Biological Aspects of Late-life Depression

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Chapter 7

General discussion

DISCUSSION

The aim of the present thesis was to explore the potential role of novel biological mechanisms in the pathophysiology of late-life depression. First, we examined whether inflammatory markers are associated with, predict or follow depressive symptoms increase. In addition, we investigated whether adherence to a Mediterranean-style diet may buffer the inflammatory process boosted by depression, and whether blood levels of carotenoids are associated and predict depressive symptoms via the mediation of inflammatory pathways. Furthermore, we studied whether hypovitaminosis D and, lastly, the phenomenon of leptin resistance may be risk factors for depression onset over time. In the present chapter, findings from Chapters 2 through Chapter 6 will be integratively reviewed and interpreted in the context of the current scientific evidence. The biological pathways underlying the different findings will be discussed in detail highlighting the potential overlapping mechanisms represented by innate immune activation. Finally, methodological considerations will be addressed, and suggestions for future research and possible clinical implications will be reviewed.

The bidirectional relationship between inflammation and depression

An increasing body of evidence points to a complex, bidirectional relationship between inflammation and depression. Clinical depression diagnosis and depressive symptoms severity have been shown to be associated with elevated inflammation (1, 2). The largest meta-analysis (1) to date of the relationship between depression and prominent inflammatory markers confirmed that CRP, IL-6 and IL-1 (including IL-1ra) are positively associated with depression. These findings, however, stem from cross-sectional association data that are unable to clarify whether systemic inflammation precedes the onset of depressive symptoms, occurs as a consequence of depression or as a part of the somatic manifestations of depression. In this thesis we presented the results from longitudinal analyses based on the InCHIANTI Study that allowed us to test the directionality of the relationship between inflammatory process and depression. While available longitudinal studies in older persons are limited, we were able to examine in the same sample of older persons both directions of the association between major inflammatory mediators and depressive symptoms.

In **Chapter 2**, we tested the hypothesis that in older persons higher plasma levels of inflammatory mediators predict the development of clinically relevant depressed mood over time. Among the different inflammatory mediators, we showed that participants with higher serum concentrations of IL-1ra had higher depressive symptoms at baseline and had a higher risk of developing depressed mood after 6 years. The association between IL-1ra and depression has been reported also by previous clinical and experimental studies (3-9). IL-1ra, which is considered an acute phase protein (10), is a reliable marker of IL-1 signaling network activation even more reliable than IL-1α and IL-1β (11-13). IL-1 network molecules candirectly communicate with the brain directly crossing the blood brain barrier through saturable active transport systems not affected by other inflammatory mediators (14, 15).

In rodents, cytokine activation of sickness behavior - an animal model for depression - has been shown to be mediated in particular by IL-1β (16), although this response appears to be augmented by co-expression of IL-6 (17) and TNFα (18). Finally, among cytokines, particularly IL-1βhas been shown to activate the hypothalamic–pituitary–adrenal (HPA) axis (19), which in turn has been associated with depression.

One of the aims of the study presented in **Chapter 3** was to examine whether depressive symptoms were prospectively associated with increases in levels of inflammatory markers. We indeed found indeed evidence of an association between high depressive symptoms at baseline and an increase of IL-6 levels over six years of follow-up. This association was not found for CRP levels, and could not be examined for other inflammatory markers such as IL-1ra and TNFα due to unavailability of longitudinal measures. These findings seem consistent with data from the few available longitudinal studies on older persons. For example, chronic stress in participants who were caregivers for a spouse with dementia, as compared to non-caregivers, was associated with a four times higher annual rate of IL-6 increase over six years (20). Another prospective study (21) on 263 healthy older adults showed that greater baseline depressive symptoms severity was associated with a 6-year increase in serum IL-6, while only a non-significant weak bidirectional relationship with CRP over time was found. The reason for this specific pattern of prospective associations between depressive symptoms and IL-6 but not CRP is unknown. CRP is an acute phase protein whose production is regulated by IL-6 (22). Although mostly upregulated by IL-6, the synthesis of CRP is strongly influenced by mechanisms of post-transcriptional regulation that are independent of IL-6 (23). Therefore, CRP may be less specific for the low-grade inflammatory processes associated with depression in older age.

