TGF-β, IL-10, and arginase 1, make a significant contribution to tumoral immunosuppression [7].

Hepatic damage, including metabolic disorders, is linked to activation of Kupffer cells (the tissue resident macrophages in the liver) and other stromal cells, which sustain inflammatory cytokine secretion [10]. These resident cells are also responsive to inflammatory factors and adipokines secreted by adipocytes. Thus, synergic events can perpetuate a vicious cycle that amplifies inflammatory processes, which will sustain both steatosis and inflammation while maintaining macrophage activation and the associated expression of arginase-1. Altogether, these events might favour disease progression, worsen hepatic damage and increase the risk of tumorigenesis.

In this context, we fully agree that a better understanding of cell autonomous and non-autonomous effects of arginase 1 and/or macrophage polarization could open new therapeutic horizons for liver diseases, particularly in the setting of NAFLD and HCC.

Conflict of interest

The authors declared that they do not have anything to disclose regarding funding or conflict of interest with respect to this manuscript.

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Protective effects of moderate alcohol consumption on fatty liver: A spurious association?

To the Editor:

We read with interest Moriya and colleagues' [1] paper on the longitudinal role of alcohol intake in relation to fatty liver. We were somewhat surprised by their findings and overall conclusion that light-to-moderate and even higher alcohol intake protects against developing fatty liver over time. They point out that several other studies also allege that moderate consumption is associated with a lower risk of non-alcoholic fatty liver disease (NAFLD) but acknowledge in their introduction that the apparent protective effects may be spurious associations induced by confounding factors. However, their analysis is also prone to a number of biases that have been well documented in the field of alcohol epidemiology [2,3].

Firstly, their choice of past week non-drinkers as a referent group is unsound. Past week non-drinkers include a mixture of lifelong abstainers, former drinkers (who may have quit for multiple reasons, including poor health) and infrequent drinkers. This misclassification error is known to negatively bias the health status of the aggregate group of non-drinkers and has been demonstrated to produce protective effects; studies that actively separate these distinctive non-drinking groups show none [3]. There is evidence that even if lifetime abstainers are separated from other non-drinkers they remain a poor choice of referent category for several reasons [3,4], including misclassification bias and that lifetime non-drinking may itself be indicative of poor health earlier in the life-course. Additionally, declines in consumption (including reductions to infrequent drinking) are more common amongst former heavy drinkers [3]. This is a potential source of bias, even in studies that are able to disentangle less than

weekly drinkers from lifelong non-drinkers, leading to spurious beneficial effects of moderate intake being observed. Combined, these studies indicate that alleged protective effects of light-to-moderate alcohol intake should be treated with caution.

Moriya and colleagues [1] provide many possible biological mechanisms for the protective effect they observe, ranging from insulin sensitivity to antioxidant agents. However, the association between alcohol intake and these posited mediators share the same limitations noted above with respect to combining current non-drinkers into a single aggregate group [2].

Additionally, large scale studies have shown that alcohol consumption is causally related to obesity [5] and that obesity is a risk factor for NAFLD [6]. Therefore adjustment for obesity is likely to have produced biased estimates. Adjusting for a variable on the causal pathway between alcohol intake and fatty liver is a form of over adjustment bias [7] – unless controlled direct causal effects are the estimate of interest which in this case seems unlikely. This typically biases estimates towards the null. Applied in this case, one might imagine that those who consume larger amounts of alcohol are more likely to become obese [5] which in turn increases their odds of developing fatty liver [6], therefore adjustment for obesity may have attenuated the estimated effect of higher levels of alcohol on fatty liver development.

While we do not have ultrasonography data to illustrate the potential impact of these biases with reference to the specific outcome used in Moriya and colleagues [1] paper, we have data on triglycerides, body mass index, waist circumference and γ-glutamyltransferase, which can be used to derive the fatty liver index (FLI) [8]. The FLI has been shown to be moderately associated with an increased occurrence of fatty liver on ultrasonography (scores of $\geqslant$60). In Fig. 1 we show that amongst 3531 men participating in the two most recent data collection phases (2007–2009 and 2012–2013) of the Whitehall II prospective cohort study [9], when the group of non-drinkers is decomposed further, never drinkers have similar odds of fatty liver approximately 5 years later as those consuming 0.1–69.9 grams of alcohol on a weekly basis (OR 1.05, CI 0.48–2.27) after adjustment for age, smoking status and physical activity. An increased risk is observed amongst former drinkers (OR 1.43, CI 0.93–2.19) and monthly/special occasion drinkers (OR 1.37, CI 1.08–1.73) – the two groups with the largest sources of bias present as described above. Furthermore, unlike like Moriya and colleagues [1], we show that higher levels of alcohol intake are associated with greater odds of fatty liver in a dose-dependent manner which is consistent with prior knowledge and current public health advice to limit alcohol intake [10].

