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The Effect of Alcohol Consumption on Cardiovascular Risk Factors, Liver Function Tests and Cancer-Related Growth Factors

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Abstract

Objective: To assess the effects of abstinence from alcohol on metabolic risk factors and cancer-related growth factors.

Design: Prospective, observational study.

Setting: Single tertiary centre.

Participants: Healthy subjects were recruited based on intention to: (i) abstain from alcohol for one month (abstinence group), or (ii) continue to drink alcohol (control group). Inclusion criteria were baseline alcohol consumption >64g/week (males) or >48g/week (females). Exclusion criteria were known liver disease or alcohol dependence.

Primary and secondary outcome measures: The primary outcome was change in insulin resistance (HOMA score). Secondary outcomes were changes in weight, blood pressure, VEGF, EGF, and liver function tests. Primary and secondary outcomes were adjusted for changes in diet, exercise, and cigarette smoking.

Results: The abstinence group comprised 94 participants (mean age 45.5 years, SD±1.2) and the control group 47 participants (mean age 48.7 years, SD±1.8). Baseline alcohol consumption in the abstinence group was 258.2g/week, SD±9.4, and in the control group 233.8g, SD±19.0. Significant reductions from baseline in the abstinence group (all $p<0.001$) were found in: HOMA score (-25.9%, IQR -48.6 to +0.3%), systolic blood pressure (-6.6%, IQR -11.8% to 0.0%), diastolic blood pressure (-6.3%, IQR -14.1% to +1.3%), weight (-1.5%, IQR -2.9% to -0.4%), VEGF (-41.8%, IQR -64.9% to -17.9%) and EGF (-73.9%, IQR -86.1% to -36.4%). None of these changes were associated with changes in diet, exercise or cigarette smoking. No significant changes from baseline in primary or secondary outcomes were noted in the control group.

Conclusion: These findings demonstrate that abstinence from alcohol in moderate-heavy drinkers improves insulin resistance, weight, blood pressure and cancer-related growth factors. These data support an independent association of alcohol consumption with cancer risk, and suggest an increased risk of metabolic diseases such as type 2 diabetes and fatty liver disease.

Strengths and limitations of this study

Strengths:

- Prospective study design
- Recruitment of a control group
- Thorough characterization of the biological and lifestyle confounders

Limitations:

- Lack of randomization to groups
- Study cohort all from university teaching hospital or science magazine.

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Data sharing statement: All raw data is available on request from the corresponding author.

Introduction

Alcohol is a major cause of disability and preventable death. Globally, alcohol is the 3rd commonest cause of lost years due to ill health, accounting for a greater burden of disease than tobacco smoking, hypertension or poor sanitation.[1] European countries have amongst the highest alcohol consumption. Eastern Europe has the highest *per capita* consumption worldwide,[2] and in the UK over 25% of the adult population drink in excess of recommended guidelines.[3]

Aside from liver disease, which is the 3rd commonest cause of preventable death in the UK, there is also a significant burden from alcohol-related cancer and metabolic syndrome.[3]

It has long been recognized that there is an important interaction between alcohol misuse and fatty liver disease. One of the main factors driving the development of fatty liver disease and steatohepatitis is insulin resistance. Thus, any action that improves insulin resistance will have a major impact on the development and severity of fatty liver disease.

In this climate of increased awareness of alcohol-related morbidity, the UK Chief Medical Officers have revised downwards their weekly guidance limits.[4] Additionally, public health campaigns, where non-dependent drinkers are encouraged to commit to one month of abstinence from alcohol, are increasingly common. However, the biological effects of short-term abstinence in this group remain unknown. The aim of this study was to assess the effects of short-term

abstinence on biochemical and physiological parameters, as well as on longer term drinking behaviour.

Methods

Study Design: This was a single-centre, prospective, observational study conducted at the Royal Free London NHS Foundation Trust. Ethical approval was granted by the NRES Committee (14/NW/1510). Study recruitment was initiated through email advertising within University College London, Queen Mary University of London, and New Scientist Magazine. The entry criteria were baseline alcohol consumption of >64g/week (8 units) for males or >48g/week (6 units) for females. Exclusion criteria were >3 days abstinence from alcohol prior to commencement of the study, the presence of known liver disease or alcohol dependence. Participants were not randomized to group, but were allocated based on intention to maintain abstinence for one month (abstinence group) or to continue alcohol consumption (control group).

Participants were assessed at baseline, and after one-month. The primary outcome was change in insulin resistance (HOMA score) at baseline and one-month. Secondary outcomes were changes in weight, blood pressure, VEGF, EGF and liver function tests. Information on diet, exercise, and smoking history were obtained by self-reporting using components of the SLIQ lifestyle questionnaire[5]. Self-reported alcohol intake was assessed at baseline using the full AUDIT questionnaire, and a direct interview by a single interviewer (KM) was also conducted to assess alcohol intake over the preceding two-months, using the timeline follow back method.[6] Additionally, a follow-up telephone interview was conducted at 6-8 months to

determine drinking habits following the study period, using the full AUDIT questionnaire (modified to capture data for the preceding 6-8 months).

Sample size calculation based on interim analysis of data from the abstinence group indicated a control group of 47 was required to adequately detect a statistically significant change in HOMA score (80% power, alpha 5%, 2-sided test).

Blood pressure (BP) was measured seated, following a 2-minute rest period, and the mean of three measurements was recorded. Fasting blood was taken, between 8am and midday, for measurement of glucose, insulin, liver function tests, lipids, carbohydrate deficient transferrin (abstinence group only) and VEGF (isoforms 165, 145 and 121) and EGF (Randox Investigator, Randox, Belfast, UK). The HOMA score was calculated according to the methods of Matthews et al.[7] Participants with diabetes requiring treatment were excluded from HOMA measurements.

Statistical analysis: Baseline and one-month differences were analysed by paired t-test for normally distributed differences in continuous variables, by Wilcoxon signed rank test for not normally distributed differences in continuous variables, and differences in categorical variables by Chi-square test. Differences between abstinence and control groups were analysed by unpaired t-test for normally distributed variables, and Mann-Whitney test for variables that were not normally distributed. Lifestyle factors were categorically graded (better/same/worse), and delta change in biological variables between lifestyle groups was assessed by Kruskal-Wallis test. Correlation between biological variables was assessed by Spearman's correlation. All analyses were performed using STATA version 13.1 and

SPSS Statistics version 21.0. Standard deviation (SD) is reported for means and interquartile range (IQR) for medians where applicable. All p values are 2 sided; p<0·01 was considered significant to account for multiple comparisons.

Results

Ninety-seven participants were recruited to the abstinence group, and forty-eight participants to the control group. Three subjects in the abstinence group and one subject in the control group did not attend for follow-up. Thus, the final abstinence group comprised ninety-four participants (43 male, 51 female) mean age 45·5 years, SD±1·2, and the control group comprised 47 participants (22 male, 25 female) mean age 48·7 years, SD±1·8. Mean baseline alcohol intake for the abstinence group was 258·2g/week, SD±9·4 (men 275·9, SD±25·5; women 243·1, SD±12·8). All subjects in this group, except one individual, remained abstinent for the study period - this participant was included in all analyses. Mean weekly baseline alcohol intake for the control group was 233·8g, SD±19·0 (men 270·2, SD±26·6; women 200·2, SD±25·8), and was not significantly different at one-month 260·1g, SD±20·8 (men 286·4, SD±26·6; women 235·8, SD±31·1), p=0·11. A flowchart of participants and observations is shown in figure 1.

Baseline and one-month variables for the abstinence and control groups are listed in table 1. There were no significant differences in baseline characteristics between abstinence and control groups, aside from baseline blood pressure which was significantly lower in the control group (systolic bp: 135·8 SD±1·9mmHg *vs* 125·7 SD±2·0mmHg, p<0·01; diastolic bp: 87·7 SD±1·2mmHg *vs* 74·3·7 SD±1·5mmHg, p<0·01). Anti-hypertensives were used in one participant in the abstinence group,

and one participant in the control group. Lipid-lowering agents were used in two participants in the abstinence group, one subject in the control group. These participants were excluded from analyses for blood pressure and lipids respectively. Significant reductions from baseline (pre *vs* post) in the abstinence group were observed in: HOMA score (-25·9%, IQR -48·6 to +0·3%), systolic blood pressure (-6·6%, IQR -11·8% to 0·0%), diastolic blood pressure (-6·3%, IQR -14·1% to +1·3%) and weight (-1·5%, IQR -2·9% to -0·4%). HOMA score was not performed due to type 1 diabetes in one participant in the abstinence group. By chance, no participants had type 2 diabetes. Levels of VEGF and EGF also markedly reduced in the abstinence group, at -41·8% (IQR -64·9% to -17·9%) and -73·9% (IQR -86·1% to -36·4%) respectively (figure 2). Serum lipids (pre *vs* post) also improved in the abstinence group: fasting total serum cholesterol (-13·4%, IQR -18·9% to -2·7%), LDL cholesterol (-9·4%, IQR -20·1% to +4·8%), HDL cholesterol (-16·7%, IQR -25·0% to 0·0%). All the above variables were significantly reduced from baseline, $p<0·001$. By contrast, the control group did not show significant changes from baseline in any of the above variables. Changes from baseline in HOMA score, VEGF, EGF, weight, systolic and diastolic blood pressure are shown in figure 3.

Table 1: Baseline and one-month variables for abstinence and control groups.

