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ORIGINAL ARTICLE

Elevated alanine aminotransferase independently predicts new onset of depression in employees undergoing health screening examinations

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Background. Non-alcoholic fatty liver disease (NAFLD) is the most common cause of elevated alanine aminotransferase (ALT). NAFLD is associated with insulin resistance and hepatic inflammation. Similarly, patients with depression exhibit insulin resistance and increased inflammatory markers. However, no study has shown a clear association between elevated ALT and the development of depression. The aim of the study was to test whether elevated ALT, a surrogate marker for NAFLD, predicts the development of depression.

Method. The present prospective cohort study investigated 12 180 employed adults referred for health examinations that included fasting blood tests and anthropometric measurements between 2003 and 2010. Exclusion criteria were: baseline minor/major depression, excessive alcohol consumption and other causes for ALT elevation. Depression was evaluated by the eight-item Patient Health Questionnaire (PHQ-8) score.

Results. The final cohort included 5984 subjects [69.4% men, aged 45.0 (S.D. = 10.24) years]. The incidence rate of minor and major depression was 3.8% and 1.4%, respectively. Elevated ALT was a significant independent predictor for the occurrence of minor [odds ratio (OR) 2.02, 95% confidence interval (CI) 1.40–2.92] and major (OR 3.132, 95% CI 1.81–5.40) depression after adjusting for age, gender, body mass index, education level, serum levels of lipids, glucose, smoking and physical activity. Adding subjective health and affective state parameters (sleep disturbances, self-rated health, anxiety and burnout) as potential mediators only slightly ameliorated the association. Persistently elevated ALT was associated with the greatest risk for minor or major depression as compared with elevation only at baseline or follow-up (p for trend <0.001).

Conclusions. Elevated ALT was associated with developing depressive symptoms, thus suggesting that NAFLD may represent an independent modifiable risk factor for depression.

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Key words: Alanine aminotransferase, depression, non-alcoholic fatty liver disease, prospective cohort studies.

Introduction

Non-alcoholic fatty liver disease (NAFLD) is emerging as one of the most common liver diseases in the Western world affecting as many as 30% of the adult western population (Bellentani *et al.* 2000; Browning *et al.* 2004; Zelber-Sagi *et al.* 2006; Baumeister *et al.* 2008). About 2–3% of the general population is also suspected of having non-alcoholic steatohepatitis (NASH), characterized by hepatic inflammation, which increases the risk for hepatic fibrosis, cirrhosis and hepatocellular carcinoma (Wong *et al.* 2010). NAFLD/NASH is closely associated with the metabolic syndrome (Bugianesi *et al.* 2010) and with poor nutritional and physical activity habits (Zelber-Sagi *et al.* 2011).

Recent studies suggest that NAFLD is also associated with impaired mental well-being, and specifically with depression. NAFLD patients have been shown to have a decreased quality of life (QOL) (David *et al.* 2009), as manifested by worse physical and mental health scores, compared with an American population

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with and without chronic illness (Elwing *et al.* 2006; Newton *et al.* 2008). NAFLD patients have also been demonstrated to have a significantly increased lifetime risk for major depression and generalized anxiety disorder compared with controls (Elwing *et al.* 2006). However, the retrospective design of that study prevented inference regarding the direction of the association and did not account for the possible confounding effect of other affective states. Similarly, a study that looked at the rate of depression in patients with chronic liver disease showed the rate to be highest (30%) in hepatitis C virus (HCV), followed closely by NAFLD (27%) (Weinstein *et al.* 2011). Another study assessed the psychosocial outcomes of children with NAFLD as compared with obese controls and found that children with NAFLD had higher levels of depression than obese controls (Kerkar *et al.* 2013). Several recent abstracts, yet to be published in full, from international meetings have reported an association between NAFLD and psychological states such as depression, psychiatric illness and attention deficit hyperactivity disorders (Lee & Jonas, 2007; Sayuk *et al.* 2007; Suzuki *et al.* 2007). Specifically, Sayuk *et al.* (2007) reported an association between elevated transaminases and depression among NASH patients.

Given the high prevalence of depression (Simon *et al.* 2002) and its physiological manifestations such as the metabolic syndrome (Toker *et al.* 2008) or cardiovascular morbidity and mortality (Lett *et al.* 2004), finding out the physiological antecedents of depression may help identify populations at risk. Nevertheless, no study to date has focused on liver enzymes, a marker of hepatocellular damage, as a risk factor for new-onset depressive symptoms.

