Role of intestinal permeability and inflammation in the biological and behavioral control of alcohol-dependent subjects

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ABSTRACT

Background and aims: Mood and cognition alterations play a role in the motivation for alcohol-drinking. Lipopolysaccharides are known to stimulate inflammation that was shown to induce mood and cognitive changes in rodents and humans. Enhanced intestinal permeability and elevated blood LPS characterize alcohol-dependent mice. However, no data have been published in non-cirrhotic humans. Our first goal was to test whether intestinal permeability, blood LPS and cytokines are increased in non-cirrhotic alcohol-dependent subjects before withdrawal and if they recover after withdrawal. Our second goal was to test correlations between these biochemical and the behavioral variables to explore the possibility of a role for a gut–brain interaction in the development of alcohol-dependence.
Methods: Forty alcohol-dependent-subjects hospitalized for a 3-week detoxification program were tested at onset (T1) and end (T2) of withdrawal and compared for biological and behavioral markers with 16 healthy subjects. Participants were assessed for gut permeability, systemic inflammation (LPS, TNFα, IL-6, IL-10, hsCRP) and for depression, anxiety, alcohol-craving and selective attention.
Results: Intestinal permeability and LPS were largely increased in alcohol-dependent subjects at T1 but recovered completely at T2. A low-grade inflammation was observed at T1 that partially decreased during withdrawal. At T1, pro-inflammatory cytokines were positively correlated with craving. At T2 however, the anti-inflammatory cytokine IL-10 was negatively correlated with depression, anxiety and craving.
Conclusion: Leaky gut and inflammation were observed in non-cirrhotic alcohol-dependent subjects and inflammation was correlated to depression and alcohol-craving. This suggests that the gut–brain axis may play a role in the pathogenesis of alcohol-dependence.

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1. Introduction

Alcohol-dependence is a disorder that is present in 5–7% of the population of developed countries (Anderson and Baumberg, 2006; Grant et al., 2004). In addition to its familial, social and professional consequences, it is also a major risk factor for several pathologies including liver or pancreatic diseases, cardiac diseases, cancers and neurological or psychiatric disorders. This addiction is also the first cause of malnutrition in developed countries. Indeed, both human and animal studies have shown that heavy chronic alcohol consumption leads to mucosal damages that will interfere with the absorption of micro and macronutrients and with mucosal enzyme activities contributing to malnutrition (Bode and Bode, 2003). Alcohol-fed animals are characterized by an intestinal hyperpermeability and possibly a Gram-negative bacterial overgrowth in the upper small intestine that contribute to increased leakage of lipopolysaccharides (LPS) into the circulation (Adachi et al., 1995; Yan et al., 2010). LPS are potent pro-inflammatory agents that through a CD14-TLR4 receptor mechanism (Akira and Hemmi, 2003) activate the transcriptional factor NFκB to induce the production of inflammatory mediators such as pro-inflammatory cytokines, chemokines, nitric oxide and reactive oxygen species

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(Wheeler, 2003). This LPS activated inflammatory process has been thought to play an important role in the development of alcoholic liver disease (Wheeler, 2003). These mechanisms have been mainly studied in rodent models of alcohol-dependence (Adachi et al., 1995; Mathurin et al., 2000; Rao et al., 2004; Wheeler, 2003; Yan et al., 2010) but data on whether this also occurs in alcohol-dependent humans are scarce.

Among the human studies, increased circulating LPS levels have been reported in subjects that have already developed liver damage and cirrhosis (Fukui et al., 1991; Parlesak et al., 2000) where the increased LPS levels have been also be attributed to dysfunctional Kupffer cells with reduced ability to detoxify endotoxins (Rao et al., 2004). Therefore, the rise in plasma LPS and subsequent inflammation in these patients can also be the consequence of the liver disease. The analysis of the effects of alcohol consumption in non-cirrhotic alcoholics would allow us to test the hypothesis that changes in intestinal permeability, LPS and low-grade inflammation may not only be a consequence but also play a role in continuing alcohol-dependence.

The influence of circulating cytokines on the human brain functions and the development of depression is supported by several studies (Dantzer and Kelley, 2007; Dantzer et al., 2008; Maes, 1999). Furthermore, an infection (Dantzer et al., 2008; Konsman et al., 2002) or the injection of LPS (Reichenberg et al., 2001) will induce several symptoms (fever, fatigue, anorexia, sleep abnormalities, loss of interest in social activities), and cognitive disturbances (e.g. memory or attention dysfunctions) that are close to symptoms of depression. These symptoms are induced by the effect of inflammation on the brain through both humoral and autonomic pathways (Konsman et al., 2002). These observations support the idea that inflammation induces a Sickness Behavior, which may play a role in the pathophysiology of some psychiatric diseases. Consistent with this idea, depressed subjects have higher plasma pro-inflammatory cytokines concentrations than controls (Dowlati et al., 2010; Maes, 1999). On the other hand, the anti-inflammatory cytokine IL-10 seems to prevent LPS-mediated mood and cognition disturbances (Bluthe et al., 1999; Richwine et al., 2009; van den Boogaard et al., 2010). In addition, depression plays an important role in the behavioral mechanism of addiction (Schuckit, 1994) and has been shown to be strongly and consistently correlated to alcohol craving (Andersohn and Kiefer, 2004) which could be defined as the appetitive urge to drink alcohol (Anton, 1999). Also, recent psychological studies indicated that negative affects (depression, anxiety) and craving largely improve during the alcohol-withdrawal (Cordovil De Sousa Uva et al., 2010; De Timary et al., 2008). Selective attention also improved during the detoxification programme whereas other cognitive functions such as executive functions (inhibition, flexibility, decision making) remained unchanged (Cordovil De Sousa Uva et al., 2010).

Our first goal was to show that heavy chronic alcohol consumption induced an increase in intestinal permeability. Then, we tested whether this hyperpermeability was associated with a rise in plasma LPS levels (Cani et al., 2007, 2008) and systemic inflammatory response. Finally, we hypothesized that these biological changes would in turn influence the severity of depressive symptoms, the intensity of craving and consequently alcohol consumption. To test these hypotheses, alcohol-dependent subjects were carefully selected upon diagnosis as non-cirrhotic. They were assessed for intestinal permeability, plasma LPS levels, plasma pro- and anti-inflammatory cytokines levels, high-sensitivity C-reactive protein (hsCRP) levels and for behavioral measures both at the onset and end of alcohol-withdrawal. This alcohol-dependent group was compared to a control group consisting of individuals matched for age and body mass index (BMI) and who socially consume low amounts of alcohol.

2. Material and methods

2.1. Participants and procedure

The study protocol was approved by the ethical committee of the hospital and all subjects signed an informed consent form prior to the investigation (B40320096274, Commission d'éthique biomédicale hospitalo-facultaire de l'UCL). A total of 52 subjects with diagnosis of alcohol-dependence according to the DSM-IV criteria (APA, 1994) were clinically evaluated by a psychiatrist (P.d.T.) and admitted to the gastroenterology ward for a 3-week detoxification and rehabilitation programme. This programme consists of 2 weeks of hospitalization separated by 1 week where the patients return back home. All patients had kept on drinking until the day of admission to the detoxification ward or the day before. Patients were tested twice, on the day of or the day following their admission (T1) and on day 18–19 (T2) corresponding to the last days of the detoxification program. Among the 52 patients who were included, only 40 participated at both times of the testing. The subjects who abandoned their treatment or who relapsed during the week at home were excluded. Also excluded prior to the study entry were patients who suffered from diabetes and Crohn's disease or other chronic inflammatory diseases (such as rheumatoid arthritis), those who presented gastric bypass or other bariatric surgery, those who took antibiotics, probiotics or glucocorticoids and non-steroidal anti-inflammatory drugs during the 2 months preceding enrollment, and subjects who presented with a diagnosis of cirrhosis. Transient liver elastrography (Fibroscan®) was applied to all patients in order to quantify liver stiffness which correlates with fibrosis grades, according to the METAVIR classification system of fibrosis (Bedossa and Poynard, 1996). Based on this evaluation, only patients without significant fibrosis (F0 and F1 scores) were included in the study (see Supplementary material section 1). Alcohol-dependent patients were compared to 16 age- and BMI-matched controls who consume low amounts of alcohol (<20 g/day) (Table 1).

