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## AN INVERSE ASSOCIATION BETWEEN SERUM LEPTIN CONCENTRATION AND REPORTED ALCOHOL INTAKE IN PATIENTS WITH MANIFEST VASCULAR DISEASE.

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► **To cite this version:**

Otto Mayer, Jan Filipovsky, Marketa Galovcová, Jan Bruthans, Jana Hrbkova, et al.. AN INVERSE ASSOCIATION BETWEEN SERUM LEPTIN CONCENTRATION AND REPORTED ALCOHOL INTAKE IN PATIENTS WITH MANIFEST VASCULAR DISEASE.. European Journal of Clinical Nutrition, 2010, 10.1038/ejcn.2010.153 . hal-00567031

**HAL Id: hal-00567031**

**<https://hal.science/hal-00567031>**

Submitted on 18 Feb 2011

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full title:

AN INVERSE ASSOCIATION BETWEEN SERUM LEPTIN CONCENTRATION AND  
REPORTED ALCOHOL INTAKE IN PATIENTS WITH MANIFEST VASCULAR DISEASE.

short title: Leptin and alcohol intake

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previous presentation:

conference of International Society of Atherosclerosis, Boston, 2009

source of funding: Internal Grant Agency, Ministry of Health of Czech Republic (IGA 9333-3)

Conflict of interests: none

total word count (incl. abstract, tables and references): 4497

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## Abstract

**Background/Objectives:** It has been reported that alcohol stimulates appetite. We aimed to establish the association between leptin, as a major food intake regulating factor, and alcohol intake in patients with chronic manifest coronary artery or cerebrovascular disease.

**Subjects/Methods:** A cross-sectional study of 820 subjects after acute coronary syndrome, coronary revascularisation or after first ischemic stroke. (Czech part of EUROASPIRE III surveys). Leptin concentrations were evaluated among predefined categories of reported weekly alcohol intake: abstainers, light drinkers (up to 2 drinks weekly, 1-44 g of pure alcohol), mild regular drinkers (3-14 drinks weekly, 45-308 g) and moderate or heavy drinkers (more than 15 drinks,  $\geq 309$  g of alcohol).

**Results:** The leptin showed a clear negative trend among alcohol intake categories. Mild regular drinkers showed significantly lower leptin [9.3(8.2)ng/mL], than abstainers [18.7(18.7) ng/mL,  $p < 0.0001$ ] and than light occasional drinkers [14.2(17.8) ng/mL,  $p = 0.00064$ ]. The negative association between leptin and alcohol intake as dependent variable remained significant even after adjustment for potential confounders in multiple linear regression [ $p = 0.00032$ ].

**Conclusions:** Drinking of small amounts of alcohol was in our setting associated with decreased serum leptin concentration, with possible benefit in terms of cardiovascular risk.

**Key words:** leptin; alcohol intake; coronary heart disease; stroke; secondary prevention; EUROASPIRE

## Introduction

Leptin is a polypeptide hormone, secreted mainly from adipocytes (and from gastric mucosa and placenta as well) as a product of the “obese” (*ob*) gene and is primarily biologically active in hypothalamus (Zhang *et al.*, 1994, Schwartz *et al.*, 1996). The endogenous leptin is known as major regulatory factor of appetite and food intake. Administration of leptin reduces feeding and body weight and increases energy expenditure (Schwartz *et al.*, 1996, Pelleymounter *et al.*, 1995) while paradoxically, obese subject showed high expression of leptin mRNA in adipose tissue and increased concentrations of leptin in systemic circulation (Considine *et al.*, 1996), probably because of phenomena known as “leptin resistance”. The possibility that increased leptin levels might be relevant to many aspects of cardiac and vascular regulation has been raised, suggesting, that leptin promotes athero-thrombotic processes involved in the pathophysiology of vascular diseases (Bodary *et al.*, 2002, Bouloumie *et al.*, 1999, Donahue *et al.*, 1999, Soderberg *et al.*, 1999, Singhal *et al.*, 2002, Bullo *et al.*, 2003).