Variable [units]	Abstinence Group				Control Group			
	Number of paired values[a]	Baseline	One-month	p-value (pre *vs* post)	Number of paired values[a]	Baseline	One-month	p-value (pre *vs* post)
HOMA score [median (IQR)]	86	1·4 (1·0-2·1)	1·0 (0·7-1·4)	p<0·001	47	1·2 (0·9-1·5)	1·1 (0·9-1·5)	p=0·42
Systolic blood pressure [mmHg, median (IQR)]	93	136·0 (121·0-147·5)	125·0 (115·0-141·0)	p<0·001	47	121·0 (118·0-134·0)	122·0 (116·0-131·0)	p=0·17
Diastolic blood pressure [mmHg, mean (SD)]	93	89·0 (80·0-95·5)	82·0 (77·0-89·0)	p<0·001	47	71·0 (68·0-80·0)	75·0 (68·0-80·0)	p=0·77
Weight [kg, median (IQR)]	89	81·1 (67·0-89·7)	79·5 (66·7-87·2)	p<0·001	47	73·8 (65·2-85·0)	72·6 (64·9-84·6)	p=0·09
VEGF [ng/L, median (IQR)]	81	7·6 (5·5-16·0)	4.0 (3·1-5·8)	p<0·001	41	13·4 (9·0-17·9)	12·9 (8·3-18·5)	p=0·34
EGF [ng/L, median (IQR)]	81	7·2 (3·6-13·5)	1·5 (1·0-2·5)	p<0·001	41	3·8 (1·0-10·3)	4·9 (1·0-9·5)	p=0·55
Total Cholesterol [mg/dL, median (IQR)]	88	228·7 (198·4-258·4)	202·1 (176·3-227·4)	p<0·001	47	208·8 (177·9-232·0)	205·0 (177·9-239·8)	p=0·25
LDL [mg/dL, median (IQR)]	88	131·5 (112·3-163·1)	127·0 (97·4-153·8)	p<0·001	47	112·1 (88·9-139·2)	112·1 (85·1-143·1)	p=0·16
HDL [mg/dL, median (IQR)]	88	74·4 (±22·3)	60·9 (±17·8)	p<0·001	47	73·5 (58·0-88·9)	73·5 (58·0-92·8)	p=0·95
Triglycerides [mg/dL, median (IQR)]	88	79·3 (57·6-115·8)	76·6 (60·4-103·2)	p=0·05	47	79·7 (62·0-124·0)	79·7 (62·0-106·3)	p=0·70
Fasting glucose [mg/dL, median (IQR)]	92	86·4 (79·2-93·6)	82.8 (77·4-90·0)	p<0·01	47	91·8 (86·4-95·4)	90·0 (84·6-96·8)	p=0·92
Gamma GT [IU/L, median (IQR)]	92	22·5 (14·0-34·8)	15·0 (10·3-24·0)	p<0·001	46	39·8±8·3	44·8±11·0	p=0·26
ALT [IU/L, median (IQR)]	94	25·0 (18·0-37·0)	20·0 (16·0-29·3)	p<0·001	47	24·0 (18·0-31·0)	25·0 (20·0-33·0)	p=0·06
AST [IU/L, median (IQR)]	94	23·0 (19·0-28·0)	21·0 (18·0-27·0)	p=0.03	47	20·0 (17·0-24·0)	22·0 (19·0-27·0)	p<0·01
Carbohydrate deficient transferrin [%, median (IQR)]	81	0·7 (0·5-1·1)	0·6 (0·4-0·8)	p<0·001	n/a	n/a	n/a	n/a

Abbreviations: HOMA, homeostatic model assessment; VEGF, vascular endothelial growth factor; EGF, epidermal growth factor; LDL, low density lipoprotein; HDL, high density lipoprotein; ALT, alanine aminotransferase; AST aspartate aminotransferase.

[a]*Where the number of paired observations is less than 94 (abstinence group) or 47 (control group), this is due to missing data points.*

Liver function tests also improved in the abstinence group, thus, there was a significant reduction in serum ALT (-14·5%, IQR -28·9 to +6·7%, p<0·001) and gamma GT (-28·6%, IQR -43·5 to -14·4%, p<0·001), and a trend towards reduction in serum AST (-5·4%, IQR -16·2 to +9·5%, p=0·03). No significant change in these variables was seen in the control group, aside from a small rise in AST (+4·5%, IQR -5·6 to +23·1%, p<0·01).

Lifestyle factors did not account for changes in the abstinence group. No changes were seen in exercise score (10·9 SD±4·7 *vs* 10·7 SD±4·6, p=0·82) or cigarette smoking (1·3 SD±0·7 *vs* 1·4 SD±0·7, p=0·17). A small change in diet score was noted (from 8·2 SD±3·3 to 8·8 SD±3·0, p=0·03). The pre/post differences in HOMA score, weight, VEGF, EGF, triglycerides and HDL were distributed with a left (negative) skew, and could not be transformed for regression analysis. Therefore, non-parametric approaches were adopted to account for lifestyle variables. Changes in HOMA score, BP, and weight in the abstinence group were not associated with changes in any lifestyle score (supplemental table 1). There was also no association between changes in HOMA score and weight (r=0·04, p=0·73). However, changes in total cholesterol and LDL cholesterol attained borderline significance between groups when compared by change in diet in the abstinence group (supplemental table 1; p=0·01 and p=0·02 respectively).

A further important result relates to follow-up questionnaire data, obtained in 77 individuals (81·9%) in the abstinence group and 40 (83·3%) in the control group, at 6-8 months following the study period. In the abstinence group, a significant reduction in alcohol consumption was maintained from their pre-study assessment.

Thus, there was a significant reduction in overall AUDIT score from 10·0 (IQR 7·0 to 15·0) to 7·0 (IQR 5·0 to 9·0), p<0·001, and in the proportion of individuals with harmful use of alcohol (AUDIT score>8) (61·0% *vs* 28·5%, p<0·001) at 6-8 months compared with baseline. By contrast, in the control group there was a non-significant trend to reduction in overall AUDIT score from 8·5 (IQR 6·3 to 12·0) to 8·0 (IQR 6·0 to 10·8), p=0·06), and no significant change in the proportion with harmful use (50% *vs* 40%, p=0·37).

Discussion

This study is the first to comprehensively assess the effects of short-term abstinence from alcohol in a population of 'healthy' individuals, who are representative of the 25% of the wider population who drink alcohol above national guidelines. The key findings of this study are improvements in insulin resistance, blood pressure, body weight, and a decrease in circulating concentrations of cancer-related growth factors following a month of abstinence from alcohol.

The strengths of this study are the prospective study design, the recruitment of a control group, and the thorough characterization of the participant's biological and lifestyle data. A weakness is the lack of randomization of groups, although for ethical reasons the allocation of individuals to a pre-defined alcohol consumption regimen was inappropriate. A further weakness relates to the study cohort, who were recruited through staff at university teaching hospitals and a science magazine, and thus probably had higher educational attainment and health-related motivation than the average population. A further confounder is the possibility of lifestyle change in the abstinence group, alongside abstinence from alcohol. We have tried to minimize

the impact of these using the SLIQ questionnaire, a self-reported measure of lifestyle factors contributing to metabolic risk with good re-test reliability.[5] As such, changes in HOMA score, weight and blood pressure were independent of changes in lifestyle as measured by the SLIQ score. Nevertheless, it remains possible that the questionnaire scoring for diet, exercise and cigarette smoking has inadequate sensitivity for all lifestyle changes within this cohort.

The primary endpoint of insulin resistance, measured by HOMA score, showed a marked decrease (~25%) following the cessation of alcohol consumption. Previous epidemiological data has supported a protective effect of low-dose alcohol use on the risk of type 2 diabetes,[8] although prospective alcohol intervention studies have provided mixed results.[9,10] Our data suggest that alcohol use above recommended guidance markedly increases the risk of type 2 diabetes. Moreover, the observed effects of abstinence on HOMA score noted in this study are too dramatic to be accounted for by weight loss alone, and no specific association was found between change in HOMA score and weight. To our knowledge, this is the first paper to prospectively demonstrate a link between excess alcohol consumption and insulin resistance.

A major novel finding of this study is the rapid decrease in serum VEGF and EGF with short-term abstinence from alcohol, which was seen in 90% of subjects in the abstinence group. Importantly, these changes were not seen in the control group with continued alcohol consumption. Alcohol is thought to be causally related to the development of several cancers, including the digestive tract, nasopharynx, liver and breast.[11] The increased risk caused by alcohol persists even at low-levels of

consumption. The mechanism remains unknown. We chose to study VEGF and EGF, since they are the both highly expressed in the solid tumours listed above, and are common therapeutic targets for these tumours.[12] VEGF plays a key role in tumour progression through angiogenic pathways, and VEGF expression is driven by oncogene expression (eg. Ras, src, HER2, EGFR).[13] EGF signalling contributes to oncogenesis by directly promoting cell proliferation,[14] and expression levels are correlated with progressive tumour growth and metastasis.[15-17]

Mechanistically, rodent models have demonstrated that alcohol exposure directly promotes the progression of several cancers, including breast cancer. Lu et al have shown, in a mouse model of breast cancer, that alcohol directly induces tumor angiogenesis and accelerated tumor growth through a VEGF-dependent mechanism.[18] Similar evidence for an alcohol-VEGF pathway exists in mouse models of colon cancer and melanoma.[19,20] The EGF pathway has also been implicated in alcohol-related breast cancer.[21-23] The baseline levels of VEGF and EGF reported in this study are lower than reported in other studies exploring associations of circulating VEGF/EGF levels with the occurrence of solid tumours.[24-26] These differences are explained by the method of sample collection. The collection of blood into EDTA tubes, as in this study, leads to reduced contribution of platelet-derived VEGF and EGF, and thus lower plasma concentrations.[27.28]

Here, we demonstrate for the first time in humans a marked effect of abstinence on circulating concentrations of VEGF and EGF, which suggests that alcohol consumption *per se* increases the concentrations of these growth factors. There is

strong evidence that these growth factors play an important role in oncogenesis. However, it would be wrong to speculate further on this observation without longitudinal study in subjects who continue moderate alcohol consumption.

These data also show the dynamic effect of regular alcohol consumption on blood pressure, an effect that is maintained in healthy individuals with no history of hypertension requiring medication. An effect of alcohol on blood pressure has long been recognized, with consumption greater than two daily doses considered to be one of the most common reversible causes of hypertension.[29]

Collectively, the above findings have implications for the risk of synergistic liver injury amongst individuals with risk factors for alcohol-related liver disease (ALD) and fatty liver disease. Previous studies have emphasized an association between these pathways of liver injury, since serum ALT amongst moderate drinkers is elevated to a greater extent in those with higher BMI, and ALD and fatty liver are pathologically similar. Two prospective cohort studies from Scotland have demonstrated an increased risk of liver disease with alcohol use and elevated BMI.[30] More recently, a large prospective study of over 100,000 women in the UK confirmed a synergistic association between alcohol and high BMI and risk of chronic liver disease.[31] Since alcohol use and insulin resistance are both directly implicated in the development of steatohepatitis, the results of this study provide further support for this common causal pathway. Further, changes in the gut microbiome have also been implicated in the pathogenesis of steatohepatitis and obesity,[32] and therefore changes in gut microbe populations following abstinence from alcohol are a further

possible explanation for the biological changes observed in this study. These hypotheses merit further attention in subsequent mechanistic studies.