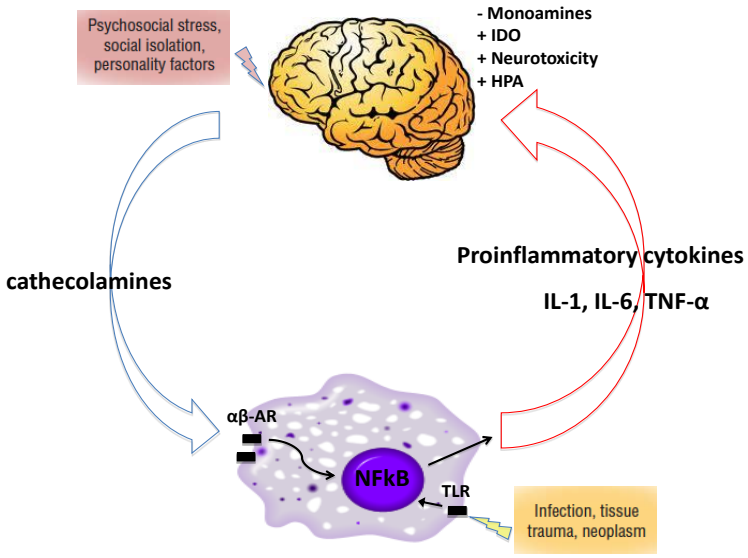
In all, the longitudinal analyses on the InCHIANTI sample presented in this thesis support the view of a bidirectional relationship between inflammation and late-life depression. That is to say that not only inflammatory process may have a role in pathophysiology of depression, but also depressive symptoms may determine an increased inflammatory response over time. Interestingly, the studies presented found different molecules associated with the two directions of this association. Whether this represents different inflammatory processes or is simply a chance finding is not yet known.This finding may be primarily due to the unavailability of longitudinal measures for all the inflammatory markers. However, our findings are consistent with the latest findings obtained by the few available prospective studies. We may speculate that different patterns of the innate immune activations may be associated to different aspects of late-life depression. However, further research is needed in order to test this hypothesis.

Biological pathways linking depression and inflammation

Figure 1 depicts the biological pathways linking inflammatory process and depression that consistently emerged from research on animal models and human depression. Environmental adversity (whether via α- or β-adrenoreceptor (α/β-AR) stimulation by

catecholamines in response to psychosocial stress or via Toll-like receptors (TLRs) stimulation in response to tissue trauma or neoplasm) activates the inflammatory signaling cascade in innate immune cells (e.g. macrophages and dendritic cells) leading to production and release of inflammatory cytokines. Although the initial immune activation may be primarily peripheral, this response can access and ultimately influence the brain through direct – IL-1 network inflammatory mediators can cross the blood brain barriers through saturable active transport molecules (14, 15) - and indirect communication systems. The latters include activation of brain microglia through stimulation of the vagus nerve afferent projections (24), diffusion into the brain of the local immune response of the leukocytes in the choroid plexus and circumventricular region (25), and entrance in the brain of monocytes attracted by monocyte chemoattractant protein-1 (MCP-1) released by microglia (26).

Figure 1. Biological pathways linking inflammatory process and depression.



Therefore, peripheral immune activation can set up a brain inflammatory response that subsequently participates in neurochemical and neuroendocrine processes known to be involved in the development of depression. For instance, inflammatory processes then have been shown to influence central serotonin signaling through increased uptake of serotonin after phosphorylation of the serotonin transporter (5HTT) via p38 mitogen-activated protein kinases (p38 MAPK) (27), and throughtryptophan depletion via the activation of the enzyme indoleamine 2,3-dioxygenase (IDO) (28). More importantly, the degradation of tryptophan induces the synthesis of the intermediate kynurenine that is further degraded into different

metabolites, the nature of which depends on the cell hosting the process (microglia synthesize neurotoxic metabolites). It has been recently hypothesized that inflammation-associated depression is dependent on the shift of kynurenine metabolism toward neurotoxic byproducts (28). Inflammatory signaling has also been linked also to overactivation of the hypothalamic–pituitary–adrenal (HPA) axis and its impaired feedback regulation, which are some of the most consistently replicated findings in patients with depression (29). Cytokines, primarily IL-1β, stimulate the release from the paraventricular nucleus of the hypothalamus of corticotrophin releasing hormone (CRH), which starts the cascade that induces the release of cortisol (19). Moreover, cytokines have been shown toinhibit glucocorticoid receptors (GR) function and responsiveness to cortisol leading to glucocorticoid resistance (30).

In addition, inflammatory processes affect also all the other potential biological mechanisms examined in this thesis, as it will be illustrated in detail in the next sections.

The interplay between nutrition (and nutritional biomarkers), inflammation and depression

In a recent cutting-edge review (31), Kiecolt-Glaser underlined the need of further research aimed at understanding the complex interrelationship between depression, nutrition and inflammation. Using the InCHIANTI Study, we were able to examine different interactive combinations between nutrient and nutritional biomarkers, depression and inflammation.