The mechanisms governing the association between NAFLD/NASH and depressive disorders are poorly understood and complex (Elwing *et al.* 2006). The possible mechanisms include cytokine-mediated inflammation, activation of the hypothalamic–pituitary–adrenal (HPA) axis, and the effects of insulin resistance on neurotransmission. An accumulating body of evidence suggests that NASH is a chronic low-grade inflammatory state (Fujii & Kawada, 2012). It has been postulated that several cytokines, including interleukin (IL)-1α/β, tumour necrosis factor-α and IL-6 are responsible for depressive changes in patients with inflammatory conditions (Raison *et al.* 2006). It was further shown in a mouse model that injection of these cytokines induces 'sickness behavior' that is similar to depressive symptoms in humans (Dantzer *et al.* 2008). Indeed, these same cytokines were shown to be involved in the pathogenesis of NASH (Tilg, 2010). These cytokines may induce malfunctioning of the serotonergic neurotransmission in the brain, resulting in depression (Milaneschi *et al.* 2009; Dantzer *et al.* 2011). Insulin resistance may also contribute to the diminished serotonergic activity in the central nervous system (Muldoon *et al.* 2006; Herrera-Marquez *et al.* 2011). Cortisol excess is often associated with insulin resistance and may result in a state resembling HPA axis hyperactivity also encountered in depression (Brown *et al.* 2004; Cowen, 2010). Furthermore, adipokines (leptin, adiponectin and resistin) which are altered in NASH are also considered to be involved in the pathogenesis of depression (Taylor & Macqueen, 2010).

Liver function tests are commonly used to screen large populations for the presence of liver disease. Although elevated ALT is estimated to have low sensitivity and thus underestimates patients with NAFLD, NAFLD patients presenting with elevated ALT may represent patients with a higher inflammatory component (Fracanzani *et al.* 2008, 2011; Kashyap *et al.* 2009). Compared with aspartate aminotransferase (AST) that is not specific to liver disease and is present in a wide variety of tissues, alanine aminotransferase (ALT) is present mostly in the liver and is more commonly used as a specific marker of hepatocyte damage (Pratt & Kaplan, 2000; Green & Flamm, 2002). A recent report suggests that NASH/NAFLD is the most common cause of abnormal liver function in a primary care setting, accounting for more than a quarter of the patients (Armstrong *et al.* 2012). Our aim was to explore whether elevated ALT, as a surrogate marker for NAFLD, predicts the incidence of depression in a large prospective cohort of employed men and women.

Method

Study sample and design

Study participants were 12 180 employed men and women, aged 20 to 69 years, referred for periodic health examination at the Tel-Aviv Sourasky Medical Center, Tel Aviv, Israel. Each patient underwent a baseline examination between 2003 and 2010 during which study participants were enrolled to the Tel-Aviv Medical Center Inflammation Survey cohort study, representing 92% of the center's examinees.

Participants were followed until 2010. The period between the baseline examination and follow-up ranged from 6 months to 8 years (median of 29 months). Follow-up data were obtained for 9622 participants (79% of the original sample). No follow-up data were available for the remaining sample owing to change of employer or health care provider.

We excluded from our analysis participants with minor or major depression at baseline [according to the Patient Health Questionnaire (PHQ) score] or who

were taking one or more of the following antidepressant medications: clomipramine, mianserin, escitalopram, citaloparm, duloxetine, tolterodine, bupropion, fluoxetine, imipramine, opipramol, dibenzepin, maprotiline, anafranil, pregabalin, trazodone, paroxetine, extract of hypericum perforatum, sertraline, lithium and reboxetine, regardless of the indication for the medication, either at baseline or during follow-up (n=1462). In addition, we excluded participants with alcohol consumption ⩾14 drinks per week for both genders (n=62), participants that had other diseases known to cause elevations of liver enzymes (e.g. celiac, history of jaundice, fever of unknown origin, inflammatory bowel disease, familial Mediterranean fever, systemic lupus erythematosis, history of Epstein–Barr virus, cytomegalovirus or fungal infections, osteoarthritis or gout or psoriasis treated with medications throughout the study period) (n=116). We also excluded participants taking medications that are known to increase liver enzymes (e.g. steroids, immunosuppressant drugs and amiodarone) (n=81). Participants with outlier values that were thought to imply measurement error or morbidities other than NAFLD (n=1251), and participants with missing data for one of the study variables were also excluded (n=666). Overall, the included participants were healthier and had a higher socio-economic status compared with the excluded participants.

