Relation between the *tumor necrosis factor-α (TNF-α)* gene and protein expression, and clinical, biochemical, and genetic markers: age, body mass index and uric acid are independent predictors for an elevated TNF-α plasma level in a complex risk model

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ABSTRACT. Background: Tumor necrosis factor-alpha (TNF-α) has been implicated in the pathogenesis of numerous complex diseases. The plasma level of this pro-inflammatory cytokine is associated with a variety of different risk factors, but little is known about the genetic background and the complex interactions. Methods: in this clinical study, correlations were studied between plasma levels of circulating TNF-α protein (ELISA), its mRNA expression in monocytes (RT-PCR) and genetic variants of TNF-α gene (SSCP), with several diseases, including obesity, atherosclerosis, diabetes mellitus, hypertension, as well as risk factors such as age, gender, inflammatory markers, the coagulation/fibrinolysis balance, and lipid metabolism. One hundred and ninety four clinically and biochemically well-characterized patients were enrolled. Results: At the transcriptional level, measured in monocytes, no association with any clinical or biochemical parameter investigated was found, including TNF-α protein level. Investigating the influence of genetic variants of the TNF-α gene on mRNA and protein levels, only one promoter polymorphism, namely c.-238G > A, was shown to be associated with transcriptional but not with translational expression. However, at the translational level, significant positive, but weak associations were determined for obesity ($P = 0.037$), age ($P = 0.038$), uric acid ($P < 0.001$), body mass index ($P = 0.01$), plasminogen ($P = 0.013$), and fibrinogen ($P = 0.002$) in bivariate regression analyses, whereas HDL-cholesterol ($P = 0.005$) was shown to be negatively correlated. However, investigating confounding effects in stepwise multivariate regression analysis, body mass index ($P = 0.009$), uric acid ($P = 0.026$) and age ($P = 0.037$) turned out to be significantly associated with plasma levels of circulating TNF-α (adjusted $R^2 = 0.117$; SE: 0.688).

Keywords: TNF-α, mRNA level, protein level, genetic background, clinical and biochemical parameters

INTRODUCTION

Tumor necrosis factor alpha (TNF-α) is produced by a wide range of cell types in response to various stimuli including viruses, bacteria, parasites, cytokines and mitogens. Originally defined by its cytostatic and cytotoxic antitumor activity, TNF-α is now also considered to be a primary cytokine which exerts a potent negative, inotropic effect; the latter might be of special importance when considering the role of TNF-α in patients with heart disease. TNF-α is an extremely pleiotropic cytokine due to the ubiquity of its receptors and its ability to activate a number of signal transduction pathways and to affect the expression of a broad range of genes [1].

In view of its biological effects, TNF-α, has been implicated in the pathogenesis of a large number of complex diseases such as autoimmune diseases [2, 3], diabetes mellitus [4, 5], Alzheimer's disease [6], cardiac diseases [7-9], depression [10], metabolic syndrome [11] or obesity [12], and sepsis [13, 14]. TNF-α was shown to be involved in different biological processes, including endothelial cell activation [15], monocyte adhesion [16], procoagulant activity [17] or oxidative processes [18].

Circulating levels of TNF-α may be regulated at different stages: transcriptional level, post-transcriptional control of mRNA stability and half life, post-translational cleavage of the receptor bound to the soluble form, and regulation due to metabolic parameters including the expression of its receptors.

In several studies, correlations between TNF-α expression and different risk factors such as age [19, 20], body mass index [21], biochemical parameters of lipid metabolism

[22], and uric acid as an inflammatory marker [23] have been demonstrated. There is also evidence that TNF-α expression is modulated by genetic variants, as polymorphic sites in the promoter region of TNF-α are associated with differences in gene expression. Numerous studies on the functional relevance of these promoter polymorphisms have been carried out using transient transfection of reporter genes controlled by allelic variants [24] and by investigating the expression in plasma or cells derived from individuals with different genotypes [25, 26]. However, the results concerning genotype-phenotype relations are highly controversial [27]. Little is known concerning the complex correlation of the TNF-α gene and protein expression, with factors influencing the development of complex, multifactorial diseases. Therefore, the aim of the present clinical study was to investigate, in a complex model, correlations between TNF-α expression at the transcriptional and translational level, and diseases including atherosclerosis (angiographically-proven coronary artery stenoses), diabetes mellitus, hypertension, and obesity, as well as clinical and biochemical risk factors and the genetic background of TNF-α. In stepwise multivariate regression analysis, possible confounding effects were evaluated.