Alcohol has been recognized for centuries as an appetizer, but the interaction between alcohol and leptin has not been well understood and infrequent studies showed equivocal results. In this study we aimed to establish the relation between alcohol intake and serum leptin concentration in the pattern of chronic non-excessive mild drinking.

## Methods

The present study represents a cross-sectional analysis. The study population consisted from pooled samples of two surveys of patients with stabilized manifest coronary heart disease (CHD) or after first ischemic stroke. CHD patients were examined in 2006-7 as Czech part of EUROASPIRE III survey. The sample selection and standard protocol of examination were in details described elsewhere (Mayer *et al.*, 2008). Briefly, patients aged  $\leq 80$  years hospitalized for any of following discharge diagnosis were retrospectively identified from hospital records: first coronary bypass graft (CABG), first percutaneous transluminal coronary angioplasty (PTCA) and acute myocardial infarction or ischemia. Recruitment of patients started with most recent hospital record and proceeded backwards until the required sample of 600 subjects was achieved. The interview of patients was done 6-24 months after the index event (coronary event or revascularisation) The stroke patients were examined in 2007 as Czech part of EUROASPIRE III-stroke survey and again, the sample selection and standard protocol of examination were in details described elsewhere (Bruthans *et al.*, 2008). A total of 507 consecutive patients aged  $\leq 80$  years hospitalized for first ischemic stroke were selected and the responders were interviewed 6-36 months after the event. Both surveys were conducted in same two centers in Czech Republic: University Hospital Pilsen and Cardio-Centre of Institute of Clinical and Experimental Medicine in Prague.

The standard protocol of EUROASPIRE survey was followed, as described elsewhere (Kotseva *et al.*, 2009). Briefly, patients were interviewed at least 6 months after their index event. Information on personal and demographic characteristics, personal and family history of coronary heart disease, life-style and pharmacotherapy were obtained. Following standardized examinations were performed: Height and weight were measured in light indoor clothes without shoes using SECA 220 scales and measuring sticks. Waist circumference was measured using tape measure. Blood pressure (BP) was measured twice in the sitting position

on the right arm using standard mercury sphygmomanometer. Breath carbon monoxide was measured by Smokerlyser (model EC 50, Bedford Scientific, U.K). Venous blood samples were drawn after at least 12 hours of overnight fast. All laboratory examinations were done in series from aliquots stored at -80 degrees by use of commercial kits and included: estimation of total (TCHOL) and HDL cholesterol (HDL), using ARCHITECT c800 analyser (Abbott Laboratoires, Germany) and DOT Diagnostics kits (Czech Republic), while triglycerides (TG) and glucose (GLU) using the same analyser and Abbott Laboratoires standard kits. HbA1c (glycohaemoglobin) was estimated using by ionex liquid chromatography using G7 analyser (TOSOH, Japan), while serum leptin and insulin using commercial radioimmunoassay kits (Millipore-Linco Reseach, USA). Weekly alcohol intake was assessed by one week follow-back (a questionnaire used for INTERSALT study (Elliot & Stamler, 1988)) and the subjects were asked, whether it is their typical alcohol intake (if not, the correction was done using typical week). The total weekly alcohol intake was calculated as follows: one beer 12° (half liter) 22 g, one beer 10° 20.5 g, one glass of wine (two deciliters) 22 g, one large glass of spirit (half of deciliter) 20g and one small glass of spirit (0.2 of deciliter) 8g of pure alcohol. The whole sample was categorized by reported weekly alcohol intake into abstainers, light irregular drinkers (1-44 g of pure alcohol weekly), mild regular drinkers (45-308 g) and moderate or heavy drinkers ( $\geq 309$  g).

Statistical analysis was done using software STATISTICA 8. All data were stored under the provisions of the Czech data protection act. The whole study was carried under Good Clinical Practice regulations, the study protocol was approved by local Ethical Committee and all study participants signed the Informed consent form.

