

HIGH ADIPONECTIN AND TNF- α LEVELS IN MODERATE DRINKERS SUFFERING FROM LIVER STEATOSIS: COMPARISON WITH NON-DRINKERS SUFFERING FROM SIMILAR HEPATOPATHY

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Moderate alcohol consumption is associated with increased insulin sensitivity and a reduced risk for type 2 diabetes. An important endogenous mediator of insulin sensitivity is adiponectin (AN), an adipokine that displays numerous antiatherogenic, antidiabetogenic and antiinflammatory effects. Recently, acute increase in alcohol consumption has been shown to be associated with increase in plasma adiponectin and, concomitantly, insulin sensitivity. Whether chronic alcohol consumption predicts an increase in plasma AN and whether this is independent of adiposity, markers of liver dysfunction, and plasma adipokines such as tumor necrosis factor (TNF)- α is not known. We, therefore, investigated these relationships in 75 men who were diagnosed with liver steatosis using ultrasound/liver biopsy.

We examined 75 men, who were diagnosed for having liver steatosis (ultrasound/liver biopsy). Each filled in a questionnaire on alcohol intake. Subjects were divided into two subgroups according to alcohol history and CDT concentrations- drinkers and non-drinkers. All individuals were examined for serum concentrations of AN, glucose, triglycerides, alanine aminotransferase (ALT), aspartate aminotransferase (AST) and glutamate tranferase (GMT) activity; carbohydrate-deficient transferrin (CDT %) a marker of chronic alcohol consumption, insulin and TNF- α . The Quicki insulin sensitivity index was calculated.

Forty-eight individuals were found to be moderate drinkers and 27 subjects non-drinkers. Moderate drinkers had significantly higher concentrations of AN (13.8 ± 3.7 versus 9.1 ± 5.4 mg/l, means \pm SD, $p = 0.012$) compared with non-drinkers, independent of adiposity. Plasma AN concentrations in the whole group were positively correlated with TNF- α concentrations ($r = 0.6$; $p = 0.0001$), CDT ($r = 0.26$; $p = 0.0084$), AST/ALT index ($r = 0.3$, $p = 0.009$), AST ($r = 0.29$; $p = 0.011$) and GMT ($r = 0.29$; $p = 0.011$) and negatively with BMI ($r = - 0.48$; $p = 0.0002$) and glycemia ($r = - 0.22$; $p = 0.049$).

The positive associations of AN with TNF- α (0.8 ; $p = 0.001$), CDT (0.55 ; $p = 0.017$), AST/ALT index (0.55 ; $p = 0.019$) and the negative correlation with glycemia ($- 0.35$; $p = 0.0158$) were independent of BMI.

Stratified according to alcohol intake, in moderate drinkers, a positive correlation was found between AN and TNF- α concentrations ($r = 0.6$, $p = 0.0001$, AST/ALT index ($r = 0.34$, $p = 0.0295$) whereas in non-drinkers no such correlations were found. The concentration of AN and BMI displayed a negative correlation in both drinker and non-drinker patients ($r = - 0.42$, $p = 0.01$ and $- 0.61$; $p = 0.012$, respectively).

We concluded that plasma AN is higher in moderate drinkers compared to non-drinkers, even after correction for BMI. Drinkers suffering from liver steatosis were found to have a positive correlation between AN concentrations, laboratory markers of liver disease and TNF- α . Such correlation was absent in non-drinkers suffering from liver steatosis. This suggests that alcohol may modulate the inhibitory effect of TNF- α on AN production, and thus, increase its plasma concentrations.

INTRODUCTION

Adiponectin (AN) is a protein secreted by adipose tissue which displays several antiatherogenic, antidiabetogenic and antiinflammatory effects. In target tissues, it is an antagonist of TNF- α ¹⁻³. AN inhibits the production of glucose in the liver, enhances lipoprotein clearance and increases beta oxidation of fatty acids^{3-7,13}.

Experimental and clinical studies have repeatedly confirmed that AN concentration shows a positive correlation with insulin sensitivity and a negative correlation with amount of adipose tissue. Low AN values have been associated with a high basal and reduced insulin-induced phosphorylation of tyroxin receptor for tyrosine kinase in muscle, resulting in progression of insulin resistance⁸.