The final sample consisted of 5984 healthy participants, representing a wide variety of occupations: high and low technology (28%), teaching or academia (21%), administration (21%), sales and services (7%), blue collar (15%) and health care (2%).

The study protocol was approved by the ethics committee of the Sourasky Medical Center and participants signed an informed consent form.

Measures

Depressive symptoms

Depressive symptoms were assessed by the eight-item PHQ (PHQ-8), an abbreviated version of the earlier nine-item PHQ (PHQ-9) measure of depression. This measure is a patient-oriented, self-administered instrument derived from the Primary Care Evaluation of Mental Disorders (PRIME-MD; Kroenke *et al.* 2009). It lists eight potential symptoms of depression in accordance with the DSM-IV criteria. The validity of the PHQ-8 and PHQ-9, which are highly correlated (see Pressler *et al.* 2011), as a diagnostic and severity measure for depressive disorders has been confirmed in large clinical and non-clinical studies (Kroenke *et al.* 2009). This measure has strong psychometric properties (Kroenke *et al.* 2010; Pressler *et al.* 2011) and high sensitivity and specificity for detecting depressive disorders (Kroenke *et al.* 2010). The PHQ-9 and the PHQ-8 have been used in several studies among Israelis (Hobfoll *et al.* 2006, 2011; Toker & Biron, 2012) and a recent study, conducted among elders in Israel, concluded that the PHQ-9 has a sensitivity of 66.6% and specificity of 98.6% relative to the Structured Clinical Interview for DSM-IV (SCID-I) (Ayalon *et al.* 2010).

In the present study, three levels were used at follow-up: no or minimal symptoms (scores 0–9), minor depression (scores 10–14) and moderately severe or major depression (scores 15 and above).

Biochemical parameters

Biochemical parameters included fasting blood tests (12 h fast) of: serum lipids, glucose and high-sensitivity C-reactive protein (hs-CRP) as a marker of inflammation (Schillinger *et al.* 2003), serum ALT, AST and γ-glutamyl transpeptidase. Serum ALT levels were analysed by a commercially available ADVIA 1650 chemistry system (Siemens Healthcare Diagnostics Inc., USA). The upper limits of normal were defined as ⩾39 international units (IU) for men and ⩾35 IU for women (as defined by the Tel-Aviv Sourasky Medical Center laboratories) and analyses with ALT as a continuous variable were also performed.

Covariates

In the analysis we controlled for several factors that were found to be associated with either depression or ALT.

(1) Sociodemographic measures: age, gender and years of education;
(2) Other affective states: job burnout was assessed using the Shirom–Melamed Burnout Measure (Shirom, 1989, 2003), due to the reciprocal relationship found between burnout and depression in past studies (Toker & Biron, 2012). Anxiety was measured based on validated questionnaires adapted from the Institute of Social Research, University of Michigan (French *et al.* 1982);
(3) Life-style behaviors and health status: physical activity intensity based on self-reported hours of strenuous leisure time physical activity per week (Richardson *et al.* 2004; Andersen *et al.* 2006), self-rated health (DeSalvo *et al.* 2006), smoking status (current or not) and sleep disturbances based on a validated modified Brief Athens Insomnia Scale (AIS-5) (Soldatos *et al.* 2000);
(4) Anthropometrics: weight and height were measured by a trained nurse, and body mass index (BMI; kg/m^2) was calculated.