## Results

A total of 820 vascular patients, 479 with manifest coronary heart disease and 341 after first ischemic stroke were analyzed in the present study. The baseline characteristics of pooled data and separately for CHD and stroke patients are given on table 1. Patients after stroke were significantly older and were more often female than CHD patients. Stroke patients showed in average higher heart rate, total, LDL, HDL cholesterol and serum leptin, while lower waist to hip ratio and reported weekly alcohol intake. Stroke patients were also less often treated with betablockers and statins, while more often with thiazide diuretics.

The association between alcohol intake categories and large set of risk factors or characteristics are given on table 2; the statistical differences were tested between mild regular drinkers (category III) against all three other categories. Comparing mild regular drinkers with abstainers, the latter were older, more often females, showed higher total cholesterol, HDL, LDL and HbA1c, whereas they were less often treated with betablockers and statins.

Serum leptin concentrations significantly decreased with increasing alcohol intake among defined categories (table 2 and figure 1). The Spearman's coefficient for correlation between leptin and weekly alcohol intake was  $-0.32$  ( $p < 0.0001$ ). The independency of association between leptin and alcohol intake was tested by multiple linear regression (table 3). Using weekly alcohol intake as dependent continuous variable, leptin concentration entered significantly negatively the regression as independent variable, also after adjustment for conventional risk factors as potential confounders. Moreover, age, gender, body mass index and waist circumference were identified as significant predictors in this setting. The second regression model (not shown in the table section) consisted from alcohol intake as dependent variable, and age, gender, leptin and concomitant treatments (i.e. betablockers, angiotensin-converting enzyme inhibitors or angiotensin II blockers, thiazide diuretics, statins and

antidiabetics). Any concomitant treatment did not enter this regression as significant confounding factor.

Table 4 expressed the leptin concentration by alcohol intake categories among pre-specified subgroups of risk factors and characteristics. The majority of these factors themselves influenced leptin significantly (first column), however the overall negative trend in leptin concentration among alcohol intake categories remained statistically significant in all subgroups. The effect of alcohol intake on leptin levels were significantly more pronounced in obese subjects than in non-obese and more in females than in males (last column, table 4).

## Discussion

In our study we found a clear, highly statistically significant and independent negative association between serum leptin concentrations and reported weekly alcohol intake. The association remained statistically significant even after adjustment for potential confounders, namely body constitution, glucose metabolism and current smoking. Our study sample consisted of middle aged and elderly patients, with stable manifest vascular disease and rather non-excessive drinking habits. The whole intake of alcohol (if any) resulted generally from regular drinking of small amounts of alcohol, nobody tended to binge drinking habit. The maximal individual reported alcohol intake was 792 g of alcohol weekly, i.e. 36 alcoholic drinks (beers) weekly (or ca. 5 daily). **The stroke patient showed significantly lower alcohol consumption than cardiac patients, probably because of higher age and seriousness of co-morbidities.**

The effect of alcohol on leptin as major appetite regulating factor is not well understood and remains to be elucidated in detail. Alcohol is often taken before meal as an aperitif. It has been reported (Hetherington *et al.*, 2001) that an alcoholic beverage taken before the meal had a stimulatory effect on food intake and increased the energy intake by 30%. Chronic alcoholics showed low leptin concentrations (Santolaria *et al.*, 2003), probably because of frequent chronic malnutrition leading to reduced fat tissue mass (as primary organ of leptin synthesis). Leptin also positively correlate with alcohol-craving in patients admitted for detoxification treatment (Hillemacher *et al.*, 2007). The interaction between alcohol and leptin on background of only non-excessive chronic alcohol drinking is probably more complex and relative sparse studies of this association showed equivocal results. One interventional study (Djurovic *et al.*, 2007) reported that a three-week regular intake of one glass of red wine per day increased leptin levels by about 17%, but only in females. Recent general population-based cross sectional study (Marques-Vidal *et al.*, 2009) did not find any significant