One of the aims of the study presented in **Chapter 3** was to test the hypothesis that the prospective association found between high depressive symptoms and increase of inflammatory mediators over time would be stronger in participants non-adherent to a healthy diet. We observed that adherence to a Mediterranean-style diet modified the association between depressive symptoms and IL-6 increase: in participants non-adherent to a Mediterranean-style diet, as compared to those adherent, higher depressive symptoms were associated with a steeper increase of IL-6 levels over time. These findings suggest that a healthy (Mediterranean-style) diet is able to buffer the inflammatory response boosted by depression in older adults. These results are consistent with single emerging from studies on different populations. A recent study based on 345 middle-aged male twins (32) showed that adherence to a Mediterranean-style diet was associated with reduced levels of interleukin-6, but not CRP, after adjustment for total energy intake, other nutritional factors, known cardiovascular risk factors, and use of supplements and medications.A large-scale prospective study examined the association between adherence to a Mediterranean-style diet and the incidence of depression in sample of 10,094 healthy young adults in Spain (33). Participants highly adherent to this type of diet, as compared to those less adherent, had a 26-42% lower risk of incident depression after a median follow-up of 4.4 years.

The positive effects of a Mediterranean-style diet on a wide array of health outcome have been confirmed by a growing number of epidemiological studies conducted in different

countries (34). From the InCHIANTI Study, we recently also demonstrated that high adherence to a Mediterranean-style diet is associated with a slower decline of mobility and lower risk of mobility disability (35). Mediterranean diet is characterized by high intake of vegetables, legumes, fruits and nuts, cereals and olive oil with low intake of saturated fat, moderately high intake of fish, low to-moderate intake of dairy products, low intake of meat, and a regular but moderate intake of alcohol primarily in the form of wine during meals (36). Among the components of this type of diet, major anti-inflammatory effects are supposed to be exerted by the antioxidant components of fruit, vegetables and olive oil. Observational studies have shown an inverse association between dietary total antioxidant capacity, fruit and vegetables intake and markers of inflammation (37-40).

In **Chapter 4** we evaluated the interplay between depression, inflammation and an important component of the antioxidant system, carotenoids, which are considered a good indicator of fruit and vegetable intake. We examined the cross-sectional and longitudinal relationship between plasma carotenoids and depressive symptoms in older persons. Moreover, we tested whether inflammatory markers mediated this relationship. We found that participants with lower total carotenoids level had higher depressive symptoms at baseline and were more likely to develop incident depressed mood after six years of follow up. Moreover, plasma carotenoids were inversely associated with levels of CRP, IL-6 and IL-1ra at baseline. Among these inflammatory molecules, we found evidence that IL-1ra partially mediated the relationship between carotenoid concentrations and development of depressed mood after 6 years. To our knowledge, this is the first study examining this relationship. However, these findings seem to be consistent with emerging indications from different studies. Recent preliminary evidence (41) from the Boston Puerto Rican Health Study, among 1216 Puerto Rican adults, showed a cross-sectional inverse association between intake and plasmastatus of total carotenoids with depressive symptoms. Among older disabled women in the Women's Health and Aging Study (WHAS) (42), participants with low serum levels of carotenoids were significantly more likely to have higher IL-6 at baseline and increasing IL-6 levels over a period of 2 years. In general, studies in older persons, and in particular data from the InCHIANTI Study, show associations between carotenoids with phenotypes that characterize age-related frailty and that have been linked with inflammation, such as sarcopenia, mobility disability and mortality (43-46).

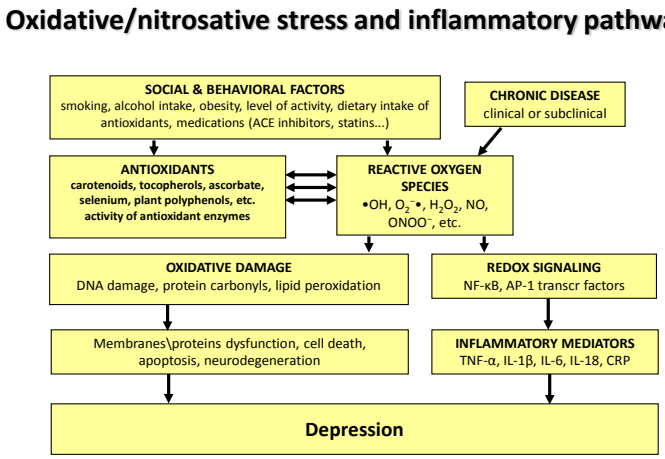
We believe that evidence emerged in the prospective studies presented in this thesis provide empirical support to the hypothesis of a complex reciprocal relationships between depression, nutritional factors and inflammatory process. A broader and deeper interface between bio-behavioral and nutritional camps is essential to shed light on the mechanisms promoting diseases commonly associated with late-life depression.