Low AN values typically occur in obese individuals, type 2 diabetic patients, persons with metabolic syndrome and persons with coronary artery disease (CAD). High AN values are associated with good insulin sensitivity, lower frequency of type 2 diabetes mellitus and CAD⁹⁻¹⁷.

It has been confirmed that administration of recombinant AN to mice corrected hyperglycemia (reduction of liver gluconeogenesis), decreased insulin resistance and reduced plasma atherogenicity^{3,4,9,18}. AN also inhibits cell adhesion and neointimal formation in vessels, thus inhibiting the progression of atherosclerotic alterations¹⁹.

Moderate alcohol consumption influences many metabolic parameters positively. It is known to be associated with higher HDL concentrations, lower atherogenic risk, lower oxidative stress and higher insulin sensitivity²⁰.

The effect of alcohol consumption on AN production and concentration in blood has not yet been investigated. At the time this paper was being prepared, two communications have been published. One was based on a mice experiment in which mice had been administered ethanol as a substantial component of caloric supply. This resulted in a significant decrease of AN in blood²¹. Another investigation made use of human volunteers who consumed alcohol for several weeks which resulted, in contrast to the mice experiment, in increased AN concentrations²⁰.

Alcohol consumption may cause liver steatosis. Both alcohol-induced (ASH) and non-alcoholic liver steatosis (NASH) are chronic liver diseases. Their incidence is increasing and is becoming a significant cause of mortality in developed countries.²¹ NASH occurs mainly in indi-

viduals with metabolic syndrome; almost 20 % of affected individuals develop liver cirrhosis^{22,23}.

The aim of the present study was to find relations between AN and long-term alcohol consumption with regard to TNF- α concentrations, insulin sensitivity and laboratory markers of liver injury in subjects with liver steatosis.

METHODS

We examined 75 men with liver steatosis diagnosed by sonography, increased laboratory markers and liver biopsy. The subjects filled in a questionnaire on frequency and quantity of alcohol intake. The study was approved by the ethical commission of the Hospital Šternberk.

The group under study was divided into two subgroups – drinkers and non-drinkers according to the following two criteria defined:

- mean alcohol intake > 40 g of alcohol daily for over 4 weeks versus absolute non-drinking for the same period
- CDT concentration (cut-off > 2.75 %)

If CDT values discriminated patients into the other group than the one they specified in the questionnaire (non-drinkers with CDT > 2.75 %, drinkers with CDT < 2.75 %), such individuals were excluded from the study.

Blood samples was drawn under aseptic precautions from vena cubiti, samples were centrifuged 10 min in

Table 1. Principal statistic in whole group, subgroups of drinkers and non-drinkers.

Parameter	Unit	Drinkers n = 47				Non-drinkers n = 28				Statistical difference	
		Mean	Median	SD	Normality	Mean	Median	SD	Normality	P	F
AN	mg/l	13.800	13.600	5.400	Yes	9.100	9.500	3.700	Yes	0.012	7.300
AN/BMI	j	0.500	0.460	0.200	Yes	0.260	0.190	0.120	Yes	0.008	8.200
AST	ukat/l	1.200	0.990	0.770	Yes	0.600	0.400	0.500	Yes	0.045	4
ALT	ukat/l	1.000	0.850	0.590	Yes	0.750	0.440	0.740	Yes	NS	
AST/ALT	j	1.250	1.210	0.470	Yes	1.000	0.880	0.590	Yes	NS	
Triglycerides	mmol/l	2.400	1.900	1.500	Yes	1.700	1.400	0.700	Yes	NS	
MCV	fl	93.800	94.000	6.600	Yes	85.800	86.000	4.900	Yes	0.003	10.300
BMI	j	26.200	26.500	4.000	Yes	29.300	27.500	6.600	Yes	NS	
CDT	%	3.900	3.300	1.400	Yes	2.000	2.100	0.340	Yes		
GI	mmol/l	6.200	5.900	3.900	Yes	6.700	5.900	1.900	Yes	NS	
GMT	ukat/l	4.300	1.700	5.300	Yes	0.900	0.370	0.950	Yes	0.02	4.000
TNF- α	pg/ml	11.300	7.000	9.200	Yes	9.500	5.500	9.000	Yes	NS	
Insulin	U/ml	10.000	8.900	4.900	Yes	8.700	5.700	12.000	Yes	NS	
Quicki	j	0.600	0.490	0.130	Yes	0.550	0.420	0.300	Yes	NS	
Age	years	53.700	55.000	10.880	Yes	58.500	58.000	9.700	Yes	NS	

