80 fL					19%
% Protoporphyrin > 70 µg/dL					37%
% Iron depletion with anemia					25%
% MCV or protoporphyrin beyond cutoff; but hemoglobin ≥ 12.3 g/dL					12.5%
% MCV>94 fL ^a					23%

^a indicator of macrocytosis, therefore potential folate and/or vitamin B12 deficiency [154]

Table 4.3: Subject characteristics, demographics; mean (SD), median, 25th and 75th percentile, or %; n=48.

Variable	Mean (SD)	Median	25th p.	75th p.	% of whole sample
Age yrs	28.9 (8.7)	27.5	21	36	
BMI kg/m ²	26.1 (4.2)	25.2	24.0	29.2	
Factor 2 (crowding, SES)	-1 (1.0)	0.2	-0.8	0.6	
% BMI > 25 kg/m ²					58%
% BMI > 30 kg/m ²					17%
% Household income < 4377 pesos ^a					92%
% Dirt floor					12.5%
% Dirt floor, walls and roof plastic, asbestos, or metal					2.1%

^a equivalent of US \$157/month in summer of 2001; national poverty line level 1 for Mexican households in 2000 [232]

Interaction model: Stress as a modifier

AUC3E cortisol was a borderline significant ($p=0.08$) modifier of the effect of hemoglobin on severe depressive mood (Figure 4.2). While the low-cortisol group had a significant ($p<0.05$) increase in the risk of severe depressive mood with decreasing hemoglobin values, the high-cortisol groups showed no such association. With every unit increase in hemoglobin (g/L), the odds ratio of severe depressive mood decreased by 6% (Table 4.4, Model 1). This association was also demonstrated with IDeA, which operationalized hemoglobin as a categorical variable: women with low cortisol and IDeA had an odds ratio of severe depressive mood that was 6.3 times greater than the reference (no IDeA, high cortisol). All other groups with only one or no risk factor did not differ from the reference (Table 4.4, Model 2; Appendix Figure C.1).

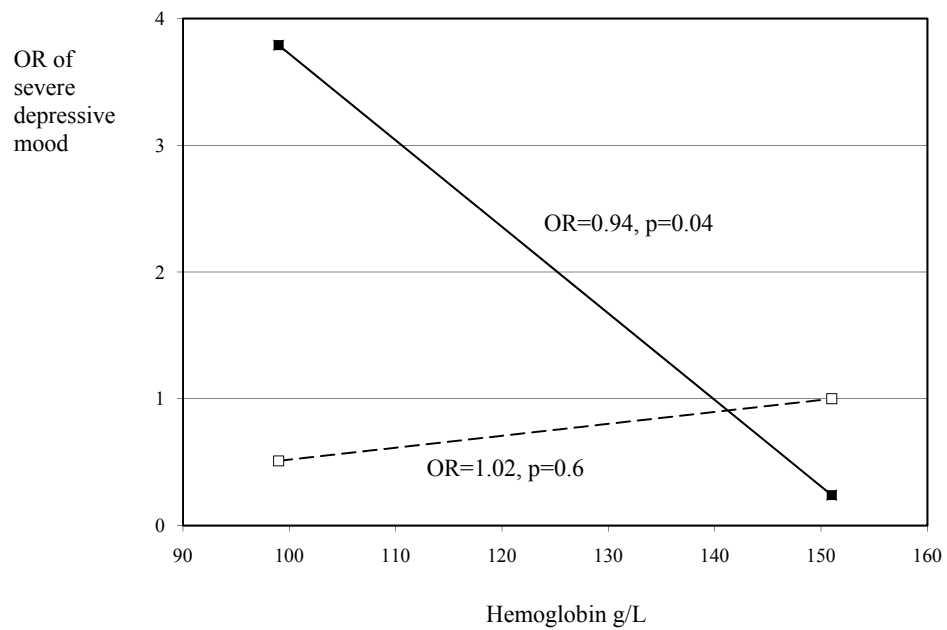


Figure 4.2: Odds ratio (OR) of severe depressive mood; modifying effect of AUC3E cortisol below (continuous line) or equal to and above the median (dashed line). Statistical model includes centered hemoglobin, AUC3E cortisol, fatigue, and factor 2. p-value of interaction = 0.08. 10th–90th percentile of hemoglobin.

Table 4.4: Logistic regression models, with outcome Beck Depression Inventory (BDI) severe depressive mood (≥ 29); modifier of hemoglobin is the dichotomous AUC3E cortisol ($<$ or \geq median); log OR (log SE); p-value; n=48; all fixed factor models; n=48. c = area under the ROC curve.

Variables	Model 1	Model 2
I	-3.511 (1.487)	-3.364 (1.376)
Centered hemoglobin g/L (continuous)	-0.066 (0.033); p=0.04	
Iron depletion with anemia (IDeA) (categorical)		1.854 (1.18); p=0.1
Fatigue	0.540 (0.278); p=0.05	0.509 (0.262); p=0.05
Factor 2 (crowding, SES)	-1.182 (0.500); p=0.018	-1.088 (0.456); p=0.01
AUC3E cortisol nmol/L	-0.160 (0.781); P=0.8	0.226 (0.847); p=0.7
Interaction (cortisol*hemoglobin)	0.082 (0.048); p=0.08	-3.263 (1.89); p=0.08
N	48	48
c	0.806	0.824
-2LL	45.2	46.4

Comparison between mixed and fixed factor models demonstrated that no additional explanatory power is added by inserting factory as a random factor (Appendix Table C.6). MCV $>$ 94 fL was not significant. Thus, the most parsimonious model was a simple logistic regression fixed factor model. The effect for hemoglobin was not curvilinear (not shown).

While the interaction between AUC3E cortisol and hemoglobin was significantly associated with risk of severe depressive mood, its association with moderate depressive mood was not significant (Appendix Table C.7).

Moreover, since hypocortisolism is associated with fatigue [120, 121, 123], the influence of fatigue on the interaction in the model with severe depressive mood was assessed (Appendix Table C.8). However, fatigue had no important effect, whereas

factor 2 (crowding, SES) acted as a negative confounder. The impact of factor 2 was further explored: associations of factor 2 with depressive mood, hemoglobin levels, and AUC3E cortisol were assessed. We found that inclusion of factor 2 (crowding, SES) controlled for an SES difference that improved depressive mood and hemoglobin values in women with high AUC3E cortisol. Specifically, mean scores of factor 2 differed by severity of depressive mood; women with severe depressive mood had a mean score of factor 2 of -0.52 , while those with BDI scores indicating no to moderate depressive mood had a mean score of factor 2 of 0.09 (p of t-test = 0.05 ; not shown). Moreover, mean hemoglobin differed significantly, with factor 2 being below or above the median, but only in the group with high AUC3E cortisol (t-test $p < 0.05$). Women with AUC3E cortisol below the median had a t-test p -value of 0.4 for the difference in means of hemoglobin due to factor 2. Mean hemoglobin for women with high AUC3E cortisol and factor 2 below the median (indicating lower SES) was 126 g/l, while the mean value of hemoglobin for women with high AUC3E cortisol and factor 2 equal to or above the median (indicating higher SES) was 143 g/l. Thus, the introduction of factor 2 into the regression equation controlled for confounding due to SES differences in women in the high AUC3E cortisol group; as a result, the distinct effect of hemoglobin on risk of severe depressive mood within the cortisol groups could emerge.

Since age and marital status were significantly different in this subgroup compared with the larger sample and the screening group, the two variables were included in the analysis. They were not significant. Likewise, oral contraceptive use did not affect the interaction.

Discussion

In summary, the biological marker of stress, cortisol, was indeed a modifier of the effect of hemoglobin on risk of severe depressive mood in the present sample of

Mexican factory workers. Women who had low cortisol experienced a 6% decreased odds of depressive mood for every g/L rise of hemoglobin. However, women with high cortisol showed no association between hemoglobin values and risk of severe depressive mood.

Limitations and plausibility

Causality

Because of its observational nature, the present research is a study of the plausibility of a relationship between iron status and depressive mood. No causal inferences between the exposure to iron deficiency and an increased risk of depressive mood can be drawn, since directionality cannot be established. While it is assumed that iron deficiency causes depressive mood, only 1 out of 5 supplementation trials has shown this [42-46]. Moreover, depressive disorders can be associated with weight gain or weight loss [53], which implies a change in diet and/or nutrient composition of the diet (e.g., a shift away from iron-rich foods, or vitamin-C-rich foods that increase absorption of iron). The effect of depression on healthy eating or supplement use has – to our knowledge – been explored once. Bowen et al. [233] found no association between depression and fruit and vegetable consumption. In addition, observational studies are vulnerable to confounding, and associations may be spurious if confounder control was inadequate. Last, we cannot exclude the possibility that hemoglobin was a modifier of the association between cortisol and risk of depressive mood, which would be contrary to our assumption.

Past depression

Past depression was not assessed in the present study. It is known that a severe mood disorder in the past increases the likelihood of recurrence of depression [178]. In addition, past depression might affect eating behavior and, consequently, iron status.

To our knowledge, no published study has investigated this relationship. It should be noted that the present sample was relatively young (on average 29 years of age). Since the first episode of major depressive disorder (MDD) occurs between 25 and 31 years of age in international samples [2], it is possible that some women in the present sample had not had a past episode of MDD; they were either free of depressive mood at the time of the interview or experienced their first bout of the disorder at that time. In comparison, in the US, lifetime prevalence of MDD is 15% between 18 to 29 years of age [62]. In conclusion, confounding by past depression is probably not as severe as it would be in an older sample.

Validity of sample selection

The final sample is a subset of the women working in the garment factories, and of the screened participants. Sixty-nine percent of all women in the four factories volunteered to take part in the screening stage. While the average participation between the four factories is acceptable, the individual percentages indicated that the factory Avi had a much lower percentage (32%), which is probably too low to be representative of a broader population. However, differences between factories were controlled for in several ways during the analysis, with no significant effect. Thus, even though the group from Avi was small, it apparently did not differ from the other three factories with respect to the variables of interest.

Random selection of participants from the screening sample most likely reduced the likelihood of additional bias (aside from potential self-selection bias during the screening stage). Nevertheless, women in the final subsample differed from women in the screening stage and from the larger sample in age and marital-status distribution. These two variables were added to the models and did not impact the coefficient of hemoglobin. Moreover, when comparing the main effect model in the larger sample of $n=100$ (outcome severe depressive mood; independent variables were

hemoglobin, FSS, factor 2 for SES and crowding, and MCV>94fL; Chapter 3) to the present subsample of n=48, the effect of hemoglobin was comparable (Table 3.2; Appendix Table C.6; consult also the internal validity section of this chapter).

Validity of Mexican garment-worker sample

The present sample of Mexican female factory workers showed a strong modifying effect of stress on the relationship between hemoglobin and depressive mood. The result might not be representative for other populations, specifically since the overall exposure to the stress of poverty in this population was very high. However, in light of the paucity of information in developing countries, this study adds valuable information and should be a good reference for future research conducted in similar settings.

Validity of the Beck Depression Inventory (BDI)

General

Originally, the BDI was developed with a non-Hispanic White and African American sample [223, 224]. The BDI-II is relatively new (from 1996); therefore, the number of studies evaluating it is not as extensive.

English-speaking samples without a prior diagnosis of current depression show good validity of the BDI (consult the internal validity section of this chapter).

Hispanic

A disadvantage of the Spanish version of the BDI-II is that cultural terms for mood disorders are not addressed. In Mexican culture, for example, the term “nervios” (loosely translated as “nerves”) indicates somatic disorders (such as gastrointestinal problems, vomiting, lump in throat, and headaches), dysthymia, MDD, and anxiety [184]. Moreover, somatization of dysthymia or major depression is frequent in Hispanic populations [157, 186, 187].

A number of BDI validation studies have recently been undertaken in Hispanic population, including Mexicans. The validity of the Spanish BDI-II appears to be as high as the English-language version (consult the internal validity section of this chapter). Importantly, language of interview does not appear to affect test scores [238-242]. It is also noteworthy that the exclusion of somatic questions did not improve the performance of the Spanish BDI-II in a sample of Mexican patients [241]. This indicates that somatic symptoms might not be necessary in order to assess depression severity with the BDI in Mexicans accurately, even though somatization of depressive mood in Hispanics is frequent [157, 186, 187]. However, our present sample of younger, healthy Mexican women is not well comparable to that of Novy et al. [241].

In summary, the BDI-II appears to be a good tool to assess depressive mood in a Spanish-speaking population. Drawbacks are 1) the high item difficulty, which might result in a lack of accuracy in assessing depression severity in a Mexican population with little education; 2) the lack of culturally specific terms for depression; and 3) a potentially low discriminant validity between depression and anxiety. However, misclassification by the BDI-II would result in a weaker association between hemoglobin and severe depressive mood in women with low cortisol values. Nevertheless, we were able to show a significant relationship.

Validity of the Fatigue Severity Scale (FSS)

The fatigue measure we employed in the present study is an established tool; however, it has been developed for English-speaking patients [227]. Validity of the FSS is generally evaluated by comparing its scores with the clinical diagnosis of fatigue. In 2001, no fatigue scale existed – to our knowledge – in Spanish. Thus, translation of the FSS was required. The English version of the FSS has good validity in terms of internal consistency (Chronbach's alphas); discriminant validity (distinction between fatigued and depressed); known group validity (discrimination

between fatigued and non-fatigued); test-retest reliability; and criterion validity (correlations with other fatigue scales) [227, 243-246]. However, our Spanish translation has not been validated in a broader population. Thus, the present study might not have measured fatigue severity accurately. It should be kept in mind, though, that our assessment of the Spanish FSS indicated good internal validity of the scale (consult internal validity section, Chapter 3). Moreover, this study aimed at producing a short measure of fatigue severity, for which the FSS was deemed to be appropriate.

Substance abuse

We did not assess legal substance abuse or illegal drug use, but participants were asked about their alcohol and medication use. Substance abuse is associated with malnutrition and depression [53, 179, 189, 190, 193-198, 204-206]; thus it is a potential confounder. None of the women reported drinking more than 1.5 glasses of alcohol per day, and women who took mood-altering drugs such as valium or antidepressants were excluded from the sample. However, abuse of alcohol or medication might have been underreported. Past traumatic events can cause both hypocortisolism and self-medicating substance abuse [266]. Past substance abuse is also associated with impaired cortisol responsiveness to stress [267]. Thus, more substance abuse might have occurred in the low-cortisol group. It should be noted, though, that abuse of illegal substances in semirural settings close to Mexico City occurs predominantly in young Mexican males [247-249]; in young women, prevalence of abuse of substances other than medications ranges from less than 1% to 2.81% [247, 249, 268]. Illegal-substance abuse for both genders was 1.5% in metropolitan areas, and not significantly different or lower for the region that would include the area where the present study took place [247, 249, 268]. Moreover, during

the analysis phase, cases with high depression scores had been removed to test influential data points; results did not differ substantially.

In summary, substance abuse in the present sample might have strengthened the association between hemoglobin and depressive mood in hypocortisolaemic women. However, the likelihood of this occurring is low, since the prevalence of illegal substance use in Mexican women is low.

Perimenopause, premenstrual syndrome (PMS), and premenstrual dysphoric disorder (PMDD)

Although menopausal women were excluded from the present sample, we collected no information related to perimenopause. However, the present sample is relatively young (mean of 29 years; 75th percentile 36 years), which means that it is unlikely that any woman is in perimenopause. PMS and PMDD are associated with depressive mood [223, 234-236]. However, no information on either disorder or on exact menstruation cycle was gathered. A literature review did not provide information on the association between iron status and PMS or PMDD. Women who had PMS or PMDD could also have had lower hemoglobin values, but this would have to be a chance occurrence. Generally, iron status is not lower at time of menstruation than at any other time during the monthly cycle [207]. It appears, in summary, unlikely that the association between depressive mood and hemoglobin was affected by perimenopause, PMS, or PMDD.

Other nutrients that may cause depression

Other nutrient deficiencies are considered potential causes of depressive mood, namely deficiencies of zinc, folate, vitamin B12, and ω -3 polyunsaturated fatty acids (specifically docosahexanoic acid) [158-163, 168]. An indicator for vitamin B12 and folate deficiency is macrocytic anemia [8, 154]. Therefore, we excluded

macrocytic anemias and controlled statistically for macrocytosis without anemia (MCV>94 fL). However, the final model did not include this control variable, because of non-significance ($p>0.3$, not shown).

Intake of zinc or ω -3 polyunsaturated fatty acids was not assessed. The prevalence of zinc deficiency was 30% in rural and urban Mexican women in 1999 [9]; considering the frequency of occurrence, a sizeable proportion of women in this sample might have been zinc deficient. However, Chapter 2 of this dissertation did not report an association between iron status, zinc intake below the recommended amount, and severe depressive mood in US Hispanic women. No information on ω -3 polyunsaturated fatty acid status for Mexicans is available except in breast milk [250]. Mean concentration in breast milk in Mexico is not significantly different from that in industrialized countries (mean % of total fatty acids is 0.26%) [250], albeit lower than the worldwide mean of 0.32% [208]. It is important to keep in mind that results about the depression-lowering properties of ω -3 polyunsaturated fatty acids are inconsistent and controversial [210-212].

In summary, while folate or vitamin B12 deficiency probably did not affect the results of this analysis, low intake of zinc or ω -3 polyunsaturated fatty acids might have. The most likely impact would have been from zinc deficiency. If low hemoglobin values correlated with zinc deficiency, zinc would have acted as a positive confounder.

Cortisol measure

We employed a biological marker of stress in the present analysis. Cortisol values are known to vary greatly between subjects [269, 270], to be responsive to acute stressors [215, 271], to be modified by affect [106, 272] and to show high day-to-day variation within subjects [269, 273]. Moreover, two phenomena, hypocortisolism and hypercortisolism, have been observed to be associated with stress

and mood disorder. Cortisol concentrations increase with acute stress [215, 264] and/or melancholic MDD [96, 113, 122, 144], while chronic stress [102, 119] and atypical MDD are associated with lower than normal cortisol values and/or decreased stress responsivity [96, 113, 122, 144, 215]. Consequently, we explored a large number of analysis methods to determine the complex relationship between cortisol and depressive mood. The traditional way of analyzing salivary or plasma cortisol is to calculate the area under the concentration curve over time [260, 273, 274]. However, other methods have been used, namely reporting only morning cortisol [100, 102, 259, 273, 275], evening cortisol [100, 276], averages throughout the day [102, 106, 277], and calculating intercepts [102] and change of cortisol (slopes) over time [99, 102, 276, 278]. Inaccurate self-reporting of time of sampling in a free-living sample has also been demonstrated [263].