2.2. Measurement of intestinal permeability with ^{51}Cr-EDTA

We measured intestinal paracellular permeability with ^{51}Cr-EDTA. This non-toxic probe was neither degraded by gut flora nor metabolized by the body and not naturally present in the urine (Bjarnason et al., 1995; Peeters et al., 1994). After an overnight fast and emptying of the bladder, patients drank a Nutridrink® (200 ml, 150 kcal/100 ml) (Nutricia, Brussels, Belgium) containing 50 μCi (1.85 MBq) ^{51}Cr-EDTA. Urine was collected for 24 h during two periods that were expected to reflect small (0–4 h) and large (4–24 h) bowel permeability, respectively. Urine creatinine was measured to ensure completeness of the collections and subjects with very low creatinine clearance rates (< 8 mg/kg/24 h) were excluded (De et al., 1993). Complete collections at T1 and T2 were

Table 1
Characteristics of the alcohol-dependent and the control groups.

Variable	AD-T1	AD-T2	Controls
Number of participants	52	40	16
Age	47 ± 11[a]	49 ± 11[a]	50 ± 11[a]
Gender, n (%)			
Male	42 (81%)	32 (80%)	9 (56%)
Female	10 (19%)	8 (20%)	7 (44%)
BMI (kg/m^2)	24.7 ± 4.1[a]	25.3 ± 3.9[a]	27.4 ± 4.1[a]
Alcohol intake (g/day)	195 ± 79[a]	0	10 ± 7[a]

AD-T1 and AD-T2 refer to alcohol-dependent subjects tested at onset and end of the detoxification program, respectively.
[a] Data are means ± SD.

obtained in 26 alcohol-dependent subjects and 16 controls. The radioactivity was measured with a gamma counter (Cobra5003 Canberra Packard, Downers Grove, IL, USA). Results were expressed as percentages of the ingested dose normalized for creatinine.

2.3. Biochemical analyses

The LPS concentration was measured with the Limulus amebocyte lysate kinetic chromogenic methodology that measures the color intensity directly related to the endotoxins concentration in a sample, using Endosafe-MCS (Charles River laboratories, Lyon, France). Plasma were diluted 1/10 with endotoxin-free buffer to minimize interferences in the reaction (inhibition or enhancement) and heated for 15 min at 70 °C. Each sample was diluted 1/70 with endotoxin-free Limulus amebocyte lysate reagent water (Charles River Laboratories, Wilmington, MA) and treated in duplicate, and 2 spikes per sample were included in the determination. All samples have been validated for recovery and coefficient of variation determination. The lower limit of detection was 0.01 EU/mL (Everard et al., 2011; Luoto et al., 2011).

Plasma cytokines (interleukin (IL)-6, IL-10 and tumor necrosis factor (TNF) α) were determined in duplicate by multiplex immunoassay (Millipore, Belgium) and measured using Luminex®xMap® technology (Biorad, Nazareth, Belgium) following the manufacturer's instructions. Plasma hsCRP was measured by an automated turbidimetry method (DxC 800, Beckman Coulter).

2.4. Psychological tests

All patients were tested for depression, anxiety, craving for alcohol and selective attention at T1 and T2 (see Supplementary material section 2).

The Beck Depression Inventory (*BDI*) is a 21-item self-report inventory designed to measure the severity of depressive symptoms, with a maximum score of 63 (Beck et al., 1996). The validated French translation of the second version of the BDI (BDI-II) was used in this study (Bourque and Beaudette, 1982).

The state report of the *State-Trait Anxiety Inventory* (*STAI* Form YA) is a valid and reliable 20-item self-report inventory for measuring the state of anxiety (Spielberger et al., 1983). The scores range from 20 to 80 where higher scores indicate greater anxiety. A valid French version was administered (Bruchon-Schweitzer and Paulhan, 1993).

The Obsessive–Compulsive Drinking Scale (*OCDS*) is a questionnaire that assesses the cognitive aspects of alcohol craving during the preceding 7 days.(Anton et al., 1995) This 14-question questionnaire provides a global craving score, as well as two subscores: an obsessive score (6 items) and a compulsive score (8 items). A valid French version was used in this study (Ansseau et al., 2000).

Selective attention was evaluated with the validated double binary computerized task from the "Batterie d'Attention de William Lennox" (BAWL) in its version 4.0 (Leclercq, 2007). Briefly, the subject was asked to react when a specific target appeared on the screen by pressing as quickly as possible the response button on the computer. The reaction time needed to press the button is calculated for each of the 40 targets.

2.5. Statistical analyses

For each variable, normal distribution was tested with the Kolmogoroff–Smirnov test. As all biological measures exhibited non-normal distributions, a log-transformation was applied to obtain normality and statistical analyses were performed on log-transformed data. The data presented in the graphs are, however, non-transformed means ± standard error of the mean (SEM).

Biological and psychological measures were analyzed using *t*-tests. Paired *t*-tests were performed to compare alcohol-dependent (AD) subjects at T1 and T2. Unpaired *t*-tests were performed to compare AD to controls (CT). Concerning selective attention, to detect a practice effect as a result of test–retesting, the control group was tested twice and data were analyzed with repeated-measures analysis of variance (ANOVA), with time as a within factor (T1 vs. T2) and group as a between factor (AD vs. CT). Correlations were calculated using Pearson's moment correlations. Statistical analyses were performed using SPSS version 18.0 and graphs with GraphPad Prism version 4.0. Statistical significance was defined for a *p*-value lower than 0.05.

3. Results

3.1. Increased intestinal permeability and LPS in alcohol-dependent subjects and recovery after withdrawal

Intestinal permeability was measured by calculating the quantity of ^{51}Cr-EDTA found in urines. At T1, the small bowel permeability was significantly higher in alcohol-dependent subjects than in controls, $t(40) = 2.44$, $p < .05$. It decreased significantly from T1 to T2, $t(25) = 5.63$, $p < .001$, and this decrease was observed in 92.5% of patients. At T2, it did not differ anymore from controls, $t(39) = 1.01$, $p = .32$. At T1, alcohol-dependent subjects had higher colon and total intestinal permeability than those of controls but the difference between groups did not reach significance. However, both colon and total intestinal permeability decreased significantly during withdrawal, $t(25) = 2.22$, $p < .05$ and $t(25) = 3.66$, $p < .01$ (Fig. 1A). These observations suggest that the effects of alcohol on gut permeability are more prominent at the level of the small bowel than the colon.

Patients at T1 also exhibited higher plasma LPS concentrations than controls, $t(37) = 2.21$, $p < .05$. It significantly decreased during withdrawal, $t(23) = 2.27$, $p < .05$, and at T2, LPS levels did not differ from controls (Fig. 1B).