Biological pathways linking nutrition, inflammation and depression

Figure 2 shows the hypothesized biological network underlying the complex interrelationship between nutrition and nutritional biomarkers, inflammation and depression. This network is characterized by the integration of the inflammatory pathways and oxidative

and nitrosative (O&NS) stress mechanisms (47). Under normal conditions reactive oxygen species (ROS) and reactive nitrogen species (RNS) are tightly regulated by the antioxidants defense system that remove ROS and RNS through scavenging their radicals. Dietary carotenoids, which are an important part of this defense system, are embedded within the cell membrane lipid bi-layers and function to quench free radicals generated by intracellular oxidative processes (48). Oxidative and nitrosative stress occurs when there is an imbalance between a relative shortage in antioxidants defenses with regard to an increased production of ROS/RNS. The latter process may cause dysfunction in the cell through damage and mutation of proteins, lipids (lipid peroxidation) and mitochondrial DNA, which in turn leads to impaired mitochondrial function and further generation of free radicals, with increased lipid peroxidation and impaired oxidative DNA repair in the nucleus. Moreover, O&NS damage may alter the chemical structure of otherwise ubiquitous molecules to generate a variety of modified neoepitopes highly immunogenic, which therefore trigger an autoimmune response. These processes together may cause dysfunctioning of the cell, apoptosis, cell death and eventually neurodegeneration. Some organs like the brain are more vulnerable to the detrimental effects of O&NS because it has a high metabolic rate and lower antioxidant levels (47). It has been hypothesized that the inability to buffer the effects of this oxidative stress may be responsible for age-related neuronal decrements and neurodegenerative disease (49, 50). Brain imaging studies have linked depression with structural and functional alterations of limbic and cortical structures, particularly in the hippocampus (51). Oxidative and nitrosative stress has also another important effect. Superoxide radicals or hydrogen peroxide may trigger the NFkB transcription activity, enhancing ultimately the inflammatory processes linked to depression. The close integration of these two pathways has been recently labeled the "inflammatory and neurodegenerative/neuroprogressive hypothesis of depression" (52). An emerging body of evidence is developing the empiric ground for this hypothesis, as showed by clinical studies demonstrating an association between major depression and biomarkers of ROS levels (53), lipid peroxidation (54, 55), oxidative DNA damage (56) and mitochondrial disturbances (57).

Figure 2. Biological network underlying the interrelationship between nutrition inflammation and depression.



Hypovitaminosis D as a risk factor for depression

In the last years vitamin D has been increasingly implicated in the pathophysiology of cognitive decline and mental illness (58-62). More recently, it has been hypothesized that hypovitaminosis D may contribute to depression (61-63) in older age, when vitamin D insufficiency and deficiency is highly prevalent. Until recently, longitudinal studies examining the association between vitamin D concentrations and development of depressive symptoms in older persons were not available.

In **Chapter 5**, we reported the first longitudinal study examining the relationship between vitamin D, as measured by serum 25(OH)D, and depressive symptoms over a 6-year follow-up in a representative group of older adults. Participants with low 25(OH)D serum levels experienced a grater increase in depressive symptoms over the follow-up period. Moreover, among participants free of clinically relevant depressive symptoms at baseline, a higher risk of depressed mood onset was found for those with low serum 25(OH)D.The strength of this prospective association was more distinctive in women than in men. More recently, a second prospective study of 939 community-dwelling Chinese older men was published (64). At baseline, higher levels of 25(OH)D were inversely associated with depressive symptoms and with lower odds of having depression. However, no association was observed between serum 25(OH)D and incident depression after 4 years. Previous cross-sectional epidemiological studies based on different international community groups identified associations between depression and low vitamin D levels, although there were a number of non-significant studies (65). The study reported in this thesis provided

the first evidence of a prospective association between low vitamin D levels and the onset of depressive symptoms, therefore sustaining recent hypotheses about the involvement of hypovitaminosis D in late-life depression. However, to date the evidence does not yet appear to be sufficient to conclude with certainty that there is a causal connection and this research should be extended to younger populations including psychiatric cases of depression.