In light of the analysis and interpretation issues of this measure, we set out to employ a conservative, hypothesis-driven approach in order to reduce the probability of type I error. Each participant had, at a minimum, 3 samples per day. Averaging of cortisol values over 2 days was used in order to increase accuracy. We preferred to dichotomize participants into two broad groups in order to avoid the influence of extreme mean AUC3E cortisol values. Acute stressor exposure during the day was recalled at the end of the workday in order to account for spikes in the cortisol curve due to acute stress. Time of sampling in this sample was only self-reported in the morning; especially on day 2 it had a very low variation around the projected time of sampling (Appendix Table C.1), which might indicate either exceptionally timely sampling or untruthful reporting. In addition, time of sampling had been estimated in a subset of the data set. Thus, some inaccuracies in the AUC3E cortisol variable might have been introduced. However, variation around the morning sample of the first day of sampling was less than 15 minutes.

Classification of cases into high- and low-cortisol groups based on the median necessarily resulted in misclassification of participants with “normal” cortisol levels, which presumably would be around the median. Thus, both groups were probably a mix of extreme and normal values of AUC3E cortisol. Nevertheless, the dichotomous variable AUC3E cortisol modified the association between hemoglobin and severe depressive mood, indicating that the effect was strong enough to be detected despite the misclassification.

Last, a different interpretation of low AUC3E cortisol is noteworthy: while hypocortisolism is thought to be a result of chronic stress, it also occurs with past severe trauma, potentially resulting in depression and/or posttraumatic stress disorder [279-281]. Thus, women with low AUC3E cortisol values in our sample might in fact have suffered from the consequences of trauma. In this case, the interpretation of the modifier changes slightly because low cortisol values might be a result not only of poverty but also of trauma. However, since higher trauma frequency is associated with poverty [282], the two are difficult to differentiate.

In summary, sampling and summarization methods of low and high cortisol values were used that increase accuracy. However, because of the variable nature of cortisol assessments, misclassification was possible. Nevertheless, the interaction between hemoglobin and AUC3E cortisol was significant, and internal validity assessment showed consistency of results.

Internal validity

Hemoglobin

All iron status measures including hemoglobin were assessed with established methods of laboratory analysis. The responsible laboratory at INSP had received excellent ratings by the Centers for Disease Control for its precision of measurements [251]. Hemoglobin, MCV, and protoporphyrin correlated significantly ($p < 0.0001$),

indicating that low hemoglobin values are accompanied by low MCV and high protoporphyrin values; correlations were of the magnitude of $r=|0.67|$ to $|0.70|$ (not shown). Indeed, having hemoglobin values <123 g/L is significantly associated with IDeA ($p<0.0001$) and IDA ($p<0.0001$).

Iron depletion anemia (IDeA)

IDeA was defined as a hemoglobin level < 123 g/L, plus MCV and/or protoporphyrin beyond cutoff (MCV < 80 fL, protoporphyrin > 70 $\mu\text{g/dL}$). However, the conventional definition of iron deficient anemia (IDA) is anemia (hemoglobin < 123 g/L), plus at least two additional indicators beyond cutoff (using, e.g., ferritin < 12 $\mu\text{g/L}$, transferrin saturation $< 16\%$, protoporphyrin > 70 $\mu\text{g/dL}$, and MCV < 80 fL) [8]. In a small sample of factory workers, all four iron status measures were available, and the effect of the interaction between IDA and AUC3E cortisol was assessed. However, although all variables showed the effect in the expected direction, effect size was much diminished. The log OR of the interaction was only 0.323, and the p-value of the interaction was 0.8. This result might be due to the small sample size ($n=32$); only 7 of the 32 cases had IDA, thus splitting them in the interaction with the dichotomous AUC3E cortisol into a cell size of 4 cases with low and 3 cases with high cortisol.

Beck Depression Inventory (BDI)

The validity of the BDI has been extensively explored. The internal consistency of the English version of the BDI-II is high (Chronbach's alphas are between 0.73 and 0.95) [223, 234, 236]. Assessments of known group validity generally show that BDI scores are independent of ethnicity [223, 283] and age [234-236, 283]; however, one study identified an association between age and scores [223]. Studies are inconsistent concerning gender differences in scores: either women have higher scores [223] or no difference was observed [234, 236, 283]. The BDI-II

correlates well with other depression scales (e.g., the Revised Hamilton Psychiatric Rating Scale for Depression has a correlation with the BDI-II of $r=0.71$), an indicator of good criterion validity [172, 237]; factor analysis consistently groups symptoms into 2 factors, affective-cognitive and somatic [172, 223, 237]; and the scale has also been shown to differentiate between psychiatric and non-psychiatric populations, and to be able to distinguish levels of depression severity [223, 237].

All identified review articles concerned the older version of the BDI, and they dispute a number of the aforementioned validity assessments. Described limitations are 1) that the factor structure is variable depending on the study population (between 2 and 9 factors); 2) that the high difficulty of the items reduces the usefulness as a simple field tool [237]; and 3) the insufficient discriminant validity in relation to anxiety [237].

Correlations between anxiety scales and BDI can be high in non-psychiatric populations ($r=0.22$ to 0.82) [223, 237]. However, Beck et al. [223], who developed the instrument, state that the scale discriminates well when comparing mean scores of depressed (BDI-II) and anxious participants; correlation between the BDI-II and anxiety scales (Beck Anxiety Inventory, Revised Hamilton Anxiety Rating Scale) is between $r=0.47$ and 0.60 . Test-retest reliability is likewise controversial since it is dependent on time interval [238-240].

For the older Spanish version of the BDI, validity assessments took place in Puerto Rican, Spanish, and Argentinian populations [238-240]. They confirm 1) high internal consistency (Chronbach's $\alpha=0.87$ to 0.91) [238, 239, 284]; 2) high test-retest reliability [238]; 3) that the scale has high criterion validity with other depression assessment instruments (Symptoms Scale Checklist-90) [238]; 4) that, likewise, in terms of known group validity, groups at higher risk (women, young adults, and low SES) had higher scores [238-240]; and 5) that factor analysis generates

2 to 4 factor solutions [240-242]. Only recently has the newer Spanish version of the BDI-II been validated in Hispanic Americans of predominantly Mexican descent [240-242]. Chronbach's alphas in those studies are high in exclusive Spanish speakers (0.89 to 0.95) [240, 242]. Scores are independent of age, gender, and SES [242].

Acculturation results in slightly lower BDI scores in one of the studies [241], while the other shows no association [242]. Test-retest reliability is good [240, 242], and a 2-factor solution was favored [240, 242]. Language of interview makes no significant difference in test scores [241]. Novy et al. [241] assessed discrimination between anxiety and depression and found a correlation of 0.60 between the Spanish versions of the Beck Anxiety Inventory and the BDI-II. The authors interpret the correlation as low and as an indicator of good discriminate validity. Only one study with middle-aged Mexican rheumatology patients assesses the validity of the Spanish BDI in Mexico [252]. The agreement in this study between clinical diagnosis of any depressive disorder and BDI was as follows: sensitivity=92%, specificity=86%, positive predictive value=0.80. Interestingly, the exclusion of somatic symptoms did not improve the validity of the BDI. This is of importance, since somatization is an important feature of depression in Hispanics [157, 186, 187].

Chapter 3 describes the assessment of validity of the Spanish version of the BDI-II for the present population; we concluded that the instrument was a valid measure of depression severity in the larger sample; therefore, no extensive assessments will be conducted in the present subsample. The Chronbach's alpha in the present subsample of 48 cases was 0.90.

Fatigue Severity Scale (FSS)

Validity assessments of the English version of the FSS show a high internal consistency (Chronbach's alpha is between 0.88 and 0.94) [227, 243, 244] and a good discrimination between fatigued and healthy subjects [227, 243-245]. The correlation

with other fatigue scales is moderate to high (0.47 to 0.81) [243, 244], except for the Fatigue Impact Scale ($r=0.15$) [244]. The scale is unidimensional (1 factor only), as expected in one study [227]; however, 2 factors were demonstrated by Hagell et al. [243]. The FSS showed substantially reduced scores with improvement of fatigue in clinical patients [227]; a good test-retest reliability [227, 244]; and its association with depression (CESD) is weak to moderate (0.2 to 0.46), depending on the population tested [227, 245]. The scale's scores were independent of gender, but not of age [243]. In a Spanish-speaking population, the FSS correlated significantly with the Fatigue Impact Scale ($r=0.68$) [246].

We extensively validated our Spanish version of the FSS (Chapter 3), and concluded that the FSS is a valid tool for assessing fatigue severity in the present sample. The Chronbach's alpha in the present subsample of $n=48$ was 0.8.

Cortisol

Saliva cortisol varies greatly within and between subjects [269, 270, 273]. The overall diurnal pattern of cortisol should be a negative slope throughout the day [260]. We confirmed this overall pattern with our sample. The slope of cortisol over time was estimated with mixed regression methodology, controlling for intercept, day, and factory as random factors. Cortisol values were log-transformed because of a skewed distribution and a curvilinear relationship with time. Overall slope was -0.13 when using 3 timed samples, assuming a non-linear decrease ($p<0.001$); when using 4 timed samples the slope was -0.08 ($p<0.001$). Thus, cortisol values show the expected diurnal decline in the overall sample.

AUC3E cortisol's association with chronic stressors and with physical and mental health indicators was investigated to assess the validity of the biological marker of perceived stress. Specifically, association with household composition, paternal help with children, marital status, SES indicators, C-reactive protein (CRP),

white blood cell count, BMI, fatigue, age, crowding, symptoms from the GHQ,²⁰ and symptoms from the medical checklist were assessed (Appendix Table C.10). Chi-square tests showed that a significantly ($p < 0.05$) higher proportion of women in the low-cortisol group felt more “nervous and uptight all the time” than usual; in addition, they suffered from “pains in [their] head” and “a feeling of tightness and pressure in [their] head” more often than usual (for statement and answer choices, consult Appendix E, General Health Questionnaire). Women with low cortisol levels also experienced more or about the same amount of satisfaction with “how they have carried out [their] task[s].” Hypocortisolism is frequently associated with chronic pain [120, 285]. In fact, Elwan et al. [286] demonstrate lower cortisol levels in the cerebrospinal fluid in headache patients compared to controls. While the medical checklist requested recall of illnesses, only respiratory infections had a sufficient number of cases to attempt analysis ($n=14$).²¹ The association was not significant. No other variables were significantly related to AUC3E cortisol.

Time of sampling was predicted rather than measured in 33% of the cases. Hence, models were rerun with the group ($n=36$) that had all measured data on cortisol and time of sampling. Appendix Figure B.2 demonstrates that hemoglobin and risk of severe depressive mood have a significant inverse association only in women with low cortisol (p -value of interaction < 0.05 ; Appendix Table C.11, Model 4). Thus, logistic regression results between the 36- and 48-case cortisol samples are virtually the same (see also Figure 4.2).

Factor 2 incorporated crowding and SES; it was a strong negative confounder of the interaction between AUC3E cortisol and hemoglobin (Appendix Table C.7). Factor 2 originated from a factor analysis with the larger sample of $n=100$ (Chapter 3).

²⁰ The GHQ statements were in this case considered separately (Appendix E, General Health Questionnaire).

²¹ Illnesses reported were asthma ($n=2$), lung disease ($n=2$), respiratory illness ($n=14$), cardiovascular illness ($n=2$), rheumatic illness ($n=2$), hypertension ($n=7$), diarrhea ($n=8$), diabetes ($n=0$), other ($n=0$).

There was no significant difference between the mean of factor 2 of the present subsample and factor 2 of the excluded cases of the larger sample (Appendix Table C.3). When using the variable crowding as a replacement for factor 2, neither crowding nor the interaction was significant. That leads us to conclude that the importance of factor 2 lies not only in its information on household composition, but also in its socioeconomic dimension (see results of factor analysis, Chapter 3).

We also assessed whether the impact of iron status in severe depressive mood was altered as a result of subsample selection. Therefore, the main effects of hemoglobin, fatigue, and factor 2 (crowding, SES) on severe depressive mood in the cortisol subsample (n=48) were compared with the effects in the larger sample (Appendix Table C.6). While hemoglobin did not reach significance, coefficients of all variables had comparable values (see Table 3.4).

Mechanisms

In the low-cortisol group, lower hemoglobin levels were associated with a higher risk of severe depressive mood, while in the high-cortisol group no such relationship was found (Figure 4.2; Appendix Figure C.1). According to our hypothesis, we expected to see this association: more highly stressed (in this case chronically stressed) individuals with ID should have a higher risk than all the other groups.

Depressive disorder is associated with alterations in neurotransmitter concentrations in the brain. Specifically, serotonin, norepinephrine, and dopamine are implicated [114, 122, 124-129].

ID is correlated with higher depression scores in a number of human observational studies [37-40]. Iron is a cofactor in the synthesis of serotonin, dopamine, and norepinephrine [25-27]; consequently, ID should result in altered concentrations of one or several of these neurotransmitters in the brain. In animal

studies, though, findings for serotonin and norepinephrine, at least, are inconsistent [28-32]. Serotonin concentrations have been shown to decrease with ID [28] or to remain unchanged [29]. Activity of tryptophan hydroxylase, the iron-dependent enzyme that is part of the pathway that converts tryptophan to serotonin, remains unchanged [28, 30]. Serotonin metabolites increase [30] or decrease [30] with ID. Norepinephrine has rarely been studied. It remains unchanged [30] or is higher [31, 32] in ID rats. Indeed, dopamine gives the best indication that ID induces changed nerve-signal transmission as a result of lower dopamine receptor density and dopamine transporter downregulation [29, 32-36]. A number of animal studies also show that the activity of monoamine oxidase, which is involved in the breakdown of serotonin, dopamine, and norepinephrine, is reduced [28, 30].

Hypocortisolism has been associated with chronic stress [102, 119, 123, 215, 216] and a higher risk of atypical MDD [96, 113, 122]. A biological explanation for the association between hypocortisolism and MDD is that chronic upregulation of the immune system mediates these two phenomena [115, 130-134]. It is thought that hypocortisolemic individuals experience an upregulation of their non-specific plasma immune response, and thus of their proinflammatory cytokine, tumor necrosis factor, interleukin 1, and interleukin 6 [115, 119, 120, 130, 136, 140]. This upregulation finds its counterpart in the brain [125, 133, 137]. Elevated cytokine concentrations in the brain reduce serotonin availability [121, 122, 145]. Norepinephrine concentrations, on the other hand, appear to be higher [6, 7, 9, 130, 132].

As a consequence, the increase in the risk of severe depressive mood in individuals with exposure to both low hemoglobin and low cortisol levels might have been due to a critical change in neurotransmitter concentrations in the synaptic cleft. Individuals with exposure to only one or no risk factor did not experience this

alteration of available neurotransmitters to the same extent. Thus, their risk of depression was not significantly different.

External validity

Hemocue was used to assess anemia (hemoglobin < 120 g/L), and its prevalence was 11% in the screening sample (see Chapter 3). This value was lower than the prevalence of 16 to 21% in rural and urban non-pregnant women in Mexico City and the central region of Mexico in 1999 [13]. Mundo et al. [21] reported a prevalence of anemia of 14.7 to 16.8% in 2006. Iron status values of the venous blood sample analysis were not representative of the population of factory workers as a whole, since women who were identified as anemic by Hemocue were oversampled. In addition, since anemic women were oversampled, and ID was associated with depressive mood in this analysis, the percent of women suffering from severe depressive mood in the present sample (31%) is not representative of the overall population in the factories. Lifetime prevalence of MDD in unacculturated female Mexican American immigrants is 8.4%, and of dysthymia 1.6% (assessed with the Composite International Diagnostic Interview Schedule [CIDI]) [13, 20]. In the Mexican state of Morelos, where the present study took place, the prevalence of MDD (based on the Diagnostic and Statistical Manual of Mental Disorders [DSM-IV]) in women is 5% within the year prior to interview [21]. In all of Mexico, prevalence of MDD in women for the last 12 months prior to interview is 7.6%, and for the last 6 months is 5.7% (measured with the Composite International Diagnostic Interview [CIDI]) [20]; lifetime prevalence is 15.9% [20].

In the present sample, the mean fatigue score was 4.1. Compared with other studies, this value is between the means of non-fatigued subjects and fatigued subjects. Non-fatigued participants have an average score of 2.3 to 3.1 [227, 246]. Fatigued and/or ill subjects have a mean FSS score of 3.9 to 6.9 [227, 243-246].

AUC3E cortisol modified the effect of hemoglobin on severe depressive mood, insofar as only individuals with low cortisol levels had a significant inverse association of hemoglobin with risk of depressive mood. To our knowledge, no published study assessed this relationship; however, we found that Hispanic American women of the HHANES who were exposed to both chronic stressors and ID showed a higher risk of severe depressive mood compared to their peers (Chapter 2). When we revisit the results of the 5 iron supplementation trials that investigated the decrease of depressive mood after supplementation, we can find further corroboration of our findings. While 4 of the 5 trials had negative results [42-45], the one study finding a decrease in depression scores after iron supplementation compared to placebo was undertaken with postpartum South African women [46]. This specific sample is not only poor, but also focuses on mothers with young infants; both poverty and early postpartum status are known to raise the risk of MDD [53, 64-66, 86, 253-256].

Few studies publish information on mean AUC cortisol. Moreover, comparisons of the values of AUC3E cortisol of our sample to the published literature are affected by the length of the sampling day. In other words, in the same sample AUC cortisol estimates for a sampling day that lasts from 8 a.m. to 8 p.m. are likely to be higher than for a sampling day that lasts from 8 a.m. to 3 p.m. Nevertheless, mean AUC3E cortisol (16.0 nmol/L; range 1.53 to 84.61) appears to be low in our sample, when comparing it to Li et al.'s [274] work. The authors report an AUC cortisol of 44.75 in their sample of 3276 British women, with a range of 6 to 300 nmol/L. The authors sampled within a 3-hour interval (45 minutes after awakening to 3 hours after the first sample).