Normality assessed by Komolgorov Smirnov test

4 °C with 3000 g and subsequently frozen at - 80 °C. All persons were examined for serum adiponectin concentration (Adiponectin, validated sandwich ELISA, Biovendor, Brno, The Czech Republic) with satisfying analytical characteristics (intrassay coefficient of variation (CV) = 5.4 %, interassay CV = 6.8 %), glucose (Glucose GOD-POD, Biovendor), triglycerides (Triglycerides, Biovendor), activity of ALT (ALT, Biovendor), AST (AST, Biovendor) and CDT % (Axis-Shield, Oslo, Norway), insulin (Insulin, DPC, USA, California, Los Angeles) and TNF- α (TNF- α , DPC, California, Los Angeles). Insulin and glycemia were taken for calculating the insulin sensitivity index (Quicki).

Body weight of patients was measured in their underwear, without any belongings (keys, shoes etc.).

Statistical data were processed by the Medcalc software (Medcalc, Mariakerke, Belgium).

RESULTS

Forty eight probands were classified as moderate drinkers, twenty seven as non-drinkers. Four subjects were excluded due to differences between anamnestic information on alcohol consumption and corresponding CDT % values (see Methods).

Normal distribution of all parameters was found in both subgroups (drinkers and non-drinkers).

Moderate drinkers had significantly higher serum adiponectin concentrations (mean 13.8 vs 9.1 mg/l) and adiponectin after correction to BMI compared to non-drinkers (0.5 vs 0.26). They also showed higher activities of AST, GMT as well as higher medium erythrocyte volume (MCV) compared to non-drinkers.

Other parameters did not differ significantly (Table 1, Fig. 1, Fig. 2).

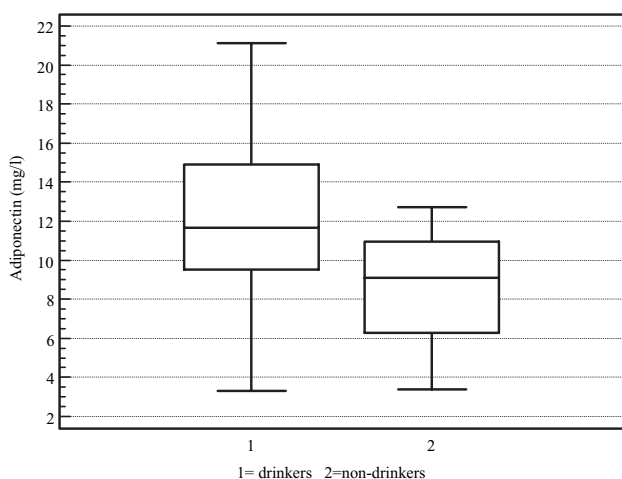


Fig. 1. Serum adiponectin levels in drinkers and non-drinker patients with liver steatosis.

The results are represented by a whisker box plot where the upper and the lower boundary of the box and the line within the box indicate the 75th and 25th percentiles and

the median, respectively. The error bars above and below the box indicate the 90th and 10th percentiles.

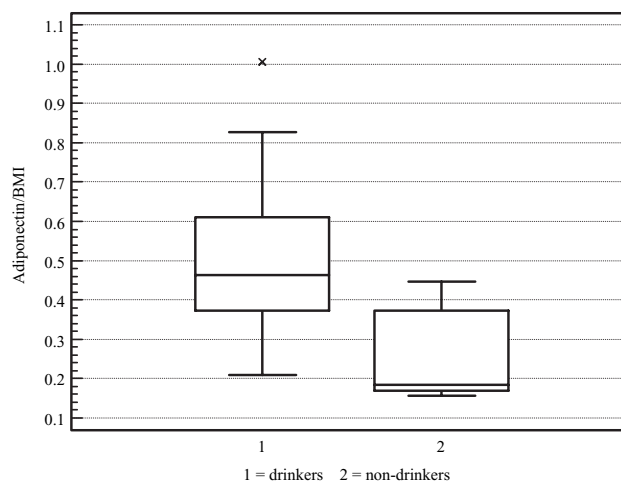


Fig. 2. Body mass index (BMI) - normalized serum adiponectin levels in drinkers and non-drinker patients with liver steatosis.