association between alcohol intake and leptin levels, confirming similar conclusion of two other reports (Lagiou *et al.*, 1999, Pischon *et al.*, 2009). On the contrary, an interventional study (Röjdmarm *et al.*, 2001) observed in healthy subjects that acute intake of ethanol (0.45 g/ kg, 3 doses, 2 hours apart) inhibited the leptin secretion. Similar study (Otaka *et al.*, 2008) reported that serum leptin significantly decreased in rats 3-6 hours after alcohol ingestion, while gastric levels remained unaffected and leptin concentration in fat tissue significantly increased. This study suggested that decreased serum leptin after alcohol administration might be due to suppression of leptin secretion from adipose tissue into systemic circulation. Our study is in line with these two reports, confirming its conclusions in the subjects with chronic, non-excessive drinking on mild alcohol amounts. **The proportion of “more-than-mild” drinkers was in our sample rather low (only 5.1% of sample) and this is probably the reason why the difference in leptin concentration between mild and heavy drinkers did not reach statistical significance (in spite of the clear negative trend).**

We may only speculate about pathophysiological mechanism of observed alcohol-leptin interaction. Leptin expression is modulated by several factors, including glucocorticoids, insulin, serotonin which stimulate leptin release, and catecholamines, which inhibit it (Fruhbeck *et al.*, 1998, Donahoo *et al.*, 1997). A plausible hypothesis is that alcohol suppressed leptin release from adipose tissue into systemic circulation via increased release of catecholamines. Several indirect mechanisms are also feasible. It is evident that insulin stimulates leptin secretion (Malmström *et al.*, 1996) and that alcohol-drinkers show lower insulinaemia and glucose concentrations (Röjdmarm *et al.*, 2001, Šimon *et al.*, 1997). However, in our study abstainers did not show any differences in insulin, glucose or HOMA index, despite their leptin concentration was almost doubled than in mild regular drinkers. This is in accordance with the above mentioned study, showing no changes in insulin in connection with acute alcohol intake (and leptin decrease as well) (Röjdmarm 1979).

Because beer was the most common alcoholic beverage, we may also speculate, that observed negative trend in leptin among increased alcohol intake also depends on type of alcoholic beverage. Xanthohumol, a compound from beer hops (*Humulus lupulus*) lowered in mice (beside plasma glucose and triglycerides) the mass of adipose tissue and increased levels of plasma adiponectin (Nozawa *et al.*, 2008), both with very close metabolic relation to leptin concentration. The prevailed type of alcoholic beverage could also be the source of equivocal results of existing alcohol-leptin association studies. Unfortunately, we cannot test this hypothesis since the sample of patients drinking other beverages than beer is very limited.

The crucial question remains whether markedly lower leptin concentration, observed in mild regular drinkers, represents any benefit in terms of individual cardiovascular risk. It is well evident that drinking of mild amounts of alcohol was associated with lower cardiovascular mortality (Keil *et al.*, 2008) in general population, but whether the same holds true for cardiovascular patients and whether leptin plays any role in this causality is not clear. In our setting, subjects with regular drinking habit showed slightly lower body mass index than abstainers. This is a paradox because the majority of alcohol intake was covered by beer (a rather high-caloric beverage). **On the other hand, we found a significant positive association of alcohol intake with waist circumference (table 3). This pattern, i.e. (sub)standard BMI, while increased waist circumference (“beer belly” with slim leg) was typically associated with alcohol consumption in several epidemiological studies (Filipovský *et al.*, 1993). Mild regular alcohol drinkers showed also significantly lower HbA1C levels than abstainers, which could also be hypothetically accounted for by low leptin concentrations. In contrast, in our setting we did not observe usual positive association between alcohol and HDL cholesterol. The possible explanation is very frequent statin treatment (about 70%) “masking” this probably only very slight HDL increase.** The more favorable lipid profile of mild drinkers could also be probably explained better by more frequent lipid-lowering treatment than by effect of alcohol