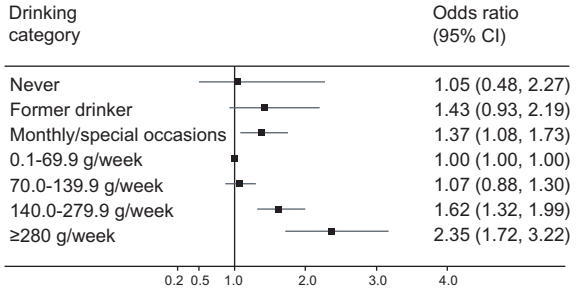

Fig. 1. Odds ratios and 95% confidence intervals for fatty liver in men in the Whitehall II Study by drinking category (N = 3531). Adjusted for age, smoking status and physical activity level.

While it is acknowledged that Moriya and colleagues [1] give a somewhat more balanced conclusion at the end of their article that the decision to drink should be tailored to an individual – this is not reflected throughout the rest of the manuscript. We illustrate that the alleged protective effect of moderate alcohol intake they describe is likely to be due to a combination of overadjustment bias and failure to appropriately stratify the current non-drinking group. As such we would recommend that it is ill-advised to take up light-to-moderate drinking to prevent fatty liver, or any other disease for that matter.

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Conflict of interest

The authors who have taken part in this letter declared that they do not have anything to disclose regarding funding or conflict of interest with respect to this manuscript.

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Reply to: "Protective effects of moderate alcohol consumption on fatty liver: A spurious association?"

To the Editor:

We would like to thank Bell *et al.* for their comments regarding our recently published article [1]. They suggested some possible concerns to be considered carefully.

Concerning the possibility that non-drinkers may not always be lifetime abstainers, it was difficult to separate former drinkers from lifetime abstainers based on the questionnaire used in the study. We recognized this limitation and pointed it out in our previous study [2], although we were unable to do so in our recent article due to limited space. It is likely that drinkers are advised to stop drinking because of fatty liver. Actually, 4% of men and 15% of women drinking at baseline abstained by the end of the follow-up. However, we have data regarding the changes in drinking habits during the observation period; the longitudinal analysis with the generalized estimating equation can partly take the changes in the drinking status into account. Bell *et al.* also suggested that lifetime abstainers might have had poor health status. Although we cannot rule out the possibility that such bias may distort the results, the participants of our study were generally healthy enough to work and we suppose that the number of those who had health problems earlier in their life-course, if any, was small enough not to affect the statistical analyses.

We cited some articles that suggest the possible mechanisms of the inverse association between alcohol consumption and fatty liver [1]. Bell *et al.* also expressed concerns regarding them. Observational studies may have similar limitations; however, we also cited an experimental study in mice [3], which is supposed to be free from those limitations. We cited some more such articles in our previous study [2], although due to limited space we were unable to cite all of these in our current article.

Additionally, Bell *et al.* mentioned the causal relationship between alcohol consumption and obesity. However, this association was not observed in our cohort and rather the reverse was noted; for example, in men, the body mass index values were significantly lower in the drinkers than in the current non-drinkers (23.4 ± 2.9 kg/m^2 *vs.* 23.8 ± 3.2 kg/m^2, $p < 0.001$) and the prevalence of obesity was lower in the drinkers than in the current non-drinkers (26.4% *vs.* 32.5%, $p < 0.001$). Therefore, there appears to be no rationale concerning overadjustment bias in our study. However, we re-calculated the odds ratio for fatty liver in each category of the average weekly alcohol consumption in men without adjusting for obesity and found similar results to those obtained after adjusting for obesity, suggesting that there was almost no impact of taking obesity into consideration on the results in our cohort (Fig. 1A).

We thank Bell *et al.* for presenting their data of the Whitehall II prospective cohort study demonstrating an association between a higher alcohol intake and greater odds of fatty liver using the fatty liver index (FLI) [4]. To compare their results with ours, we again re-calculated the odds ratio for fatty liver in a similar manner to the analysis by Bell *et al.*; that is, we analyzed with drinking 0.1–69.9 g/week as reference and adjusted for age, smoking status, and regular exercise. As shown in Fig. 1B, the non-drinker category had a higher risk for fatty liver than drinking 0.1–69.9 g/week. Since the non-drinker group was a mixture of former drinkers and lifetime abstainers, it is difficult to discuss further concerning this relationship. On the other hand, higher levels of alcohol intake were not associated with greater odds of fatty liver in our cohort; it was different from the results by Bell *et al.* We are not sure whether the discrepancy is due to the difference in ethnicity or in the modality to assess fatty liver. Ultrasonography is more reliable than FLI in assessing fatty liver, since FLI was developed to predict the presence of fatty liver diagnosed on ultrasonography [4]. Further studies in different ethnicities with ultrasonographic assessment would be warranted.

Finally, we have not stated the safety of alcohol consumption for all individuals nor encouraged alcohol consumption. In our paper, we simply described the evidence obtained from a longitudinal analysis showing that alcohol consumption is inversely associated with fatty liver in a major part of the general population. However, it is hard to claim a causal relationship between alcohol consumption and fatty liver, since our study was observational and not interventional. Moreover, although we hypothesize that alcohol may have a somewhat protective effect against fatty liver within a certain threshold and that this