Clinical relevance

One way to determine the clinical significance of our findings is to compare them with the effect on antidepressant therapy on remission of MDD. We found one

randomized controlled trial assessing the efficacy of antidepressant therapy with the outcome being the probability of remission. The treatment trial uses a different depression scale (Hamilton Rating Scale for Depression). The authors [220] investigate the probability of remission after therapy, testing the antidepressants Duloxetine and Paroxetine on a total of 367 patients with MDD. Depending on the dosage, Duloxetine increases the probability of remission by 21 to 28% compared to a placebo, while Paroxetine increases it by 17%. In the present study, when defining a BDI score below 29 as remission (reference BDI score ≥ 29), we were able to estimate the potential effect of improving hemoglobin status from 100 to 123 g/L (from moderate anemia to not clinically anemic) in hypocortisolemic individuals. Covariates were fatigue, and factor 2 (representing crowding and SES). The variable $MCV > 94$ fL was not significant. The odds ratio of remission of severe depressive mood with a unit increase of hemoglobin was 1.07 (CI 1.001, 1.14; not shown). One interpretation of this finding is that the change from a hemoglobin value of 100 g/L to 123 g/L would increase the probability of remission of severe depressive mood by 28%. This magnitude is comparable to the effect of antidepressant treatment in the Detke et al. [220] study. However, the definition of remission in Detke et al.'s trial (depression scale score of ≤ 7 points after 8 weeks of treatment) and in our study are very different; thus, comparison of outcomes is impaired.

Conclusion

In conclusion, we found that a biological marker of perceived stress modified the effect of hemoglobin on severe depressive mood. Specifically, in women with low cortisol, the risk of severe depressive mood decreased with increasing hemoglobin levels. No significant effect could be found in hypercortisolemic women.

This observational study provides promising implications for future research and treatment.

First, it suggests target groups for the exploration of the biological association between ID and depression, namely women who experience chronic stress. Including measures of chronic stress is advisable in order to tease out the effect of iron supplementation in subgroups.

Second, supplementation trials should be undertaken that focus on stressed populations in order to further elucidate the association between ID and depressive mood. Specifically, the issue of spurious associations due to uncontrolled confounders and reverse causality needs to be resolved. A comparison to antidepressant therapy as a control condition is suggested.

Third, the results suggest that severely depressed women could benefit from iron supplementation, provided they are ID as well. Conversely, iron sufficiency could protect against the development of severe depressive mood, even in the face of stressor exposure. The public health relevance of this finding is high, since iron supplementation might reduce the risk of severe depressive mood in stressed women. Since women living in poverty might not be able to afford antidepressant therapy that reduces the severity of their depression, iron supplementation provides an inexpensive alternative.

Chapter 5: CONCLUSION AND RECOMMENDATIONS

In conclusion, iron deficiency (ID) is associated with depressive mood, despite the statistical control of potential confounders and fatigue. Moreover, stressor exposure and cortisol as a biological marker of chronic stress modify the association of iron status and the risk of depressive mood substantially.

Suggestions addressing the design of future studies would be to

- use both categorical and continuous measures of iron status. This includes composite measures such as iron stores [155, 217] or the categorical mean corpuscular volume (MCV) or ferritin model of ID determination [8]
- further explore whether fatigue might be a mediator between iron status and depressive mood with samples and scales that are different from the ones in the present dissertation
- include moderate as well as severe levels of depressive mood in the analysis. This would preclude a depression assessment tool, such as the CESD, that does not offer cut-points for severity of depressive mood

For future research, an inclusion of stress assessment and a focus on specific subgroups is suggested. We propose that iron supplementation trials be undertaken to confirm and extend our findings.

- Iron supplementation trials should be conducted with women who are exposed to chronic stressors, in order to ensure a measurable supplementation effect compared to placebo. Stress might be defined as poverty, or a more sophisticated screening process might be used.
- Control conditions in iron supplementation trials might not only be placebo, but standard therapy (antidepressants).
- Iron supplementation trials would also help to minimize the threat of unmeasured confounders to the results, since randomization usually

ensures equal distribution of confounders in both groups. Additionally, it addresses the question of reverse causality, since a time order of change in iron status is imposed by the iron supplementation.

- It is advisable to measure stress and immunological markers at the beginning and end of the iron supplementation, in order to enhance the biological explanation of the mechanisms. Stress should be assessed via questionnaire and/or cortisol, in order to measure perceived stress. A convenient immunological marker may be C-reactive protein, which is now a convenient field method. Best would be pro-inflammatory cytokines, since they appear to be the direct mediators between stress and depressive mood.
- Specifically, we would expect that in a stressed sample the decline of depression scores would be more pronounced with iron supplementation than in a non-stressed sample. In a chronically stressed sample, perceived stress and stressor exposure should remain high, and cortisol levels should remain low throughout the study period. Likewise, the immune system should remain upregulated. Improvement in iron status should reflect successful supplementation, and the magnitude of the iron status improvement should be reflected by a significant decrease in depression scores.
- While iron supplementation addresses one component of the synergistic relationship between stress and iron deficiency, another option would be to modify the contribution of stress. This might be possible with behavioral intervention, or by treated depressed participants with proinflammatory cytokine antagonists.

Appendix A: IRON DEFICIENCY AND DEPRESSIVE MOOD IN HISPANIC AMERICAN WOMEN (HHANES)

1.1 Figures and Tables

Table A.1: Odds ratios of depressive mood when covariates were included in the basic model (depressive mood = intercept + iron stores/100 + previous-week’s fatigue). Logistic regression model with CESD-17 as dependent variable (event defined as being in the upper 90th percentile of the distribution). Odds ratios (OR), lower and upper limit of confidence intervals (CI) of odds ratio. The models are adjusted for stratification, clustering (PSUs), and relative weights. Ref PR=reference Puerto Rican.

Covariates included into basic model	OR of iron stores/100	CI of iron stores/100	OR of covariates	CI of covariates
Living space	0.90	0.83,0.97	0.85	0.76,0.95
Spouse in house	0.89	0.82,0.96	0.50	0.33,0.76
Ethnic group (Ref PR):	0.89	0.81,0.97		
Cuban			0.64	0.25, 1.62
Mexican			0.39	0.24, 0.64
Undetermined			0.28	0.12, 0.64
All covariates out	0.89	0.82, 0.97		
All covariates in	0.89	0.82, 0.97		

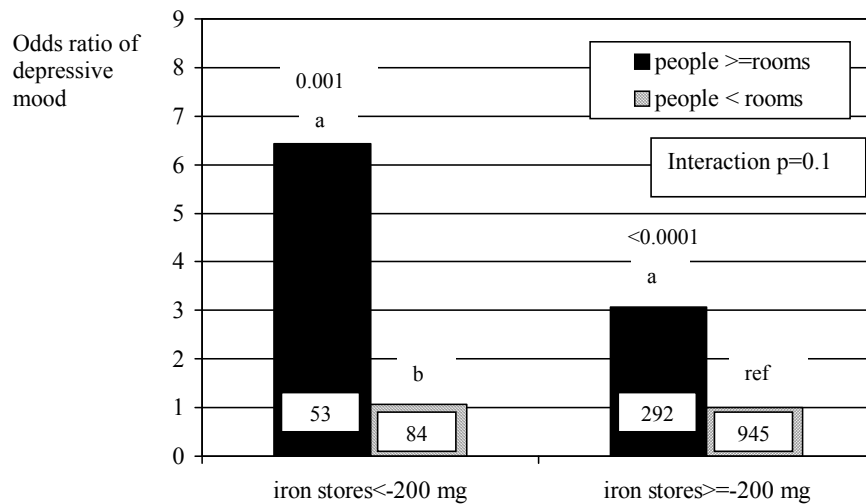


Figure A.1: Odds ratio of depressive mood with iron stores < -200 mg or ≥ -200 mg, grouped by space availability. Logistic regression model with CESD-17 as dependent variable (event defined as being in the upper 90th percentile of the distribution). Covariates were previous-week's fatigue, marital status, and ethnic group. The model is adjusted for stratification, clustering (PSUs), and relative weights. Group size in bars. P-value of difference from reference group (ref), and any difference with significance below 0.05 shown by different letters.

Crowded living space (number of persons in household equal or greater than number of rooms) and iron stores smaller than -200 mg increased the odds of depressive mood by 600% when compared to the reference group. The overall interaction had a p-value of 0.1, while the group comparison between high risk and reference is significant at a p-value of 0.001. Having less space available was identified as an indicator of low SES, based on the results of the factor analysis.

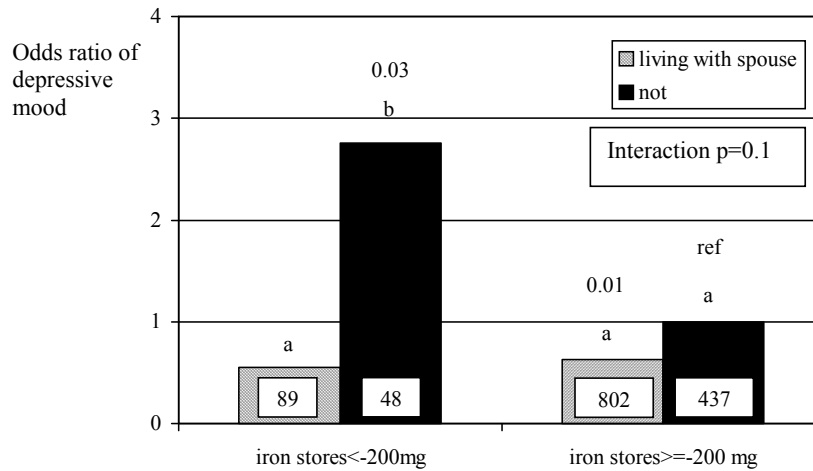


Figure A.2: Odds ratio of depressive mood with iron stores < -200 or \geq -200 mg, grouped by living with spouse or not. Logistic regression model with CESD-17 as dependent variable (event defined as being in the upper 90th percentile of the distribution). Covariates were previous-week's fatigue, space available, and ethnic group. The model is adjusted for stratification, clustering (PSUs), and relative weights. Group size in bars. P-value of difference from reference group (ref), and differences with significance below 0.05 shown by different letters.

Women who do not have the spousal support in the same household and are severely ID have odds of depressive mood that are more than 400% greater than the reference group. The overall interaction was significant at a p-value of $p=0.1$; the comparison between highest risk group and reference group had a p-value of 0.03. Living with the spouse in the house was found to be an indicator of higher SES when performing a factor analysis.

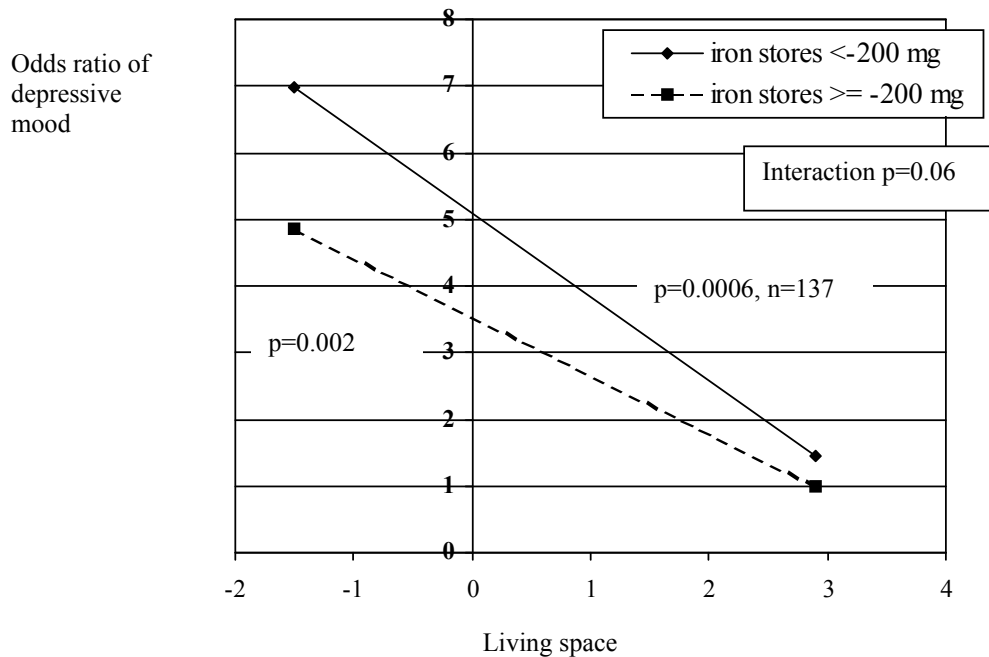


Figure A.3: Odds ratio of depressive mood with space in household, grouped by iron stores (mg) < -200mg or ≥ -200 mg. Logistic regression model with CESD-17 as dependent variable (event defined as being in the upper 90th percentile of the distribution). Covariates were previous-week’s fatigue, marital status, and ethnic group. The model is adjusted for stratification, clustering (PSUs), and relative weights.

Women with iron stores less than -200 mg have a greater increase of risk of depressive mood with reduced space in their household than women with iron stores greater than or equal to -200 mg.

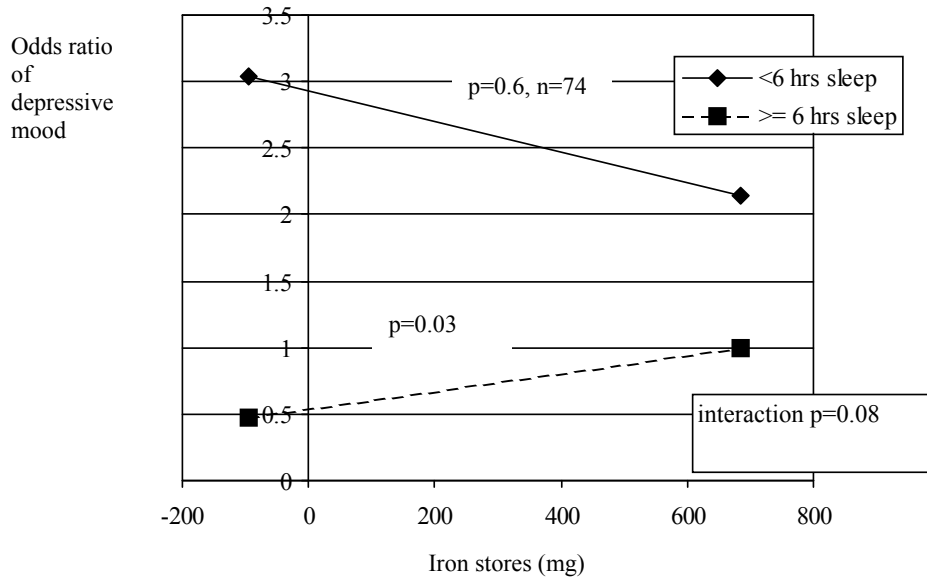


Figure A.4: Odds ratio of depressive mood with iron stores (mg), grouped by hours of sleep less than 6 or greater or equal to 6. Logistic regression model with CESD-17 as dependent variable (event defined as being in the upper 90th percentile of the distribution), $n=1373$. Covariates were previous-week’s fatigue, space available, marital status, and ethnic group. The model is adjusted for stratification, clustering (PSUs), and relative weights.

Women who slept less than 6 hours over the last 24 hours had a significantly different slope of iron stores’ effect on depressive mood than women who slept 6 or more hours ($p=0.08$, Appendix Figure A.6). While women with less than 6 hours of sleep over the last 24 hours showed an increase of depressive mood with lowering iron stores, women who slept 6 hours or more appeared to have the opposite relationship. Note that the overall interaction p -value addresses the difference in slopes, while the p -values of the subgroup slopes inform about the difference from a slope from 0. The slope of the sleep-deprived subgroup was not significant from 0, albeit the sample size was only 74 cases.

Table A.2: Odds ratios of depressive mood when covariates were included in the basic model (depressive mood = intercept + iron stores/100 + previous-week’s fatigue). Logistic regression model with CESD-17 as dependent variable (event defined as being in the upper 90th percentile of the distribution). Odds ratios (OR), lower and upper limit of confidence intervals (CI) of odds ratio. The models are adjusted for stratification, clustering (PSUs), and relative weights. Ref PR=reference Puerto Rican.

Covariates included in basic model	OR of iron stores/100	CI of iron stores/100	OR of covariates	CI of covariates
Living space	0.90	0.83,0.97	0.85	0.76,0.95
Spouse in house	0.89	0.82,0.96	0.50	0.33,0.76
Ethnic group (Ref PR):	0.89	0.81,0.97		
Cuban			0.64	0.25, 1.62
Mexican			0.39	0.24, 0.64
Undetermined			0.28	0.12, 0.64
All covariates out	0.89	0.82, 0.97		
All covariates in	0.89	0.82, 0.97		

Table A.3: Comparison between final sample of 1375 cases and the excluded 1528 respondents. Means or percentages. Sample size varies in the excluded group depending on availability of variables. No adjustment for sampling procedures. Significance testing with t-test or Chi-square test.

Variables	Final sample n=1375	Excluded respondents total n=1528	
			N of variable
Mean age (yrs)	33 *	35	1528
Mean hemoglobin (g/L)	134	133	758
Mean ferritin µg/L	39	35	488
Mean CESD score	10	10	472
% Above CESD 16	18	19	472
% Above CESD 23	11	11	472
% Above 90th percentile of CESD-17	9	9	472
% Ethnic group			1522
Puerto Rican	23	23	
Cuban	12	15	
Mexican	52	50	
Undetermined	13	12	
% Poverty index ratio < 130%	44	44	1262

*p<0.05

Table A.4: Classification as depressed by the CESD-20 with cutoff of 23 points, and the CESD-17 with cutoff above the 90th percentile.

CESD-20	CESD-17	
	≤90th percentile	>90th percentile
≤23	1248	0
>23	20	108

Table A.5: Classification as depressed by the CESD-20 with cutoff above the 90th percentile, and the CESD-17 with cutoff above the 90th percentile.

CESD-20	CESD-17	
	≤90th percentile	>90th percentile
≤90th percentile	1216	3
>90th percentile	6	105

Table A.6: Comparison of cases of CESD-17 (90th percent cutoff) and DIS (current major depression).

	DIS current major depression	DIS no current major depression	Total
CESD-17 > 90 percentile	32	99	131
CESD-17 ≤ 90 percentile	8	1236	1244
Total	40	1335	1375

Table A.7: Logistic regression model with high previous-week’s fatigue as dependent variable (event defined as being in the upper quartile of the distribution, score > 5), n=1375. Odds ratios (OR), and lower and upper limit of confidence intervals (CI) of odds ratio. The models are adjusted for stratification and clustering (PSUs), and relative weights. c = area under the ROC curve.