A frequent criticism of public health strategies of short-term abstinence (eg. Dry January) has been the lack of evidence of health benefits, or even negative effects on longer-term alcohol consumption. This study demonstrates a durable effect on drinking behaviour following a short-term period of abstinence, albeit we cannot exclude the behavioural effect of participation in the study.

Although this study has demonstrated health benefits from short-term abstinence, a possible misrepresentation of these results is the concept that a 'detox' period is all that is required to 'refresh' the liver or achieve other health gains. This is clearly untrue, since the durability of the observed biological effects remains to be established. The data presented here represent an important public health message, providing supportive mechanistic evidence for the recent changes in alcohol guidance due to cancer risk, and the synergistic relationship between alcohol and metabolic syndrome. Further attention should be directed to determining the durability of these biological effects of abstinence, and conveying these complex public health messages to the public.

Acknowledgements / Contributors:

GM contributed to study design, participated in data collection, wrote the analytical plan, and drafted and revised the paper. He is guarantor. SM participated in study design, participated in data collection, and drafted and revised the paper. AC and TKB analysed the data, and drafted and revised the paper. CS participated in study design, and revision of the paper. MR, SAK, AJ, CC, JM, AG and TH participated in data collection and revision of the paper. CJ, RS, DN and RJ contributed to study design, and revision of the paper. KM supervised the study, contributed to study design, participated in data collection and drafted and revised the paper. Administrative support for the study was provided by Ms Patricia Langley.

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Supplemental Table 1a: Change from baseline in biological variables between categories of change in SLIQ DIET score (better / same / worse) in the abstinence group.

		Categorical change from baseline in DIET score in Abstinence Group			p-value[a]
		Better (n=42)[b]	Same (n=16)[b]	Worse (n=32)[b]	
Change from baseline in biological variable [%, median (IQR)]	HOMA	-14·1 (-42·6 to -0·6)	-33·7 (-48·9 to +1·7)	-34·9 (-53·1 to -1·7)	p=0·45
	Systolic blood pressure	-6·6 (-13·3 to -1·4)	-6·9 (-11·8 to +3·5)	-6·8 (13·8 to +2·5)	p=0·83
	Diastolic blood pressure	-7·3 (-15·1 to +1·3)	-5·6 (-16·1 to +5·5)	-5·9 (-12·5 to +1·2)	p=0·71
	Weight	-1·5 (-2·8 to 0·0)	-1·1 (-2·8 to +0·4)	-1·4 (-3·0 to -0·3)	p=0·95
	VEGF	-36·2 (-63·6 to -10·1)	-57·5 (-73·9 to -30·1)	-50·0 (-75·5 to -23·7)	p=0·15
	EGF	-73·0 (-85·9 to -31·8)	-75·8 (-91·2 to -54·3)	-76·7 (-88·5 to -37·5)	p=0·54
	Cholesterol	-9·2 (-16·7 to +3·5)	-17·3 (-25·0 to -13·6)	-14·0 (-17·1 to -7·6)	p=0·01
	LDL	-5·1 (-19·2 to +16·8)	-18·4 (-25·8 to -8·8)	-12·2 (-18·4 to -3·8)	p=0·02
	HDL	-15·4 (-23·0 to -5·8)	-18·9 (25·5 to -7·8)	-21·0 (-29·7 to -10·9)	p=0·26
	Triglycerides	-5·1 (-27·2 to +23·0)	-19·9 (-33·4 to -2·1)	+1.2 (-13·6 to +24·8)	p=0·03

Supplemental Table 1b: Change from baseline in biological variables between categories of change in SLIQ EXERCISE score (better / same / worse) in the abstinence group.

		Categorical change from baseline in EXERCISE score in Abstinence Group			p-value[a]
		Better (n=42)[b]	Same (n=16)[b]	Worse (n=32)[b]	
Change from baseline in biological variable [%, median (IQR)]	**HOMA**	-12·2 (-45·1 to +1·8)	-26·5 (-46·3 to -1·1)	-38·9 (-56·2 to -8·7)	p=0·21
	Systolic blood pressure	-6·0 (-11·1 to 0·0)	-2·7 (-12·9 to +4·6)	-8·0 (15·0 to -3·7)	p=0·36
	Diastolic blood pressure	-4·2 (-13·9 to +1·3)	-3·9 (-13·6 to +7·9)	-8·5 (-14·9 to +0·3)	p=0·31
	Weight	-1·7 (-3·1 to -0·4)	-1·4 (-2·9 to +0·1)	-1·1 (-2·5 to -0·1)	p=0·51
	VEGF	-28·5 (-53·2 to -10·7)	-57·4 (-75·4 to -34·4)	-55·5 (-68·6 to -20·2)	p=0.11
	EGF	-74·9 (-88·5 to -32·7)	-75·0 (-91·2 to -38·2)	-71·4 (-82·4 to -41·4)	p=0.67
	Cholesterol	-13·7 (-18·5 to -3·2)	-12·6 (-20·1 to -4·5)	-14·1 (-17·7 to -2·7)	p=0·93
	LDL	-10·8 (-18·9 to +1·9)	-10·2 (-21·0 to +5·4)	-8·7 (-22·3 to +8·6)	p=0·99
	HDL	-16·7 (-25·3 to -7·6)	-16·2 (26·6 to -8·3)	-18·3 (-25·7 to -6·9)	p=0·97
	Triglycerides	-14·6 (-31·1 to +15·3)	-4.4 (-15·9 to +49·2)	+-4·7 (-16·7 to +14·4)	p=0·43

Abbreviations: SLIQ, simple lifestyle indicator questionnaire; HOMA, homeostatic model assessment; VEGF, vascular endothelial growth factor; EGF, epidermal growth factor; LDL, low density lipoprotein; HDL, high density lipoprotein; bp, blood pressure.

a. *Participants were graded as better, same or worse based on changes in SLIQ diet score (supplemental table 1a) and exercise score (supplemental table 1b). Data for changes in cigarette smoking is not shown – only 5 individuals had a change from baseline score in the abstinence cohort. Changes from baseline in the abstinence cohort in biological variables were compared between the better / same / worse groups using the Kruskal-Wallis test. P<0.01 was considered significant to account for multiple comparisons.*

Complete baseline and one-month lifestyle questionnaire data was available on 90 participants in the abstinence cohort. The number of paired values analysed for each variable are: HOMA n=82; systolic bp n=89; diastolic bp n=89; weight n=85; VEGF n=78; EGF n=78; cholesterol n=84; LDL n=84; HDL n=84; triglycerides n=84.

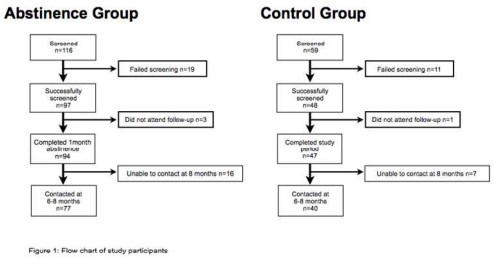
Figure 1: Flow chart of study participants.

Figure 2: Baseline and one-month data for the abstinence group presented as pre/post scatterplot (left) and bar chart chart (right).

Bar chart data are presented as median (IQR). Panels (clockwise from top right): HOMA score, weight, diastolic bp, EGF, VEGF, systolic bp. Baseline and one-month values were compared with Wilcoxon signed rank test, p<0·01 taken as level of significance. Abbreviations: HOMA, homeostatic model assessment; VEGF, vascular endothelial growth factor; EGF, epidermal growth factor; bp, blood pressure.

Figure 3: Percentage change from baseline in HOMA score, VEGF, EGF, systolic bp, diastolic bp and weight in abstinence (dark bar) and control (light bar) groups.

Data are presented as median (IQR). Changes from baseline in abstinence and control groups were compared with Mann Whitney test, p<0·01 taken as level of significance. Abbreviations: HOMA, homeostatic model assessment; VEGF, vascular endothelial growth factor; EGF, epidermal growth factor; bp, blood pressure.



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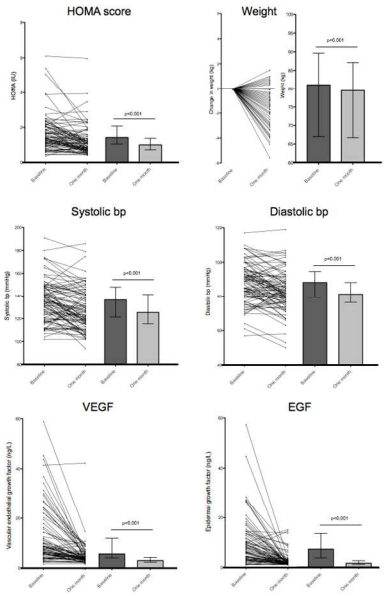

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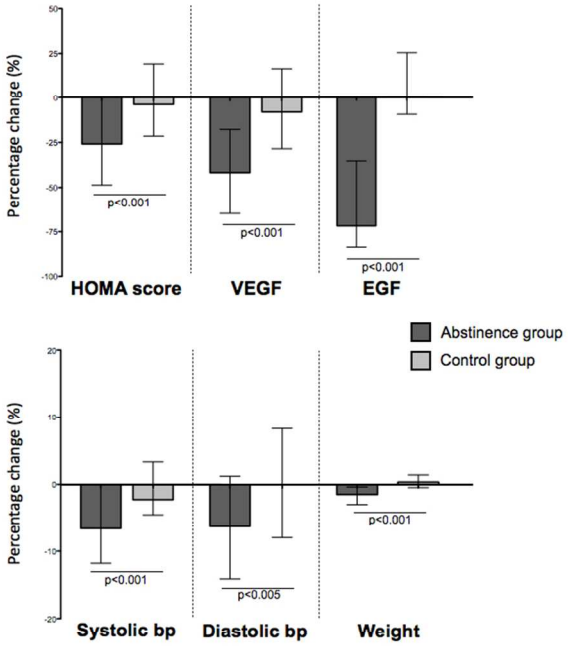

Figure 3: Percentage change from baseline in HOMA score, VEGF, EGF, systolic bp, diastolic bp and weight in abstinence (dark bar) and control (light bar) groups.