The results are represented by a whisker box plot where the upper and the lower boundary of the box and the line within the box indicate the 75th and 25th percentiles and the median, respectively. The error bars above and below the box indicate the 90th and 10th percentiles.

All probands showed a significant positive correlation between adiponectin and TNF- α ($r = 0.6$, $p = 0.0001$, Spearman), CDT % ($r = 0.26$, $p = 0.0084$, Spearman), AST/ALT index ($r = 0.3$; $p = 0.009$, Spearman), and AST ($r = 0.29$; $p = 0.011$, Spearman) as well as GMT ($r = 0.29$; $p = 0.011$, Spearman) and a negative correlation with BMI ($r = - 0.48$; $p = 0.0002$, Spearman) and glycemia ($r = - 0.22$, $p = 0.049$, Spearman).

Normalisation of adiponectin values to BMI further strengthened the correlations: TNF- α ($r = 0.8$; $p = 0.001$, Spearman), CDT ($r = 0.55$; $p = 0.017$, Spearman), AST/ALT index ($r = 0.55$; $p = 0.019$, Spearman), glycemia ($r = - 0.35$; $p = 0.0158$, Spearman) (Table 2, Fig. 3-4).

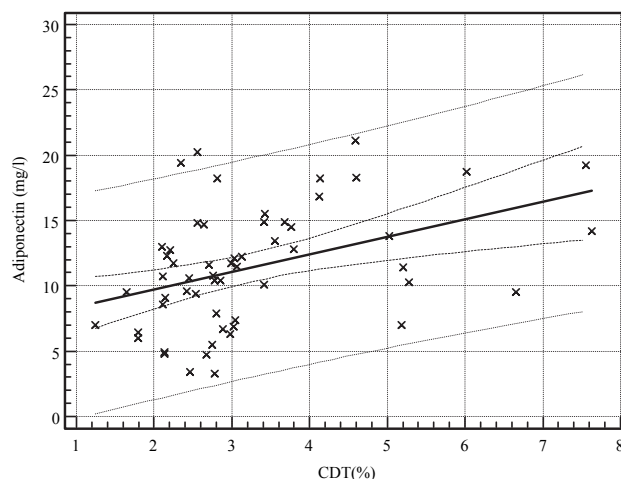


Fig. 3. Scatter, adiponectin and CDT %.

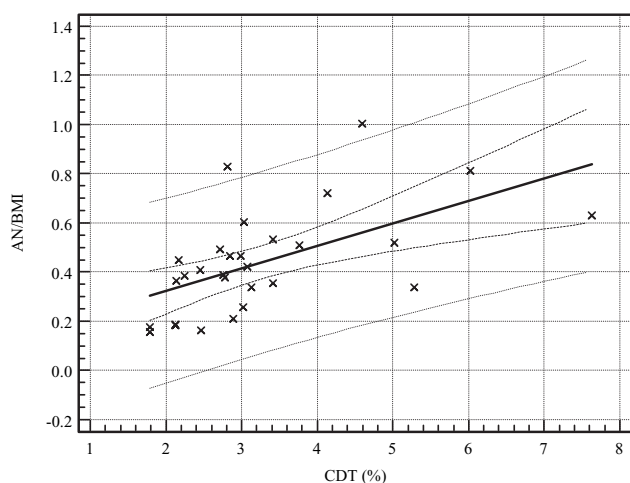


Fig. 4. Scatter, adiponectin/BMI and CDT%.

Dividing the cohort according to alcohol intake showed that a positive correlation between serum adiponectin with TNF- α ($r = 0.6$; $p = 0.0001$) and between serum AN and AST/ALT index ($r = 0.34$; $p = 0.0295$) was found in drinkers only. A negative correlation of AN with BMI was found in both drinkers ($r = -0.42$; $p = 0.01$) and non-drinkers ($r = -0.61$; $p = 0.012$) (Table 3).