Table 1. *Main baseline characteristics of the study population and comparison between subjects with and without elevated ALT*

Parameter	Total population (n=5984)	Normal ALT (n=5316)	Elevated ALT (n=668)	p
Age, years	45.0 (10.24)	45.07 (10.37)	44.38 (9.15)	0.07
Gender, % males	69.4	67.5	84.6	<0.001
Education, years	15.83 (2.82)	15.82 (2.83)	15.86 (2.71)	0.70
BMI, kg/m^2	26.35 (3.82)	26.11 (3.76)	28.24 (3.80)	<0.001
Triglycerides, mg/dl (50–175)	117.87 (60.57)	114.13 (58.43)	147.62 (68.57)	<0.001
Cholesterol, mg/dl (150–200)	199.80 (36.35)	199.26 (36.21)	203.98 (37.22)	0.002
Glucose, mg/dl (70–110)	93.31 (16.02)	92.80 (15.55)	97.42 (18.87)	<0.001
hs-CRP, mg/l	2.56 (4.03)	2.55 (4.14)	2.64 (2.96)	0.48
Current smoking, %	14.8	14.8	14.7	0.92
Use of statins, %	10.0	9.4	14.4	<0.001
Sport, h per week	2.24 (2.63)	2.28 (2.68)	1.90 (2.12)	<0.001
Sleep disturbances, mean score	2.10 (0.63)	2.08 (0.63)	2.19 (0.66)	<0.001
Self-rated health, mean score	4.18 (0.59)	4.20 (0.59)	4.09 (0.59)	<0.001
Anxiety, mean score	1.66 (0.69)	1.66 (0.69)	1.66 (0.69)	0.85
Burnout, mean score	2.02 (0.74)	2.02 (0.74)	2.01 (0.75)	0.92
Baseline depression, mean score	0.22 (0.27)	0.22 (0.28)	0.23 (0.28)	0.37

Data are given as mean (standard deviation) or as percentage.
ALT, Alanine aminotransferase; BMI, body mass index; hs-CRP, high-sensitivity C-reactive protein.

Statistical analysis

Statistical analyses were performed using SPSS version 19 (SPSS Inc., USA) software. Continuous variables are presented as mean values and standard deviations. To test differences in continuous variables between two groups the independent-samples t test was performed. Associations between nominal variables were performed with the Pearson χ^2 test and p for trend was calculated by the Mantel–Haenszel test. A multivariate logistic regression analysis was performed to test the adjusted association between ALT as a continuous or dichotomous variable and the incidence of minor or major depressive symptoms. All models were performed in steps starting with crude association, then adjusting for universal variables and then adding lifestyle parameters that may be confounders. To further elaborate on the association, potential mediators were entered into the model as job burnout, anxiety and sleep disturbances. For all analyses, $p<0.05$ was considered statistically significant.

Results

Main baseline characteristics of the study population and comparison between subjects with and without elevated levels of ALT (Table 1)

The final cohort included 5984 subjects: 4154 men and 1830 women (69.4% men, mean age 45.0, S.D.=10.24 years). Of the participants, 11% (n=668) had elevated levels of ALT, *versus* 5316 with normal ALT. A flowchart summary describing the exclusion of participants and the final sample is depicted in Fig. 1.

Subjects with elevated ALT had higher BMI, fasting serum glucose and lipids, were more likely to be men and users of statins. Subjects with elevated ALT performed less physical activity than those with normal ALT, had lower self-rated health scores along with higher sleep disturbances scores, but had similar anxiety and burnout scores.

There was no difference between groups in baseline levels of the depression score based on the mean score of the eight items.

Association between baseline ALT and incidence of minor depression (Table 2)

The cumulative incidence rate of minor depression was 3.8% (226/5984) in a median follow-up of 29 months (major depression included). Among subjects with elevated ALT at baseline, the incidence rate of minor depression was 6.3% as compared with 3.5% in those with normal ALT ($p<0.001$). We assessed the association of ALT and depression using several models. ALT, both as a dichotomous variable (i.e. elevated above normal levels) and as a continuous variable, was a significant independent predictor for the development of minor depression. The predictive value was maintained following adjustment for universal variables and time of follow-up (model 2), biochemical parameters and use of statins (model 3) and health behavior (model 4). Adding subjective health and