Biological pathways linking vitamin D to depression

Although the extent to which vitamin D and depression are related remains unclear, the biological plausibility of various pathways on which this relationship may rely is well supported by studies in animal models or humans. Novel studies in human brains confirmed the presence of vitamin D receptors (VDR) and 25(OH)D3 1α-hydroxylase, the enzyme that catalyzes the hydroxylation of calcidiol to calcitriol (the bioactive form of vitamin D), in both neuronal and glial cells within brain structures implicated in the regulation of cognition, mood and behavior such as the amygdala and in pyramidal neurons of the hippocampus (66, 67). It has been shown that genetic variance in the VDR gene influences the susceptibility to age-related changes in cognitive functioning (68). In a sample of 563 individuals aged 85 years and older, those who carried one of three specific VDR polymorphisms experienced impairment in memory and attention. Interestingly, another genetic variant associated with better cognitive outcome was also associated with less depressive symptoms. Furthermore, vitamin D may protect the structure and integrity of neurons through different mechanisms. Calcitriol upregulates neurotrophin factors, such as nerve growth factor (NGF), neurotrophin-3 (NT-3), mainly present in hippocampus and neocortex, where they affect neurotransmission and synaptic plasticity (69). Calcitriol, by down-regulating the expression of voltage-sensitive calcium channel transcripts, modulates cell calcium homeostasis.Dysregulation of cell calcium is a characteristic of the neurotoxicity of neurodegenerative disorders (70). Finally, vitamin D exerts important antioxidant function, enhancing innate antioxidant pathways resulting in increase of glutathione, which protects oligodendrocytes and the integrity of the nerve conduction pathways (71).

An interesting hypothesis yet to be tested is that the influence exerted on depression by vitamin D may partially be explained by its effect on inflammatory pathways. Macrophages and some dendritic cells express the VDR as well as the enzymes required to produce the active form of vitamin D. Calcitriol is known to be synthesized by microglial cells (72), which are the primary mediators of proinflammatory immune responses in the brain. Moreover, CD4-positive T lymphocytes (Th1, Th2) have been shown to be the preferential target of vitamin D. Calcitriol appears to participate in regulating Th1:Th2 balance by down-regulating the Th1 pathway (producing the proinflammatory cytokines IL-2, IFN-**γ**, and TNF-**α)** and promoting the Th2 pathway (producing the anti-inflammatory cytokines IL-4 and IL-10) through a VDR mediated inhibition of gene transcription (73, 74). Moreover, vitamin D has been shown to downregulate the activity of NFkB (75), which triggers the inflammatory signaling cascade and is the key mediator linking stress-induced increases in

IL-1β with impaired hippocampal neurogenesis and depressive-like behaviors (76).It has also been demonstrated that vitamin D reversesthe age-related increase in microglial activation and the accompanying increase in IL-1β concentration in rat hippocampus (77). In a RCT of patients with congestive heart failure, vitamin D supplementation was associated with decreased serum concentrations of the proinflammatory cytokine TNF-α and increased concentrations of the anti-inflammatory cytokine IL-10 (78).

Leptin resistance, abdominal obesity and depression

The "leptin hypothesis of depression" contends that leptincontributes to the regulation of affective status (79). Leptin was initially identified as an anti-obesity hormone acting as a negative feedback aimed at controlling energy homeostasis. Recently, new effects of leptin have been observed. Leptin has been shown to improve cognition and mood in animal models of depression, suggesting that leptin may have antidepressive properties (80). However, the availability of only a limited number of small cross-sectional studies with highly conflicting results doesn't allow to reach definitive conclusions on this hypothesis. In particular, the role of different mechanisms proposed, such as leptin insufficiency versus leptin resistance, needs to be clarified.

In **Chapter 6**, we present the results of the first longitudinal study on a large sample of older adults, using data from the Health ABC Study, examining whether serum leptin concentrations in older adults are associated with an increased risk of developing clinically relevant depressive symptoms. We found that in older men high serum levels of leptin were associated with an increase in depressive symptoms over a 5-year period. Moreover, the impact of high serum levels on depression onset was especially evident in men with abdominal obesity. Since, the presence of hyperleptinemia in obese persons may be considered an indicator of leptin resistance, the latter finding may suggest that leptin resistance may contribute to alterations of affective status. These findings, not only provide for the first time evidence of a prospective association between leptin and late-life depression, implying a possible causal role for leptin in the pathophysiology of depression, but also suggest that leptin resistance may serve as a common biological factor for the well-established comorbidity of obesity and depression. Increasing evidence confirms that abdominal obesity, as compared to overall obesity, is specifically linked with the risk of depression and health consequences commonly associated with depression, such as metabolic and cardiovascular disturbances (81-84).