Data are presented as median (IQR). Changes from baseline in abstinence and control groups were compared with Mann Whitney test, p<0·01 taken as level of significance. *Abbreviations: HOMA, homeostatic model assessment; VEGF, vascular endothelial growth factor; EGF, epidermal growth factor; bp, blood pressure.*

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STROBE Statement—checklist of items that should be included in reports of observational studies

	Item No	Recommendation	Completed (page no.)
Title and abstract	1	(*a*) Indicate the study's design with a commonly used term in the title or the abstract	Yes (2)
		(*b*) Provide in the abstract an informative and balanced summary of what was done and what was found	Yes (2)
Background/rationale	2	Explain the scientific background and rationale for the investigation being reported	Yes (4)
Objectives	3	State specific objectives, including any prespecified hypotheses	Yes (4.5)
Study design	4	Present key elements of study design early in the paper	Yes (5)
Setting	5	Describe the setting, locations, and relevant dates, including periods of recruitment, exposure, follow-up, and data collection	Yes (5,6)
Participants	6	(*a*) *Cohort study*—Give the eligibility criteria, and the sources and methods of selection of participants. Describe methods of follow-up *Case-control study*—Give the eligibility criteria, and the sources and methods of case ascertainment and control selection. Give the rationale for the choice of cases and controls *Cross-sectional study*—Give the eligibility criteria, and the sources and methods of selection of participants	Yes (5.6)
		(*b*) *Cohort study*—For matched studies, give matching criteria and number of exposed and unexposed *Case-control study*—For matched studies, give matching criteria and the number of controls per case	n/a
Variables	7	Clearly define all outcomes, exposures, predictors, potential confounders, and effect modifiers. Give diagnostic criteria, if applicable	Yes (5.6)
Data sources/ measurement	8*	For each variable of interest, give sources of data and details of methods of assessment (measurement). Describe comparability of assessment methods if there is more than one group	Yes (5,6)
Bias	9	Describe any efforts to address potential sources of bias	Yes (5.6)
Study size	10	Explain how the study size was arrived at	Yes (6)
Quantitative variables	11	Explain how quantitative variables were handled in the analyses. If applicable, describe which groupings were chosen and why	Yes (6)
Statistical methods	12	(*a*) Describe all statistical methods, including those used to control for confounding	Yes (6)
		(*b*) Describe any methods used to examine subgroups and interactions	Yes (6)
		(*c*) Explain how missing data were addressed	Yes (6)
		(*d*) *Cohort study*—If applicable, explain how loss to follow-up was addressed *Case-control study*—If applicable, explain how matching of cases and controls was addressed	Yes (6)

		Cross-sectional study—If applicable, describe analytical methods taking account of sampling strategy	
		(*e*) Describe any sensitivity analyses	n/a

Participants	13*	(a) Report numbers of individuals at each stage of study—eg numbers potentially eligible, examined for eligibility, confirmed eligible, included in the study, completing follow-up, and analysed	Yes (7)
		(b) Give reasons for non-participation at each stage	Yes (7)
		(c) Consider use of a flow diagram	Yes (figure 1)
Descriptive data	14*	(a) Give characteristics of study participants (eg demographic, clinical, social) and information on exposures and potential confounders	Yes (7, table 1, supplemental tables)
		(b) Indicate number of participants with missing data for each variable of interest	Yes (table 1)
		(c) *Cohort study*—Summarise follow-up time (eg, average and total amount)	Yes (7-10)
Outcome data	15*	*Cohort study*—Report numbers of outcome events or summary measures over time	Yes (7-10)
		Case-control study—Report numbers in each exposure category, or summary measures of exposure	*n/a*
		Cross-sectional study—Report numbers of outcome events or summary measures	*n/a*
Main results	16	(*a*) Give unadjusted estimates and, if applicable, confounder-adjusted estimates and their precision (eg, 95% confidence interval). Make clear which confounders were adjusted for and why they were included	Yes (7-10)
		(*b*) Report category boundaries when continuous variables were categorized	Yes (7-10)
		(*c*) If relevant, consider translating estimates of relative risk into absolute risk for a meaningful time period	n/a
Other analyses	17	Report other analyses done—eg analyses of subgroups and interactions, and sensitivity analyses	Yes (7-10)
Key results	18	Summarise key results with reference to study objectives	Yes (7,8)
Limitations	19	Discuss limitations of the study, taking into account sources of potential bias or imprecision. Discuss both direction and magnitude of any potential bias	Yes (11)
Interpretation	20	Give a cautious overall interpretation of results considering objectives, limitations, multiplicity of analyses, results from similar studies, and other relevant evidence	Yes (11)
Generalisability	21	Discuss the generalisability (external validity) of the study results	Yes (11)
Funding	22	Give the source of funding and the role of the funders for the present study and, if applicable, for the original study on which the present article is based	Yes (16)

*Give information separately for cases and controls in case-control studies and, if applicable, for exposed and unexposed groups in cohort and cross-sectional studies.

Note: An Explanation and Elaboration article discusses each checklist item and gives methodological background and published examples of transparent reporting. The STROBE checklist is best used in conjunction with this article (freely available on the Web sites of PLoS Medicine at http://www.plosmedicine.org/, Annals of Internal Medicine at http://www.annals.org/, and Epidemiology at http://www.epidem.com/). Information on the STROBE Initiative is available at www.strobe-statement.org.

BMJ Open

Short-Term Abstinence from Alcohol and Changes in Cardiovascular Risk Factors, Liver Function Tests and Cancer-Related Growth Factors: A Prospective Observational Study

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Primary Subject Heading:	Gastroenterology and hepatology
Secondary Subject Heading:	Gastroenterology and hepatology, Public health
Keywords:	Alcohol, Insulin resistance, Cancer, Fatty liver

Short-Term Abstinence from Alcohol and Changes in Cardiovascular Risk Factors, Liver Function Tests and Cancer-Related Growth Factors: A Prospective Observational Study

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Abstract

Objective: To assess changes in metabolic risk factors and cancer-related growth factors associated with short-term abstinence from alcohol.

Design: Prospective, observational study.

Setting: Single tertiary centre.

Participants: Healthy subjects were recruited based on intention to: (i) abstain from alcohol for one month (abstinence group), or (ii) continue to drink alcohol (control group). Inclusion criteria were baseline alcohol consumption >64g/week (males) or >48g/week (females). Exclusion criteria were known liver disease or alcohol dependence.

Primary and secondary outcome measures: The primary outcome was change in insulin resistance (HOMA score). Secondary outcomes were changes in weight, blood pressure, VEGF, EGF, and liver function tests. Primary and secondary outcomes were adjusted for changes in diet, exercise, and cigarette smoking.

Results: The abstinence group comprised 94 participants (mean age 45.5 years, SD±1.2) and the control group 47 participants (mean age 48.7 years, SD±1.8). Baseline alcohol consumption in the abstinence group was 258.2g/week, SD±9.4, and in the control group 233.8g, SD±19.0. Significant reductions from baseline in the abstinence group (all $p<0.001$) were found in: HOMA score (-25.9%, IQR -48.6 to +0.3%), systolic blood pressure (-6.6%, IQR -11.8% to 0.0%), diastolic blood pressure (-6.3%, IQR -14.1% to +1.3%), weight (-1.5%, IQR -2.9% to -0.4%), VEGF (-41.8%, IQR -64.9% to -17.9%) and EGF (-73.9%, IQR -86.1% to -36.4%). None of these changes were associated with changes in diet, exercise or cigarette smoking.

No significant changes from baseline in primary or secondary outcomes were noted in the control group.

Conclusion: These findings demonstrate that abstinence from alcohol in moderate-heavy drinkers improves insulin resistance, weight, blood pressure and cancer-related growth factors. These data support an independent association of alcohol consumption with cancer risk, and suggest an increased risk of metabolic diseases such as type 2 diabetes and fatty liver disease.

Strengths and limitations of this study

Strengths:

- Prospective study design
- Recruitment of a control group
- Thorough characterization of the biological and lifestyle confounders

Limitations:

- Lack of randomization to groups
- Study cohort all from university teaching hospital or science magazine.

Funding: This work was funded by the Royal Free Charity, Camden and Islington Public Health, and the Royal Free London NHS Foundation Trust.

Competing interests declaration: None of the authors, or their spouses/children, have a financial relationship with any organization(s) that might have an interest in the submitted work in the previous three years, or any other relationship/activity that could appear to have influenced this work.

Data sharing statement: All raw data is available on request from the corresponding author.

Introduction

Alcohol is a major cause of disability and preventable death. Globally, alcohol is the seventh leading risk factor overall in terms of disability-adjusted life years (DALYs), and is the leading risk factor globally in working age individuals (ages 15-59). Moreover, alcohol use attributable DALYs have increased by over 25% in the last 25 years.[1] European countries have amongst the highest alcohol consumption. Eastern Europe has the highest *per capita* consumption worldwide,[2] and in the UK over 25% of the adult population drink in excess of recommended guidelines.[3]

Aside from liver disease, which is the 3rd commonest cause of preventable death in the UK, there is also a significant burden from alcohol-related cancer and metabolic syndrome.[3] Alcohol has been classified by the WHO as a class I carcinogen for some decades, and a report from the World Cancer Research Fund/American Institute for Cancer Research states that there is convincing evidence that alcohol is causally related to cancers of the oral cavity, pharynx, larynx, oesophagus, breast and colorectum.[4]

Moreover, it has long been recognized that there is an important interaction between alcohol misuse and fatty liver disease.[5] One of the main factors driving the development of fatty liver disease and steatohepatitis is insulin resistance. Thus, any action that improves insulin resistance will have a major impact on the development and severity of fatty liver disease. However, there remains debate as to the impact of

alcohol consumption on fatty liver disease driven predominantly by insulin resistance and metabolic factors. [6,7]

In this climate of increased awareness of alcohol-related morbidity, the UK Chief Medical Officers have revised downwards their weekly guidance limits.[8] Additionally, public health campaigns, where non-dependent drinkers are encouraged to commit to short-term abstinence from alcohol, are increasingly common. The aim of this study was to assess changes in insulin resistance, metabolic risk factors and cancer-related growth factors with short-term abstinence from alcohol in moderate drinkers.

Methods

Study Design: This was a single-centre, prospective, observational study conducted at the Royal Free London NHS Foundation Trust. Ethical approval was granted by the NRES Committee (14/NW/1510), and written informed consent was obtained from all participants. Study recruitment was initiated through email advertising within University College London, Queen Mary University of London, and New Scientist Magazine. The entry criteria were baseline alcohol consumption of >64g/week (8 units) for males or >48g/week (6 units) for females. Exclusion criteria were >3 days abstinence from alcohol prior to commencement of the study, the presence of known liver disease or alcohol dependence. Participants were not randomized to group, but were allocated based on intention to maintain abstinence for one month (abstinence group) or to continue alcohol consumption (control group).