itself. Several clinical and experimental studies suggested that leptin promotes atherosclerosis and thrombosis and reported positive associations with established vascular risk factors, such as lipids, blood pressure (Donahue *et al.*, 1999), impaired fibrinolysis or prothrombotic status (Soderberg *et al.*, 1999), endothelial dysfunction or increased vessel wall stiffness (Bouloumie *et al.*, 1999, Singhal *et al.*, 2002), and inflammation (Bullo *et al.*, 2003). Particular attention is given to possible role of leptin in pathophysiology of type II diabetes and pre-diabetic disorders (Leyva *et al.*, 1998, Zimmet *et al.*, 1998). Studies dealing with leptin and incident vascular disease are relatively sparse and heterogenous. Systematic review of 7 prospective studies (Sattar *et al.*, 2009) reported that adjusted risk ratio of CHD among subject with leptin in the top tertile was 1.44, however, it did not reach statistical significance. The authors suggest that the association between leptin and CHD risk is largely dependent on BMI. Thus, the causality of leptin in vascular diseases etiology remains to be elucidated in detail.

Our study has several limitations. Namely, the estimated ethanol intake is based on reported amounts of alcohol drinks and only during one week time-frame (moreover, we asked, whether the reported alcohol intake is the typical one), however similar limitation had probably the majority of epidemiological studies. Nobody showed an excessive increase in gamma-glutamyl transferase. Thus, we can presume that reported alcohol intake is generally correct. Our study sample consisted from very specific population of middle-aged or elderly patients with stable manifest vascular diseases and the whole distribution of alcohol intake markedly differed from general population. The usual recommendations for secondary prevention of vascular diseases is to tolerate (but not to encourage) the voluntary intake of mild amounts of alcohol (up to 2 drinks daily) and ca 95% of our subjects was compliant with this rule. Therefore, in contrast to usual standards, the category of light occasional drinkers was taken into account separately. These subjects reported alcohol intake up to 2 drinks weekly (usually during weekend) and we wanted to test whether such a low intake may also

play a role in their risk profile. Indeed, their average leptin concentration was between abstainers and mild drinkers.

In conclusion, in our study we found that leptin concentration showed a clear negative trend among alcohol intake categories in vascular disease patients. Whether rather low levels of leptin in subjects with regular mild intake of alcohol represents any benefit in terms of their cardiovascular risk remains to be solved.

### **Acknowledgement**

The present study was founded by Internal Grant Agency of Czech Ministry of Health (grant no. 9333-3) and by Ministry of Education (grant no. 0021620819). We also used several data from EUROASPIRE III and EUROASPIRE III-stroke surveys; we would like to acknowledge the cooperation of all investigators who participated on the EUROASPIRE surveys in the Czech Republic.

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**Table 1.** Baseline characteristics of study sample [mean (standard deviation) or factor proportion]