3.2. Induction of plasma cytokines in alcohol-dependent subjects and partial decrease after short-term withdrawal

The levels of pro-inflammatory cytokines TNFα and IL-6 were significantly higher in alcohol-dependent subjects than in controls at T1, $t(44) = 3.77$, $p < .001$ and $t(31) = 2.185$, $p < .05$, and at T2, $t(44) = 3.50$, $p < .01$ and $t(28) = 2.20$, $p < .05$ (Fig. 2A and B). The mean plasma cytokine concentrations decreased during withdrawal, although not significantly. The levels of plasma IL-6 decreased in only half of the patients. The TNFα concentrations decreased in 66% of patients but increased in 33% of them. HsCRP, which is one of the acute phase proteins that increases during systemic inflammation, was significantly higher in alcohol-dependent subjects than in controls at both T1, $t(47) = 2.45$, $p < .05$, and T2, $t(48) = 3.12$, $p < .01$ (Fig. 2D). There was no significant decrease of hsCRP levels from T1 to T2. The anti-inflammatory cytokine IL-10 was 2-fold higher in alcoholics at T1 than in controls but this difference did not reach significance (Fig. 2C). Surprisingly, plasma IL-10 decreased significantly during withdrawal, $t(26) = 4.20$, $p < .001$, and this decrease was observed in 85% of the patients. At the end of withdrawal, IL-10 returned to the same level as controls. These results permit us to conclude that alcohol-dependent subjects indeed present with a low-grade inflammation, which is defined by a 2- to 3-fold increase in inflammatory cytokines and acute phase proteins (Petersen and Pedersen, 2005; Ross, 1999).
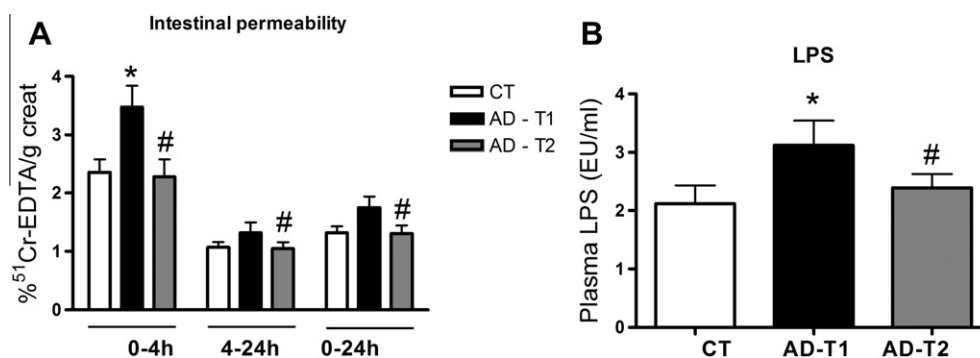
Fig. 1. A 3-week detoxification program induced a total recovery of intestinal permeability ($n = 26$) and blood LPS ($n = 27$). (A) Intestinal permeability was assessed by measurement of ^{51}Cr-EDTA excreted in urine. The first 4-h urinary collection reflects the small bowel permeability and the second 20-h collection large bowel permeability. Total intestinal permeability was also calculated. (B) Plasma LPS concentrations measured in alcohol-dependent subjects and in controls. Results are means ± SEM but corresponding p-values are derived from log-transformed data. Creat: creatinine; EU: endotoxin units; LPS: lipopolysaccharide; CT: control group; AD-T1 and AD-T2 refer respectively to the alcohol-dependent group at the onset and at the end of withdrawal. *$p < 0.05$ as determined by a two-tailed Student's unpaired t-test (comparison between alcoholics and controls). #$p < 0.05$ as determined by a two-tailed Student's paired t-test (comparison of alcohol-dependent subjects during withdrawal).
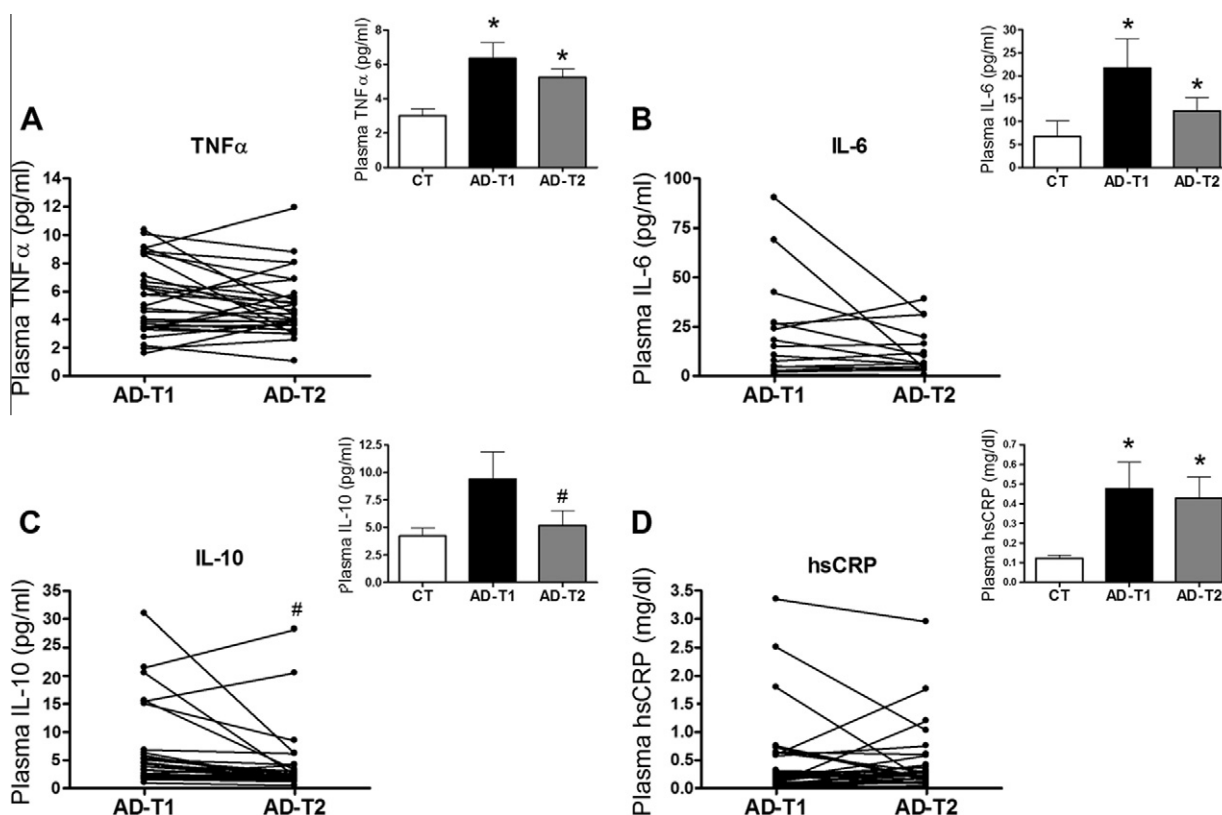

Fig. 2. Change in inflammatory state during alcohol-withdrawal. Plasma (A and B) pro-inflammatory and (C) anti-inflammatory cytokines levels and (D) plasma hsCRP in alcohol-dependent and in control group. Results are means ± SEM but corresponding p-values are derived from log-transformed data. TNFα: tumor necrosis factor α ($n = 30$); IL-6: interleukin-6 ($n = 16$); IL-10: interleukin-10 ($n = 26$); hsCRP: high-sensitive C-reactive protein ($n = 31$); CT: control group; AD-T1 and AD-T2 refer respectively to the alcohol-dependent group at the onset and at the end of withdrawal. *$p < 0.05$ as determined by a two-tailed Student's unpaired t-test (comparison between alcohol-dependents and controls. #$p < 0.05$ as determined by a two-tailed Student's paired t-test (comparison of alcohol-dependent subjects during withdrawal).