Participants were assessed at baseline, and after one-month. The primary outcome was change in insulin resistance (HOMA score) at baseline and one-month. Secondary outcomes were changes in weight, blood pressure, VEGF, EGF and liver function tests. Information on diet, exercise, and smoking history were obtained by self-reporting using components of the SLIQ lifestyle questionnaire[9]. Self-reported alcohol intake was assessed at baseline using the full AUDIT questionnaire, and a direct interview by a single interviewer (KM) was also conducted to assess alcohol intake over the preceding two-months, using the timeline follow back method.[10] Additionally, a follow-up telephone interview was conducted at 6-8 months to determine drinking habits following the study period, using the full AUDIT questionnaire (modified to capture data for the preceding 6-8 months).

Sample size calculation for the control group was performed, based on pre/post data acquired from the abstinence group (table 1). Specifically, based on this data, a power calculation determined that the following sample sizes were required to detect statistically significant differences of the same magnitude (80% power, alpha 5%, 2-sided test): HOMA score n=47, weight n=21, VEGF n=31, EGF n=30.

Blood pressure (BP) was measured seated, following a 2-minute rest period, and the mean of three measurements was recorded. Fasting blood was taken, between 8am and midday, for measurement of glucose, insulin, liver function tests, lipids, carbohydrate deficient transferrin (abstinence group only) and VEGF (isoforms 165, 145 and 121) and EGF (Randox Investigator, Randox, Belfast, UK). The HOMA score was calculated according to the methods of Matthews et al.[11] Participants with diabetes requiring treatment were excluded from HOMA measurements.

Statistical analysis: Baseline and one-month differences were analysed by paired t-test for normally distributed differences in continuous variables, by Wilcoxon signed rank test for not normally distributed differences in continuous variables, and differences in categorical variables by Chi-square test. Differences between abstinence and control groups were analysed by unpaired t-test for normally distributed variables, and Mann-Whitney test for variables that were not normally distributed. Lifestyle factors were categorically graded (better/same/worse), and delta change in biological variables between lifestyle groups was assessed by Kruskal-Wallis test. Multivariable logistic regression analysis was also used to test the effect of abstinence on improvement in HOMA, weight, blood pressure, VEGF and EGF once other lifestyle factors (diet and exercise) were taken into account. Correlation between biological variables was assessed by Spearman's correlation. All analyses were performed using STATA version 13.1 and SPSS Statistics version 21.0. Standard deviation (SD) is reported for means and interquartile range (IQR) for medians where applicable. All p values are 2 sided; $p<0{\cdot}01$ was considered significant to account for multiple comparisons.

Patient and Public Involvement: The research question was developed following public feedback to a pilot project, conducted in collaboration with, and published by, New Scientist magazine (New Scientist, 31st December 2013). Additionally, the research question was informed by focus groups, funded through the NIHR Enabling Involvement Fund. No specific patient advisers were involved in the design or conduct of the study. Results of the study will be disseminated to all participants by email.

Results

Ninety-seven participants were recruited to the abstinence group, and forty-eight participants to the control group. Three subjects in the abstinence group and one subject in the control group did not attend for follow-up. Thus, the final abstinence group comprised ninety-four participants (43 male, 51 female) mean age 45·5 years, SD±1·2, and the control group comprised 47 participants (22 male, 25 female) mean age 48·7 years, SD±1·8. Mean baseline alcohol intake for the abstinence group was 258·2g/week, SD±9·4 (men 275·9, SD±25·5; women 243·1, SD±12·8). All subjects in this group, except one individual, remained abstinent for the study period - this participant was included in all analyses. Mean weekly baseline alcohol intake for the control group was 233·8g, SD±19·0 (men 270·2, SD±26·6; women 200·2, SD±25·8), and was not significantly different at one-month 260·1g, SD±20·8 (men 286·4, SD±26·6; women 235·8, SD±31·1), p=0·11. A flowchart of participants and observations is shown in figure 1.

Baseline and one-month variables for the abstinence and control groups are listed in table 1. There were no significant differences in baseline characteristics between abstinence and control groups, aside from baseline blood pressure which was significantly lower in the control group (systolic bp: 135·8 SD±1·9mmHg *vs* 125·7 SD±2·0mmHg, p<0·01; diastolic bp: 87·7 SD±1·2mmHg *vs* 74·3·7 SD±1·5mmHg, p<0·01). Anti-hypertensives were used in one participant in the abstinence group, and one participant in the control group. Lipid-lowering agents were used in two participants in the abstinence group, one subject in the control group. These participants were excluded from analyses for blood pressure and lipids respectively. Significant reductions from baseline (pre *vs* post) in the abstinence group were

observed in: HOMA score (-25·9%, IQR -48·6 to +0·3%), systolic blood pressure (-6·6%, IQR -11·8% to 0·0%), diastolic blood pressure (-6·3%, IQR -14·1% to +1·3%) and weight (-1·5%, IQR -2·9% to -0·4%). HOMA score was not performed due to type 1 diabetes in one participant in the abstinence group. By chance, no participants had type 2 diabetes. Levels of VEGF and EGF also markedly reduced in the abstinence group, at -41·8% (IQR -64·9% to -17·9%) and -73·9% (IQR -86·1% to -36·4%) respectively (figure 2). Serum lipids (pre *vs* post) also improved in the abstinence group: fasting total serum cholesterol (-13·4%, IQR -18·9% to -2·7%), LDL cholesterol (-9·4%, IQR -20·1% to +4·8%), HDL cholesterol (-16·7%, IQR -25·0% to 0·0%). All the above variables were significantly reduced from baseline, $p<0·001$. By contrast, the control group did not show significant changes from baseline in any of the above variables. Changes from baseline in HOMA score, VEGF, EGF, weight, systolic and diastolic blood pressure are shown in figure 3.

Table 1: Baseline and one-month variables for abstinence and control groups.

Variable [units]	Abstinence group					Control Group				
	Number of paired values[a]	Baseline	One-month	p-value (pre vs post)	Effect size	Number of paired values[a]	Baseline	One-month	p-value (pre *vs* post)	Effect size
HOMA score [median (IQR)]	86	1·4 (1·0-2·1)	1·0 (0·7-1·4)	p<0·001	-0.40	47	1·2 (0·9-1·5)	1·1 (0·9-1·5)	p=0·42	-0.08
Systolic blood pressure [mmHg, median (IQR)]	93	136·0 (121·0-147·5)	125·0 (115·0-141·0)	p<0·001	-0.57	47	121·0 (118·0-134·0)	122·0 (116·0-131·0)	p=0·17	-0.14
Diastolic blood pressure [mmHg, mean (SD)]	93	89·0 (80·0-95·5)	82·0 (77·0-89·0)	p<0·001	-0.34	47	71·0 (68·0-80·0)	75·0 (68·0-80·0)	p=0·77	-0.03
Weight [kg, median (IQR)]	89	81·1 (67·0-89·7)	79·5 (66·7-87·2)	p<0·001	-0.49	47	73·8 (65·2-85·0)	72·6 (64·9-84·6)	p=0·09	-0.18
VEGF [ng/L, median (IQR)]	81	7·6 (5·5-16·0)	4.0 (3·1-5·8)	p<0·001	-0.55	41	13·4 (9·0-17·9)	12·9 (8·3-18·5)	p=0·34	-0.10
EGF [ng/L, median (IQR)]	81	7·2 (3·6-13·5)	1·5 (1·0-2·5)	p<0·001	-0.56	41	3·8 (1·0-10·3)	4·9 (1·0-9·5)	p=0·55	-0.07
Total Cholesterol [mg/dL, median (IQR)]	88	228·7 (198·4-258·4)	202·1 (176·3-227·4)	p<0·001	-0.85	47	208·8 (177·9-232·0)	205·0 (177·9-239·8)	p=0·25	-0.12
LDL [mg/dL, median (IQR)]	88	131·5 (112·3-163·1)	127·0 (97·4-153·8)	p<0·001	-0.42	47	112·1 (88·9-139·2)	112·1 (85·1-143·1)	p=0·16	-0.15
HDL [mg/dL, median (IQR)]	88	74·4 (±22·3)	60·9 (±17·8)	p<0·001	-0.57	47	73·5 (58·0-88·9)	73·5 (58·0-92·8)	p=0·95	-0.02
Triglycerides [mg/dL, median (IQR)]	88	79·3 (57·6-115·8)	76·6 (60·4-103·2)	p=0·05	-0.15	47	79·7 (62·0-124·0)	79·7 (62·0-106·3)	p=0·70	-0.05
Gamma GT [IU/L, median (IQR)]	92	22·5 (14·0-34·8)	15·0 (10·3-24·0)	p<0·001	-0.53	46	39·8±8·3	44·8±11·0	p=0·26	-0.12
ALT [IU/L, median (IQR)]	94	25·0 (18·0-37·0)	20·0 (16·0-29·3)	p<0·001	-0.29	47	24·0 (18·0-31·0)	25·0 (20·0-33·0)	p=0·06	-0.21
AST [IU/L, median (IQR)]	94	23·0 (19·0-28·0)	21·0 (18·0-27·0)	p=0.03	-0.16	47	20·0 (17·0-24·0)	22·0 (19·0-27·0)	p<0·01	-0.27
Carbohydrate deficient transferrin [%, median (IQR)]	81	0·7 (0·5-1·1)	0·6 (0·4-0·8)	p<0·001	-0.32	n/a	n/a	n/a	n/a	n/a

Effect size for normally distributed variables is calculated as [mean change in variable]/SD. Effect size for non-normally distributed variables is calculated as Wilcoxon signed rank [test statistic]/ √ [number of observations].

Abbreviations: HOMA, homeostatic model assessment; VEGF, vascular endothelial growth factor; EGF, epidermal growth factor; LDL, low density lipoprotein; HDL, high density lipoprotein; ALT, alanine aminotransferase; AST aspartate aminotransferase.