Variable	Model	
	OR	CI
Intercept	0.0009	0.0002, 0.003
Iron stores (mg)/100	1.06	1.002,1.12
Depressive mood (CESD-17)	1.19	1.16,1.22
Poverty index ratio < 130%	1.96	1.24, 3.09
Age (yrs)	1.05	1.02,1.07
-2LL	376	
c	0.901	

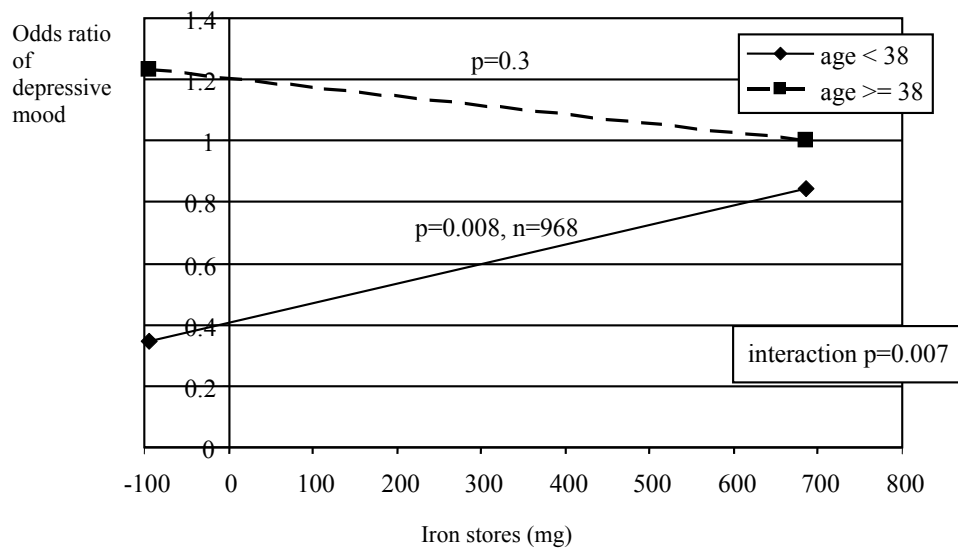


Figure A.5: Odds ratio of high previous-week's fatigue with iron stores (mg), grouped by age less than 38 years or greater or equal to 38. Logistic regression model with previous-week's fatigue as dependent variable (event defined as being in the upper 90th percentile of the distribution, score > 5). Covariates were CESD-17, and poverty index ratio < 130%. The model is adjusted for stratification, clustering (PSUs), and relative weights.

Table A.8: Logistic regression model with CESD-20 as dependent variable (event defined as a score greater than 23), n=1375. Odds ratios (OR), and lower and upper limit of confidence intervals (CI) of odds ratio. The models are adjusted for stratification and clustering (PSUs), and relative weights. c = area under the ROC curve.

Variable	Model	
	OR	CI
Intercept		
Iron stores (mg)/100	0.95	0.91, 0.99
Living space	0.84	0.78, 0.92
Spouse in house (Ref not)	0.45	0.29, 0.68
Ethnic group (ref PR)		
Cuban	0.41	0.21, 0.78
Mexican	0.35	0.23, 0.53
Undetermined	0.33	0.16, 0.68
-2LL	789	
c	0.705	

1.2 Supportive Analysis

1.2.1 Factor analysis

Methods

In order to explore the relationship between covariates, and to address the problem of colinearity, a factor analysis was performed with SAS Statistical Software [155]. It included variables coding for SES, stressors, and ethnic status with sufficient sample size (greater than 1000 cases). Specifically, the following variables were included: whether received food stamps were not sufficient, and additional money had to be spent (yes/no); any health insurance (yes/no); married with spouse in the household (yes/no); poverty index ratio (<130%, 130–300%, ≥300%); education (no high school, high school, college, graduate); crowding in household (same number of rooms and household members or more rooms than members vs. more members than rooms); residence (urban, central city; central city; rural); ethnic group (Puerto Rican, Cuban, Mexican); language of interview (Spanish vs. English); whether a complete kitchen was available (yes/no); past depression (yes/no); and less than 6 hours of sleep (yes/no). All variables were coded in such a way that the higher values corresponded to potentially better emotional health status than the lower values. For example, Puerto Ricans are known in this sample to have higher rates of depression than Mexicans and Cubans, and were therefore coded as “1,” Cubans as “2,” and Mexicans as “3.” Likewise, a poverty index ratio above 300% received the categorical code “3,” while a poverty index ratio below 130% was coded “1.” Variables with a factor loading smaller than |4| were not considered high enough to be part of the interpretation. Factors were rotated with the Promax function, allowing for correlation between factors. Best factor models were decided on the basis of successful summarization of variables, and interpretability of factors.

Results and discussion

Factor analysis was performed as a confirmation of the covariates *living space*, *spouse in house*, and *ethnic group* that were included in the final model (Table 2.4, Model 4). A total of 1141 cases had complete information on all the variables included in the factor analysis. Three factors emerged: SES associated with being married and having the spouse in the same household, SES associated with the subject's own achievements, and a third factor related to ethnic group (Appendix Table A.9).

While both SES measures included poverty index ratio, the factor *SES based on spouse in the same household* is also associated with receiving food stamps, and with access to health care. The factor *SES based on the participant's achievements* includes years of education completed, spouse not in household, poverty index ratio, language of the interview, and number of rooms available to the persons living in the household. The third factor, *ethnic status*, is as much a reflection of the ethnic group as of the sampling design: Puerto Ricans and Cubans were approached in the metropolitan regions of New York City and Miami, while Mexicans in the Southwest were more likely to live in rural settings. Language of interview loads high in the factors *SES based on the participant's achievements* and *ethnic status*, meaning that Spanish speakers are more likely to have fewer years of education and to have a lower poverty index ratio; and to be Puerto Ricans and live in an urban center. The variables *past depression*, *complete kitchen*, and *hours of sleep less than six* dropped out of the analysis because of low factor loadings ($<|4|$). The three factors coincided with the three significant covariates included into the final logistic model (Table 2.4, Model 4); thus *ethnic status*, *marital status*, and *crowding* appear to each be a representative of these factors. The resulting factor scores were weakly correlated, between 0.02 and 0.18 (Appendix Table A.10), as a result of the performed oblique rotation method.

Table A.9: Factor analysis, factor loadings.

Variable	Factor poverty based on marital status	Factor poverty based on subject's achievements	Factor ethnic status
Foodstamp recipient, not sufficient to cover food expenses	0.75	0.09	0.04
Any health insurance	0.74	0.21	-0.06
Spouse in household vs. other options	0.68	-0.38	0.03
Poverty index ratio (<130%, 130 to 300%, ≥300%)	0.65	0.45	-0.03
Education	0.26	0.71	0.05
Living space in household	0.03	0.69	-0.09
Area of residence	-0.07	0.03	0.76
Ethnic group	0.29	-0.26	0.69
Language of interview	0.16	-0.51	-0.60
Variance explained	2.1	1.7	1.4

Table A.10: Correlation between factor scores. Correlation coefficients and p-values.

	SES marital status	SES subject's achievements	Ethnic status
SES marital status	1	0.10 (0.0003)	0.18 (<0.0001)
SES subject's achievements		1	0.02 (0.4)
Ethnic group			1

1.3 Effect of Iron Stores with Severity of Depressive Mood

An additional analysis investigated whether the relationship between depressive mood and iron stores was the same at all levels of severity of depressive mood. To this purpose, the outcome CESD-17 was split into three levels: scores from 0 to 9 (which corresponds to below the 70th percentile of the CESD-17), mid-range scores from 10 to 20 (percentiles 70 to 90 in the CESD-17), and greater than or equal to 21 (greater than the 90th percentile). Corresponding scores of the CESD-20 would be scores from 0 to 11 (below the 70th percentile), scores from 11 to 25 (the 70th to 90th percentile), and scores greater than 25 (greater than the 90th percentile). Cases with low-level depression were used as a reference group in a logistic regression model. Two models with two outcome variables were run: one with the event group as mid-level depression, and one with the event group as high-level depression (effect of iron stores with severity of depressive mood, Appendix Table A.11). All models were adjusted for stratification, clustering, and probability of selection. All models contained the independent variables selected for the final Model 4 shown in Table 2.4. The group that qualified as having no depressive mood contained 972 cases, the mid-level depressive mood group had 266 cases, and the high level depressive mood group had 131 cases. The odds of depression in the logistic regression model decreased by 3% with every 100 g increase of iron stores in the model with mid-level depressive mood. The odds of depressive mood in the logistic regression model decreased by 8% with every 100 g increase of iron stores in the model with high-level depressive mood. Thus, there is a progression in the effect of ID on depressive mood. It should be noted that the strongest effect (an 11% decrease) of iron stores is found when combining low-grade and moderate depressive mood, and contrasting those groups with high-level depressive mood (Table 2.4, Model 4).

Table A.11: Results of analysis of low versus moderate or high depressive mood. Logistic regression model with CESD-17 as dependent variable. Odds ratios (OR), lower and upper limit of confidence intervals (CI) of odds ratio. The models are adjusted for stratification, clustering (PSUs), and relative weights. Ref PR=reference Puerto Rican; c = area under the ROC curve.

Variable	Low and moderate depressive mood			Low and high depressive mood		
	OR	CI	DEF	OR	CI	DEF
Intercept	2.70	1.92, 3.79		.049	0.03, 0.08	
Iron stores (mg)/100	0.97	0.94, 0.99		0.92	0.84, 0.99	
Last week's fatigue	1.57	1.47, 1.68		2.74	2.46, 3.06	
Living Space	0.91	0.84,0.99		0.79	0.73, 0.86	
Spouse in house (Ref other)	0.64	0.43, 0.96		0.36	0.18, 0.75	
Ethnic group (ref PR)	Cuban	0.43	0.25, 0.73		0.49	0.13, 1.82
	Mexican	0.59	0.39, 0.89		0.32	0.19, 0.54
	Other	0.46	0.27, 0.77		0.13	0.034, 0.49
-2LL	1147			300		
c	0.743			0.960		

Since the effect size of iron stores on moderate depressive mood was very small (OR=0.97), we decided to instead test interactions between iron stores and potential stressors while using moderate depressive mood as an outcome (Appendix Table A.12). Hypothetically, the association between iron stores and moderate depressive mood should be stronger in groups exposed to stressors. Only two of the

tested interactions (Appendix Table A.12, Model 1), with the stressors *having < 6 hours of sleep over the last 24 hours and being married with the partner living in the same household vs. other*, turned out to be significant.

Table A.12: Results of interactions between select potential stressors and iron stores. Logistic regression model with CESD-17 as dependent variable. Outcome reference is CESD-17 0 to 69th percentile; Model 1 presents moderate depressive mood (CESD-17 70th–90th percentile) as event of interest; Model 2 presents high depressive mood (CESD > 90th percentile). Covariates were previous-week’s fatigue, ethnic group, and – depending on model – marital status and crowding. Displayed are p-values of interaction between iron stores (continuous iron stores mg/100; or categorical iron stores < –200 mg vs. ≥ –200 mg) and potential stressors. The models are adjusted for stratification, clustering (PSUs), and relative weight.

Stressor variable	Model 1 (n=1238) Low versus moderate depressive mood		Model 2 (n=1103) Low versus high depressive mood	
	Continuous iron stores mg/100	Categorical iron stores <≥ –200 mg	Continuous iron stores mg/100	Categorical iron stores <≥ –200 mg
Poverty index < 130%	0.7	0.14	0.2	0.06
Married vs. other	0.001	0.7	0.5	0.28
Crowding	0.2	0.4	0.4	0.18
Post-pregnant	0.6	0.4	0.66	0.26
Postpartum	0.7	0.7	0.9	0.7
Ethnic group	0.7	0.9	0.6	n/a (0 cells)
High control over health vs. other	0.9	0.4	0.7	0.06
Hours of sleep < 6	0.006	0.28	0.2	0.3
Food stamps not enough for food expenses (yes/no)	0.5	0.7	0.4	0.3

Appendix Figure A.6 shows that living with a spouse increased the risk of moderate depressive mood for Hispanic women by 6 percent for every 100 mg decrease in iron stores. This result is in contrast to the results with severe depressive mood: living with one's spouse had a protective effect against severe depressive mood when iron stores were low (Appendix Figure A.2). Appendix Figure A.7 shows that having less than 6 hours of sleep during the previous 24 hours increased the risk of moderate depressive mood by 14% for every 100 mg decrease in iron stores. Compared to results with severe depressive mood (Appendix Figure A.8), this result is more consistent with our expectations that the stressor lack of sleep would increase the risk of depressive mood. Note, though, that this relationship was based on a subsample size of n=55 and concurrent short-term assessments of mood and sleep deprivation.

In summary, the effect of stressors – when comparing the effect of iron stores on severe and moderate depressive mood – was inconsistent. Moreover, in models with moderate depressive mood as an outcome, only two interactions were significant. Thus, the consistency between interactions with the outcome of moderate depressive mood was limited.

Model 2 in Table A.12 showed that the reduced sample size (the 266 cases with moderate depressive mood were excluded) affects the significance of the interactions with the full sample size. Only the interactions with the stressors *poverty index < 130%* and *low control over one's health* demonstrated a low p-value.

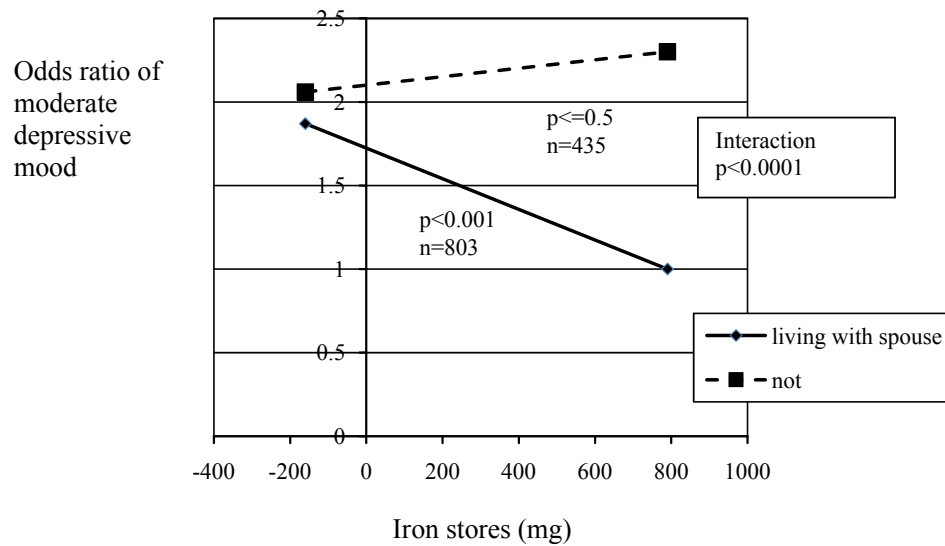


Figure A.6: Odds ratio of moderate depressive mood with iron stores (mg), grouped by married living with spouse or other. Logistic regression model with CESD-17 as the dependent variable (event being defined as the 70th–90th percentile of distribution). Covariates were previous-week’s fatigue, space available, marital status, and ethnic group. Model was adjusted for stratification, clustering, and relative weights.

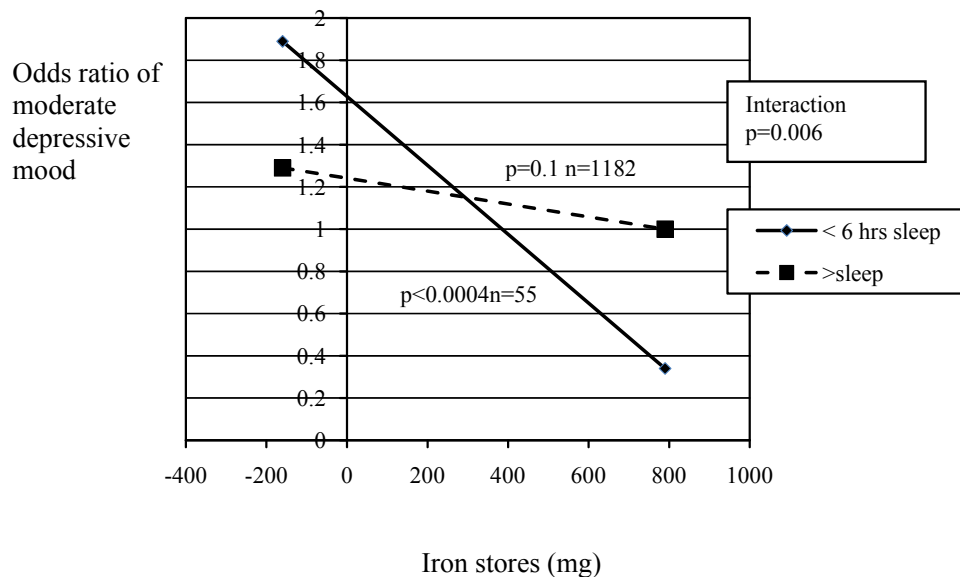


Figure A.7: Odds ratio of moderate depressive mood with iron stores (mg), grouped by hours of sleep (<6, or ≥6). Logistic regression model with CESD-17 as the dependent variable (event being defined as the 70th–90th percentile of distribution), n=1298. Covariates were previous-week’s fatigue, space available, marital status, ethnic group, and hours of sleep < 6. Model was adjusted for stratification, clustering, and relative weights.

1.4 Role of Relative Weight

In the present analysis it became apparent that the probability of selection based on chronological age (which is expressed in the survey design variable “relative weight”) was important. Statistically adjusting for relative sampling weight increased the strength of the effect of iron stores on depressive mood: the odds ratio of iron stores/100 mg changed from 0.92 without relative weight adjustment (Appendix Table A.13) to 0.89 with adjustment for weight (Table 2.4, Model 4). Iron stores remained significant in both models. A possible explanation is that participants 20 to 44 years of age had a lower probability of selection [155], and that adjustment for relative sampling weight increased their influence on the analysis. An interaction between chronological age and iron stores ($p=0.06$) showed that women younger than 35 have an increasing probability of depressive mood with decreasing iron stores compared to a weaker relationship in women older than 35 years of age (Appendix Figure A.8). The cutoff of age 35 was chosen because it had the highest level of significance, even though it does not coincide with the cutoff for sampling probability. The p-value of the interaction between age at 45 and iron stores was $p=0.6$ (model not shown). A number of possible explanations were explored. Chronological age might represent perimenopausal status (see Chapter 2, discussion section). Women in perimenopause could have a higher probability of depressive mood but lower prevalence of ID than women of a younger cohort. Their relationship between iron stores and depressive mood might be expected to be weaker than in younger women. Other additional variables were investigated as modifiers of the effect of iron stores in models without relative sample weight adjustment. Oral contraceptive use has been shown to increase the likelihood of depression in women with ID [287], but in our sample no significant interaction was found (model not shown). Buffering of the relationship between iron stores and depressive mood by personal characteristics was also explored. Higher

control over one’s situation has been shown to be such a buffer [288]. The HHANES survey had one variable coding for control over health (“How much control do you think you have over your health?”). No interaction between iron stores and control over one’s health was found in the unweighted model (model not shown).