From the Health ABC Study (85),Vogelzangset al. showed that visceral fat, independent of overall obesity, was a risk factor for depression onset in older men. Using the InCHIANTI Study, the same authors (86) recently showed that greater waist circumference, but not other features of metabolic syndrome, was highly predictive of developing depression, and once developed, metabolic syndrome was associated with almost threefold increase in the risk of chronicity of depression. On the other hand, the increased leptin levels commonly observed among obese persons are thought to be caused by leptin resistance, a physiological mechanism similar to the one that links type 2 diabetes and insulin resistance

(87, 88). Leptin resistance, creates a dissociation between circulating levels and central action of leptin, whose effect on inhibition of food intake and increase in metabolic turn-over are attenuated despite increasing blood concentrations. This has been clearly shown in a randomized trial (89) where treatment with recombinant leptin was more effective in terms of weight loss in lean subjects as compared to obese subjects. Based on these observations, it has been repeatedly hypothesized that leptin resistance may be one of the biological bases of the increased depression risk in obese patients. The study presented in this thesis provides for the first time a strong empiric support to this hypothesis, suggesting a potential common shared biological link between depression, obesity and their association with negative health outcomes.

Biological pathways linking leptin resistance, depression and abdominal obesity

Leptin is synthesized in white adipose tissue along with other cytokines and adipokines and interacts with receptors in the hypothalamic arcuate nucleus (90), where it exerts its homeostatic function through a biologically elegant system of neuropeptides. However leptin receptors (in particular the isoform LRb that activates intracellular transduction pathways) are expressed in limbic substrates related to mood and cognition regulation, such as the hippocampus and amygdala, suggesting a potential neuroactive function. In animal models of depression, leptin has indeed been shown indeed to improve cognition and mood (80). The systemic administration of leptin in rodents has been shown to exert antidepressant behavioral effects and to improve learning and memory in behavioral and cellular assays (91-93). Leptin has also been shown to affect hippocampal and cortical structure through its actions on neurogenesis, axon growth, synaptogensis and dendritic morphology regulation (94). Moreover, accumulating evidence shows that leptin modulates hypothalamus-pituitary-adrenal (HPA) axis activity, which has been implicated in depression and obesity (95). Chronic administration of leptin can reverse hypercortisolemia even prior to weight loss in mice (96), and there is an inverse relationship between plasma leptin glucocorticoids and circadian rhythm activity (97).

In obesity, the central effect of leptin is attenuated by leptin resistance due to impaired transport across the blood–brain barrier, reduced function of LRb and defects in leptin signal transduction (87, 88). Indeed, diet-induced obese animals have been shown to be leptin resistant, displaying decreased anorectic response and decreased amplitude of maximal LRb signaling in the hypothalamus (98, 99). For this reason it has been hypothesized that it is not the absolute serum leptin concentration but rather the ability of the hormone to induce an effect at the receptor/post-receptor level, that is correlated with mood and cognition.

Interestingly, the development of leptin resistance has been recently linked to inflammatory processes, which in turn are associated with depression and obesity. Visceral adiposity is indeed consistently associated with increased inflammatory response, with excess production of cytokines and acute phase proteins (100, 101). It has been shown that C-reactive Protein (CRP) may directly bind to leptin and attenuate its physiological functions

and this effect has been postulated to rise in proportion to the severity of obesity (102). Moreover, Interleukin-1 receptor antagonist (IL-1ra) has been shown to antagonize the action of leptin in the hypothalamus of rodents (103). Finally, leptin, which is a member of the type I cytokine superfamily, has been shown to be increased by IL-1 and IL-6 (104) and to mediate the relationship between adiposity and inflammation (105).

Other potential underlying mechanisms

Beyond the biological pathways considered in the previous paragraphs, it is likely that other somatic and behavioral mechanisms known to be associated with depression may have contributed to the relationship of depression with the biological factors studied in this thesis.

Cardiovascular diseases and diabetes are consistently shown to be associated with increased inflammation markers and depression (21, 106-109). Behavioral life-style factor,factors, known to be related to depression, may be connected to all the measures considered in the studies reported: smoking habit, excessive alcohol consumption and physical inactivity are associated with inflammatory alteration, unhealthy dietary preferences with lower antioxidants intake, and reduced outdoor activity with consequent hypovitaminosis D due to reduced sun exposure (110-114). Furthermore, disability and cognitive decline may be associated with all the major biological measures we used in each study (115-117). Finally, antidepressant drugs may have confounded the relationship studied. Indeed, antidepressants have been shown to partially exert their therapeutic effect trough negative immunoregolatory effect via stimulation of IL-10 release (118, 119). However, in the studies we used the proportion of participants using antidepressant drugs was low. Our analyses were based on data from two of the most methodologically complete epidemiological studies of aging, the InCHIANTI and the Health ABC study, allowing us to include adjustment for these other mechanism mentioned. Although residual confounding cannot be completely ruled out in epidemiological research, adjustment for a wide array of confounders decreased the possibility that other factors may have largely driven the observed associations. Other important methodological issues are considered in the next section.