3.3. Recovery of psychological markers during alcohol-withdrawal

Depression and anxiety scores were significantly higher in alcohol-dependent subjects at T1 than in controls, $t(51) = 9.95$, $p < .001$ and $t(45) = 6.74$, $p < .001$, and decreased significantly from T1 to T2, $t(39) = 10.11$, $p < .001$ and $t(39) = 6.83$, $p < .001$. However, at the end of withdrawal, both scores remained significantly higher than those of controls, $t(50) = 4.51$, $p < .001$ and $t(42) = 3.18$, $p < .01$ (Fig. 3A and B). For craving, the total OCDS score as well as the obsession and compulsion subscores were significantly

higher at T1 in alcohol-dependent subjects than in controls, $t(53) = 22.61$, $p < .001$ and $t(45) = 20.94$, $p < .001$ and $t(51) = 19.86$, $p < .001$, and all three decreased significantly during withdrawal, $t(39) = 15.63$, $p < .001$ and $t(39) = 14.33$, $p < .001$ and $t(39) = 12.93$, $p < .001$, while remaining significantly higher than those of controls at the end of withdrawal, $t(50) = 5.51$, $p < .001$ and $t(45) = 6.04$, $p < .001$ and $t(53) = 3.88$, $p < .001$ (Fig. 3D–F). Selective attention was evaluated using reaction times from the double binary task of BAWL. The results of the repeated-measures ANOVA show a main effect of group, $F(1,54) = 19.98$, $p < 0.001$,
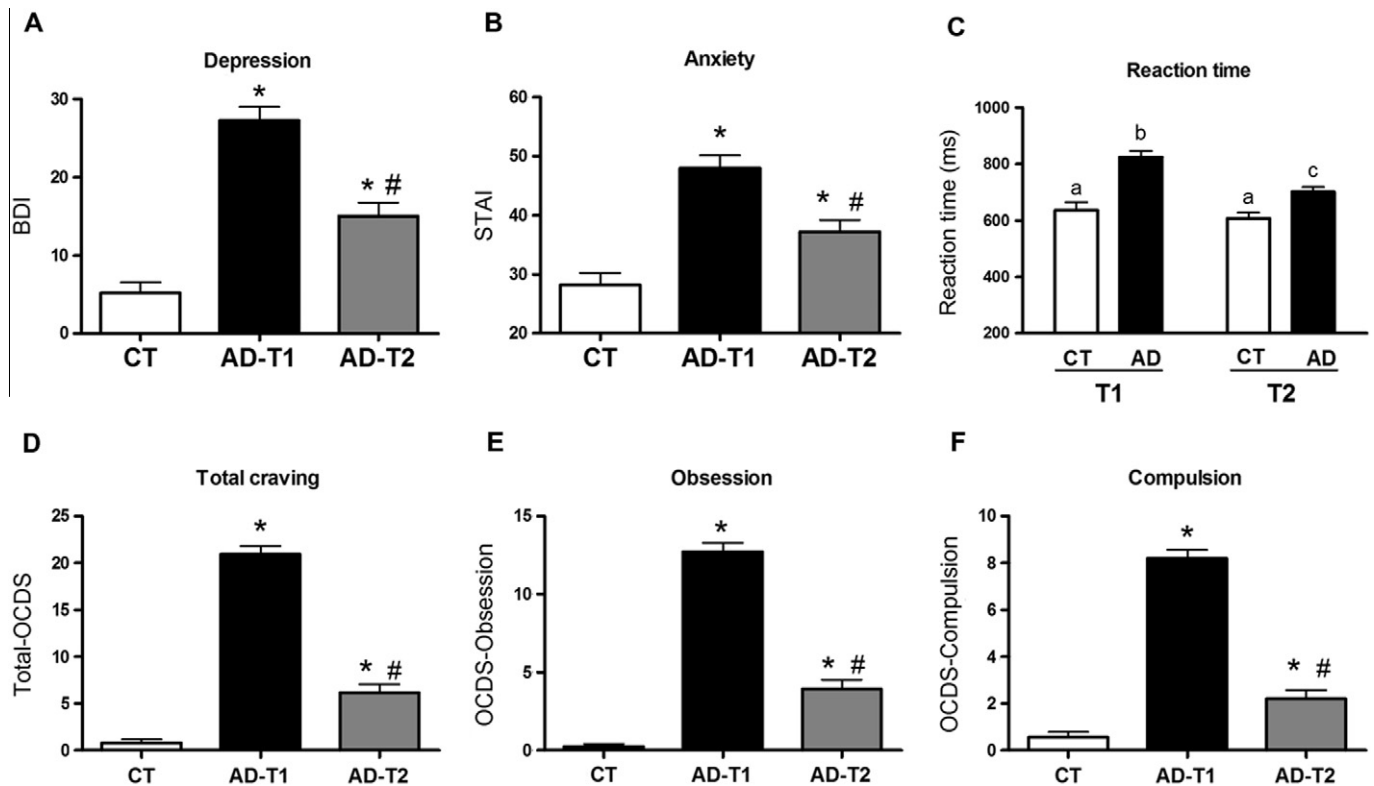
Fig. 3. Decrease in emotional, motivational and cognitive disturbances during alcohol-withdrawal ($n = 40$). (A and B) Comparison of levels of depression and anxiety in alcohol-dependent subjects and in controls. (C) Selective attention, measured by reaction times, was evaluated twice on each participant to detect a possible practice effect. Analyses were performed using repeated-measures analysis of variance (ANOVA), with time as a within factor (T1 vs. T2) and group as a between factor (alcohol-dependents vs. controls). (D–F) Comparison of total craving, obsessive and compulsive components of craving in alcohol-dependent and in control groups. Results are means ± SEM. BDI: Beck depression inventory; STAI: state-trait anxiety inventory; OCDS: obsessive–compulsive drinking scale; ms: milliseconds; ALC: alcohol-dependent group; CT: control group; AD-T1 and AD-T2 refer respectively to the alcohol-dependent group at the onset and at the end of withdrawal. $*p < 0.01$ as determined by a two-tailed Student's unpaired *t*-test (comparison between alcohol-dependents subjects and controls). $\#p < 0.001$ as determined by a two-tailed Student's paired *t*-test (comparison of alcohol-dependents during withdrawal). Data with different superscript letters were significantly different according to *t*-tests following 2×2 MANOVA statistical analysis.

$\eta^2 = 0.27$, meaning that the performances of alcohol-dependent subjects were lower than in controls. As expected, there was also a main effect of time, $F(1,54) = 17.13$, $p < 0.001$, $\eta^2 = 0.24$, indicating that subjects had a quicker reaction time at T2 than at T1 (practice effect) (Fig. 3C). Most importantly, there was a significant Time × Group interaction, $F(1,54) = 6.93$, $p = 0.011$, $\eta^2 = 0.11$, showing that reaction times in alcohol-dependent subjects improved more than those of controls from T1 to T2.