Table A.13: Unweighted final model. Logistic regression model with CESD-17 as dependent variable (event defined as being in the upper 90th percentile of the distribution). Odds ratios (OR), and lower and upper limit of confidence intervals (CI) of odds ratio. The models are adjusted for stratification and clustering (PSUs). Ref PR = reference Puerto Rican, c = area under the ROC curve.

Variable		Model	
		OR	CI
Intercept		0.038	.025, .057
Iron stores (mg)/100		0.92	0.85, 0.99
Last week’s fatigue		2.10	1.94, 2.27
Living space		0.81	0.73, 0.90
Spouse in house (Ref other)		0.46	0.26, 0.81
Ethnic group (ref PR)	Cuban	0.90	0.13, 0.74
	Mexican	0.43	
	Other	0.31	
-2LL		464	
c		0.937	

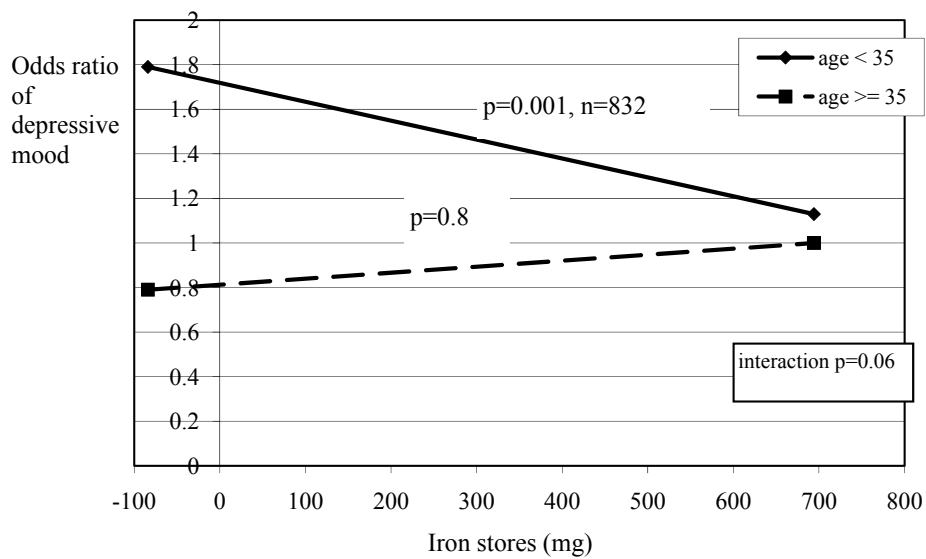


Figure A.8: Odds ratios of depressive mood in interaction between iron stores (mg) and chronological age (yrs). Logistic regression model with CESD-17 as dependent variable (event defined as being in the upper 90th percentile of the distribution). Covariates were previous-week's fatigue, space available, marital status, and ethnic group. The model was adjusted for stratification and clustering (PSUs).

Appendix B: IRON DEFICIENCY AND DEPRESSIVE MOOD IN FEMALE MEXICAN GARMENT WORKERS

1.1 Figures and Tables

Table B.1: Comparison between all participants in screening stage, and study participants; mean (SD), or %; t-test or Chi-square test.

Variable	Screening	Sample	p-value
N	237	100	
Age (years)	25.5 (8.6)	27.1 (7.8)	0.11
Height (m)	1.52 (0.57)	1.51 (0.56)	0.15
Weight (kg)	59.5 (11.8)	58.4 (9.7)	0.4
Marital status %			0.19
Married or living with partner	26	28	
Single	64	56	
Other	10	16	

Table B.2: Logistic regression models: outcome BDI moderate and severe depressive mood (BDI ≥ 20) vs. none to mild (BDI < 20); OR (CI); n=100; c = area under the ROC curve.

Variables	Fixed factor models				Mixed model
	Model 1	Model 2	Model 3	Model 4	Model 5
Intercept	1.06 (0.68, 1.66)	0.10 (0.07, 0.38)***	1.06 (0.63, 1.47)	0.10 (0.03, 0.36)***	0.08 (0.02, 0.33)***
Centered hemoglobin g/L	0.99 (0.96, 1.01)	0.99 (0.96, 1.02)	0.99 (0.96, 1.01)	0.99 (0.96, 1.02)	0.99 (0.74, 1.02)
Fatigue		1.89 (1.36, 2.63)***		1.91 (1.37, 2.67)***	1.96 (0.72, 2.78)***
Factor 2 (crowding/SES)			1.03 (0.69, 1.54)	0.90 (0.57, 1.41)	0.87 (0.55, 1.37)
MCV>94 fL	0.93 (0.36, 2.41)	0.75 (0.26, 2.15)	0.93 (0.36, 2.44)	0.73 (0.25, 2.11)	0.84 (0.28, 2.52)
N	100	100	100	100	100
c	0.522	119.8	0.517	0.736	n/a
-2LL	137.7	0.731	137.7	119.5	426.0 ^a
Random factor factory: Cov parameter estimate (std error)	n/a	n/a	n/a	n/a	1.23 E -18 (.)

^a Pseudo-log Likelihood for Proc Glimmix
*p<0.05; **p<0.001; ***p<0.0001

Table B.3: Odds ratio (OR) of depressive mood when compared to no or mild depressive mood (score < 20); assessed were moderate (20–28), or severe depressive mood (≥29); n=100. c = area under the ROC curve.

Variables	Outcome moderate (20–28) vs. none or mild (<20)	Outcome severe (≥29) vs. none or mild (<20)
Intercept	0.11 (0.02, 0.54)**	0.00 (0.00, 0.05)****
Centered hemoglobin g/L	1.02 (0.98, 1.07)	0.95 (0.91, 0.99)**
Fatigue	1.58 (1.04, 2.40)**	2.80 (1.69, 4.64)****
Factor 2 (crowding/SES)	1.39 (0.70, 2.76)	0.56 (0.30, 1.06)*
MCV>94 fL	0.25 (0.05, 1.16)*	2.21 (0.50, 9.67)
N	74	75
c	0.710	0.863
-2LL	83.6	64.3

*p-value ≥0.05 and <0.1; **p<0.05; ***p<0.001; ****p<0.0001

Table B.4: Odds ratio (OR) of severe depressive mood (≥ 29) with categorical iron depletion anemia (IDeA); fixed factor model. c = area under the ROC curve.

Variables	OR (CI)
Intercept	0.005 (0.00, 0.05)****
Iron depletion anemia ^a	3.44 (0.87, 13.63)*
Fatigue	2.41 (1.59, 3.65)****
Factor 2 (crowding/SES)	0.53 (0.30, 0.95)**
MCV>94 fL	2.49 (0.69, 9.02)*
N	100
c	0.826
-2LL	84.9

^a hemoglobin<12.3 g/L and MCV and/or protoporphyrin beyond cutoff

* p-value ≥ 0.05 and <0.1 ; ** p<0.05; ***p<0.001; ****p<0.0001

Table B.5: Results factor analysis of FSS – validation; varimax rotation^a; refer also to Appendix E, Fatigue Severity Scale; bolded numbers have factor loadings $\geq |4|$.

Variable	Factor 1	Factor 2
1. low motivation	0.19	0.50
2. exercise fatigues	-0.2	0.83
3. easily fatigued	0.33	0.62
4. interferes with physical functioning	-0.20	0.83
5. causes frequent problems	0.65	0.16
6. prevents sustained physical functioning	0.65	0.16
7. interferes with duties	0.77	0.12
8. among most disabling symptoms	0.74	0.09
9. interferes with social functioning	0.83	0.18
Variance explained	3.00	1.78

^a note that the oblique Promax rotation (not shown) gave virtually identical results

Table B.6: Odds ratio (OR) of a fatigue score (FSS) above the 75th percentile; OR (CI), p-value, n=100. c = area under the ROC curve.

Variables	OR (CI)	p-value
Intercept	9.0 (4.05, 20.12)	<0.0001
Centered hemoglobin g/L	0.98 (0.94, 1.01)	0.21
BDI \geq 29	0.05 (0.01, 0.20)	<0.0001
Factor 2 (crowding/SES)	0.49 (0.27, 0.90)	0.02
N	100	
c	0.803	
-2LL	86.5	

1.2 Supportive Analysis

1.2.1 Methods for subsample: Iron status

In addition to hemoglobin, MCV, and protoporphyrin, transferrin saturation, ferritin, and C-reactive protein were measured. Serum iron and total iron binding capacity was determined with atomic absorption spectrometry with an Analyst 300 Spectrometer (Perkin-Elmer, Norwalk, CT, USA). Transferrin saturation (%) was the ratio of serum iron and total iron binding capacity. Commercial kits were used to measure the serum concentrations of ferritin [$\mu\text{g/L}$], (Dade Behring Inc, Newark, DE 19714, USA), and C-reactive protein (CRP) [mg/dL]²² by nephelometry, using monoclonal antibodies (Behring Nephelometer 100 Analyzer, Behring Laboratories, Messer Grisheim GmbH, Frankfurt, Germany). Ferritin was set to missing for the 2 cases with C-reactive protein $> 10\text{mg/dL}$ and ferritin values $\geq 12 \mu\text{g/L}$. Thus, high ferritin levels whose elevation could be due to acute phase response were not included in the analysis.

All iron status indicators were evaluated as continuous and categorical variables. Cutoffs for the additional iron measures were ferritin $< 12 \mu\text{g/L}$ and transferrin saturation $< 16\%$. The composite indicators of iron status were iron deficiency without anemia (IDNA), iron deficiency anemia (IDA), and iron stores. IDNA was defined as hemoglobin above 12.3 and at least *two indicators* of IDNA (MCV, protoporphyrin, ferritin, and transferrin saturation) beyond the cutoff. IDA was based on a hemoglobin value below 12.3 g/L and at least 2 indicators of IDNA beyond the cutoff. All other cases with hemoglobin greater than or equal to 12.3 g/dL and no IDNA were defined as iron sufficient. A continuous composite of iron status was iron stores [mg] [276]; this measure incorporates hemoglobin, ferritin, transferrin saturation, and protoporphyrin in a 3-step formula based on cutoff. The resulting score

²² CRP is a clinical indicator of a systemic response of the body to infection (also called acute phase response).

ranges from negative to positive value. A value equal to or above 0 indicates iron sufficiency, a value between 0 and –300 mg indicates IDNA, and a value smaller than –300 mg indicates IDA.

Methods for subsample: Sample selection

The final subsample of 76 participants contained information on five iron status indicators: hemoglobin, MCV, protoporphyrin, ferritin, and transferrin saturation (Appendix Figure B.1). While no difference in iron and mood parameters was found between the sample of 100 cases and the subsample of 76 cases, age was significantly higher in the excluded group of 24 cases (Appendix Table B.7).

1.2.2 Results of subsample

Results of the analysis with the subsample are displayed in Appendix Table B.8, Appendix Table B.9, Appendix Table B.10, and Appendix Table B.11. Of the iron status measures, only hemoglobin had a significant effect on depressive mood. All other measures (singular and composite) were not significant.

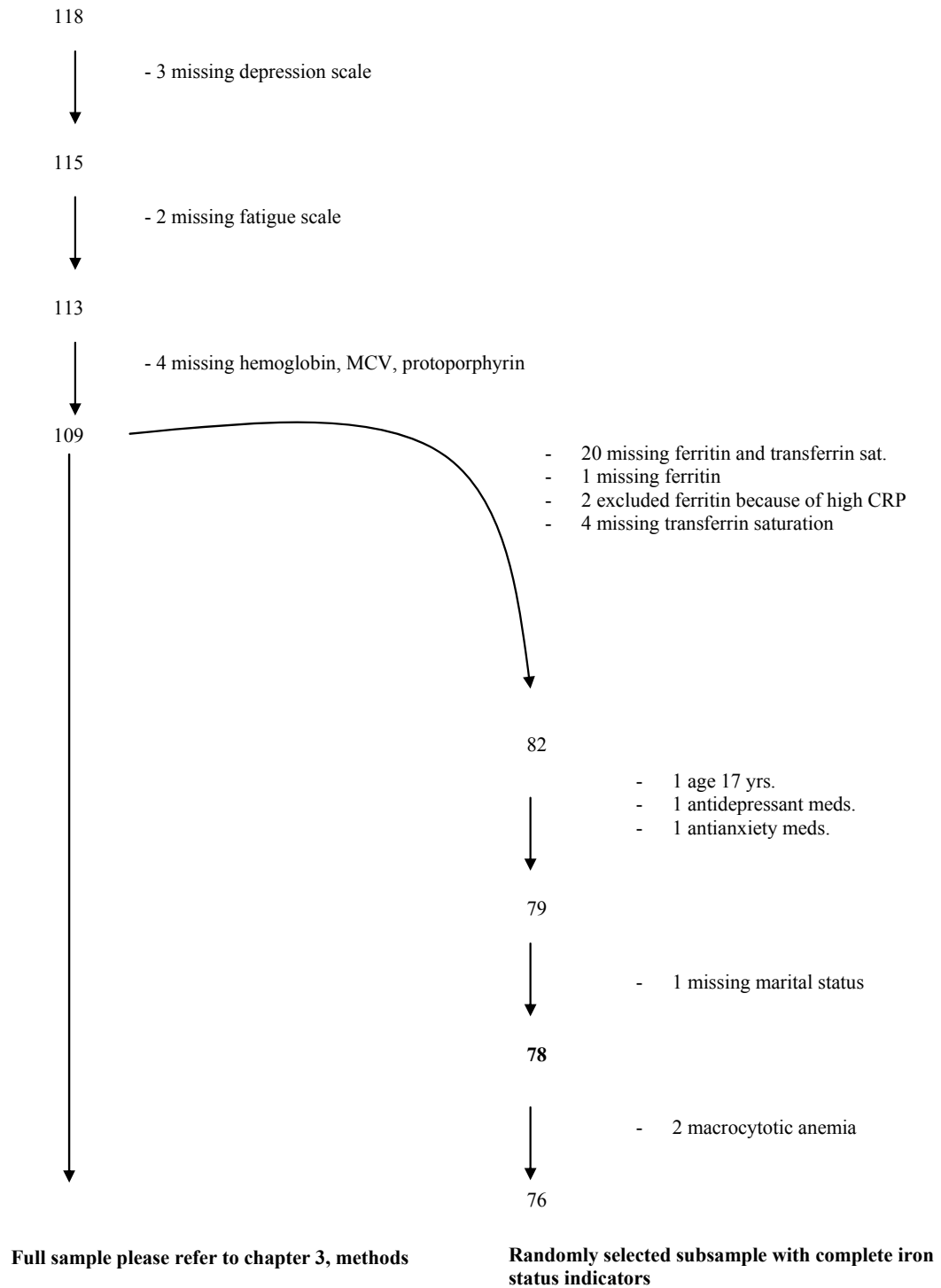


Figure B.1: Sample selection.

Table B.7: Subject characteristics comparison between samples of 100 and 76 cases; means (SD) or percentages; t-test, Chi-square test or Fisher's-Exact test.

Variable	Sample of n=76	Excluded n=24	p-value
Hemoglobin (g/L)	132 (15)	130 (18)	0.6
MCV fL	88.5 (8.7)	89.5 (8.2)	0.6
Protoporphyrin	82.7 (71.2)	67.0 (52.0)	0.3
Age (yrs)	26 (8)	31 (7)	0.01
BMI kg/m ²	25.6 (4.3)	25.8 (4.0)	0.8
Fatigue	3.7 (1.5)	4.0 (1.4)	0.3
% iron depletion with anemia ^a	23%	17%	.5
% iron depletion, but no anemia ^b	17%	8%	.2
MCV > 94 fL	22%	25%	.7
BMI > 25kg/m ²	51%	50%	.9
BMI > 30kg/m ²	12	21	.2
Severe depression ^c	26%	25%	.8
Moderate depression ^d	28%	17%	.4
Household income < 4377 pesos ^e	91%	96%	0.4
%dirt floor	9%	12%	0.7

^a MCV and/or protoporphyrin beyond cutoff, plus hemoglobin < 123g/L

^b MCV and/or protoporphyrin beyond cutoff, but hemoglobin ≥ 123g/L

^c BDI ≥ 29 points

^d BDI 20–28 points

^e equivalent of \$157/month in summer of 2001; household poverty line level 1 in 2000 in Mexico

Table B.8: Subject characteristics, general; mean (SD), median, 25th and 75th percentile, or %; n=76.

Variable	Mean (SD)	Median	25th p.	75th p.	% of whole sample
Age (yrs)	26 (7.7)	23	19	32	
BMI kg/m ²	25.6 (4.3)	25.1	22.8	28.3	
Factor 2	0.0 (0.97)	0.2	-0.5	0.7	
% BMI > 25 kg/m ²					51%
% BMI > 30 kg/m ²					12%
% Household income < 4377 pesos ^a					91%
% Dirt floor					9%
% Dirt floor, walls, and roof plastic, asbestos, or metal					1%

^a equivalent of \$157/month in summer of 2001; national poverty line for Mexican households in 2000 [232]

Table B.9: Subject characteristics, iron status; mean (SD), median, 25th and 75th percentile, or %; n=76.

Variable	Mean (SD)	Median	25th p.	75th p.	% of whole sample
Hemoglobin g/L	132 (15)	134	123	140	
MCV fL/l	88.5 (8.7)	91.5	84.3	93.6	
Protoporphyrin µg/dL	82.8 (71.2)	57.5	36.7	96.2	
Ferritin µg/L	32.4 (29.7)	20.6	14.2	41.0	
Transferrin saturation %	32.3 (9.8)	31.9	25.2	36.4	
Iron stores mg	238.6 (330.2)	208.3	41.1	473.5	
% Hemoglobin < 12.3 g/dL					24%
% MCV < 80 fL					16%
% Protoporphyrin > 70 µg/dL					41%
% Ferritin < 12 µg/L					17%
% Transferrin saturation <16%					1%
% IDA ^a					22%
% IDNA ^b					30%
% MCV>94 ^c					22%

^a iron deficiency anemia, with hemoglobin <123 g/L, plus at least 2 indicators of iron deficiency (MCV, ferritin, transferrin saturation, or protoporphyrin) beyond cutoff

^b iron deficiency without anemia, with hemoglobin ≥123 g/L, but at least 2 indicators of iron deficiency (MCV, ferritin, transferrin saturation or protoporphyrin) beyond cutoff

^c indicator of macrocytosis, therefore potential folate and/or vitamin B12 deficiency

Table B.10: Subject characteristics, mood; mean (SD), median, 25th and 75th percentile, or %; n=76.