	pooled data	CHD patients	stroke patients	p
n	820	479	341	-
age [years]	65.3 (9.33)	62.6 (8.60)	69.0 (9.1)	<0.0001
gender [% of males]	70.6	78.8	58.9	<0.0001
current smoking [%]	18.5	20.3	15.8	0.27
body mass index [kg/m <sup>2</sup> ]	29.3 (4.78)	29.4 (4.62)	29.1 (5.0)	0.14
waist circumference [cm]	101.4 (12.3)	102.2 (11.8)	100.2 (12.8)	<0.007
systolic BP [mmHg]	136.5 (18.0)	135.8 (17.0)	137.5 (19.3)	0.23
diastolic BP [mmHg]	80.4 (10.1)	80.5 (9.87)	80.1 (10.4)	0.63
heart rate [beats/minute]	67.9 (11.8)	65.6 (10.4)	71.1 (12.9)	<0.0001
total cholesterol [mmol/L]	4.82 (1.26)	4.61 (1.32)	5.11 (1.11)	<0.0001
HDL-cholesterol [mmol/L]	1.23 (0.36)	1.12 (0.29)	1.39 (0.39)	<0.0001
LDL-cholesterol [mmol/L]	2.87 (0.97)	2.71 (0.95)	3.08 (0.96)	<0.0001
triglycerides [mmol/L]	1.71 (1.61)	1.79 (1.99)	1.58 (0.80)	0.11
fasting glycaemia [mmol/L]	6.86 (2.54)	6.89 (2.51)	6.81 (2.60)	0.37
fasting insulinemia [mU/L]	13.9 (16.1)	13.7 (10.02)	14.3 (22.1)	0.28
HbA1c[%]	4.60 (1.35)	4.69 (1.46)	4.51 (1.21)	0.84
HOMA index <sup>1</sup>	4.41 (4.76)	4.44 (4.32)	4.37 (5.32)	0.36
<b>γ-GT [μkat/L]</b>	<b>0.77 (1.24)</b>	<b>0.84 (1.51)</b>	<b>0.69 (0.82)</b>	<b>&lt;0.02</b>
serum leptin [ng/mL]	14.1 (15.7)	12.2 (12.8)	16.7 (18.8)	<0.0001
Betablockers [%]	69.7	88.0	43.1	<0.0001
ACEi or ARBs [%]	71.7	73.6	68.9	0.13
Thiazide diuretics [%]	18.0	15.0	22.3	<0.008
Statins [%]	69.8	81.9	52.2	<0.0001
Antidiabetics [%]	21.1	20.3	22.3	0.48
weekly alcohol intake [g]	80.3 (117.1)	100.6 (124.1)	51.7 (100.1)	<0.0001
alcohol intake categories [%]:				
<i>abstainers</i>	43.9	33.2	58.9	<0.0001*
<i>light occasional drinkers</i> <sup>2</sup>	16.8	19.8	12.6	
<i>mild regular drinkers</i> <sup>3</sup>	34.1	40.1	25.8	
<i>moderate or heavy drinkers</i> <sup>4</sup>	5.1	6.9	2.6	
alcohol source categories[%]:				
<i>beer</i>	77.7	75.5	82.1	0.23
<i>wine</i>	17.3	19.0	13.1	
<i>spirits</i>	4.9	5.5	4.7	

CHD, coronary heart disease; BP, blood pressure; HDL, high density lipoprotein; LDL, low density lipoprotein; ACEi, angiotensin-converting enzyme inhibitors; ARBs, angiotensin II receptor blockers; γ-GT, gamma- glutaryl transferase;

p value by Mann-Whitney U test or X<sup>2</sup> test for categorical variables (CHD patients vs. stroke patients), \* p value for proportion of subjects among alcohol intake categories (X<sup>2</sup> test)

<sup>1</sup> Homeostasis Model Assessment Index, fasting insulin x fasting glucose/22.5; weekly alcohol intake of <sup>2</sup> 1-44 g, <sup>3</sup> 45-308 g and <sup>4</sup> ≥ 309 g.

**Table 2.** The association between categorized weekly alcohol intake and conventional risk factors, glucose metabolism parameters and treatment used.