[a]*Where the number of paired observations is less than 94 (abstinence group) or 47 (control group), this is due to missing data points.*

Liver function tests also improved in the abstinence group, thus, there was a significant reduction in serum ALT (-14·5%, IQR -28·9 to +6·7%, p<0·001) and gamma GT (-28·6%, IQR -43·5 to -14·4%, p<0·001), and a trend towards reduction in serum AST (-5·4%, IQR -16·2 to +9·5%, p=0·03). No significant change in these variables was seen in the control group, aside from a small rise in AST (+4·5%, IQR -5·6 to +23·1%, p<0·01).

Lifestyle factors did not account for changes in the abstinence group. No changes were seen in exercise score (10·9 SD±4·7 *vs* 10·7 SD±4·6, p=0·82) or cigarette smoking (1·3 SD±0·7 *vs* 1·4 SD±0·7, p=0·17). A small change in diet score was noted (from 8·2 SD±3·3 to 8·8 SD±3·0, p=0·03). The pre/post differences in HOMA score, weight, VEGF, EGF, triglycerides and HDL were distributed with a left (negative) skew. Therefore, non-parametric approaches were adopted to account for lifestyle variables. Changes in HOMA score, BP, and weight in the abstinence group were not associated with changes in any lifestyle score (supplemental table 1). There was also no association between changes in HOMA score and weight (r=0·04, p=0·73). However, changes in total cholesterol and LDL cholesterol attained borderline significance between groups when compared by change in diet in the abstinence group (supplemental table 1; p=0·01 and p=0·02 respectively).

Additionally, multivariable logistic regression analysis was used across the whole cohort, combining the abstinence and control groups, to determine predictors of: HOMA score reduction ≥20%, systolic bp reduction ≥5%, weight reduction ≥2%, VEGF reduction ≥20%, EGF reduction ≥20%. The model used covariates of abstinence (yes/no) or change in exercise and diet SLIQ score (better/same/worse).

Abstinence was a highly significant predictor of improvement in these biological variables (all p<0.01). By contrast, change in exercise and diet score was not associated with improvement in any of these variables (table 2).

A further important result relates to follow-up questionnaire data, obtained in 77 individuals (81·9%) in the abstinence group and 40 (83·3%) in the control group, at 6-8 months following the study period. In the abstinence group, a significant reduction in alcohol consumption was maintained from their pre-study assessment. Thus, there was a significant reduction in overall AUDIT score from 10·0 (IQR 7·0 to 15·0) to 7·0 (IQR 5·0 to 9·0), p<0·001, and in the proportion of individuals with harmful use of alcohol (AUDIT score>8) (61·0% *vs* 28·5%, p<0·001) at 6-8 months compared with baseline. By contrast, in the control group there was a non-significant trend to reduction in overall AUDIT score from 8·5 (IQR 6·3 to 12·0) to 8·0 (IQR 6·0 to 10·8), p=0·06), and no significant change in the proportion with harmful use (50% *vs* 40%, p=0·37).

Table 2: Independent predictors of improvement in HOMA score, systolic bp, weight, VEGF and EGF

Biological variable (target reduction)	All study participants (n=141)		
Covariate	Odds Ratio	95% CI	p value
HOMA score (reduction ≥20%}			
Abstinence	3.48	1.60 - 7.53	p=0.002
Exercise + diet SLIQ score	0.87	0.43 - 1.77	p=0.694
Systolic bp (reduction ≥5%)			
Abstinence	6.47	2.71 - 15.46	p<0.001
Exercise + diet SLIQ score	1.47	0.70 - 3.08	p=0.310
Weight (reduction ≥2%)			
Abstinence	15.63	3.54 - 68.95	p<0.001
Exercise + diet SLIQ score	0.74	0.323 - 1.69	0.475
VEGF (reduction ≥20%)			
Abstinence	4.35	1.93 - 9.81	p<0.001
Exercise + diet SLIQ score	2.17	0.97 - 4.86	p=0.59
EGF (reduction ≥20%)			
Abstinence	48.81	15.26 - 156.06	p<0.001
Exercise + diet SLIQ score	2.52	0.80 - 7.95	p=0.115

Results are presented as adjusted odds ratios and 95% confidence intervals using multivariable logistic regression analysis. Abbreviations: OR, odds ratio; CI, confidence interval; HOMA, homeostatic model assessment; VEGF, vascular endothelial growth factor; EGF, epidermal growth factor.

Discussion

This study is the first to comprehensively assess the effects of short-term abstinence from alcohol in a population of 'healthy' individuals, who are representative of the 25% of the wider population who drink alcohol above national guidelines. The key findings of this study are improvements in insulin resistance, blood pressure, body weight, and a decrease in circulating concentrations of cancer-related growth factors following a month of abstinence from alcohol.

The strengths of this study are the prospective study design, the recruitment of a control group, and the thorough characterization of the participant's biological and lifestyle data. A weakness is the lack of randomization of groups, although for ethical reasons the allocation of individuals to a pre-defined alcohol consumption regimen was inappropriate. A further weakness relates to the study cohort, who were recruited through staff at university teaching hospitals and a science magazine, and thus probably had higher educational attainment and health-related motivation than the average population. A further confounder is the possibility of lifestyle change in the abstinence group, alongside abstinence from alcohol. We have tried to minimize the impact of these using the SLIQ questionnaire, a self-reported measure of lifestyle factors contributing to metabolic risk with good re-test reliability.[9] As such, changes in HOMA score, weight and blood pressure were independent of changes in lifestyle as measured by the SLIQ score. Nevertheless, it remains possible that the questionnaire scoring for diet, exercise and cigarette smoking has inadequate sensitivity for all lifestyle changes within this cohort.

The primary endpoint of insulin resistance, measured by HOMA score, showed a marked decrease (~25%) following the cessation of alcohol consumption. Some previous epidemiological data has supported a protective effect of low-dose alcohol use on the risk of type 2 diabetes,[12] although more recent work suggests this may be due to incomplete adjustment for 'sick quitters'[13], and prospective alcohol intervention studies have provided mixed results.[14,15] Our data support a positive association of moderate-heavy alcohol use with an increased risk of type 2 diabetes. Moreover, the observed effects of abstinence on HOMA score noted in this study are too dramatic to be accounted for by weight loss alone, and no specific association was found between change in HOMA score and weight. To our knowledge, this is the first paper to prospectively demonstrate a link between excess alcohol consumption and insulin resistance.

A major novel finding of this study is the rapid decrease in serum VEGF and EGF with short-term abstinence from alcohol, which was seen in 90% of subjects in the abstinence group. Importantly, these changes were not seen in the control group with continued alcohol consumption. Alcohol is causally related to the development of several cancers, including the digestive tract, nasopharynx, liver and breast, and is classified as a class I carcinogen.[4,16] The increased risk caused by alcohol persists even at low-levels of consumption. The mechanism of mutagenesis is thought to relate to direct effects of the alcohol metabolite, acetaldehyde[4]. However, in this study, we chose to study VEGF and EGF, since they are key molecules in the multi-step progression of cancer, are both highly expressed in the solid tumours listed above, and are common therapeutic targets for these tumours.[17] VEGF plays a key role in tumour progression through angiogenic

pathways, and VEGF expression is driven by oncogene expression (eg. Ras, src, HER2, EGFR).[18] EGF signalling contributes to oncogenesis by directly promoting cell proliferation,[19] and expression levels are correlated with progressive tumour growth and metastasis.[20-22]

Mechanistically, rodent models have demonstrated that alcohol exposure directly promotes the progression of several cancers, including breast cancer. Lu et al have shown, in a mouse model of breast cancer, that alcohol directly induces tumor angiogenesis and accelerated tumor growth through a VEGF-dependent mechanism.[23] Similar evidence for an alcohol-VEGF pathway exists in mouse models of colon cancer and melanoma.[24,25] The EGF pathway has also been implicated in alcohol-related breast cancer.[26-28] The baseline levels of VEGF and EGF reported in this study are lower than reported in other studies exploring associations of circulating VEGF/EGF levels with the occurrence of solid tumours.[29-31] These differences are explained by the method of sample collection. The collection of blood into EDTA tubes, as in this study, leads to reduced contribution of platelet-derived VEGF and EGF, and thus lower plasma concentrations.[32,33]

Here, we demonstrate for the first time in humans an association of abstinence from alcohol with a marked reduction in circulating concentrations of VEGF and EGF, which suggests that alcohol consumption *per se* increases the concentrations of these growth factors. There is strong evidence that these growth factors play an important role in oncogenesis. However, it would be wrong to speculate further on

this observation without longitudinal study in subjects who continue moderate alcohol consumption.

These data also show the dynamic effect of regular alcohol consumption on blood pressure, an effect that is maintained in healthy individuals with no history of hypertension requiring medication. An effect of alcohol on blood pressure has long been recognized, with consumption greater than two daily doses considered to be one of the most common reversible causes of hypertension.[34]

Collectively, the above findings have implications for the risk of synergistic liver injury amongst individuals with risk factors for alcohol-related liver disease (ALD) and fatty liver disease. Previous studies have emphasized an association between these pathways of liver injury, since serum ALT amongst moderate drinkers is elevated to a greater extent in those with higher BMI, and ALD and fatty liver are pathologically similar. Two prospective cohort studies from Scotland have demonstrated an increased risk of liver disease with alcohol use and elevated BMI.[35] More recently, a large prospective study of over 100,000 women in the UK confirmed a synergistic association between alcohol and high BMI and risk of chronic liver disease.[36] Since alcohol use and insulin resistance are both directly implicated in the development of steatohepatitis, the results of this study provide further support for this common causal pathway. Further, changes in the gut microbiome have also been implicated in the pathogenesis of steatohepatitis and obesity,[37] and therefore changes in gut microbe populations following abstinence from alcohol are a further possible explanation for the biological changes observed in this study. These hypotheses merit further attention in subsequent mechanistic studies.

A frequent criticism of public health strategies of short-term abstinence (eg. Dry January) has been the lack of evidence of health benefits, or even negative effects on longer-term alcohol consumption.

Although this study has demonstrated health benefits from short-term abstinence, a possible misrepresentation of these results is the concept that a 'detox' period is all that is required to 'refresh' the liver or achieve other health gains. This is clearly untrue, since the durability of the observed biological effects remains to be established. The data presented here provide supportive mechanistic evidence for the recent changes in alcohol guidance due to cancer risk, and the synergistic relationship between alcohol and metabolic syndrome. Further attention should be directed to determining the durability of these biological effects of abstinence, and conveying these complex public health messages to the public.