Variable	Mean (SD)	Median	25th p.	75th p.	% of whole sample
Beck Depression Inventory	23.1 (14.2)	21.0	14.0	29.5	
Fatigue	3.7 (1.5)	3,3	2,4	5.1	
CESD-20 ¹	19.7 (10.7)	18	12.5	25	
% Beck Depression Inventory moderate score 20–28					28%
% Beck Depression Inventory severe ≥ 29					26%
% CESD-20 ^a > 16					58%

^a Center of Epidemiologic Studies – Depression scale

Table B.11: Logistic regression model. Odds ratio (OR) of severe depression (BDI \geq 29). N=76; fixed factor models. c = area under the ROC curve.

Variable	Model 1	Model 2	Model3
Intercept	0.002 (0.00, 0.03)****	0.002 (0.00, 0.03)****	0.003 (0.00, 0.04)****
Centered hemoglobin g/L	0.93 (0.88, 0.98)**		
IDeA (iron depletion anemia) vs. not ^a		3.30 (0.65, 16.71)	
IDA vs. not ^b			5.97 (1.02, 34.87)**
Fatigue	2.85 (1.65, 4.90)***	2.57 (1.59, 4.11)***	2.42 (1.51, 3.88)***
Factor 2 (crowding, SES)	0.85 (0.39, 1.85)	0.73, (0.35, 1.52)	0.75 (0.36, 1.58)
MCV>94	6.73 (1.16, 39.01)**	3.56 (0.75, 16.96)	3.76 (0.83, 17.06)*
N	76	76	76
c	0.890	0.854	0.879
-2LL	54.0	61.55	59.5

^a defined as hemoglobin<12.3 g/L, and MCV and/or protoporphyrin beyond cutoff

^b defined as hemoglobin<12.3 g/L, and 2 of 4 iron deficiency indicators (MCV, protoporphyrin, transferrin saturation, Ferritin) beyond cutoff

*p-value \geq 0.05 and <0.1; **p<0.05; ***p<0.001; ****p<0.0001

Appendix C: CORTISOL, IRON STATUS, AND SEVERE DEPRESSIVE MOOD IN FEMALE MEXICAN FACTORY WORKERS

1.1 Tables and Figures

Table C.1: Time in hours of cortisol sampling time by sampling day (1 and 2), and sample per day (1 to 4). Time was digitalized for easier interval calculation (e.g., 30 min = 0.5). Actual sampling time (n=36), and actual and programmed^a sampling time (n=48).

Day	Sample	Mean (SD) digitalized actual time	Mean (SD) digitalized actual and programmed time
1	1	0.49 (0.20)	0.49 (0.19)
	2	3.05 (0.24)	3.05 (0.22)
	3	7.05 (0.19)	7.05 (0.16)
	4	10.05 (0.18)	10.05 (0.17)
2	1	0.49 (0.099)	0.40 (0.09)
	2	2.99 (0.28)	2.98 (0.23)
	3	7.00 (0.29)	7.01 (0.24)
	4	9.99 (0.22)	9.99 (0.18)

^a Programmed time refers to cases that had times of actual sampling missing. The average time of available actual time values for that particular day and sampling time was inserted instead.

Table C.2: Comparison between all participants in screening stage, and study participants; mean (SD), or %; t-test or Chi-square test.

Variable	Screening	Sample	p-value
N	237	48	
Age (years)	25.5 (8.6)	28.9 (8.7)	0.01
Height (m)	1.52 (0.57)	1.51 (0.53)	0.15
Weight (kg)	59.5 (11.8)	59.7 (10.1)	0.4
Marital status %			0.02
Married or living with partner	26	28	
Single	64	44	
Other	10	19	

Table C.3: Subject characteristics comparison between sample of 100 and 48 cases; displayed are the subsample of 48 cases and the remaining 52 cases from stage 2 that were excluded; means (SD) or percentages; t-test, Chi-square test or Fisher's-Exact test.

Variable	Sample of n=48	Excluded n=52	p-value
Hemoglobin (g/L)	131 (18)	132 (14)	0.7
MCV fL	88.4 (9.8)	89.0 (7.2)	0.7
Protoporphyrin µg/dL	87.6 (82.4)	71.0 (49.0)	0.2
Age (yrs)	29 (9)	25 (7)	0.02
BMI kg/m ²	26.1 (4.2)	25.2 (4.1)	0.2
Fatigue	4.1 (1.6)	3.5 (1.4)	0.06
Factor 2 (crowding, SES)	-0.1 (1.0)	0.1 (1.0)	0.3
% Iron depletion with anemia ^a	25	17	0.3
% Iron depletion, but no anemia ^b	12.5	17	0.6
% MCV > 94 fL	23	23	0.7
% BMI > 25kg/m ²	58	44	0.16
% BMI > 30kg/m ²	17	11	0.4
% Severe depression ^c	31	21	0.2
5 Moderate depression ^d	25	25	0.9
% CESD > 16	71	48	0.02
% Household income < 4377 pesos ^e	92	92	0.9
% Dirt floor	12.5	7.7	0.4
% Marital status			0.05
Married	37.5	19	
Single	44	67	
Disrupted ^f	19	13	

^a MCV and/or protoporphyrin beyond cutoff, plus hemoglobin < 123g/L

^b MCV and/or protoporphyrin beyond cutoff, but hemoglobin ≥ 123g/L

^c BDI ≥ 29 points

^d BDI 20–28 points

^e equivalent of \$157/month in the summer of 2001; national poverty line level 1 for Mexico in 2000

^f separated, single mother, divorced, or widowed

Table C.4: Cortisol values (nmol/L) for samples 1–4 for both days; mean (SD), median, minimum, and maximum.

Sample	Mean (SD)	25th percentile	50th percentile	75th percentile	Minimum	Maximum
1	5.2 (5.5)	2	3.2	5.7	0.5	32
2	2.3 (2.6)	0.87	1.9	2.5	Undet. ^a	14.0
3	1.9 (1.8)	0.6	1.6	2.4	Undet.	12.0
4	2.4 (3.1)	0.7	1.6	2.6	Undet.	17.9

^a undetectable level

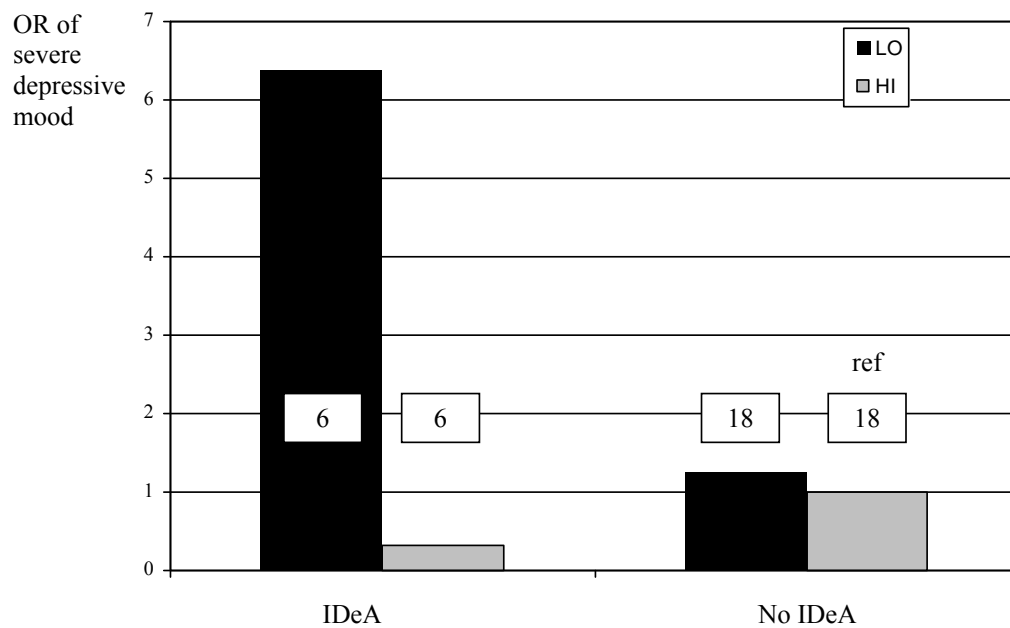


Figure C.1: Odds ratio (OR) of severe depressive mood; modifying effect of cortisol below (LO) or above (HI) median. Statistical model includes iron depletion anemia (IDeA), cortisol, fatigue, and factor 2 (crowding, SES). Cell size is textbox. P-interaction = 0.08.

Table C.5: Logistic regression models with outcome risk of severe depressive mood (≥ 29); fixed factor (Model 1) and mixed model with random factor factory (Model 2); log OR (log SE); n=48. c = area under the ROC curve.

Variables	Model 1	Model 2
Intercept	-3.511 (1.487)	-3.964 (1.651)
Centered hemoglobin g/L	-0.066 (0.033); p=0.04	-0.067 (0.034); p=0.05
Fatigue	0.540 (0.278); p=0.05	0.676 (0.309); p=0.03
Factor 2 (crowding, SES)	-1.182 (0.500); p=0.018	-1.400 (0.535); p=0.01
AUC3E cortisol nmol/L	-0.160 (0.781); P=0.8	-0.035 (0.807); p=0.9
Interaction (hemoglobin*cortisol)	0.082 (0.048); p=0.08	0.08 (0.049); p=0.1
N	48	48
c	0.806	n/a
-2LL	45.2	243.2 ^a
Random factor factory: Cov parameter estimate (std error)	N/a	0.793 (1.44)

^a Pseudo-log Likelihood for Proc glimmix

Table C.6: Logistic regression models, excluding cortisol variable, for comparison with effect sizes of large sample (n=100) in Table 3.4; outcome BDI severe depressive mood; OR (CI); n=48. c = area under the ROC curve.

Variables	Fixed factor models			Mixed model
	Model 1	Model 2	Model 3	Model 4
Intercept	0.03 (0.002, 0.45)	0.03 (0.002, 0.46)	0.03 (0.002, 0.45)	0.02 (0.001, 0.45)
Centered hemoglobin g/L	0.97 (0.93, 1.01) ^a	0.97 (0.94, 1.01) ^c		0.97 (0.93, 1.01) ^d
Iron depletion with anemia (IDeA)			1.42 (0.31, 6.52)	
Fatigue	1.78 (1.03, 3.08)	1.74 (1.02, 2.96)	1.73 (1.04, 2.89)	1.95 (1.09, 3.49)
Factor 2 (crowding, SES)	0.42 (0.19, 0.92)	0.42 (0.19, 0.93)	0.43 (0.20, 0.92)	0.34 (1.14, 0.81)
MCV>94 fL	1.55 (0.25, 9.62) ^b			
N	48	48	48	48
c	0.785	0.787	0.748	n/a
-2LL	48.6	48.9	50.6	233.4 ^e
Random factor factory: Cov parameter estimate (std error)	n/a	n/a	n/a	0.800 (1.32)

^a p=0.15

^b p=0.6

^c p=0.17

^d P=0.18

^e Pseudo-log Likelihood for Proc glimmix

Table C.7: ln OR of severe depressive mood ($BDI \geq 29$); comparison between interaction model with, and without fatigue and/or factor 2 (crowding, SES). ln OR (ln SE), p-value; independent variables in full model were centered hemoglobin, fatigue, factor 2 (crowding, SES), AUC3, and interaction; $MCV > 94$ not included due to $p\text{-value} > 0.3$; $n=48$. c = area under the ROC curve.

Variables	Model 1	Model 2	Model 3	Model 4
Intercept	-3.511 (1.487) p=0.01	-1.007 (0.559) p=0.07	-1.982 (1.103) p=0.07	-0.770 (0.489) p=0.1
Centered hemoglobin g/L	-0.066 (0.033) p=0.04	-0.062 (0.030) p=0.03	-0.047 (0.028) p=0.09	-0.050 (0.027) p=0.06
Fatigue	0.540 (0.278) p=0.05		0.286 (0.228) p=0.2	
Factor 2 (crowding, SES)	-1.182 (0.500) p=0.01	-0.847 (0.426) p=0.04		
AUC3E cortisol nmol/L	-0.1717 (0.779) p=0.8	-0.319 (0.734) p=0.6	-0.288 (0.698) p=0.6	-0.317 (0.685) p=0.6
Interaction (hemoglobin*cortisol)	0.082 (0.048) p=0.08	0.079 (0.044) p=0.07	0.039 (0.040) p=0.3	0.047 (0.039) p=0.2
c	0.806	0.765	0.690	0.612
-2LL	45.250	49.6	53.0	54.7

Table C.8: ln OR of depressive mood. Comparing none to mild depressive mood (<20) with moderate and severe depressive mood (≥ 20) (Model 1); none and mild depressive mood (<20) with severe depressive mood (≥ 29) (Model 2); and none to mild depressive mood (<20) with moderate depressive mood (20–28) (Model 3); all values ln OR (ln SE); n=48. c = area under the ROC curve.

Variable	Outcome moderate and severe depressive mood	Outcome severe depressive mood	Outcome moderate depressive mood
	Model 1	Model 2	Model 3
Intercept	-2.161 (1.119)**	-3.483 (1.531)***	-7.195 (3.602)***
Centered hemoglobin g/L	-0.021 (0.026)	-0.051 (0.033)*	0.201 (0.133)*
Fatigue	0.488 (0.238)***	0.633 (0.306)***	1.308 (0.699)**
Factor 2 (crowding, SES)	-0.312 (0.341)	-0.945 (0.485)**	1.152 (0.834)(
Cortisol AUC3E nmol/L	0.704 (0.653)	0.063 (0.832)	2.107 (1.119)**
Interaction (hemoglobin*cortisol)	0.038 (0.038)	0.068 (0.047)*	-0.049 (0.079)
c	0.698	0.808	0.857
-2LL	58.3	37.9	33.6
N	48	36	33

*p<0.2; **p<0.1; ***p<0.05

1.2 Methods and Results of All Cortisol Measures

1.2.1 Morning cortisol

The women collected morning cortisol at home, 30 minutes after waking. They also recorded time of waking and actual sampling time. When cortisol was sampled at exactly 30 minutes after waking, the true cortisol value was used. If time of sampling deviated, cortisol concentrations for the 30-minute morning waking were predicted individually per case per day from the existing values for the day (50% of the cases). When day 1 and day 2 had values for morning cortisol, the mean between those two predicted values was taken (92% of the cases). Otherwise the one available day was used. The means, standard deviations, and coefficients of variation (for log-transformed values) for partially predicted and for the unpredicted morning cortisol are shown in Appendix Table C.9. Mean and median cortisol values of the unpredicted and partially predicted morning cortisol are comparable. Morning cortisol had 49 cases (1 case more than the AUC estimates), since one participant had a morning cortisol but was not available for samples during the workday.

Morning cortisol variables were then dichotomized (below the median, or equal to or above the median) and entered as a modifier of the effect of hemoglobin into the logistic regression model (Appendix Table C.10). The interaction between morning cortisol and hemoglobin was not significant. This might be due to the high variability of morning cortisol (compare coefficient of variation in Appendix Table C.9). Higher variability of cortisol might be due to individual morning stressors and sleep quality [287, 289], time of awakening [288, 290], or time of [290] start of the workday. On the other hand, one study found no effect of time of awakening, sleep duration, and use of an alarm clock on the cortisol awakening response [259].

1.2.2 Area under the curve (AUC)

AUC was assessed twice, for a concentration curve that used the first 3 saliva samples of a day, and one that used all 4 samples. AUC was calculated with the pharmacological program *pkexamine* of the statistical Stata software version 9.0 [265]. When 2 days of sampling were available (83% of the sample), the mean between the two curves was used. Otherwise the AUC of the available day was taken.

Descriptive statistics are presented in Appendix Table C.9. While mean AUC for 4 samples had higher values, CVs were lower. Means, standard deviations, and coefficients of variation between the smaller group with both cortisol values and sampling times (n=36) were comparable to the larger sample, where some of the sampling times had to be estimated (n=48). The effect of AUC with 3 or 4 samples in the interaction with hemoglobin was equivalent (Appendix Table C.11). When plotting the interaction with AUC with 3 samples (AUC3) without inserting missing times of sampling (Appendix Figure C.2) or with AUC with 3 samples with some of the times being estimated (AUC3E; see Figure 4.1), the interaction is comparable. While the lower cortisol group has a significant increase in risk of severe depressive mood with decreasing hemoglobin, no significant effect of hemoglobin on risk of severe depressive mood can be found with the high-cortisol group.

1.2.3 Individual slopes

Another method of distinguishing individuals with low and high cortisol is by estimating the slope of their diurnal rhythm over the day [99, 102, 276, 278]. Low-cortisol subjects were assumed to have lower morning cortisol values and therefore flatter slopes [276]. Individual slopes of cortisol values over time at three sampling time points per day (30 minutes, 3 hours, and 7 hours after waking) were estimated with mixed regression model methods (SAS, version 9.1, proc mixed; [176]). Cortisol was log-transformed (natural logarithm) to adjust for skewness of the outcome and

non-linearity of the diurnal curve. Time of sampling was reported as estimated average time if reported time was not available; otherwise the actual reported time of sampling was used (total sample size was 48 observations). The method resulted in a slope and intercept estimate per subject per day; the mean of the slopes for 2 days (if available) was calculated; otherwise the slope of 1 day was used. The overall effect (slope) of time of sampling on log cortisol was -0.124 nmol/L ($p < 0.0001$); median of the slopes was -0.0085 , minimum -0.114 , and maximum 0.135 (not shown). Slope was dichotomized as smaller than the median, and greater than or equal to the median. This dichotomous variable was inserted as a modifier of hemoglobin into the logistic regression model with severe depressive mood as an outcome. Covariates were fatigue and factor 2 (crowding, SES). The interaction was not significant ($p = 0.49$; not shown). A reason for the negative result might be that cortisol values over the day are too variable in this sample to obtain an accurate estimate of the diurnal cortisol curve per subject; therefore, cases might have been misclassified. Appendix Table C.10 shows the cross-tabulation of true/false positive and negative values between the dichotomous variable of cortisol slopes and AUC3E cortisol. Correct classification of cases by the dichotomized cortisol slope variable into the low (sensitivity) and high (specificity) cortisol categories is only 54% when using AUC3E cortisol as the gold standard. Another reason could be that that evening cortisol levels – which are usually the lowest values in the curve – needed to be collected in order to get the maximum possible decline of the curve [276]. The result would be a greater variability of slopes, and a potentially better distinction between individuals with low and high cortisol.