<b>alcohol intake categories:</b>	<b>I. abstainers</b>	<b>II. light occasional drinkers</b>	<b>III. mild regular drinkers</b>	<b>IV. moderate or heavy drinkers</b>	p <sub>1</sub>	p <sub>2</sub>	p <sub>3</sub>
<i>[weekly alcohol intake]</i>	<i>[0 g]</i>	<i>[1-44 g]</i>	<i>[45-308 g]</i>	<i>[≥309 g]</i>	III.vs.I	III. vs. II.	III. vs. IV.
n	360	138	280	42	-	-	-
age	67.2 (9.27)	64.9 (8.72)	64.1 (9.27)	59.0 (8.05)	<0.0002	0.54	<0.001
gender [% of males]	55.3	73.2	84.9	92.9	<0.0001	<0.005	0.17
current smoking [%]	16.9	15.9	21.1	26.2	0.18	0.21	0.45
body mass index	29.7 (5.18)	29.5 (4.64)	28.7 (4.42)	29.0 (3.68)	<0.03	0.08	0.51
waist circumference	100.5 (12.2)	102.5 (13.0)	101.7 (12.4)	103.2 (9.29)	0.23	0.61	0.29
systolic BP	136.2 (18.9)	138.3 (18.5)	136.1 (16.7)	135.0 (17.1)	0.76	0.25	0.78
diastolic BP	79.4 (10.0)	81.5 (10.6)	80.7 (10.0)	82.1 (9.4)	<0.04	0.73	0.42
heart rate	69.6 (11.8)	66.3 (12.9)	66.5 (11.5)	68.0 (8.3)	<0.002	0.97	0.18
total cholesterol	4.95 (1.41)	4.76 (1.15)	4.68 (1.1)	4.91 (1.19)	<0.02	0.60	0.17
HDL-cholesterol	1.26 (0.38)	1.22 (0.37)	1.20 (0.36)	1.26 (0.29)	<0.03	0.59	0.13
LDL-cholesterol	2.97 (1.02)	2.79 (0.91)	2.78 (0.94)	2.91 (0.92)	<0.03	0.66	0.27
triglycerides	1.72 (2.20)	1.72 (1.08)	1.67 (0.88)	1.77 (1.16)	0.50	0.85	0.78
fasting glycaemia	7.02 (2.90)	7.13 (2.88)	6.58 (2.88)	6.58 (1.86)	0.97	0.59	0.63
fasting insulinemia	13.8 (10.7)	17.0 (32.3)	12.9 (9.5)	11.7 (7.5)	0.30	0.11	0.49
HBA1 <sub>c</sub>	4.75 (1.49)	4.80 (1.67)	4.38 (0.84)	3.98 (1.25)	<0.03	0.34	0.17
HOMA index	4.53 (4.68)	5.34 (7.12)	3.92 (3.40)	3.58 (2.65)	0.17	0.09	0.58
<b>γ-GT</b>	<b>0.73 (1.56)</b>	<b>0.85 (1.35)</b>	<b>0.94 (0.59)</b>	<b>1.84 (2.36)</b>	0.13	0.28	0.08
serum leptin	18.7 (18.7)	14.2 (17.8)	9.3 (8.2)	7.7 (6.7)	<0.0001	<0.0006	0.19
Betablockers	63.9	76.1	74.6	73.8	<0.004	0.75	0.91
ACEi or ARBs	71.4	76.1	71.4	76.2	0.99	0.32	0.52
Thiazide diuretics	19.4	18.8	16.4	16.7	0.33	0.54	0.97
Statins	64.4	72.5	76.8	69.0	<0.0008	0.34	0.28
Antidiabetics	22.2	21.7	20.4	16.7	0.57	0.74	0.58

Mean (standard deviation) or factor proportion are given; p values calculated by Mann-Whitney U test or X<sup>2</sup>\* test (for categorical variables)

**Table 3.** Multivariate association between weekly alcohol intake as dependent variable, leptin and conventional risk factor as potential confounders in multiple linear regression.

	$\beta$ coefficient (SE of $\beta$ )	odds ratio (95% CI)	p
<b>age</b>	<b>-0.127 (0.036)</b>	<b>0.88 (0.82-0.95)</b>	<b>&lt;0.0004</b>
<b>gender</b>	<b>0.136 (0.038)</b>	<b>1.15 (1.06-1.23)</b>	<b>&lt;0.0005</b>
current smoking	0.038 (0.034)	1.04 (0.97-1.11)	0.26
<b>body mass index</b>	<b>-0.154 (0.059)</b>	<b>0.86 (0.76-0.96)</b>	<b>&lt;0.01</b>
<b>waist circumference</b>	<b>0.209 (0.057)</b>	<b>1.23 (1.10-1.38)</b>	<b>&lt;0.0003</b>
systolic blood pressure	0.032 (0.034)	1.03 (0.97-1.10)	0.35
LDL-cholesterol	0.025 (0.035)	1.03 (0.96-1.10)	0.47
fasting glycaemia	-0.046 (0.038)	0.96 (0.89-1.03)	0.23
HOMA index	-0.050 (0.041)	0.95 (0.88-1.03)	0.22
<b>serum leptin</b>	<b>-0.145 (0.040)</b>	<b>0.87 (0.80-0.94)</b>	<b>&lt;0.0004</b>