In drinkers, AN/BMI index correlated positively with TNF- α concentration ($r = 0.64$; $p = 0.041$, Spearman), AST/ALT index ($r = 0.72$; $p = 0.0005$, Spearman) and negatively with triglycerides concentration ($r = -0.55$; $p = 0.045$, Spearman). Non-drinkers showed only a distinct negative correlation between glycemia and adiponectin ($r = -0.72$; $p = 0.045$, Spearman) (Table 4).

Drinkers revealed a positive correlation between CDT and TNF- α ($r = 0.5$; $p = 0.041$) and between CDT and

AST ($r = 0.43$; $p = 0.02$). In non-drinkers, a significant negative correlation was found between TNF- α and the Quicki index of insulin sensitivity ($r = -0.42$; $p = 0.032$) (Table 5).

The stepwise variable regression showed that out of all measured parameters only the adiponectin value could be used to calculate the regression formula to assess drinkers ($F 8.44$; $p = 0.0103$, $R^2 0.35$) (of course, except for CDT % which was used for defining this subgroup).

The values of AN/BMI index > 0.19 had 100 % sensitivity and 63 % specificity for identifying drinkers (Fig. 6).

To illustrate the diagnostic validity of AN/BMI for identifying drinkers, the ROC curves for adiponectin/BMI were compared with GMT and Mean Erythrocyte (Corpuscular) Volume (MCV). The resulting Areas Under Curve (AUC) did not differ significantly and all three were of diagnostic value (AUC for adiponectin/BMI was 0.89 (95% CI 0.67-0.96), AUC for GMT was 0.81 (95% CI 0.61-0.93) and AUC for MCV was 0.84 (95% CI 0.64-0.93) (Fig. 7).

DISCUSSION

Liver steatosis is characterized by overproduction of inflammatory cytokines (TNF- α , Interleukin-1 β a Interleukin-6) by activated Kupffer cells and by imbalance of the production of inflammatory and anti-inflammatory cytokines in the liver²⁴⁻²⁷ reducing adiponectin expression in adipose tissue^{28,29}.

Anti-inflammatory cytokines have hepatoprotective effect²⁷. Decreased concentration of Interleukin-10 in mice model after alcohol intake or its absence in Interleukin-10 knockout mice leads to a markedly enhanced incidence of hepatic necrosis³⁰.

Table 3. Correlation, adiponectin and others.

Drinkers	Parameter	Corr coef	p	Non-drinkers	Parameter	Corr coef	p
AN	BMI	(-) 0.420	0.01	AN	BMI	(-) 0.61	0.0120
	AST/ALT	0.340	0.0295				
	TNF- α	0.600	0.0001				

Table 4. Correlation, adiponectin BMI and others.

Drinkers	Parameter	Corr coef	p	Non-drinkers	Parameter	Corr coef	P
AN/BMI	AST/ALT	0.38	0.0381	AN/BMI	GI	(-) 0.43	0.048

Table 5. Correlation, others.

Drinkers	Parameter	Corr coef	p	Non-drinkers	Parameter	Corr coef	p
CDT	AST	0.43	0.02				
CDT	TNF- α	0.5	0.041	TNF- α	Quicki	(-) 0.42	0.032

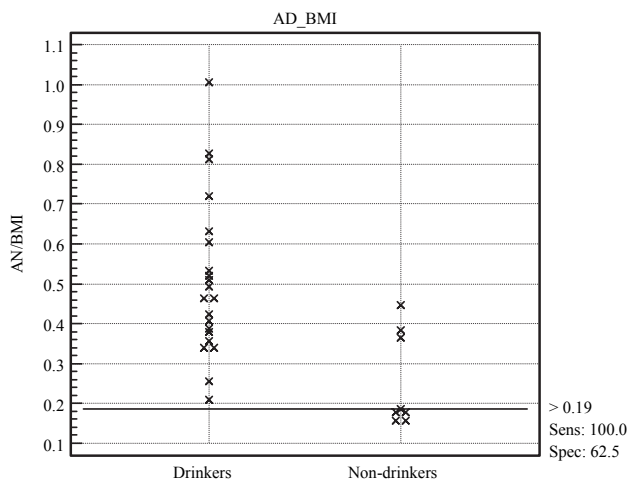


Fig. 6. ROC Adiponectin/BMI.