Methodological considerations

Causal inference - A first point to be made is that the results presented in this thesis stem from longitudinal observational studies. Although major advantages of this type of study are the prospective nature and the ability to study the natural course of diseases, a limitation is represented by the restricted ability of drawing definite causal inferences. However, demonstrating a consistent time sequenced association provides a strong empirical ground to further support the hypothesis of a causal pathway. Moreover, as we extensively discussed in this chapter the hypotheses we tested are sustained by a plausible biological rational as emerged from pre-clinical studies and animal models of depression and aging.

Furthermore, in the studies where biological factors were hypothesized as etiological aspects of depression, the phenomenon of reverse causation may have biased the results. For example, it is possible that participants who were subclinically depressed at enrollment could have had lower levels of vitamin D or carotenoids as a consequence of depression via unhealthy dietary preferences or decreased outdoor activities. Moreover, it is also possible that biological risk factors could be due to existing somatic disorders or subclinical disease which could lead to depression. For example, impaired glucose metabolism has been linked to altered circulating levels of adipokines such as leptin (109) and diabetes has been associated with an increased risk of depression (108). However, the risk of biased results were controlled by accounting for baseline levels of depressive symptoms and somatic diseases, and by performing different sensitivity analyses excluding participants who developed depressive symptoms or particular disease at intermediate time points or by setting more stringent operational criteria for the onset of depressed mood.

Definition of depression - A limitation of the studies presented in this thesis is that depressive symptoms were evaluated by the CES-D questionnaire and the diagnosis of depression was not confirmed by a clinical psychiatric diagnosis. Although the CES-D scale can detect the presence of clinically relevant depressive symptomsit does not equal a depressive disorder diagnosis. However, DSM-defined affective disorders are not highly prevalent among elderly persons in the community, while subsyndromal chronic depression is more common (120, 121). A fundamental issue that should be considered is the influence of somatic health aspects on the evaluation of depression in older persons with a symptom questionnaire. Depressive symptoms checklists in general contain a considerable number of somatic symptoms, e.g. fatigue, sleep problems and appetite or weight changes. Persons with many somatic health problems, which are especially common in older persons, could theoretically score high on these depressive symptoms questionnaires simply because of their somatic symptoms. However, by adjusting analyses for diseases and health related factors, the possible influence of depression misclassification due to somatic symptoms was systematically reduced. Furthermore, the CES-D has been widely used in population-based studies of older persons, where it has shown very good sensitivity and specificity when identifying major depressive disorder (122). Moreover, the consistency of our results with the best evidence available to date suggests a low risk of biased results due to assessment of depressive symptoms. For example, the largest meta-analysis (1) to date of the relationship between depression and prominent inflammatory markers, included also studies in community sample and with depressive symptoms measured by clinical (DSM-based) instruments in clinical settings. The association between depression and inflammation was present in both clinic- and community-based samples as well as those studies using clinical interviews or self-report measures of depression. Although the magnitude of association was substantially larger in clinical samples and when standard clinical interviews were used, continuity was found across clinic- and community-based samples.

The commonly observed connection of late–life depression with somatic conditions poses also a problem of generalization. Is it possible that depression that first emerges in

late-life might be much more entangled with somatic conditions than depression that emerges earlier in life, because competing somatic risk factors could override the effects of mental health factors. These considerations implicate that results of the present thesis cannot automatically be generalized to younger populations.

Selective survival - Finally, when dealing with research in older persons the phenomenon of selective survival should be taken into account for a comprehensive interpretation of causality between behavioral factors and health outcomes. By definition, when recruiting a sample of older persons, those who have already died are not included in the cohort. Therefore, it is certainly possible that for certain known behavioral risk factors associations are no longer found to be predictive of diminished health, solely because those who were both exposed and affected by this risk factor are no longer alive. However, if selective survival tends to decrease the strength of investigated associations, it suggests that when associations are found (as in our studies) in reality the relationship might be even stronger.