Contributors:

GM contributed to study design, participated in data collection, wrote the analytical plan, and drafted and revised the paper. He is guarantor. SM participated in study design, participated in data collection, and drafted and revised the paper. AC and TKB analysed the data, and drafted and revised the paper. CS participated in study design, and revision of the paper. MR, SAK, AJ, CC, JM, AG and TH participated in data collection and revision of the paper. CJ, RS, DN and RJ contributed to study design, and revision of the paper. KM supervised the study, contributed to study design, participated in data collection and drafted and revised the paper.

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Figure 1: Flow chart of study participants.

Figure 2: Baseline and one-month data for the abstinence group presented as pre/post scatterplot (left) and bar chart chart (right).

Bar chart data are presented as median (IQR). Panels (clockwise from top right): HOMA score, weight, diastolic bp, EGF, VEGF, systolic bp. Baseline and one-month values were compared with Wilcoxon signed rank test, p<0·01 taken as level of significance. Abbreviations: HOMA, homeostatic model assessment; VEGF, vascular endothelial growth factor; EGF, epidermal growth factor; bp, blood pressure.

Figure 3: Percentage change from baseline in HOMA score, VEGF, EGF, systolic bp, diastolic bp and weight in abstinence (dark bar) and control (light bar) groups.

Data are presented as median (IQR). Changes from baseline in abstinence and control groups were compared with Mann Whitney test, p<0·01 taken as level of significance. Abbreviations: HOMA, homeostatic model assessment; VEGF, vascular endothelial growth factor; EGF, epidermal growth factor; bp, blood pressure.

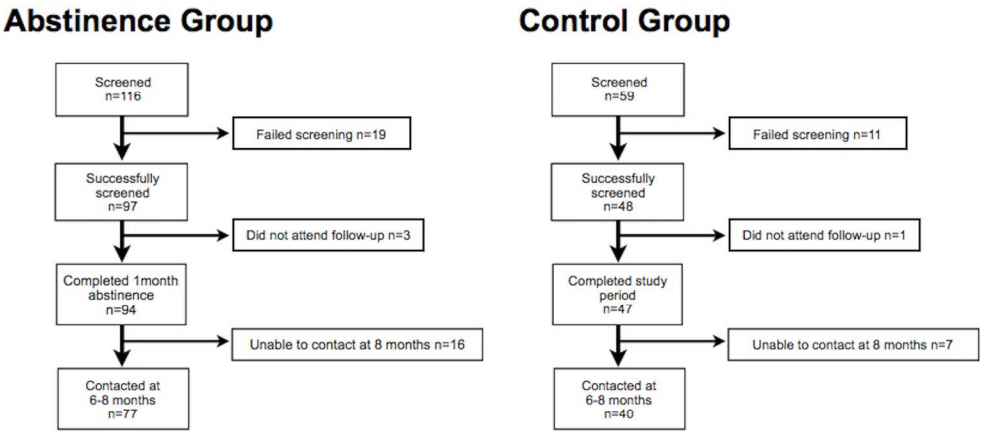

Figure 1: Flow chart of study participants

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147x78mm (300 x 300 DPI)

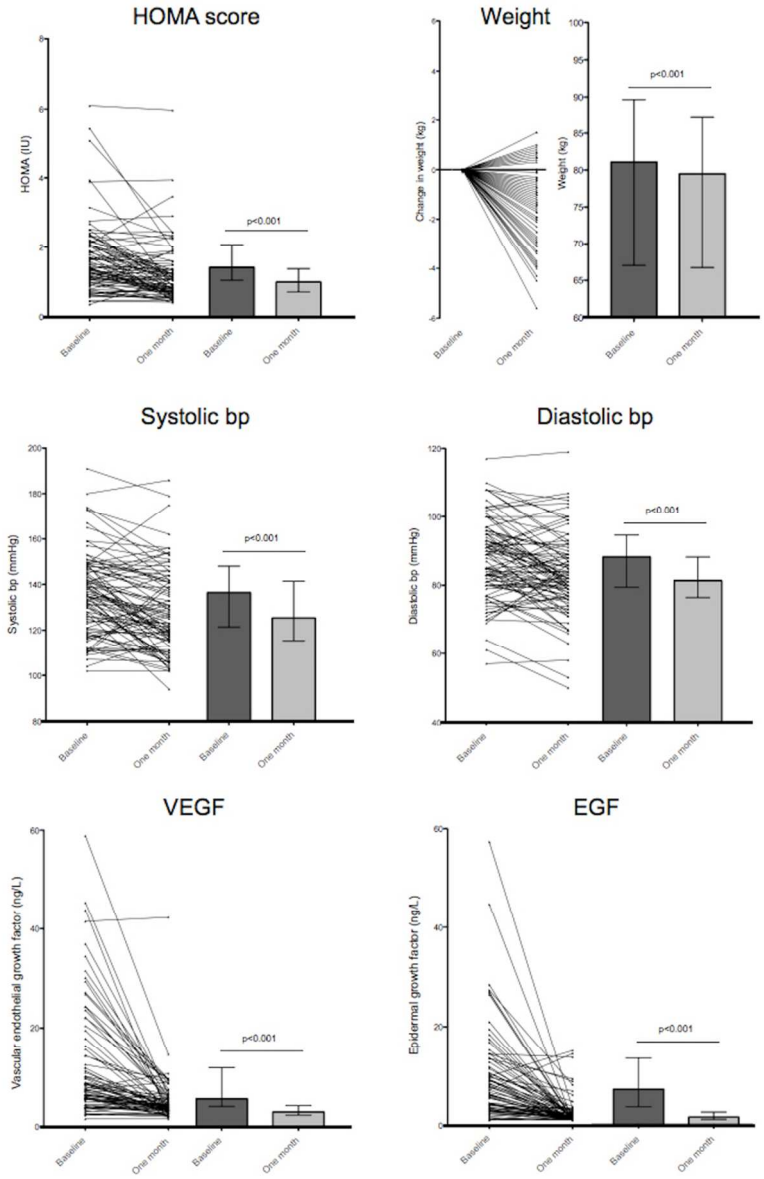

Figure 2: Baseline and one-month data for the abstinence group presented as pre/post scatterplot (left) and bar chart chart (right). ‼ ┼ Bar chart data are presented as median (IQR). Panels (clockwise from top right): HOMA score, weight, diastolic bp, EGF, VEGF, systolic bp. Baseline and one-month values were compared with Wilcoxon signed rank test, p<0·01 taken as level of significance. Abbreviations: HOMA, homeostatic model assessment; VEGF, vascular endothelial growth factor; EGF, epidermal growth factor; bp, blood pressure.‼ ┼

80x120mm (300 x 300 DPI)

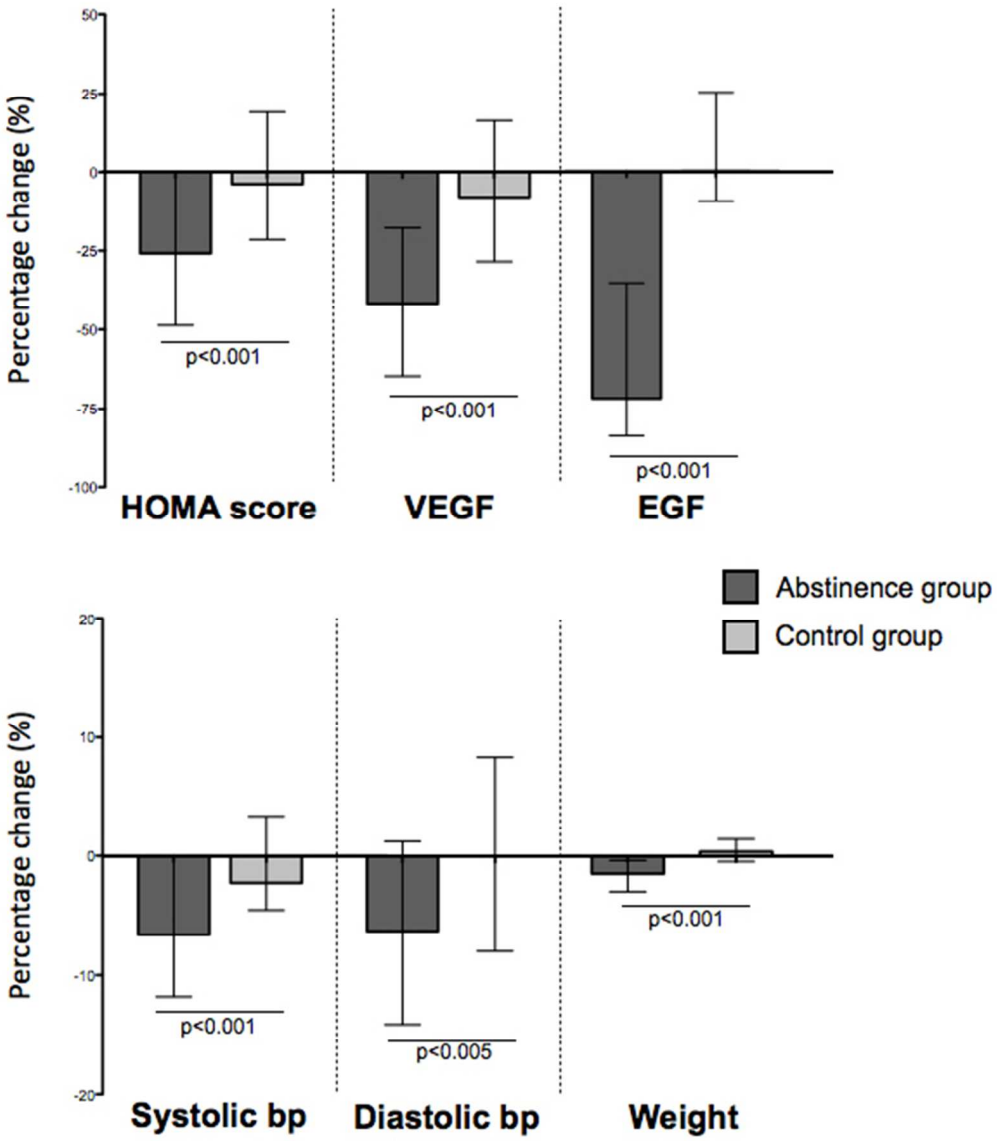

Figure 3: Percentage change from baseline in HOMA score, VEGF, EGF, systolic bp, diastolic bp and weight in abstinence (dark bar) and control (light bar) groups.
Data are presented as median (IQR). Changes from baseline in abstinence and control groups were compared with Mann Whitney test, p<0·01 taken as level of significance. Abbreviations: HOMA, homeostatic model assessment; VEGF, vascular endothelial growth factor; EGF, epidermal growth factor; bp, blood pressure.