3.4. Correlations between biological and psychological factors

Cytokines have in the past been suggested to play a role in the development of psychiatric diseases and mostly in major depression (Dantzer and Kelley, 2007; Dantzer et al., 2008; Konsman et al., 2002; Maes, 1999). Here, we decided to test the possibility that intestinal permeability and inflammation that were observed to be abnormal in alcohol-dependent subjects could be related to some behavioral symptoms that are specific to alcohol-dependence, particularly depression, anxiety, selective attention and alcohol craving.

We first examined correlations in patients at T1. We found that IL-6 was positively correlated with depression ($r = 0.44$, $p < .05$). Moreover, all cytokines measured in this study were positively and significantly correlated with alcohol craving (TNFα: $r = 0.44$, $p < .05$; IL-6: $r = 0.52$, $p < .05$; IL-10: $r = 0.52$, $p < .01$; hsCRP: $r = 0.46$, $p < .01$). We also observed that selective attention was significantly correlated with small bowel permeability ($r = 0.38$, $p < .05$) (Fig. 4).

After 3 weeks of abstinence, it was the anti-inflammatory cytokine IL-10 that was this time negatively correlated with all psychological factors: depression ($r = -0.45$, $p < .05$), anxiety ($r = -0.44$, $p < .05$), craving ($r = -0.48$, $p < .05$) and selective attention ($r = -0.39$, $p = .05$). This suggests a potent psychological role for anti-inflammatory cytokines. However, the pro-inflammatory cytokine TNFα was still positively correlated with craving ($r = 0.41$, $p < .05$). Finally, we also observed that selective attention was positively correlated with large bowel permeability ($r = 0.39$, $p < .05$) and with hsCRP ($r = 0.37$, $p < .05$) (Fig. 4).

4. Discussion

Several animal and human studies support the view that alcohol-induced increase in intestinal permeability and portal LPS play a role in the development of alcoholic liver disease (Adachi et al., 1995; Bode and Bode, 2003; Keshavarzian et al., 2009; Mathurin et al., 2000; Mutlu et al., 2009; Parlesak et al., 2000; Ramachandran et al., 2002; Rao et al., 2004; Wheeler, 2003; Yan et al., 2010). However, these previous human studies have always been done in alcohol-dependent subjects that had already developed liver damage (Parlesak et al., 2000). The first aim of our study was to test whether intestinal permeability, circulating LPS and inflammatory response would be elevated in non-cirrhotic alcohol-dependent subjects. Our results clearly showed that these patients presented with a greater intestinal permeability than control subjects, predominantly in the small intestine. Interestingly, the permeability of all intestinal segments decreased significantly during
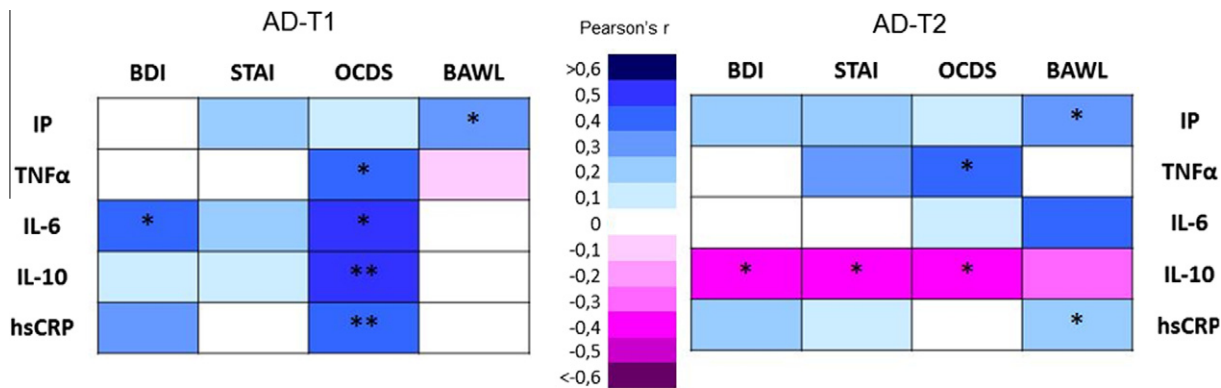
Fig. 4. Correlations between biological and psychological factors at the onset (T1) and at the end (T2) of withdrawal. BDI: Beck depression inventory; STAI: state-trait anxiety inventory; OCDS: obsessive compulsive drinking scale; BAWL: batterie d'attention de William Lennox; IP: intestinal permeability. *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$.

withdrawal and reached at T2 the level observed in controls. This confirms the deleterious effect of alcohol on the small intestine, where alcohol is mainly absorbed (Inserm, 2001). The rapid recovery suggests that 3 weeks of abstinence are sufficient to restore a functional gut barrier, probably because of the rapid turn-over of intestinal mucosa (Creamer et al., 1961).

Concerning circulating LPS that can be elevated in the case of gut impairment (Muccioli et al., 2010), we found that plasma LPS levels were significantly higher in alcohol-dependent group at T1 than in controls but returned to normal at T2. This observation supports the view that severe alcohol intake may increase LPS levels independently of the development of cirrhosis. Our study is the first to observe intestinal hyperpermeability and increased LPS levels in non-cirrhotic alcohol-dependent humans, thereby confirming the results of a recent study in rats (Keshavarzian et al., 2009). The increase in LPS levels can probably be attributed to the increase in small intestine permeability. However, we cannot exclude that small intestinal bacterial overgrowth, that characterizes alcohol-dependent subjects (Bode and Bode, 2003; Bode et al., 1993), might also contribute to the increase in LPS. As mentioned above and in contrast with a previous study performed in obese animals (Cani et al., 2009), we failed to find a significant correlation between intestinal permeability and plasma LPS concentrations. This discrepancy may be explained by two reasons: firstly, in our study, blood samples were taken from the antecubital and not from the portal vein; secondly, the probe we used to assess permeability was ^{51}Cr-EDTA, that has a molecular mass of 350 Da, whereas LPS have a molecular weight of 2000–20,000 Da (Caroff et al., 2002). Indeed, in a previous study, LPS were found to correlate with permeability only when probes of large molecular weight were used (Mathurin et al., 2000; Parlesak et al., 2000).

These bacterial lipopolysaccharides are the major components of the outer surface of Gram-negative bacteria and are considered to be powerful pro-inflammatory agents (Caroff and Karibian, 2003). We assessed the inflammatory markers of the alcohol-dependent subjects and observed that the levels of TNFα, IL-6, IL-10, and hsCRP were significantly higher than in controls at T1. Surprisingly, the anti-inflammatory cytokine IL-10 was the only one to totally recover at T2 whereas the pro-inflammatory cytokines TNFα, IL-6 and hsCRP only partially decreased. This indicated that IL-10 was the first cytokine that responded to the decrease in intestinal permeability and circulating LPS. Although the exact source of plasma cytokines remains unknown in alcohol-dependent subjects, we may hypothesize that IL-10, which is mainly produced by leukocytes (Sabat et al., 2010) and epithelial intestinal cells (Autschbach et al., 1998) could be more related to the intestine and that TNFα and IL-6 are mainly produced by the liver, after activation of Kupffer cells by LPS (Elsharkawy and Mann, 2007).