PATIENTS

For the expression study and genomic investigation, fresh blood was obtained from 194, clinically and biochemically well-characterized Caucasians from Central Germany (average age: 51.5 ± 9.1 years, 139 males, after overnight fasting between 7 am-8 am). All 194 individuals underwent detailed clinical and biochemical investigation. Reference values were taken from a local, clinical chemistry laboratory manual. Medical histories, includind former and current diseases, familial diseases, medication, and the lifestyle influencing health status were acquired. Because of suspected coronary artery disease, all patients underwent angiography (Integris H 5000S; Phillips, Germany): 30.4% of the patients underwent invasive interventions such as ACVB (aorto-coronary venous bypass) and PCI (percutaneous coronary intervention). However, none of these patients exhibited septic symptoms as a complication of the procedures. Furthermore, none of the subjects suffered from any autoimmune disease or cancer.

In this evaluation age, gender, body mass index, diseases such as obesity, coronary state, diabetes mellitus, hypertension, immunological/inflammatory markers including uric acid, C-reactive protein, α2-macroglobulin, leucocytes, factors of lipid metabolism such as HDL-cholesterol, LDL-cholesterol, total-cholesterol, apo AI, apo B, lipoprotein (a), triglycerides, and markers of the coagulation/fibrinolysis balance – plasminogen, plasminogen activator inhibitor, fibrinogen – as well as glucose and genetic variants of TNF-α, namely c.-863C > A, c.-857C > T, c.-308G > A, c.-238G > A, P84L, and IVS3 + 51A > G were investigated.

Clinical and biochemical details are given in *Table 1*. All persons included in this study had given their informed consent.

This study was approved by the local ethics committee. The investigations were in accordance with the ethical guidelines of the 1975 Declaration of Helsinki.

Table 1
Clinical and biochemical characteristics of the patient group.
Reference values were taken from a local clinical chemistry laboratory manual

Variable	Patients (n = 194)	Reference value
Age* (years)	51.5 ± 9.1	
Gender (% males)	71.6	
Body mass index* (kg/m^2)	27.11 ± 3.9	< 24.9
History of:		
Autoimmune disease (%)	0	
Cancer (%)	0	
Coronary stenosis (angiographically proven) (%)	49.5	
Diabetes mellitus (%)	15.1	
Hypertension (%)	47.1	
Invasive intervention (ACVB%, PCI%)	16.5% & 13.9%	
Obesity (%)	49	
Sepsis (%)	0	
Biochemical data		
Immunological/inflammatory markers		
C-reactive protein** (mg/l)	14.31 ± 28.79	< 5
Uric acid male* (µmol/l)	361.6 ± 83.1	200-420
Uric acid female* (µmol/l)	290.4 ± 87.9	140-340
α2-macroglobulin** (g/l)	1.5 ± 0.5	1.3-3
Leucocytes** (Gpt/l)	7.1 ± 2.14	3.8-9.8
Factors of the lipid metabolism		
Apo AI male* (g/l)	1.37 ± 0.2	0.8-1.75
Apo AI female* (g/l)	1.47 ± 0.2	0.9-1.9
Apo B* (g/l)	1.22 ± 0.3	0.8-1.4
Total-cholesterol* (mmol/l)	5.7 ± 1.2	< 5.2
HDL-cholesterol male* (mmol/l)	1.3 ± 0.4	> 0.9
HDL-cholesterol female* (mmol/l)	1.5 ± 0.4	> 1.1
LDL-cholesterol* (mmol/l)	3.5 ± 1.1	< 3.9
Lipoprotein (a)** (g/l)	0.25 ± 0.34	< 0.2
Triglycerides** (mmol/l)	2.01 ± 1.7	< 2.3
Factors of the coagulation/fibrinolysis balance		
Plasminogen* (%)	118.9 + 25.7	75-140
Plasminogen activator inhibitor** (U/ml)	3.2 ± 2.6	< 3.5
Fibrinogen* (g/l)	3.4 ± 0.9	1.5-4
Glucose** (mmol/l)	5.4 ± 1.5	3.33-5.55
Lifestyle influencing health status		
Smoking (%)	74.4	

* Values distributed normally ** Values not distributed normally

METHODS

TNF-α: transcriptional investigations

Since it is well established that TNF-α is produced primarily by monocytes, this cell system was chosen as an appropriate basis for the transcriptional investigation [28]. In order to exclude possible circadian variation in TNF-α expression, the blood was always obtained at the same time in the morning, followed by immediate preparation of plasma and monocytes. For the competitive experiments of TNF-α mRNA-expression, native monocytes were prepared by gradient centrifugation immediately after blood withdrawal, using NycoPrep™ 1.068 (Gibco BRL) in order to ensure comparable differentiation states of cells. The quality of the monocytes was tested by FACS analyses using the macrophage-specific antibody for the mannose receptor. The cells were treated with TriStar™-Reagent

(AGS), and total RNA was isolated in accordance with the manufacturer's instructions for use.