Table C.9: Comparison of means, SD, coefficients of variation (CV), and medians of cortisol measures.

Variable	N	Mean	SD	CV	Median
ln (morning cortisol 30 min after wake up) ^a nmol/L	49	1.35	0.70	52	1.25
ln (morning cortisol approx. 30 min after wake up) ^b nmol/L	49	1.26	0.72	57	1.21
ln (AUC, 3 samples) ^c nmol/L	36	2.58	.7	27	2.69
ln (AUC, 3 samples, predicted time) ^d nmol/L	48	2.47	.76	31	2.64
ln (AUC, 4 samples) ^e nmol/L	36	3.0	.7	23	3.13
ln (AUC, 4 samples, predicted time) ^f nmol/L	48	2.47	.73	25	2.97
Morning cortisol 30 min after wake up ^g nmol/L	49	5.08	4.6		3.50
Morning cortisol approx. 30 min after wake up ^h nmol/L	49	4.72	4.4		3.34
AUC, 3 samples ⁱ nmol/L	36	17.36	16.4		14.7
AUC, 3 samples, predicted time ^l nmol/L	48	15.97	15.4		14.13
AUC, 4 samples ^j nmol/L	36	25.62	20.1		22.97
AUC, 4 samples, predicted time ^k	48	22.64	17.6		19.5

^a log transformed morning cortisol, collected 30 minutes after wake up, actual time of sampling for all cases

^b log transformed morning cortisol, collected 30 minutes after wake up, actual time of sampling for cases with exactly 30 minutes after wake up collection; predicted cortisol value for cases with earlier or later time of collection

^c log transformed area under the curve (AUC) for 3 samplings per day; only values with both time of sampling and cortisol

^d log transformed AUC for 3 samplings per day; if time of sampling was missing, but cortisol assessed, mean time of sampling was inserted

^e log transformed AUC for 4 samples per day; only values with both time of sampling and cortisol

^f log transformed AUC for 4 samples per day; if time of sampling was missing, but cortisol assessed, mean time of sampling was inserted

^g morning cortisol, collected 30 minutes after wake up, actual time of sampling for all cases

^g morning cortisol, collected 30 minutes after wake up, actual time of sampling for cases with exactly 30 minutes; if time of sampling was missing, but cortisol assessed, mean time of sampling was inserted

^h AUC for 3 samplings per day; only values with both time of sampling and cortisol

ⁱ AUC for 3 samplings per day; if time of sampling was missing, but cortisol assessed, mean time of sampling was inserted

^j AUC for 4 samples per day

^k AUC for 4 samples per day; if time of sampling was missing, but cortisol assessed, mean time of sampling was inserted

Table C.10: Association of low or high AUC3E cortisol with participant characteristics; means (SD) or %; t-test for continuous variables, Wilcoxon 2-sample test for GHQ and GHQ modules; Chi-square or Fisher's-Exact test for categorical variables.

	AUC3E < median, n=24	AUC3E ≥ median, n=24	p-value
Physical health			
Age	30 (8)	27 (9)	0.2
BMI kg/m ²	26 (4)	25 (4)	0.2
FSS	4.1 (1.6)	4.0 (1.6)	0.7
CRP mg/L	1 (1)	1 (1)	0.9
White blood cells 10 ³ μL	6 (2)	8 (3)	0.1
% Oral contraceptive use	8	8	1
% BMI > 25 kg/m ²	67	50	0.2
% BMI > 30 kg/m ²	21	12	0.7
% respiratory illness	21	37	0.2
GHQ			
GHQ	28 (13)	25 (13)	0.3
GHQ anxiety	8 (5)	7 (4)	0.2
GHQ depression	4 (5)	3.5 (4)	0.8
GHQ social functioning	6 (2)	8 (3)	0.1
GHQ somatic	9 (5)	8 (4)	0.2
% more headache ^a	65	21	0.002
% more pressure in the head ^a	52	21	0.02

^a GHQ somatic

^b GHQ anxiety

^c GHQ social functioning

^d GHQ depression

^e widowed, single mother, divorced, separated

Table C.10 (Continued)

	AUC3E < median, n=24	AUC3E ≥ median, n=24	p-value
GHQ (continued)			
% more hot/cold spells ^a	52	29	0.1
% more nervous ^b	48	17	0.02
% much less satisfied ^c	22	50	0.04
% keeping busier ^c	35	21	0.2
% life is hopeless ^d	17	4	0.1
SES and household composition			
Crowding	-0.7 (2)	-1 (2)	0.6
Factor 1 (wealth, SES)	0.1 (1)	0.04 (0.8)	0.8
Factor 2 (crowding, SES)	-0.1(1)	-0.1 (0.9)	0.9
Factor 3 (life stage)	-0.2 (1)	-0.2 (1)	0.9
% kids under 5 years	21	25	0.7
% kids under 10 years	33	33	1
% kids under 18 years	46	50	0.7
% father helps with child care	33	29	1
% living alone	26	39	0.3
%disrupted marital status ^e	29	8	0.1

Table C.11: Log OR of severe depressive mood (BDI \geq 29); interaction between 6 cortisol measures and hemoglobin; log OR (log SE); MCV>94 was included when $p < 0.3$. c = area under the ROC curve.

Variable	Model 1	Model 2	Model 3	Model 4	Model 5	Model 6
Intercept	-3.296 (1.344)***	-3.821 (1.460)***	-3.521 (1.481)***	-5.755 (2.543)***	-3.531 (1.529)***	-5.392 (2.587)***
Centered hemoglobin g/L	-0.015 (0.028)	-0.038 (0.027)*	-0.066 (0.033)***	-0.108 (0.049)***	-0.062 (0.029)***	-0.107 (0.049)***
Fatigue	0.552 (0.264)***	0.616 (0.281)***	0.540 (0.278)**	0.813 (0.458)**	0.648 (0.302)***	0.780 (0.456)**
Factor 2 (crowding, SES)	-0.661 (0.394)**	-0.722 (0.382)**	-1.182 (0.500)***	-1.586 (0.671)***	-1.202 (0.464)***	-1.558 (0.668)***
MCV>94 fl				1.528 (1.229)		1.391 (1.287)
MOCP nmol/L	0.090 (0.724)					
MOC nmol/L		0.413 (0.724)				
AUC3E nmol/L			-0.177 (0.779)			
AUC3 nmol/L				0.603 (0.979)		
AUC4E nmol/L					-0.556 (0.759)	
AUC4 nmol/L						0.277 (1.044)
Interaction (hemoglobin* cortisol)	-0.018 (0.039)	0.028 (0.042)	0.082 (0.048)**	0.125 (0.06)***	0.081 (0.046)**	0.126 (0.062)***
c	0.771	0.755	0.806	0.685	0.834	0.864
-2LL	51.8	51.3	45.2	29.9	44.3	29.8
n	49	49	48	36	48	36

Note: MOCP: morning cortisol with some predicted cortisol values for 30 minutes after wake-up; MOC: morning cortisol; AUC3P: AUC, 3 samples, predicted times if necessary; AUC3: AUC, 3 samples; AUC4P: AUC, 4 samples, predicted times if necessary; AUC4, 4 samples
* p-value<0.2; ** p-value<0.1; *** p-value<0.05

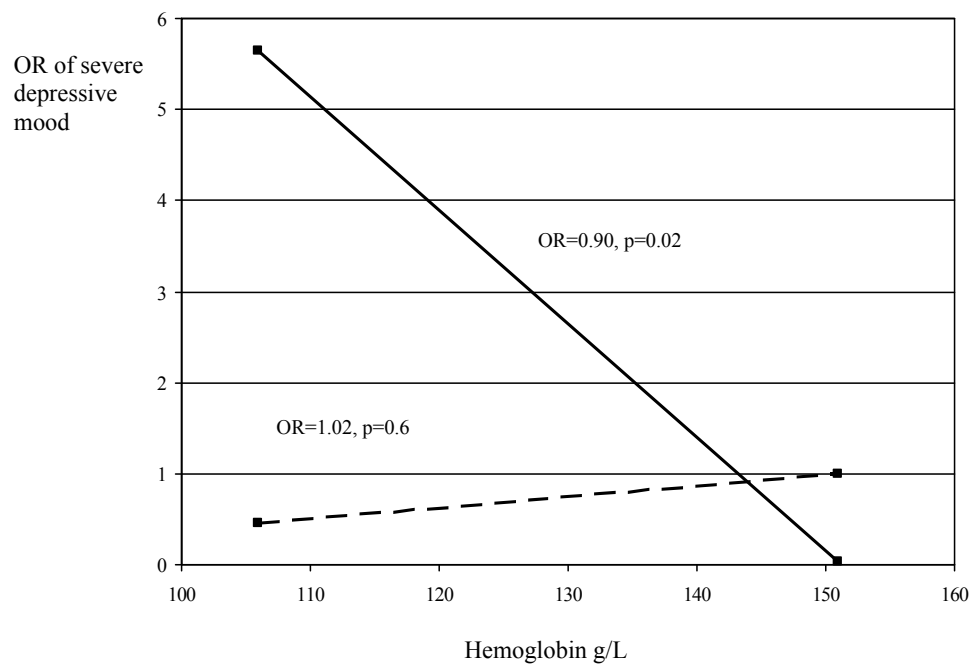


Figure C.2: Odds ratio (OR) of severe depressive mood; modifying effect of AUC3 cortisol below (continuous line) or above median (dashed line) of AUC3E cortisol. Statistical model includes centered hemoglobin, cortisol, fatigue, MCV>94 fL, and factor 2 (crowding, SES). p-interaction = 0.02. n=36; sample includes only recorded times of sampling for cortisol; 10th–90th percentile of hemoglobin.

Table C.12: Assessment of correct classification of cases into low and high cortisol by the dichotomized cortisol slope over time of day variable. Gold standard is of the dichotomized area under the curve (AUC3E) cortisol variable. Listed are number of cases that are true and false positive, and true and false negative.

Slope of cortisol concentrations over time	Area under the curve cortisol (AUC3E cortisol)		total
	<median	≥median	
<median	True positive = 13	False positive = 11	24
≥median	False negative = 11	True negative =13	24
total	24	24	48

Note: Sensitivity = true positive/(true positive + false negative) = 54%; Specificity = true negative/(true negative + false positive) = 54%

Appendix D: DIAGNOSIS OF MOOD DISORDERS RELATED TO DEPRESSION

Based on Diagnostic and statistical manual of mental disorders. DSM-IV-TR [53].

Major depressive episode (total of 5 or more symptoms)

One or more depressive episodes for at least 2 weeks per episode

Symptoms:

- At least:
 - Depressed mood
 - or
 - Loss of interest and pleasure
- Plus 3–4 additional:
 - Significant weight loss or gain
 - Insomnia or hypersomnia
 - Agitation or retardation
 - Fatigue
 - Worthlessness or guilt
 - Concentration difficulties or indecisiveness
 - Thoughts of death, suicidal ideation, suicide attempt, or plan for committing suicide
- The symptoms cause clinically significant distress or impairment in social, occupational, or other important areas of functioning
- The symptoms are not account for by bereavement

Melancholic feature specifier:

- Either one of the following:
 - loss of pleasure or lack of reactivity to usually pleasurable stimuli
- Plus 3 or more of the following:
 - Depressed mood
 - Depression worse in the morning
 - Early morning awakening
 - Psychomotor retardation or agitation
 - Anorexia or weight loss
 - Guilt

Atypical feature specifier

- Mood reactivity
- Plus 2 or more of the following
 - Weight gain or increase in appetite
 - Hypersomnia
 - Leaden paralysis
 - Sensitivity to interpersonal rejection

Postpartum onset specifier:

- Onset within 4 weeks postpartum

Minor depressive episode (total of 2–4 symptoms)

Included in depressive disorder not otherwise specified²³

One or more depressive episodes for at least 2 weeks per episode

Symptoms:

- At least
 - Depressed mood
 - or
 - Loss of interest or pleasure
- Plus 1–3 additional:
 - Weight change
 - In- or hypersomnia
 - Agitation or retardation
 - Fatigue
 - Worthlessness or guilt
 - Concentration difficulties
 - Thoughts of death

Research criteria for minor depressive disorder

- At least 2 but not more than 5 of the following symptoms present during 2-week period; one of the symptoms has to be depressed mood or loss of interest or pleasure
- Plus 1–4 additional ones:
 - Depressed mood
 - Loss of interest or pleasure
 - Weight loss or weight gain
 - Insomnia or hypersomnia
 - Psychomotor agitation or retardation
 - Fatigue or loss of energy
 - Feelings of worthlessness, guilt
 - Diminished ability to concentrate, or indecisiveness
 - Recurring thought of death, suicidal ideation, suicide attempt, plan to commit suicide

Dysthymia (total of 3 or more symptoms)

At least 2 years of depressive symptoms

Plus symptoms:

- Poor appetite or overeating
- Insomnia or hypersomnia

²³ Disorders that are included in this category include premenstrual dysphoric disorder, minor depressive episode.

- Low energy or fatigue
- Low self-esteem
- Poor concentration or difficulty making decisions
- Feelings of hopelessness

Atypical feature specifier (total of 3 symptoms):

- Mood reactivity

plus

- increased appetite or weight gain
- hypersomnia
- leaden paralysis
- extreme sensitivity to interpersonal rejection

Alternative criteria B for dysthymic disorder

- presence, while depressed of 3 or more of the following
- low self-esteem or self-confidence, feelings of inadequacy
- pessimism, despair, hopelessness
- loss of interest or pleasure
- social withdrawal
- chronic fatigue or tiredness
- guilt, brooding
- irritability or excessive anger
- decreased activity, effectiveness, productivity
- difficulty in thinking, reflected by poor concentration, memory or indecisiveness

Appendix E: INSTRUMENTS

1. *Diagnostic Interview Schedule, Depression Module*

1. Are you in one of these spells of feeling low and disinterested and having some of these problems now?
 - Yes/no
2. When did you last spell like that end?
 - Within last 2 weeks
 - Within last month
 - Within last 6 months
 - within last year
 - more than a year ago

(if more than a year ago)

3. How old were you then
 - Age
4. The lifetime DSM-III diagnosis of major depressive episode
 - No diagnosis
 - Non-severe diagnosis
 - Severe diagnosis
 - Severe bereavement
 - Non-severe bereavement

} Coded as depressed
5. Age last depressive symptoms occurred if ever met criteria for any major depressive episode
 - 10–38 years old
 - last episode within last 2 weeks
 - last episode within 2 weeks to 1 month ago
 - last episode within 1 to 6 months ago
 - last episode 7 months to 1 year ago
6. Total number of positive depression symptoms groups ever. The dysphoria symptom is also included.
 - 0–9
7. Age last depressive symptoms occurred if ever met criteria for any single episode of major depressive episode
 - 10–38 years old
 - last episode within last 2 weeks
 - last episode within 2 weeks to 1 month ago
 - last episode within 1 to 6 months ago
 - last episode 7 months to 1 year ago
8. Age last depressive symptoms occurred if ever met criteria for any recurrent episode of major depressive episode

- 10–38 years old
 - last episode within last 2 weeks
 - last episode within 2 weeks to 1 month ago
 - last episode within 1 to 6 months ago
 - last episode 7 months to 1 yr ago
- Variable coding:
 - Current major depression: suffering from current major depression requires answering yes to question 1, or within the last 2 weeks in question 2, plus a diagnosis as depressed in question 4.
 - Past major depression: requires answering within the last months to more than a year in question 2, plus a diagnosis as depressed in question 4.

2. Lifetime Fatigue Score, Diagnostic Interview Schedule

From the sleep summary

- Have you ever had a period of two weeks or longer when you were sleeping too much?
 - No [0]
 - Yes [1]

From the tired out summary

- Has there ever been a period lasting two weeks or more when you felt tired out all the time?
 - No [0]
 - Yes [1]

From the slow/restless summary

- Has there ever been a period of two weeks or more when you talked or moved more slowly than is normal for you?
 - No [0]
 - Yes [1]

Total fatigue score = sum

3. Center of Epidemiology Studies–Depression scale (CESD)

During the last week:

- I was bothered by things that usually don't bother me.
 - Rarely or some of the time (less than 1 day) [0]
 - Some or a little of the time (1–2 days) [1]
 - Occasionally or a moderate amount of the time (3–4 days) [2]
 - Most or all of the time (5–7 days) [3]

- I did not feel like eating: my appetite was poor.
 - Rarely or some of the time (less than 1 day) [0]
 - Some or a little of the time (1–2 days) [1]
 - Occasionally or a moderate amount of the time (3–4 days) [2]
 - Most or all of the time (5–7 days) [3]

- I felt that I could not shake off the blues even with the help from my family or friends.
 - Rarely or some of the time (less than 1 day) [0]
 - Some or a little of the time (1–2 days) [1]
 - Occasionally or a moderate amount of the time (3–4 days) [2]
 - Most or all of the time (5–7 days) [3]

- I felt I was just as good as other people.
 - Rarely or some of the time (less than 1 day) [3]
 - Some or a little of the time (1–2 days) [2]
 - Occasionally or a moderate amount of the time (3–4 days) [1]
 - Most or all of the time (5–7 days) [0]

- I had trouble keeping my mind on what I was doing.
 - Rarely or some of the time (less than 1 day) [0]
 - Some or a little of the time (1–2 days) [1]
 - Occasionally or a moderate amount of the time (3–4 days) [2]
 - Most or all of the time (5–7 days) [3]

- I felt depressed.
 - Rarely or some of the time (less than 1 day) [0]
 - Some or a little of the time (1–2 days) [1]
 - Occasionally or a moderate amount of the time (3–4 days) [2]
 - Most or all of the time (5–7 days) [3]

- I felt that everything I did was an effort.
 - Rarely or some of the time (less than 1 day) [0]
 - Some or a little of the time (1–2 days) [1]
 - Occasionally or a moderate amount of the time (3–4 days) [2]
 - Most or all of the time (5–7 days) [3]

- I felt hopeless about the future.
 - Rarely or some of the time (less than 1 day) [0]
 - Some or a little of the time (1–2 days) [1]