Indeed, IL-10 is commonly considered as a Th3 cytokine which is involved in immune tolerance by T cells at sites for first line of defense such as intestinal mucosa, where they have a protective role against the development of uncontrolled inflammation in the gut (Allez and Mayer, 2004). Moreover, IL-10 inhibits the production of pro-inflammatory cytokines by monocytes and macrophages (de Waal et al., 1991) and might hence play a significant role in maintaining a non-inflammatory immune status in the normal intestine (Autschbach et al., 1998). In case of inflammatory bowel disease (IBD), the immunosuppressive effect of IL-10 is probably not sufficient to control local inflammation (Autschbach et al., 1998; Kucharzik et al., 1995) since patients with IBD also have high levels of pro- and anti-inflammatory cytokines (Kucharzik et al., 1995). Our data also suggested that in alcohol-dependent subjects in whom there is systemic immune activation, the anti-inflammatory cytokines are increased to counteract the actions of pro-inflammatory cytokines. This may explain the strong correlations between IL-10 and TNFα (T1: $r = 0.50$, $p < .01$; T2: $r = 0.51$, $p < .01$) or IL-6 (T1: $r = 0.74$, $p < .001$; T2: $r = 0.45$, $p = .06$) that we found in our study. Another evidence for the role of IL-10 in the intestine immune tolerance is the observation that IL-10 knock-out mice spontaneously develop chronic enterocolitis (Kuhn et al., 1993). All of these data support the fact that the recovery of plasma IL-10 observed in alcohol-dependent subjects during withdrawal could be related to the recovery of local inflammatory state that induced intestinal permeability. On the other hand, the explanation for the lack of recovery of the pro-inflammatory cytokines is unclear. It could be due to the persistence of liver inflammation. There is evidence of ongoing liver damage at the end of a 3-week withdrawal, as assessed by the persistence of liver stiffness on transient elastography measures after 30 days of abstinence (Gelsi et al., 2011) and the absence of total recovery of cytolytic enzymes observed in our patients (data not shown). However, we can also propose the hypothesis that the incomplete resolution of the general immune response could also contribute to the continuing low-grade inflammation observed at the end of the detoxification program.

Our study also has a second important aim: to test the possible role of these markers of permeability and inflammation on psychological factors that play a central role in the development of alcohol-dependence. We therefore decided to assess psychological factors that play a role in the development of alcohol-dependence but that are also known to change during withdrawal. We observed, as expected, that scores of depression, anxiety, alcohol craving and selective attention were largely altered in alcohol-dependent subjects. All the scores recovered during withdrawal, following a pattern parallel to that of biological markers. However, despite a total recovery of intestinal permeability and circulating

LPS after alcohol-withdrawal, markers of inflammation and of psychological distress had only partially recovered. This suggests that they are not related only to the intestinal permeability.

To test this last hypothesis, we looked for correlations between biological and psychological factors. Previous animal or human studies have shown some emotional and cognitive disturbances when inflammation was artificially induced by the injection of LPS. For instance, Sparkman et al. (2006) reported that IL-6 could be a key mediator of the deficit in working memory in mice injected with LPS. Reichenberg et al. (2001) reported that LPS injection in humans induced memory dysfunctions and an increase in depression and anxiety. Moreover, LPS-induced depression and anxiety were correlated with cytokines and cortisol levels. Interestingly, we observed that alcohol-drinking is a condition that naturally induces changes in LPS, intestinal permeability and cytokines, and hence resembles the artificial situations described above. In accordance with these previous reports, we observed at T1, when LPS levels are elevated, a correlation between the pro-inflammatory cytokine IL-6 and depression. However, we also found at T1 that cytokines IL-6, TNFα and IL-10 are correlated with alcohol craving. It is, to our knowledge, the first observation of a relationship between inflammation and craving in alcohol-dependent subjects, where craving is expected to play a central role in drinking behavior. Selective attention correlated with intestinal permeability at both times of withdrawal. More importantly, we found at T2 that IL-10 was negatively correlated with depression and alcohol craving. These two markers are known to be important predictors of relapse after withdrawal (Andersohn and Kiefer, 2004) suggesting therefore an important role for the anti-inflammatory cytokine in abstinent alcohol-dependent subjects. Animal studies have suggested a role for IL-10 to prevent the effects of LPS on mood and cognition. Bluthe et al. (1999) showed that IL-10 injection in mice abrogated the depressive symptoms induced by central or peripheral LPS administration. More recently, LPS-induced fatigue and deficits in psychomotor coordination were found to be exacerbated in mice deficient in IL-10 (Krzyszton et al., 2008). Richwine et al. (2009) reported that LPS injection induced cognitive disturbances in IL-10$^{-/-}$ mice but not in wild-type mice. However, only one recent study mentioned a positive impact of IL-10 on human cognitive performances (van den Boogaard et al., 2010). Whether IL-10 influences behavior through an inhibition of the secretion of pro-inflammatory cytokines (Bluthe et al., 1999; Di et al., 1995) or through a direct effect on the brain (Mesquita et al., 2008) is still under debate.

In conclusion, our study is the first to demonstrate a relationship between heavy chronic alcohol consumption, increased intestinal permeability, increased circulating LPS and low-grade inflammation in non-cirrhotic alcohol-dependent subjects. Furthermore, the parallel changes of these biological markers and of psychological markers related to drinking behavior observed during withdrawal, suggest a role for a gut–brain interaction in alcohol-dependence. While pro-inflammatory cytokines are positively correlated with depression and craving at the onset of withdrawal, the anti-inflammatory cytokine IL-10 becomes negatively correlated with depression and craving at the end of the detoxification program, suggesting a potential protective role for this cytokine against relapse. Moreover, we can suggest that the incomplete recovery of pro-inflammatory cytokines after a short-term withdrawal could also contribute to the high number of relapses observed among these patients within several weeks or months following the detoxification program.

Because correlation does not imply causal connections, further experimental studies using agents that directly affect the levels of inflammation are definitely needed to reinforce the evidence of the important role of cytokines in the behavior of AD subjects. However, this potential gut–brain interaction opens a new field of research for pharmacological interventions in alcohol-dependent subjects, targeting the gut or inflammation. Indeed current pharmacological approaches of alcohol-dependence that only target brain neurotransmitters have proved so far to be modestly efficient (Johnson, 2010), eventually because they fail to take into account of intestinal permeability and inflammation as a driving force for the psychological components of alcoholic pathology. An interesting perspective would be to test whether nutritional factors such as probiotics or prebiotics that are known to have positive impacts on the gut barrier (Delzenne et al., 2011) will be able to reduce inflammation and improve mood and craving, as a new treatment of alcohol-dependence.

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.bbi.2012.04.001.

References

Adachi, Y., Moore, L.E., Bradford, B.U., Gao, W., Thurman, R.G., 1995. Antibiotics prevent liver injury in rats following long-term exposure to ethanol. Gastroenterology 108, 218–224.

Akira, S., Hemmi, H., 2003. Recognition of pathogen-associated molecular patterns by TLR family. Immunol. Lett. 85, 85–95.

Allez, M., Mayer, L., 2004. Regulatory T cells. Peace keepers in the gut. Inflamm. Bowel Dis. 10, 666–676.

Andersohn, F., Kiefer, F., 2004. Depressive mood and craving during alcohol withdrawal: association and interaction. German J. Psychiatry 7, 6–11.

Anderson, P., Baumberg, B., 2006. Alcohol in Europe – public health perspective: report summary. Drugs: Educ. Prev. Policy 13, 483–488.

Ansseau, M., Besson, J., Lejoyeux, M., Pinto, E., Landry, U., Cornes, M., Deckers, F., Potgieter, A., Ades, J., 2000. A French translation of the obsessive-compulsive drinking scale for craving in alcohol-dependent patients: a validation study in Belgium, France, and Switzerland. Eur. Addict. Res. 6, 51–56.

Anton, R.F., 1999. What is craving? Models and implications for treatment. Alcohol Res. Health 23, 165–173.