Competitive RT-PCR

An amount of 0.2 µg of the total RNA of each sample was reverse transcribed into cDNA (primer: 5'-caatgatcc-caaagtagacctgccc-3'), and TNF-α gene expression was determined by competitive PCR (upper primer: 5'-agc-aatgggtaggagaatgt-3'; lower primer: 5'-cagtgtgtggccata-tcttc-3'). A specific TNF-α cDNA standard of known concentration was used as a reference, for the determination of TNF-α gene expression. For minimizing errors, eight independent samples of the same concentration were co-amplified with standard dilutions (titration) for every patient. In order to reduce variation due to reverse transcription and competitive PCR, an internal laboratory, pooled RNA standard was constantly analysed as a control sample in every preparation series of patient samples. The results of the competitive PCR were visualised by UV irradiation, photographic documentation, and scanning competition fragments using a LASER densitometer with a computer-based imaging system (Image Quant™, MOLECULAR Dynamics GMBH). The TNF-α mRNA level of every sample was calculated by linear regression of the eight corresponding dilution results.

TNF-α: genomic studies

The genomic DNA was prepared from leucocytes from human venous blood (DNA Blood Kit, Quiagen). For analyzing genomic variants of the TNF-α gene, single strand conformation polymorphism (SSCP) and sequencing analyses were used.

All PCR were carried out in a total volume of 25 µl with 50 ng of genomic DNA, including varying concentrations of formamide and glycerol under standard conditions (predenaturation for 5 min at 95 °C, followed by 28 cycles: 30 sec at 92 °C, 30 sec at varying annealing temperatures (48 °C-51 °C), 30 sec at 72 °C, last extension 10 min at 72 °C, ending with cooling up to 4 °C in a thermal cycler (Mastercycler gradient, Eppendorf; PCR primers are available from authors upon request). For SSCP analyses, PCR products were precipitated and redissolved in 6 µl of a loading dye (95% formamide). The single stranded DNA fragments were separated in a polyacrylamide gel (PAA gel: C = 10.4%, T = 3.7%), and visualized by silver staining. The sequence changes, detected by an aberrant SSCP pattern, were confirmed by cycle sequencing of a mixture of four independent PCR-samples (ABI Prism™ system 373A). For sequencing, a Thermo Sequenase II dye terminator cycle sequencing premix kit (Amersham Pharmacia Biotech) was used according to the supplier's recommendations.

TNF-α: translational investigations

For determination of plasma TNF-α of human venous EDTA blood, the commercially available ELISA-Kit "Quantikine® HS-Kit for human TNF-α" (R,D-Systems) was used according to the manufacturer's manual. Eight standard dilutions supplied with the kit were applied in every preparation series. For every preparation series, two independent plasma samples were used as additional internal, laboratory standards. The optical density of the samples was determined using a microplate reader set to 490 nm (wavelength corrections: 650 nm). The duplicate readings for each standard and sample were averaged and the zero standard optical density was subtracted. The plasma TNF-α protein concentrations of the samples were calculated in relation to the applied standards (plasma samples and standard dilutions).

Statistical evaluation

Statistical analyses were carried out using SPSS 11.5. Values of $P < 0.05$ were considered to be significant. Data are reported as mean ± standard deviation. Categorical variables were plotted in contingency tables and evaluated using Pearson's Chi square analysis and Fisher's exact test. Metric parameters were analysed using the Kolmogorov-Smirnov test (test of normal distribution). For the statistical evaluation of the TNF-α protein and gene expression, Student's t-test or One-Way-Anova (normal distribution) and the Mann-Whitney U test or Kruskal-Wallis test (no normal distribution) were used. Relationships between TNF-α protein or mRNA level and other variables were assessed by bivariate regression analyses (normal distribution: Pearson correlation coefficient; no normal distribution: Spearman correlation coefficient). Confounding effects were proven in stepwise multivariate regression analysis.

GeneBank accession number

All sequence data were derived from the sequences of human TNF-α gene (Z15026).

RESULTS

TNF-α: transcriptional investigations

To investigate the possible regulatory basis of any differences in the level of circulating TNF-α protein, the mRNA level in monocytes was measured. However, no interdependence between mRNA and protein expression was detected.

Moreover, it could be shown that the TNF-α mRNA level was not associated with any clinical *(Table 2)* or biochemical risk factor investigated (non-significant, bivariate correlation). However, it turned out that one genetic variant, namely the c.-238G > a polymorphism located in the promoter of TNF-α gene, influences the transcriptional level of TNF-α *(Figure 1)*.