48x56mm (300 x 300 DPI)

Supplemental Table 1a: Change from baseline in biological variables between categories of change in SLIQ DIET score (better / same / worse) in the abstinence group.

		Categorical change from baseline in DIET score in Abstinence Group			p-value[a]
		Better (n=42)[b]	Same (n=16)[b]	Worse (n=32)[b]	
Change from baseline in biological variable [%, median (IQR)]	HOMA	-14·1 (-42·6 to -0·6)	-33·7 (-48·9 to +1·7)	-34·9 (-53·1 to -1·7)	p=0·45
	Systolic blood pressure	-6·6 (-13·3 to -1·4)	-6·9 (-11·8 to +3·5)	-6·8 (13·8 to +2·5)	p=0·83
	Diastolic blood pressure	-7·3 (-15·1 to +1·3)	-5·6 (-16·1 to +5·5)	-5·9 (-12·5 to +1·2)	p=0·71
	Weight	-1·5 (-2·8 to 0·0)	-1·1 (-2·8 to +0·4)	-1·4 (-3·0 to -0·3)	p=0·95
	VEGF	-36·2 (-63·6 to -10·1)	-57·5 (-73·9 to -30·1)	-50·0 (-75·5 to -23·7)	p=0·15
	EGF	-73·0 (-85·9 to -31·8)	-75·8 (-91·2 to -54·3)	-76·7 (-88·5 to -37·5)	p=0·54
	Cholesterol	-9·2 (-16·7 to +3·5)	-17·3 (-25·0 to -13·6)	-14·0 (-17·1 to -7·6)	p=0·01
	LDL	-5·1 (-19·2 to +16·8)	-18·4 (-25·8 to -8·8)	-12·2 (-18·4 to -3·8)	p=0·02
	HDL	-15·4 (-23·0 to -5·8)	-18·9 (25·5 to -7·8)	-21·0 (-29·7 to -10·9)	p=0·26
	Triglycerides	-5·1 (-27·2 to +23·0)	-19·9 (-33·4 to -2·1)	+1.2 (-13·6 to +24·8)	p=0·03

Supplemental Table 1b: Change from baseline in biological variables between categories of change in SLIQ EXERCISE score (better / same / worse) in the abstinence group.

		Categorical change from baseline in EXERCISE score in Abstinence Group			p-value[a]
		Better (n=42)[b]	Same (n=16)[b]	Worse (n=32)[b]	
Change from baseline in biological variable [%, median (IQR)]	**HOMA**	-12·2 (-45·1 to +1·8)	-26·5 (-46·3 to -1·1)	-38·9 (-56·2 to -8·7)	p=0·21
	Systolic blood pressure	-6·0 (-11·1 to 0·0)	-2·7 (-12·9 to +4·6)	-8·0 (15·0 to -3·7)	p=0·36
	Diastolic blood pressure	-4·2 (-13·9 to +1·3)	-3·9 (-13·6 to +7·9)	-8·5 (-14·9 to +0·3)	p=0·31
	Weight	-1·7 (-3·1 to -0·4)	-1·4 (-2·9 to +0·1)	-1·1 (-2·5 to -0·1)	p=0·51
	VEGF	-28·5 (-53·2 to -10·7)	-57·4 (-75·4 to -34·4)	-55·5 (-68·6 to -20·2)	p=0.11
	EGF	-74·9 (-88·5 to -32·7)	-75·0 (-91·2 to -38·2)	-71·4 (-82·4 to -41·4)	p=0.67
	Cholesterol	-13·7 (-18·5 to -3·2)	-12·6 (-20·1 to -4·5)	-14·1 (-17·7 to -2·7)	p=0·93
	LDL	-10·8 (-18·9 to +1·9)	-10·2 (-21·0 to +5·4)	-8·7 (-22·3 to +8·6)	p=0·99
	HDL	-16·7 (-25·3 to -7·6)	-16·2 (26·6 to -8·3)	-18·3 (-25·7 to -6·9)	p=0·97
	Triglycerides	-14·6 (-31·1 to +15·3)	-4.4 (-15·9 to +49·2)	+-4·7 (-16·7 to +14·4)	p=0·43

Abbreviations: SLIQ, simple lifestyle indicator questionnaire; HOMA, homeostatic model assessment; VEGF, vascular endothelial growth factor; EGF, epidermal growth factor; LDL, low density lipoprotein; HDL, high density lipoprotein; bp, blood pressure.

a. *Participants were graded as better, same or worse based on changes in SLIQ diet score (supplemental table 1a) and exercise score (supplemental table 1b). Data for changes in cigarette smoking is not shown – only 5 individuals had a change from baseline score in the abstinence cohort. Changes from baseline in the abstinence cohort in biological variables were compared between the better / same / worse groups using the Kruskal-Wallis test. P<0.01 was considered significant to account for multiple comparisons.*

Complete baseline and one-month lifestyle questionnaire data was available on 90 participants in the abstinence cohort. The number of paired values analysed for each variable are: HOMA n=82; systolic bp n=89; diastolic bp n=89; weight n=85; VEGF n=78; EGF n=78; cholesterol n=84; LDL n=84; HDL n=84; triglycerides n=8.

STROBE Statement—checklist of items that should be included in reports of observational studies

	Item No	Recommendation	Completed (page no.)
Title and abstract	1	(*a*) Indicate the study's design with a commonly used term in the title or the abstract	Yes (2)
		(*b*) Provide in the abstract an informative and balanced summary of what was done and what was found	Yes (2)
Background/rationale	2	Explain the scientific background and rationale for the investigation being reported	Yes (4)
Objectives	3	State specific objectives, including any prespecified hypotheses	Yes (4.5)
Study design	4	Present key elements of study design early in the paper	Yes (5)
Setting	5	Describe the setting, locations, and relevant dates, including periods of recruitment, exposure, follow-up, and data collection	Yes (5,6)
Participants	6	(*a*) *Cohort study*—Give the eligibility criteria, and the sources and methods of selection of participants. Describe methods of follow-up *Case-control study*—Give the eligibility criteria, and the sources and methods of case ascertainment and control selection. Give the rationale for the choice of cases and controls *Cross-sectional study*—Give the eligibility criteria, and the sources and methods of selection of participants	Yes (5.6)
		(*b*) *Cohort study*—For matched studies, give matching criteria and number of exposed and unexposed *Case-control study*—For matched studies, give matching criteria and the number of controls per case	n/a
Variables	7	Clearly define all outcomes, exposures, predictors, potential confounders, and effect modifiers. Give diagnostic criteria, if applicable	Yes (5.6)
Data sources/ measurement	8*	For each variable of interest, give sources of data and details of methods of assessment (measurement). Describe comparability of assessment methods if there is more than one group	Yes (5,6)
Bias	9	Describe any efforts to address potential sources of bias	Yes (5.6)
Study size	10	Explain how the study size was arrived at	Yes (6)
Quantitative variables	11	Explain how quantitative variables were handled in the analyses. If applicable, describe which groupings were chosen and why	Yes (6)
Statistical methods	12	(*a*) Describe all statistical methods, including those used to control for confounding	Yes (6)
		(*b*) Describe any methods used to examine subgroups and interactions	Yes (6)
		(*c*) Explain how missing data were addressed	Yes (6)
		(*d*) *Cohort study*—If applicable, explain how loss to follow-up was addressed *Case-control study*—If applicable, explain how matching of cases and controls was addressed	Yes (6)

		Cross-sectional study—If applicable, describe analytical methods taking account of sampling strategy	
		(*e*) Describe any sensitivity analyses	n/a

Participants	13*	(a) Report numbers of individuals at each stage of study—eg numbers potentially eligible, examined for eligibility, confirmed eligible, included in the study, completing follow-up, and analysed	Yes (7)
		(b) Give reasons for non-participation at each stage	Yes (7)
		(c) Consider use of a flow diagram	Yes (figure 1)
Descriptive data	14*	(a) Give characteristics of study participants (eg demographic, clinical, social) and information on exposures and potential confounders	Yes (7, table 1, supplemental tables)
		(b) Indicate number of participants with missing data for each variable of interest	Yes (table 1)
		(c) *Cohort study*—Summarise follow-up time (eg, average and total amount)	Yes (7-10)
Outcome data	15*	*Cohort study*—Report numbers of outcome events or summary measures over time	Yes (7-10)
		Case-control study—Report numbers in each exposure category, or summary measures of exposure	*n/a*
		Cross-sectional study—Report numbers of outcome events or summary measures	*n/a*
Main results	16	(*a*) Give unadjusted estimates and, if applicable, confounder-adjusted estimates and their precision (eg, 95% confidence interval). Make clear which confounders were adjusted for and why they were included	Yes (7-10)
		(*b*) Report category boundaries when continuous variables were categorized	Yes (7-10)
		(*c*) If relevant, consider translating estimates of relative risk into absolute risk for a meaningful time period	n/a
Other analyses	17	Report other analyses done—eg analyses of subgroups and interactions, and sensitivity analyses	Yes (7-10)
Key results	18	Summarise key results with reference to study objectives	Yes (7,8)
Limitations	19	Discuss limitations of the study, taking into account sources of potential bias or imprecision. Discuss both direction and magnitude of any potential bias	Yes (11)
Interpretation	20	Give a cautious overall interpretation of results considering objectives, limitations, multiplicity of analyses, results from similar studies, and other relevant evidence	Yes (11)
Generalisability	21	Discuss the generalisability (external validity) of the study results	Yes (11)
Funding	22	Give the source of funding and the role of the funders for the present study and, if applicable, for the original study on which the present article is based	Yes (16)

*Give information separately for cases and controls in case-control studies and, if applicable, for exposed and unexposed groups in cohort and cross-sectional studies.

Note: An Explanation and Elaboration article discusses each checklist item and gives methodological background and published examples of transparent reporting. The STROBE checklist is best used in conjunction with this article (freely available on the Web sites of PLoS Medicine at http://www.plosmedicine.org/, Annals of Internal Medicine at http://www.annals.org/, and Epidemiology at http://www.epidem.com/). Information on the STROBE Initiative is available at www.strobe-statement.org.