Anton, R.F., Moak, D.H., Latham, P., 1995. The Obsessive Compulsive Drinking Scale: a self-rated instrument for the quantification of thoughts about alcohol and drinking behavior. Alcohol Clin. Exp. Res. 19, 92–99.

APA, 1994. Diagnostic and Statistical Manual of Mental Disorders, fourth ed. American Psychiatric Association.

Autschbach, F., Braunstein, J., Helmke, B., Zuna, I., Schurmann, G., Niemir, Z.I., Wallich, R., Otto, H.F., Meuer, S.C., 1998. In situ expression of interleukin-10 in noninflamed human gut and in inflammatory bowel disease. Am. J. Pathol. 153, 121–130.

Beck, A.T., Steer R.A., Brown, G.K., 1996. Manual for the Beck Depression Inventory-II. Psychological Corporation, TX, San Antonio.

Bedossa, P., Poynard, T., 1996. An algorithm for the grading of activity in chronic hepatitis C. The METAVIR Cooperative Study Group. Hepatology 24, 289–293.

Bjarnason, I., Macpherson, A., Hollander, D., 1995. Intestinal permeability: an overview. Gastroenterology 108, 1566–1581.

Bluthe, R.M., Castanon, N., Pousset, F., Bristow, A., Ball, C., Lestage, J., Michaud, B., Kelley, K.W., Dantzer, R., 1999. Central injection of IL-10 antagonizes the

behavioural effects of lipopolysaccharide in rats. Psychoneuroendocrinology 24, 301–311.

Bode, C., Bode, J.C., 2003. Effect of alcohol consumption on the gut. Best. Pract. Res. Clin. Gastroenterol. 17, 575–592.

Bode, C., Kolepke, R., Schafer, K., Bode, J.C., 1993. Breath hydrogen excretion in patients with alcoholic liver disease – evidence of small intestinal bacterial overgrowth. Z. Gastroenterol. 31, 3–7.

Bourque, P., Beaudette, D., 1982. Etude psychométrique du questionnaire de dépression de Beck auprès d'un échantillon d'étudiants universitaires francophones. Can. J. Behav. Sci./Revue canadienne des sciences du comportement 14, 211–218.

Bruchon-Schweitzer, M., Paulhan, I., 1993. Le Manuel du STAY-Y (French version of the STAY-Y), ECPA, Paris.

Cani, P.D., Amar, J., Iglesias, M.A., Poggi, M., Knauf, C., Bastelica, D., Neyrinck, A.M., Fava, F., Tuohy, K.M., Chabo, C., Waget, A., Delmee, E., Cousin, B., Sulpice, T., Chamontin, B., Ferrieres, J., Tanti, J.F., Gibson, G.R., Casteilla, L., Delzenne, N.M., Alessi, M.C., Burcelin, R., 2007. Metabolic endotoxemia initiates obesity and insulin resistance. Diabetes 56, 1761–1772.

Cani, P.D., Bibiloni, R., Knauf, C., Waget, A., Neyrinck, A.M., Delzenne, N.M., Burcelin, R., 2008. Changes in gut microbiota control metabolic endotoxemia-induced inflammation in high-fat diet-induced obesity and diabetes in mice. Diabetes 57, 1470–1481.

Cani, P.D., Possemiers, S., Van de Wiele, T., Guiot, Y., Everard, A., Rottier, O., Geurts, L., Naslain, D., Neyrinck, A., Lambert, D.M., Muccioli, G.G., Delzenne, N.M., 2009. Changes in gut microbiota control inflammation in obese mice through a mechanism involving GLP-2-driven improvement of gut permeability. Gut 58, 1091–1103.

Caroff, M., Karibian, D., 2003. Structure of bacterial lipopolysaccharides. Carbohydr. Res. 338, 2431–2447.

Caroff, M., Karibian, D., Cavaillon, J.M., Haeffner-Cavaillon, N., 2002. Structural and functional analyses of bacterial lipopolysaccharides. Microbes Infect. 4, 915–926.

Cordovil De Sousa Uva, M., Luminet, O., Cortesi, M., Constant, E., Derely, M., de Timary, P., 2010. Distinct effects of protracted withdrawal on affect, craving, selective attention and executive functions among alcohol-dependent patients. Alcohol Alcohol. 45, 241–246.

Creamer, B., Shorter, R.G., Bamforth, J., 1961. The turnover and shedding of epithelial cells. I. The turnover in the gastro-intestinal tract. Gut 2, 110–118.

Dantzer, R., Kelley, K.W., 2007. Twenty years of research on cytokine-induced sickness behavior. Brain Behav. Immun. 21, 153–160.

Dantzer, R., O'Connor, J.C., Freund, G.G., Johnson, R.W., Kelley, K.W., 2008. From inflammation to sickness and depression: when the immune system subjugates the brain. Nat. Rev. Neurosci. 9, 46–56.

De, M.S., Cecchin, E., Basile, A., Bertotti, A., Nardini, R., Bartoli, E., 1993. Renal tubular dysfunction in chronic alcohol abuse – effects of abstinence. N. Engl. J. Med. 329, 1927–1934.

de Timary, P., Luts, A., Hers, D., Luminet, O., 2008. Absolute and relative stability of alexithymia in alcoholic inpatients undergoing alcohol withdrawal: relationship to depression and anxiety. Psychiatry Res. 157, 105–113.

de Waal, M.R., Abrams, J., Bennett, B., Figdor, C.G., de Vries, J.E., 1991. Interleukin 10 (IL-10) inhibits cytokine synthesis by human monocytes: an autoregulatory role of IL-10 produced by monocytes. J. Exp. Med. 174, 1209–1220.

Delzenne, N.M., Neyrinck, A.M., Backhed, F., Cani, P.D., 2011. Targeting gut microbiota in obesity: effects of prebiotics and probiotics. Nat. Rev. Endocrinol..

Di, S.E., Sironi, M., Pozzi, P., Gnocchi, P., Isetta, A.M., Delvaux, A., Goldman, M., Marchant, A., Ghezzi, P., 1995. Interleukin-10 inhibits lipopolysaccharide-induced tumor necrosis factor and interleukin-1 beta production in the brain without affecting the activation of the hypothalamus–pituitary–adrenal axis. Neuroimmunomodulation 2, 149–154.

Dowlati, Y., Herrmann, N., Swardfager, W., Liu, H., Sham, L., Reim, E.K., Lanctot, K.L., 2010. A meta-analysis of cytokines in major depression. Biol. Psychiatry 67, 446–457.

Elsharkawy, A.M., Mann, D.A., 2007. Nuclear factor-kappaB and the hepatic inflammation–fibrosis–cancer axis. Hepatology 46, 590–597.

Everard, A., Lazarevic, V., Derrien, M., Girard, M., Muccioli, G.G., Neyrinck, A.M., Possemiers, S., Van, H.A., Francois, P., de Vos, W.M., Delzenne, N.M., Schrenzel, J., Cani, P.D., 2011. Responses of gut microbiota and glucose and lipid metabolism to prebiotics in genetic obese and diet-induced leptin-resistant mice. Diabetes 60, 2775–2786.

Fukui, H., Brauner, B., Bode, J.C., Bode, C., 1991. Plasma endotoxin concentrations in patients with alcoholic and non-alcoholic liver disease: reevaluation with an improved chromogenic assay. J. Hepatol. 12, 162–169.