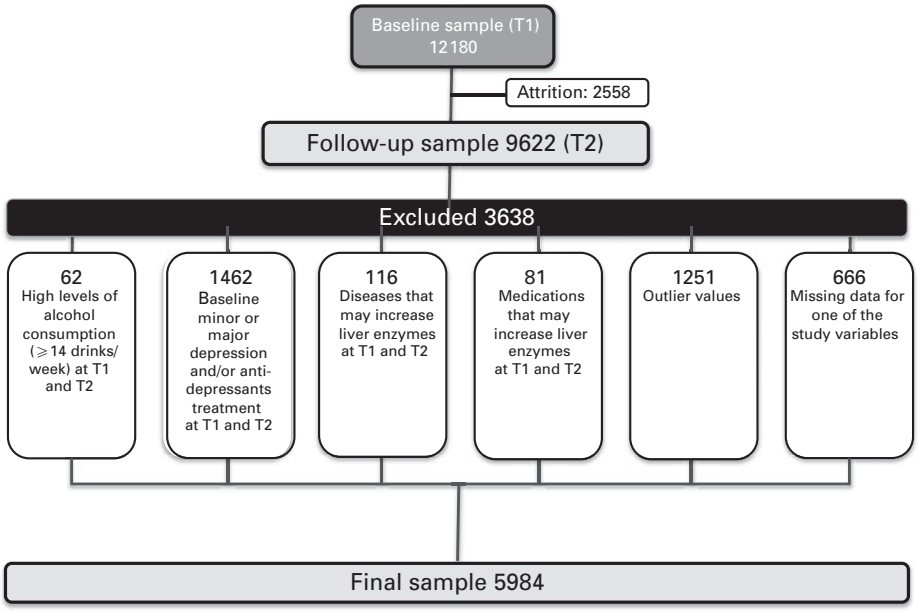

Fig. 1. Flow chart of the study population.

Table 2. *Multivariate analysis of the independent association between baseline ALT and minor depression*

	Model 1, unadjusted	Model 2[a]	Model 3[b]	Model 4[c]	Model 5[d]
ALT (IU/l, per 1 unit)	1.01 (1.003–1.02)	1.02 (1.01–1.03)	1.02 (1.008–1.02)	1.02 (1.008–1.02)	1.01 (1.006–1.02)
p	0.005	<0.001	<0.001	<0.001	0.001
Elevated ALT	1.87 (1.33–2.64)	2.19 (1.52–3.14)	2.03 (1.40–2.92)	2.02 (1.40–2.92)	1.78 (1.22–2.61)
p	<0.001	<0.001	<0.001	<0.001	0.003

Data are given as odds ratio (95% confidence interval).

ALT, Alanine aminotransferase; IU, international units; BMI, body mass index.

[a] Model 2, adjusted for: age, gender, BMI, education level, time of follow-up.

[b] Model 3. All model 2 covariates plus: serum levels of triglycerides, cholesterol, glucose and use of statins.

[c] Model 4. All model 3 covariates plus health behavior: smoking status (current yes/no), physical activity (h/week).

[d] Model 5. All model 4 covariates plus subjective health and affective states parameters: sleep disturbances, self-rated health, anxiety and burnout.

affective states parameters (sleep disturbances, self-rated health, anxiety and burnout as potential mediators; model 5) reduced the odds ratio (OR) for the association between elevated ALT and minor depression from 2.02 [95% confidence interval (CI) 1.40–2.92] to 1.78 (95% CI 1.22–2.61), but it remained highly significant ($p=0.003$). Other variables that significantly predicted minor depression in the full multivariate model (model 5) were female gender (OR 2.38, 95% CI 1.75–3.22), use of statins (OR 1.62, 95% CI 1.07–2.44), serum glucose levels (OR 1.007, 95% CI 1.00–1.01), sleep disturbances (OR 2.15, 95% CI 1.72–2.70), anxiety (OR 1.30, 95% CI 1.07–1.59) and burnout (OR 1.45, 95% CI 1.20–1.76) as risk factors. In contrast, years of education (OR 0.90, 95% CI 0.86–0.95), self-rated health (OR 0.75, 95% CI 0.57–0.97) and time of follow-up between examinations (OR 0.98, 95% CI 0.97–0.99) were significant protective factors.