SE, standard error; CI, confidence intervals

**Table 4.** Leptin concentrations by alcohol intake categories among subgroups by potential confounders

<b>alcohol intake categories:</b> <i>[weekly alcohol intake]</i>	<b>full sample</b>	<b>abstainers</b> <i>[0 g]</i>	<b>light occasional drinkers</b> <i>[1-44 g]</i>	<b>mild regular drinkers</b> <i>[45-308 g]</i>	<b>moderate or heavy drinkers</b> <i>[≥309 g]</i>	p for trend <sup>1</sup>	p for interaction <sup>2</sup>
<b>age</b>							
> 50 years	14.4 (16.2)	19.0 (19.2)	13.7 (17.8)	9.6 (8.6)	8.1 (7.3)	<0.0001	-
≤ 50 years	11.8 (11.7)	16.6 (16.2)	16.0 (17.9)	7.8 (5.3)	6.6 (4.4)	<0.02	0.41
<b>gender</b>							
males	10.8 (12.4)	13.2 (14.0)	12.8 (19.0)	8.5 (4.2)	7.8 (6.9)	<0.002	-
females	22.1 (19.6)***	25.5 (21.3)	17.7 (13.6)	13.9 (13.5)	5.7 (1.5)	<0.0002	<0.021
<b>current smoking</b>							
smokers	14.7 (16.4)	14.8 (15.7)	10.5 (7.3)	8.8 (8.0)	5.6 (0.9)	<0.0004	-
non-smokers	11.1 (11.8)**	19.5 (11.1)	14.9 (19.1)	9.4 (8.2)	8.4 (7.5)	<0.0001	0.58
<b>body mass index</b>							
≥ 30 kg/m <sup>2</sup>	21.8 (19.4)	27.9 (22.8)	19.6 (17.4)	14.5 (9.9)	9.9 (4.4)	<0.0001	-
< 30 kg/m <sup>2</sup>	9.4 (10.6)***	11.6 (10.5)	10.6 (17.1)	6.8 (5.8)	6.6 (7.4)	<0.0001	<0.0004
<b>waist circumference</b>							
≥ 102 <sup>m</sup> or 88 <sup>f</sup> cm	18.4 (17.3)	23.4 (20.8)	16.5 (15.6)	14.5 (9.9)	9.9 (4.4)	<0.0001	-
< 102 <sup>m</sup> or 88 <sup>f</sup> cm	8.3 (11.1)***	10.9 (11.0)	10.6 (20.2)	6.8 (5.8)	6.6 (7.4)	<0.0001	0.07
<b>diabetes mellitus</b>							
present	15.2 (17.4)	19.5 (18.9)	14.9 (23.4)	10.4 (8.3)	6.9 (4.8)	<0.0002	-
absent	13.4 (14.6)	18.2 (18.6)	13.5 (12.0)	8.7 (8.1)	8.1 (7.9)	<0.0001	0.96
<b>inclusion diagnosis</b>							
coronary heart disease	12.2 (12.8)	17.2 (16.0)	13.6 (14.8)	8.5 (7.3)	7.2 (6.8)	<0.0001	-
stroke	16.7 (18.8)	19.9 (20.6)	15.3 (23.1)	11.0 (9.7)	9.5 (6.3)	<0.002	0.99

Means (SD) are given.

<sup>1</sup> Kruskal-Wallis ANOVA (differences among alcohol intake categories); <sup>2</sup> two-way ANOVA

\*\*\* p<0.001, \*\* p<0.01, p value for difference among confounding factor (Mann-Whitney U test)

### **Figure legend**

**Figure 1:** Individual leptin concentrations according to the reported alcohol intake categories (median and interquartile range are expressed separately)