- Occasionally or a moderate amount of the time (3–4 days) [2]
 - Most or all of the time (5–7 days) [3]
- I felt my life had been a failure.
 - Rarely or some of the time (less than 1 day) [0]
 - Some or a little of the time (1–2 days) [1]
 - Occasionally or a moderate amount of the time (3–4 days) [2]
 - Most or all of the time (5–7 days) [3]
- My sleep was restless.
 - Rarely or some of the time (less than 1 day) [0]
 - Some or a little of the time (1–2 days) [1]
 - Occasionally or a moderate amount of the time (3–4 days) [2]
 - Most or all of the time (5–7 days) [3]
- I was happy.
 - Rarely or some of the time (less than 1 day) [3]
 - Some or a little of the time (1–2 days) [2]
 - Occasionally or a moderate amount of the time (3–4 days) [1]
 - Most or all of the time (5–7 days) [0]
- I talked less than usual.
 - Rarely or some of the time (less than 1 day) [0]
 - Some or a little of the time (1–2 days) [1]
 - Occasionally or a moderate amount of the time (3–4 days) [2]
 - Most or all of the time (5–7 days) [3]
- I felt lonely.
 - Rarely or some of the time (less than 1 day) [0]
 - Some or a little of the time (1–2 days) [1]
 - Occasionally or a moderate amount of the time (3–4 days) [2]
 - Most or all of the time (5–7 days) [3]
- People were unfriendly.
 - Rarely or some of the time (less than 1 day) [0]
 - Some or a little of the time (1–2 days) [1]
 - Occasionally or a moderate amount of the time (3–4 days) [2]
 - Most or all of the time (5–7 days) [3]
- I enjoyed life.
 - Rarely or some of the time (less than 1 day) [3]
 - Some or a little of the time (1–2 days) [2]
 - Occasionally or a moderate amount of the time (3–4 days) [1]
 - Most or all of the time (5–7 days) [0]
- I had crying spells.
 - Rarely or some of the time (less than 1 day) [0]
 - Some or a little of the time (1–2 days) [1]
 - Occasionally or a moderate amount of the time (3–4 days) [2]

- Most or all of the time (5–7 days) [3]
- I felt sad.
 - Rarely or some of the time (less than 1 day) [0]
 - Some or a little of the time (1–2 days) [1]
 - Occasionally or a moderate amount of the time (3–4 days) [2]
 - Most or all of the time (5–7 days) [3]
- I felt that people disliked me.
 - Rarely or some of the time (less than 1 day) [0]
 - Some or a little of the time (1–2 days) [1]
 - Occasionally or a moderate amount of the time (3–4 days) [2]
 - Most or all of the time (5–7 days) [3]
- I could not get going
 - Rarely or some of the time (less than 1 day) [0]
 - Some or a little of the time (1–2 days) [1]
 - Occasionally or a moderate amount of the time (3–4 days) [2]
 - Most or all of the time (5–7 days) [3]

CESD Total Score = sum

4. Beck Depression Inventory (BDI)

English

Oral administration:

This is a questionnaire. On the questionnaire are groups of statements. I will read a group of statements; then I would like you to pick out the one statement in each group that best describes the way you have been feeling during the past 2 weeks, including today. Now which of the statements best describes the way you have been feeling during the past 1 week, including today?

1. Sadness

I do not feel sad (0)

I feel blue or sad a large part of the time (1)

I feel sad the whole time (2)

I feel so sad or unhappy that I can't stand it (3)

2. Pessimism

I am not pessimistic about the future (0)

I feel more discouraged about my future than is normal (1)

I feel that things don't turn out well for me (2)

I feel that the future is hopeless and that things cannot improve (3)

3. Past failure

I do not feel like a failure (0)

I feel I have failed more than an average person (1)

As I look back at my life I can see a lot of failure (2)

I feel I am a complete failure as a person (3)

4. Loss of pleasure

I obtain as much pleasure as before in the things that I enjoy (0)

I don't enjoy the things the way that I used to (1)

I don't get satisfaction out of anything any more (2)

I am dissatisfied with anything (3)

5. Guilty feelings

I don't feel I am being punished (0)

I feel guilty for many things that I have done or should to have done and did not I do (1)

I feel bad or unworthy practically all the time (2)

I feel as though I am very bad or worthless (3)

6. Punishment feelings

I don't feel as I am punished (0)

I feel like am being punished or will be punished (1)

I feel I deserve to be punished (2)
I feel that I am being punished for life (3)

7. Self dislike

I do not feel disappointed in myself (0)
I am disappointed with myself (1)
I am disgusted with myself (2)
I hate myself (3)

8. Self criticalness

I don't feel any worse than anybody else (0)
I am very critical of myself for my mistakes (1)
I blame myself for everything that goes wrong (2)
I have many bad faults (3)

9. Suicidal thoughts or wishes

I do not have any thoughts of harming myself (0)
I have thoughts of harming myself but I would not carry them out (1)
I would like to kill myself (2)
I would kill myself if I could (3)

10. Crying

I don't cry anymore than usual (0)
I cry more now than I used to (1)
I cry all the time now (2)
I feel like I would like to cry, but I can't (3)

11. Agitation

I am no more restless or tense than normal (0)
I get restless or tense more easily than I used to (1)
I am that restless or agitated that it is difficult for me to remain quit (2)
I am that restless or agitated that I have to keep moving or doing something constantly (3)

12. Loss of interest

I have not lost interest in other people or activities (0)
I am less interested in other people or activities than I used to be (1)
I have lost most of my interest in other people or activities (2)
I have difficulties to be interested in anything (3)

13. Indecisiveness

I make decisions about as well as ever (0)
It is more difficult for me to make decisions than usual (1)
I have much greater difficulties making decisions than usual (2)
I can't make decisions at all anymore (3)

14. Worthlessness

I do not feel as if I am worthless (0)

I do not think of myself as worthy and useful as before (1)

I feel worthless in comparison to others (2)

I feel completely worthless (3)

15. Loss of energy

I have as much energy as before (0)

I have less energy than I used to (1)

I have not enough energy to do many things (2)

I have not enough energy left for anything (3)

16. Changes in sleeping patterns

I can sleep as well as usual (0)

I sleep a bit more than usual *or* I sleep a bit less than usual (1)

I sleep much more than usual *or* I sleep much less than usual (2)

I sleep all day *or* I wake up 1–2 hours earlier and cannot go back to sleep (3)

17. Irritability

I am no more irritable than usual (0)

I am more irritable than usual (1)

I am much more irritable than usual (2)

I am irritable most of the time (3)

18. Changes in appetite

I did not experience any change in appetite (0)

My appetite is a little less than it used to be *or* my appetite is a little bigger than it used to be (1)

My appetite is much worse now than it used to be *or* my appetite is much bigger now than it used to be (2)

I have no appetite at all anymore *or* I want to eat all the time (3)

19. Concentration difficulty

I can concentrate as well as always (0)

I cannot concentrate as well as I am used to (1)

It is difficult for me to focus for most of the time (2)

I feel like I cannot concentrate on anything (3)

20. Tiredness or fatigue

I do not get any more tired or fatigued than usual (0)

I get tired or fatigued more easily than I used to (1)

I get tired or fatigued by many things even before I start doing them (2)

I get tired or fatigued by most of the things even before I start doing them (3)

21. Loss of interest in sex

I have not noticed any recent change in my interest in sex (0)

I am less interested in sex than I used to be (1)

I am much less interested in sex now (2)

I have lost interest in sex completely (3)

BDI Total Score = sum

5. Fatigue Severity Scale (FSS)

Over the last 2 weeks:

1. My motivation is lower when I am fatigued.
2. Exercise brings on my fatigue.
3. I am easily fatigued.
4. Fatigue interferes with my physical functioning.
5. Fatigue causes frequent problems for me.
6. My fatigue prevents sustained physical functioning.
7. Fatigue interferes with carrying out certain duties and responsibilities.
8. Fatigue is among my three most disabling symptoms.
9. Fatigue interferes with my work, family, or social life.

FSS Total Score = sum/9

6. *General Health Questionnaire (GHQ)*

Over the past few weeks, have you:

- Been feeling perfectly well and in good health?
 - Better than usual (0)
 - Same as usual (1)
 - Worse than usual (2)
 - Much worse than usual (3)
- Been feeling in need of some medicine to pick you up?
 - Not at all (0)
 - No more than usual (1)
 - Somewhat more than usual (2)
 - Much more than usual (3)
- Been feeling run down and out of sorts?
 - Not at all (0)
 - No more than usual (1)
 - Somewhat more than usual (2)
 - Much more than usual (3)
- Felt that you are ill?
 - Not at all (0)
 - No more than usual (1)
 - Somewhat more than usual (2)
 - Much more than usual (3)
- Been getting pains in your head?
 - Not at all (0)
 - No more than usual (1)
 - Somewhat more than usual (2)
 - Much more than usual (3)
- Been getting a feeling of tightness or pressure in your head?
 - Not at all (0)
 - No more than usual (1)
 - Somewhat more than usual (2)
 - Much more than usual (3)
- Been having hot and cold spells?
 - Not at all (0)
 - No more than usual (1)
 - Somewhat more than usual (2)
 - Much more than usual (3)
- Lost much sleep over worry?
 - Not at all (0)
 - No more than usual (1)
 - Somewhat more than usual (2)
 - Much more than usual (3)
- Had difficulty staying asleep?
 - Not at all (0)
 - No more than usual (1)
 - Somewhat more than usual (2)
 - Much more than usual (3)

- Felt constantly under strain?
 - Not at all (0)
 - No more than usual (1)
 - Somewhat more than usual (2)
 - Much more than usual (3)
- Been getting edgy and bad tempered?
 - Not at all (0)
 - No more than usual (1)
 - Somewhat more than usual (2)
 - Much more than usual (3)
- Been getting scared or panicky for no good reason?
 - Not at all (0)
 - No more than usual (1)
 - Somewhat more than usual (2)
 - Much more than usual (3)
- Found everything getting on top of you?
 - Not at all (0)
 - No more than usual (1)
 - Somewhat more than usual (2)
 - Much more than usual (3)
- Been feeling nervous and uptight all the time?
 - Not at all (0)
 - No more than usual (1)
 - Somewhat more than usual (2)
 - Much more than usual (3)
- Been managing to keep yourself busy and occupied?
 - More so than usual (0)
 - Same as usual (1)
 - Somewhat less than usual (2)
 - Much less than usual (3)
- Been taking longer over the things you do?
 - Quicker than usual (0)
 - Same as usual (1)
 - Longer than usual (2)
 - Much longer than usual (3)
- Felt on the whole you were doing things well?
 - Better than usual (0)
 - About the same (1)
 - Less well than usual (2)
 - Much less well (3)
- Been satisfied with the way you've carried out your task?
 - More satisfied (0)
 - About the same as usual (1)
 - Less satisfied than usual (2)
 - Much less satisfied (3)
- Felt that you are playing a useful part in things?
 - More so than usual (0)
 - Same as usual (1)
 - Somewhat less than usual (2)

- Much less than usual (3)
- Felt capable of making decisions about things?
 - More so than usual (0)
 - Same as usual (1)
 - Somewhat less than usual (2)
 - Much less than usual (3)
- Been able to enjoy your normal day-to-day activities?
 - More so than usual (0)
 - Same as usual (1)
 - Somewhat less than usual (2)
 - Much less than usual (3)
- Been thinking of yourself as a worthless person?
 - Not at all (0)
 - No more than usual (1)
 - Somewhat more than usual (2)
 - Much more than usual (3)
- Felt that life is entirely hopeless?
 - Not at all (0)
 - No more than usual (1)
 - Somewhat more than usual (2)
 - Much more than usual (3)
- Felt that life isn't worth living?
 - Not at all (0)
 - No more than usual (1)
 - Somewhat more than usual (2)
 - Much more than usual (3)
- Thought of the possibility that you might do away with yourself?
 - Definitely not (0)
 - I don't think so (1)
 - Has crossed my mind (2)
 - Definitely have (3)
- Found at times you couldn't do anything because your nerves were too bad?
 - Not at all (0)
 - No more than usual (1)
 - Somewhat more than usual (2)
 - Much more than usual (3)
- Found yourself wishing you were dead and away from it all?
 - Not at all (0)
 - No more than usual (1)
 - Somewhat more than usual (2)
 - Much more than usual (3)
- Found that the idea of taking your own life kept coming into your mind?
 - Not at all (0)
 - No more than usual (1)
 - Somewhat more than usual (2)
 - Much more than usual (3)

GHQ Total Score = sum

7. Mexico 2001

Screening (Stage 1)

1. Age:
2. Age of you children younger than 18 years (from youngest to oldest)
 - 1.
 - 2.
 - 3.
 - 4.
 - 5.
 - 6.
3. Marital status: _____
4. Factory:
5. Type of activity in factory:
 1. Sewing
 2. Inspection
 3. labels
 4. sleeves
 5. making pockets
 6. ironing
 7. packaging
 8. Sales department
 9. quality control
 10. embroidering
 11. cutting the clothes
 12. cleaning the facility
 13. other (_____)
6. How long do you do this kind of activity? _____
7. Do you have another employment?
Yes: No: Type: _____
8. Do you use oral contraceptives?
Yes No
9. Date of last menstruation: _____
10. Cycle is regular?
Yes No

11. Are you currently:
Pregnant
Breast feeding
None of the above

12. Weight (kg): _____

13. Height (m): _____

2nd Stage:

Ms.(*name of participant*), thank you for participating in our study. My name is(*name of interviewer*). During this interview we will talk about how you have been feeling recently. In addition, I will ask you a few questions about your home. This information will help us to understand how your nutrition affects you. Before we start, I would like to remind you that we can stop this interview at any time, if you wish so. Please feel free to interrupt and ask me, if my questions are not clear. It is important to us that we understand your life as best as we can.

First I would like to ask you a few general questions in the beginning:

1. How old are you? _____
2. What is your birth date? _____

I would like to learn a bit more about your family. I am going to read you a few answer choices to my questions. Please indicate which option describes your situation best:

3. Do you have children?
Yes _____ No _____
(if answer is no please skip to question 7)

4. Are you
Married
Married but separated
Divorced
Not married, but father of child/children lives with you
Not married, father does not live in house

5. Does the father help you with the child care?

6. Do you live
By yourself

- With a friend
- With your parents
- With your parents-in-law
- With siblings
- Other person (_____)

(only ask if children were indicated) Now I would like to ask a few questions about your children and other persons who live in the house:

- 7. How many children under 18 live in the household? _____
- 8. How many rooms do you have? _____
- 9. How many persons sleep in your household? _____

Now I would like to ask you a few questions about your time use. This includes the time at work and at home. I will give you the answer choices, please indicate which one describes best your situation.

- 10. How many hours did you work in the factory during a typical day?
 - 1-4 hours _____
 - 5-6 hours _____
 - 7-8 hours _____
 - More than 8 hours _____

- 11. Did you have a second job in the previous month?
 - Yes No (if no, skip to q.14)

- If yes: 12. For you second job, do you work
- Outside of home
 - At home
 - At home and outside of your home

- 13. How many days a week do you work in your second job?
 - 1-3 days _____
 - 4-5 days _____
 - 6 days _____
 - 7 days _____

Before continuing, I would like to remind you that all the information you are giving us is completely confidential.

- 14. Out of which material is the floor of your household?
 - Soil
 - Concrete

Tiles, wood, or other
Don't know

15. Out of which material are most of the walls of your household?

Carton
Bamboo or palm tree
Mud or plaster
Wood
Sheets of asbestos or metal
Brick (clay)
Brick (e.g. concrete)
Other _____
Don't know

16. Out of which material is the roof of your home?

Carton
Bamboo or palm tree
Sheet of asbestos or metal
Tile
Concrete, wood
Other
Don't know

17. Do you have a separate room for cooking in your home?

Si No

18. Do you use that room for cooking and for sleeping?

Si No

19. How many rooms do you have in total in your house, without counting walkways, the bath and the kitchen?

20. What type of water do you have in your house?

Tap in the kitchen or bath
Tap outside of home, right next to it
Water form a public bath or hydrant
Water from a well or pump
Water from a spring, river or lake
Water from a pipe
Other.....

21. The people in your home use

A toilet

- A latrine
- An outhouse
- A hole in the ground
- Do not use a special place

22. What do you do with the waste? Do they go in the

- Ground
- River or lake
- Septic tank
- Public system
- There is no system for it

23. Does your home have a (*number*)

- Radio or recorder.....
- TV.....
- VCR.....
- Telephone or cell.....
- Refrigerator.....
- Washing machine.....
- Car, truck or motor cycle.....

24. Your monthly income is

- <4377²⁴ pesos
- 4377–8754
- >8754

25. The household income is

- <4377 pesos
- 4377–8754
- >8754

Health Check list

26. Smoking
 Yes No

If yes: 27. Number of cigarettes per day: _____

28. Drinks alcohol
 Yes No

If yes: 29. daily weekly monthly Number of glasses: _____

²⁴ Equivalent of \$147 in summer 2001

30. Takes medication:

Yes No

If yes: 31. Specify _____

32. Diarrhea (this or last week):

Yes No

33. Chronic diseases (*over the last 3 months*)

Hypertension

Yes No

Diabetes

Yes No

Rheumatisms

Yes No

Cardiovascular

Yes No

Cancer

Yes No

34. Respiratory disease:

Upper respiratory

Yes No

Bronchitis

Yes No

Pulmonary

Yes No

Asthma

Yes No

35. Other illness, accidents or surgery: _____

36. Amenorrhea:

Yes no

37. Date of last menstruation:

Yes no

38. Regular cycle:

Yes No

3rd stage (stress):

Initial information

Before we begin, I would like to ask you some questions, in order to whether there might be factors that affect the concentrations of hormones in you saliva.

1. Are you pregnant?

Yes No

2. During the last month, did you take oral contraceptives?

Yes No

Form for participant for record keeping of 1st saliva sample at home

3. Time of wake-up

Hour s Minutes

4. Time of saliva sample at home (30 minutes after wake-up)

Hour Minute

Reminder form for participant of sampling time

5. Time of 2nd sample

Hour Minute

6. Time of 3rd sample

Hour Minute

7. Time of 4th sample

Hour Minute

Form for interviewer for record keeping of sampling

8. Time of wake up

Hour Minute

9. Time of sampling of first sample

Hour Minute

10. Programmed time of 2nd sample (+3 hours after wake up)

Hour Minute

11. Actual time 2nd sample

Hour Minute

12. Programmed time of 3rd sample (+7 hours after wake up)

Hour Minute

13. Actual time of 3rd sample

Hour Minute

14. Programmed time of 4th sample (+10 hours after wake up)

Hour Minute

15. Actual time of 4th sample

Hour Minute

Quality Control

16. Did anything unusual happen today?

.....

17. Did you think that you experienced something particularly stressful or difficult today?

Yes No

If yes: Could you please explain?

.....

When did this event occur?

Hour Minute

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