Association between baseline ALT and incidence of major depression (Table 3)

The cumulative incidence rate of major depression was 1.4% (86/5984) in a median follow-up of 29 months. Among subjects with elevated ALT at baseline the incidence rate of major depression was 3.0% as compared with 1.2% in those with normal ALT ($p<0.001$). ALT, both as elevated above the normal limit or as a continuous variable, was a significant independent predictor for the development of major depression. The predictive value was maintained following adjustment for universal variables and time of follow-up (model 2),

Table 3. *Multivariate analysis of the independent association between baseline ALT and major depression*

	Model 1, unadjusted	Model 2[a]	Model 3[b]	Model 4[c]	Model 5[d]
ALT (IU/l, per 1 unit)	1.01 (1.001–1.02)	1.02 (1.01–1.03)	1.02 (1.009–1.03)	1.02 (1.009–1.03)	1.02 (1.006–1.03)
p	0.03	<0.001	<0.001	<0.001	0.003
Elevated ALT	2.46 (1.48–4.08)	3.15 (1.84–5.37)	3.07 (1.79–5.29)	3.13 (1.82–5.40)	2.61 (1.49–4.58)
p	0.01	<0.001	<0.001	<0.001	0.001

Data are given as odds ratio (95% confidence interval).

ALT, Alanine aminotransferase; IU, international units; BMI, body mass index.

[a] Model 2, adjusted for: age, gender, BMI, education level, time of follow-up.

[b] Model 3. All model 2 covariates plus: serum levels of triglycerides, cholesterol, glucose and use of statins.

[c] Model 4. All model 3 covariates plus health behavior: smoking status (current yes/no), physical activity (h/week).

[d] Model 5. All model 4 covariates plus subjective health and affective states parameters: sleep disturbances, self-rated health, anxiety and burnout.

biochemical parameters and use of statins (model 3) and health behavior (model 4). Adding subjective health and affective states parameters as potential mediators (model 5) reduced the OR for the association between elevated ALT and major depression from 3.13 (95% CI 1.82–5.40) to 2.61 (95% CI 1.49–4.58), but it remained highly significant ($p=0.001$).

Other variables that significantly predicted major depression in the full multivariate model (model 5) were female gender (OR 2.17, 95% CI 1.35–3.49), smoking (OR 1.89, 95% CI 1.15–3.10), sleep disturbances (OR 2.11, 95% CI 1.48–3.01) and burnout (OR 1.54, 95% CI 1.15–2.06) as risk factors. In contrast, years of education (OR 0.90, 95% CI 0.83–0.97), self-rated health (OR 0.51, 95% CI 0.34–0.77) and time of follow-up between examinations (OR 0.98, 95% CI 0.97–0.99) were significant protective factors.

As NAFLD has been shown to be associated with inflammation, we studied the possible mediating role of hs-CRP, by adding it to all the models presented. This addition did not change the strength of the association with ALT, and no significant association was observed between CRP and major or minor depression ($p \geqslant 0.17$ in all models).

Risk for minor and major depression by ALT status both at baseline and follow-up (Fig. 2)

ALT status combined for both baseline and follow-up measurements was categorized into four groups: never elevated (as the reference group), elevated only at follow-up, elevated only at baseline and persistently elevated. For the prediction of minor depression, persistently elevated ALT was associated with a greater risk (OR 2.35, 95% CI 1.29–4.29) compared with elevation only at follow-up or at baseline (p for trend <0.0001) adjusted for all variables in model 5 (Tables 2 and 3). Similarly, for the prediction of major depression, persistently elevated ALT was associated with a greater risk (OR 3.11, 95% CI 1.30–7.43) compared with elevation only at follow-up or at baseline (p for trend <0.0003) adjusted for all variables in model 5.

Discussion

Depression is a highly prevalent, multi-systemic chronic disorder that displays early age onset (Insel & Charney, 2003; Moussavi *et al.* 2007; Richards, 2011). In the present study, elevated ALT was a significant, independent predictor for the development of minor or major depression after adjustment for a wide range of potential confounders. ALT is a well-established marker of liver inflammation and hepatocellular injury. Even within the normal range, ALT predicts the development and regression of fatty liver (Omagari *et al.* 2011). Elevated ALT levels were shown to have a predictive value in assessing insulin resistance in obese patients (Chen *et al.* 2009) and in the incidence of diabetes (Nguyen *et al.* 2011).