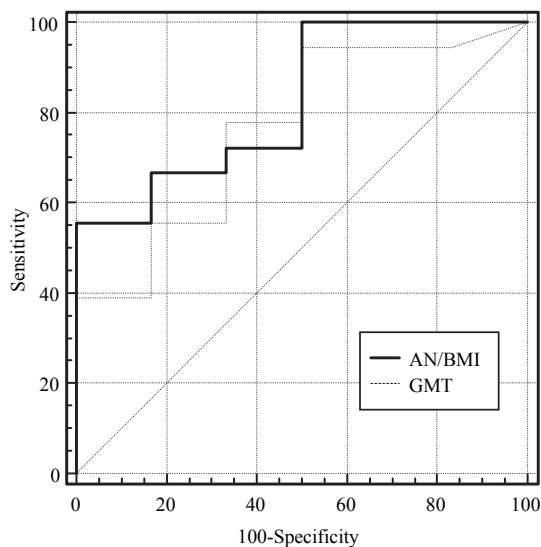


Fig. 7. Comparison of ROC curves for AN/BMI and GMT.

The alcohol-induced hepatic involvement is also markedly reduced by administration of antibodies against TNF- α ³¹ and completely eliminated in TNF- α receptor 1 knock-out mice model³². In addition, therapy with thalidomid (inhibitor of TNF- α production in Kupffer cells) inhibits alcohol-induced liver injury¹¹.

High concentrations of TNF- α lead to high basal phosphorylation of muscle insulin receptor and is manifested as insulin resistance^{34,35}.

In general, AN and TNF- α are antagonist proteins. TNF- α is considered a causal factor of insulin resistance. By contrast, AN enhances insulin sensitivity^{2,3}. AN displays antiatherogenic effects^{36,37}, TNF- α leads to progression of atherosclerosis^{33,38}.

One experimental study showed that administration of AN resulted in decreased production of TNF- α by the liver and reduced concentration of TNF- α in circulation³⁹. These findings were also confirmed in adiponectin-knock-out mice models, where high expression and production of TNF- α were demonstrated⁴.

Enhanced expression of cytokines and chemokines, such as TNF- α , Interleukin-6, Interleukin-18, MCP-1 and resistin is also typical of obese individuals. Obese adipose tissue is infiltrated by cells of monocyte origin producing such peptides²⁰.

This suggests that the conditions leading to enhanced production and activity of cytokines and chemokines (e.g. liver steatosis or obesity) are associated with low levels of adiponectin. However, the very opposite was recorded in our group of alcohol drinkers.

These findings lead us to the hypothesis that alcohol blocks the inhibitory effect of one or more anti-inflammatory factors (e.g. TNF- α) on adiponectin expression and secretion.

Extensive literature documents that chronic alcohol consumption in humans is related to enhanced insulin sensitivity. Many prospective studies proved have shown regular alcohol intake (so-called moderate drinking) is associated with augmented type 2 diabetes and cardiovascular mortality⁴⁰⁻⁴⁴. We found three experimental studies dealing with a direct impact of chronic alcohol abuse on insulin sensitivity. One of them reported a significant increase in insulin sensitivity after moderate alcohol intake in 51 postmenopausal women⁴⁴, two other studies proved that alcohol had no significant effect on the parameters of insulin sensitivity^{12, 42-43}.

The absence of an effect of chronic alcohol intake on insulin sensitivity in these studies might be due to an insufficient daily alcohol dose, short follow-up and many other factors.

A recent study employing healthy volunteers²⁰ revealed that regular alcohol intake did not only lead to a decrease but to an increase of AN concentrations and enhanced insulin sensitivity (by 11% after a 4-week consumption of 40 g alcohol/day). TNF- α levels did not change significantly due to drinking. This makes us conclude that similar TNF- α levels in both drinkers and non-drinkers lead to attenuated inhibition of adiponectin expression and/or secretion in drinkers and, subsequently, to higher adiponectin levels measured in circulation.

If alcohol intake results in reduction of the inhibitory effect of TNF- α on adiponectin expression and/or secretion, it may be speculated that alcohol also eliminates or blocks other effects of TNF- α which are causal or supportive on the emergence and propagation of metabolic syndrome and atherosclerosis.

To clarify all these relations, extensive experiments should be carried out, particularly at the molecular level.

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