Gelsi, E., Dainese, R., Truchi, R., Mariné-Barjoan, E., Anty, R., Autuori, M., Burroni, S., Vanbiervliet, G., Evesque, L., Cherikh, F., Tran, A., 2011. Effect of detoxification on liver stiffness assessed by Fibroscan® in alcoholic patients. Alcohol. Clin. Exp. Res. 35, 566–570.

Grant, B.F., Dawson, D.A., Stinson, F.S., Chou, S.P., Dufour, M.C., Pickering, R.P., 2004. The 12-month prevalence and trends in DSM-IV alcohol abuse and dependence. United States, 1991–1992 and 2001–2002. Drug Alcohol Depend. 74, 223–234.

Inserm, 2001. Alcool. Effets sur la santé. Expertise collective. Paris.

Johnson, B.A., 2010. Medication treatment of different types of alcoholism. Am. J. Psychiatry 167, 630–639.

Keshavarzian, A., Farhadi, A., Forsyth, C.B., Rangan, J., Jakate, S., Shaikh, M., Banan, A., Fields, J.Z., 2009. Evidence that chronic alcohol exposure promotes intestinal oxidative stress, intestinal hyperpermeability and endotoxemia prior to development of alcoholic steatohepatitis in rats. J. Hepatol. 50, 538–547.

Konsman, J.P., Parnet, P., Dantzer, R., 2002. Cytokine-induced sickness behaviour: mechanisms and implications. Trends Neurosci. 25, 154–159.

Krzyszton, C.P., Sparkman, N.L., Grant, R.W., Buchanan, J.B., Broussard, S.R., Woods, J., Johnson, R.W., 2008. Exacerbated fatigue and motor deficits in interleukin-10-deficient mice after peripheral immune stimulation. Am. J. Physiol. Regul. Integr. Comp. Physiol. 295, R1109–R1114.

Kucharzik, T., Stoll, R., Lugering, N., Domschke, W., 1995. Circulating antiinflammatory cytokine IL-10 in patients with inflammatory bowel disease (IBD). Clin. Exp. Immunol. 100, 452–456.

Kuhn, R., Lohler, J., Rennick, D., Rajewsky, K., Muller, W., 1993. Interleukin-10-deficient mice develop chronic enterocolitis. Cell 75, 263–274.

Leclercq, M., 2007. Batterie d'attention William Lennox (BAWL) version 4.0. Louvain-la-Neuve, Belgium.

Luoto, R., Kalliomaki, M., Laitinen, K., Delzenne, N.M., Cani, P.D., Salminen, S., Isolauri, E., 2011. Initial dietary and microbiological environments deviate in normal-weight compared to overweight children at 10 years of age. J. Pediatr. Gastroenterol. Nutr. 52, 90–95.

Maes, M., 1999. Major depression and activation of the inflammatory response system. Adv. Exp. Med. Biol. 461, 25–46.

Mathurin, P., Deng, Q.G., Keshavarzian, A., Choudhary, S., Holmes, E.W., Tsukamoto, H., 2000. Exacerbation of alcoholic liver injury by enteral endotoxin in rats. Hepatology 32, 1008–1017.

Mesquita, A.R., Correia-Neves, M., Roque, S., Castro, A.G., Vieira, P., Pedrosa, J., Palha, J.A., Sousa, N., 2008. IL-10 modulates depressive-like behavior. J. Psychiatr. Res. 43, 89–97.

Muccioli, G.G., Naslain, D., Backhed, F., Reigstad, C.S., Lambert, D.M., Delzenne, N.M., Cani, P.D., 2010. The endocannabinoid system links gut microbiota to adipogenesis. Mol. Syst. Biol. 6, 392.

Mutlu, E., Keshavarzian, A., Engen, P., Forsyth, C.B., Sikaroodi, M., Gillevet, P., 2009. Intestinal dysbiosis: a possible mechanism of alcohol-induced endotoxemia and alcoholic steatohepatitis in rats. Alcohol Clin. Exp. Res. 33, 1836–1846.

Parlesak, A., Schafer, C., Schutz, T., Bode, J.C., Bode, C., 2000. Increased intestinal permeability to macromolecules and endotoxemia in patients with chronic alcohol abuse in different stages of alcohol-induced liver disease. J. Hepatol. 32, 742–747.

Peeters, M., Hiele, M., Ghoos, Y., Huysmans, V., Geboes, K., Vantrappen, G., Rutgeerts, P., 1994. Test conditions greatly influence permeation of water soluble molecules through the intestinal mucosa: need for standardisation. Gut 35, 1404–1408.

Petersen, A.M., Pedersen, B.K., 2005. The anti-inflammatory effect of exercise. J. Appl. Physiol. 98, 1154–1162.

Ramachandran, A., Prabhu, R., Thomas, S., Reddy, J.B., Pulimood, A., Balasubramanian, K.A., 2002. Intestinal mucosal alterations in experimental cirrhosis in the rat: role of oxygen free radicals. Hepatology 35, 622–629.

Rao, R.K., Seth, A., Sheth, P., 2004. Recent advances in alcoholic liver disease I. Role of intestinal permeability and endotoxemia in alcoholic liver disease. Am. J. Physiol. Gastrointest. Liver Physiol. 286, G881–G884.

Reichenberg, A., Yirmiya, R., Schuld, A., Kraus, T., Haack, M., Morag, A., Pollmacher, T., 2001. Cytokine-associated emotional and cognitive disturbances in humans. Arch. Gen. Psychiatry 58, 445–452.

Richwine, A.F., Sparkman, N.L., Dilger, R.N., Buchanan, J.B., Johnson, R.W., 2009. Cognitive deficits in interleukin-10-deficient mice after peripheral injection of lipopolysaccharide. Brain Behav. Immun. 23, 794–802.

Ross, R., 1999. Atherosclerosis – an inflammatory disease. N. Engl. J. Med. 340, 115–126.

Sabat, R., Grutz, G., Warszawska, K., Kirsch, S., Witte, E., Wolk, K., Geginat, J., 2010. Biology of interleukin-10. Cytokine Growth Factor Rev. 21, 331–344.

Schuckit, M.A., 1994. Alcohol and depression: a clinical perspective. Acta Psychiatr. Scand. Suppl. 377, 28–32.

Sparkman, N.L., Buchanan, J.B., Heyen, J.R., Chen, J., Beverly, J.L., Johnson, R.W., 2006. Interleukin-6 facilitates lipopolysaccharide-induced disruption in working memory and expression of other proinflammatory cytokines in hippocampal neuronal cell layers. J. Neurosci. 26, 10709–10716.

Spielberger, C.D., Gorsuch, R.L., Lushene, R., Vagg, P.R., Jacobs, G.A., 1983. Manual for the State-Trait Anxiety Inventory. Consulting Psychologists Press, Palo Alto. CA.

van den Boogaard, M., Ramakers, B.P., van, A.N., van der Werf, S.P., Fick, W.F., Hoedemaekers, C.W., Verbeek, M.M., Schoonhoven, L., van der Hoeven, J.G., Pickkers, P., 2010. Endotoxemia-induced inflammation and the effect on the human brain. Crit. Care 14, R81.

Wheeler, M.D., 2003. Endotoxin and Kupffer cell activation in alcoholic liver disease. Alcohol Res. Health 27, 300–306.

Yan, A.W., Fouts, E., Brandl, J., Starkel, P., Torralba, M., Schott, E., Tsukamoto, H., Nelson, D., Brenner, A., Schnabl, B., 2010. Enteric dysbiosis associated with a mouse model of alcoholic liver disease. Hepatology.