Which mechanisms could account for our findings? First, NAFLD patients have a significantly lower QOL scores compared with patients with hepatitis B virus (HBV) or HCV (Dan *et al.* 2007) and reported a poorer health-related QOL compared with a healthy US population both on physical and mental health scores (David *et al.* 2009). The reason for this reduced QOL in NAFLD patients is unclear, especially since NAFLD is usually asymptomatic. However, fatigue is a common symptom in NAFLD patients (Newton *et al.* 2008), and they report low scores for vitality (David *et al.* 2009). Fatigue has been demonstrated to reduce QOL of patients with other types of liver disease (Younossi *et al.* 2001; Stanca *et al.* 2005; Gutteling *et al.* 2006; Swain, 2006). Interestingly, in our study adjustment for sleep disturbances score and for

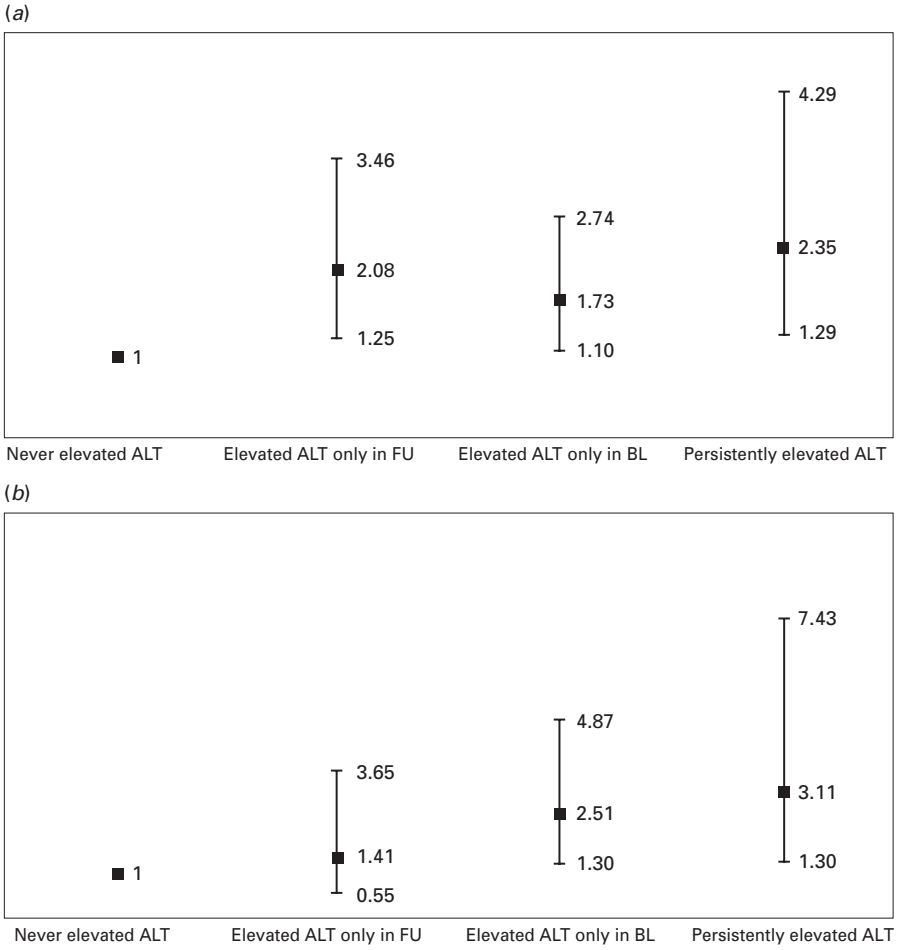

Fig. 2. Risk for minor (*a*) and major (*b*) depression by alanine aminotransferase (ALT) status at baseline (BL) and follow-up (FU). For both levels of depression the odds ratios (95% confidence intervals) presented are adjusted for all variables in model 5 (see Tables 2 and 3). The Mantel–Haenszel test was used to calculate p for trend: minor depression, $p<0.0001$; major depression, $p<0.0003$.

burnout, that include elements of fatigue, did not attenuate the association significantly.

A second mechanism may be related to metabolic processes. The association of NAFLD with chronic metabolic diseases and cardiovascular complications may restrict our ability to define the specific role of liver damage in the development of depressive symptoms in these patients. Diabetes, the overt manifestation of insulin resistance, is prevalent among NAFLD patients. The prevalence of depression in diabetes is increased twofold compared with the general population (Anderson *et al.* 2001; Silva *et al.* 2011). However, the association with depression remained significant even with adjustments for BMI, serum glucose, serum lipids and use of statins and health-related parameters such as smoking and physical activity. Thus, metabolic abnormalities are not likely to mediate this relationship. Furthermore, adjusting for the potential mediators of self-rated health and anxiety, which may be influenced by the fear of having liver disease or other chronic metabolic diseases, only modestly attenuated the association.