Public health and clinical implication

The results of the present thesis showed that late-life depression is linked to biological factors that do not belong to the pathways considered in the classical hypotheses. These results may have implications that go beyond the simple re-appraisal of the biological underpinning of depression. We studied a series of immunitary and metabolic dysregulated markers that may identify, within the extreme phenotypic heterogeneity of depression, specific subgroups of subjects at higher risk for negative health outcomes typical of advanced age. A consistent body of evidence now indicates that increased inflammation is only found in a subset of depression, typically depression associated with late-onset and/or cardiovascular disorders (123). Recent studies based on latent class methods (124) suggest that symptoms driving atypical depression may be more related to somatic or metabolic conditions. The recent introduction of the "metabolic depression" research hypothesis suggests that metabolic alterations may identify a chronic subtype of this disease (86). This implicates that, if these results are confirmed, a significant proportions of depressed patients may benefit from the assessment of these biomarkers that may become valuable clinical tools for risk assessment.

The relevance of these endophenotypes, however, depends on the ability to develop and test interventions that are specifically effective. The results of the present thesis highlight different biological pathways that can be targeted for intervention. For example, there is a pressing need to investigate strategies to disrupt inflammatory signaling in persons with depression who show indication of a proinflammatory state. Results from this thesis suggest strategies that may be relevant from a public health standpoint. Evidence emerging from the study of the nutritional determinants of late-life depression suggests that intervention aimed at improving the quality of diet, especially through a Mediterranean-style dietary pattern rich in antioxidant, may be effective in buffering depression through modulation of inflammatory pathways. Furthermore, promotion of physical activity can

decrease visceral fat and promote a more positive inflammatory and metabolic profile. Indeed, increasing evidence suggests that physical exercise can improve depressive symptoms to some degree (125). Finally, assessment and normalization of vitamin D levels may in the future become part of the treatment of older depressed patients who are at risk of deficiency or insufficiency as a consequence of geographical or lifestyle factors.

Future research

With the studies included in this thesis we showed that new biological mechanisms may have a potential role in the pathophysiology of late-life depression. These results suggest that future directions for research should be focused to a better understanding of the exact mechanisms of these associations. For some of the biomarkers examined (e.g. carotenoids, vitamin D and leptin) the studies presented in this thesis represent the first evidence available of a longitudinal association with depression. Therefore, the first aim of future research should be the consistent confirmation of this temporal pattern of association. Then, further steps will be needed in order to confirm a causal role: 1) the demonstration of a dose-response relationship using studies including also patients with current and remitted established psychiatric diagnosis of different depressive disorders; 2) the demonstration of the ability to modify the course of late-life depression through experimental manipulation of the pathways identified in appropriately design randomized controlled trials.

Along this process several specific issues for the different biological aspects we investigated should be addressed. We confirmed a bidirectional relationship between inflammation and late-life depression and we found different molecules associated with the two directions of this association. Future studies with appropriate measures should examine whether different patterns of immune activations are associated with different aspects of late-life depression. Other key issues in the study of the inflammation-depression link include the identification of the best biomarkers and the differential effect of this relationship across gender, race and endophenotype of depression. The consistent body of evidence emerging from the study of the nutritional determinants of inflammation and late-life depression provides a rationale for the development of intervention studies aimed to test whether improving the quality of diet may be especially effective in improving depression in older persons.

Research on the role of hypovitaminosis D in late-life depression should face the methodological challenge affecting the general research on vitamin D, including the development of reliable assays and the consensus on the optimal levels of vitamin D. Moreover, if intervention trials will be undertaken, important questions about type and dosage of vitamin D supplemented and association with other compounds (e.g. calcium) should be addressed.

Future research on leptin will have to definitively clarify the role of leptin insufficiency versus leptin resistance in depression, and in the latter case a difficult challenge will be represented by the development of treatment effective in overcoming central leptin

resistance. Moreover, further studies are warranted on the role of other adipokines commonly dysregulated in obesity, such as adiponectin and resistin.

Furthermore, systematic studies across sample of different ages are needed to confirm whether these new biological aspects are specifically linked to late-life depression.

Finally, the identification of a clear-cut depressive endophenotype presumed to lie between a particular genotype and a more complex (and elusive) disease phenotypes, will benefit the research on the genetic determinants of depression.

In conclusion

This thesis aimed to explore the role in late-life depression of new molecular mechanisms beyond the classical hypothesis of a deficiency in monoaminergic neurotransmission. The results of this thesis identified new biological aspects related to late-life depression, including inflammation, dietary patterns and dietary antioxidant biomarkers, hypovitaminosis D and leptin resistance. Depression is a major public health problem worldwide and especially in older persons may results in lethal health consequences, complicating chronic illness and increasing the risk of disability and death. Nevertheless, treatments available nowadays are far from ideal in terms of efficacy. We believe that the results presented in this thesis significantly contribute to the re-appraisal of the biological underpinning of late-life depression, identifying new risk factors that may become target for interventions aimed at reducing depressive mood in the elderly and prevent its deleterious consequences on morbidity and mortality.

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