According to the 'two-hit' theory (Marchesini *et al.* 2001), NAFLD is a result of insulin resistance that leads to the accumulation of triglycerides within the hepatocytes followed by oxidative stress (Basaranoglu *et al.* 2010). Depression has been demonstrated to be associated with both insulin resistances (Timonen *et al.* 2005; Pearson *et al.* 2010) and inflammation (Miller *et al.* 2003; Raison *et al.* 2006; Dantzer *et al.* 2011).

The association between ALT and depression may also involve inflammatory processes. In response to inflammation, innate immune cells produce pro-inflammatory cytokines that act on the brain to cause 'sickness behavior'. When activation of the peripheral immune system continues, the ensuing immune signaling to the brain can lead to the development of

symptoms of depression in vulnerable individuals (Dantzer *et al.* 2008). Furthermore, increasing evidence indicates that cytokine/adipokine/chemokine-mediated inflammation may be an underlying mechanism leading to symptoms of depression, anxiety and fatigue (Lotrich *et al.* 2011) that have all been described in NAFLD patients (Elwing *et al.* 2006; Newton *et al.* 2008). The mechanism by which cytokines induce depression can be explained by their ability to access the brain and to interact with pathophysiological domains relevant to depression and influence the synthesis, release, and reuptake of mood-relevant neurotransmitters, including serotonin, norepinephrine and dopamine (Anisman *et al.* 2008; Miller, 2009; Dantzer *et al.* 2011; Lotrich *et al.* 2011). Similarly, it has been suggested that diminished serotonergic activity in the central nervous system may mediate the association between insulin resistance and the development of depressive symptoms (Herrera-Marquez *et al.* 2011).

There are several methodological limitations to our study. The first concerns the external validity of the study population that represents workers that are referred for periodic examinations and thus may be at a higher socio-economic level and healthier as compared with the general population. Future studies should confirm these observed associations in lower socio-economic status populations.

The major limitation of this study stems from the lack of imaging to confirm the presence of NAFLD. It has been demonstrated that assessing the prevalence of NAFLD based on elevated liver enzymes leads to gross underestimation when compared with ultrasonography (Zelber-Sagi *et al.* 2006) and that the normal range for ALT should be reduced in order to better detect NAFLD patients (Prati *et al.* 2002; Clark & Diehl, 2003; Browning *et al.* 2004; Bedogni *et al.* 2005; Kariv *et al.* 2006). However, NAFLD patients presenting with elevated ALT may represent patients with NASH or with a higher NAFLD Activity Score (NAS) (Fracanzani *et al.* 2008, 2011; Kashyap *et al.* 2009), which may be clinically more important. This misclassification bias in the diagnosis of NAFLD is non-differential, thus leading to underestimation of the true strength of the associations presented.

Another limitation is the absence of serologic tests to exclude chronic viral hepatitis. Several studies indicated that the prevalence of HCV in the Israeli adult population is as low as 0.9%, with its prevalence in Israeli-born individuals being 0.1% and reaching a peak of 5.7% in immigrants from central Asia (Sermoneta-Gertel *et al.* 2001). The prevalence of chronic HBV infection among Jewish Israelis (99% of our study population) is very low, ranging from 0.44–0.85% in healthy blood donors (Bar-Shany *et al.* 1995) to 2% in the general population (Andre, 2000). Most of our study population (73.8%) was born in Israel, and only 16% (108/668) of the subjects with elevated ALT were born in moderate or high endemic countries (central Asia and northern Africa). It is, therefore, reasonable to assume that most of the elevated ALT in that study population cannot be attributed to chronic viral hepatitis infection.

In summary, this prospective study indicates a temporal, dose–response, association between ALT and the development of depression even after a wide range of adjustments, and therefore marks treatment of NAFLD as a possible beneficial way for the prevention of depression. Assuming a causal relationship, based on the difference between the incidence rates in our study, the numbers needed to treat of persons with NAFLD-related elevated ALT in order to reduce one case of depression is 36 for minor depression and 56 for major depression. These results need to be confirmed by further prospective studies and clinical trials to translate the findings into clinical treatment implications.

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Declaration of Interest

None.

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