Carriers of the mutant A allele exhibit significantly higher levels of TNF-α mRNA than GG-carriers (GG-carriers versus AG-carriers: 6.5 ± 6.3 *versus* 8.94 ± 6.24 ag/cell, $P = 0.033$). This increase in mRNA level was not associated with a change in plasma levels of circulating protein. Furthermore, none of the genetic markers investigated were associated with age or gender.

TNF-α: translational investigations

The investigations of possible correlations of TNF-α plasma protein level with the clinical history of different diseases, including coronary artery disease (angiographically-proven severe stenosis), diabetes mellitus, hypertension, or obesity revealed a significant association between obesity and TNF-α expression ($P = 0.037$, *Table 2*).

Table 2
TNF-α mRNA and protein expression according to clinical parameters

Parameter	mRNA expression in ag/cell		Protein expression in pg/ml plasma	
	Mean values ± SE	*P*-value*	Mean values ± SE	*P*-value**
Gender				
Male (*n* = 139)	6.86 ± 6.54	0.936	2.48 ± 0.65	0.74
Female (*n* = 55)	5.68 ± 4,97		2.44 ± 0.92	
Smoking				
Smoker (*n* = 120; male *n* = 102)	6.79 ± 6.25	0.880	2.43 ± 0.66	0.819
Non-smoker (*n* = 41; male *n* = 20)	7.48 ± 7.65		2.46 ± 0.72	
Coronary stenosis				
Yes (*n* = 96; male *n* = 85)	6.91 ± 6.24	0.436	2.43 ± 0.61	0.385
No (*n* = 98; male *n* = 54)	6.14 ± 6.06		2.52 ± 0.84	
Diabetes Mellitus				
Yes (*n* = 29; male *n* = 21)	5.88 ± 5.02	0.786	2.7 ± 0.59	0.079
No (*n* = 162; male *n* = 116)	6.56 ± 6.27		2.44 ± 0.75	
Hypertension				
Yes (*n* = 73; male *n* = 53)	6.96 ± 6.82	0.636	2.54 ± 0.68	0.094
No (*n* = 82; male *n* = 64)	7.04 ± 6.66		2.35 ± 0.68	
Obesity				
Yes (*n* = 95; male *n* = 69)	6.49 ± 6.67	0.219	2.59 ± 0.82	0.037
No (*n* = 99; male *n* = 70)	6.55 ± 5.63		2.36 ± 0.62	

* Mann-Whitney U test (values of mRNA expression not distributed normally)
** Student's t-test (values of protein expression distributed normally)

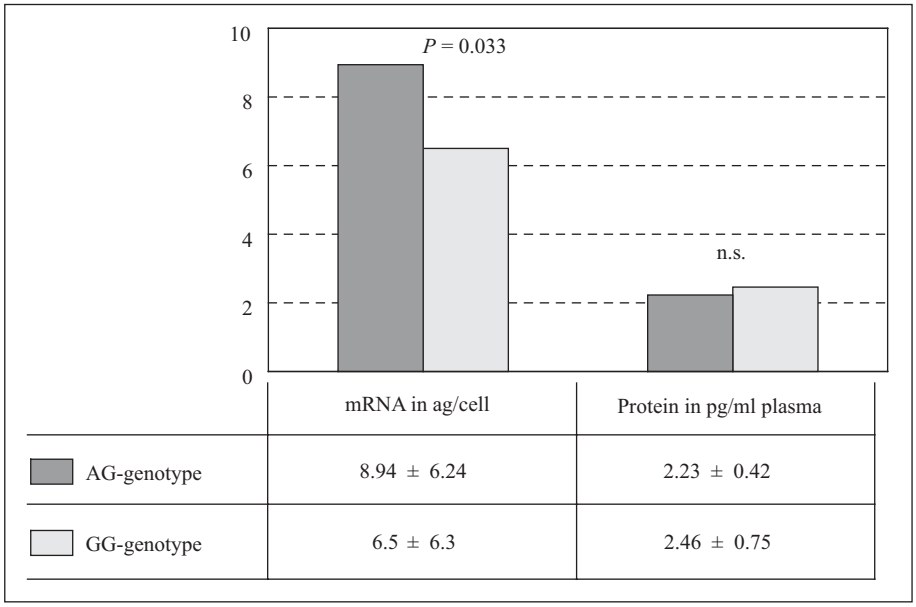

Figure 1
TNF-α mRNA and protein levels and the c.-238G > a polymorphism located in the promoter region of TNF-α gene.

When investigating the TNF-α protein level in relation to age, body mass index and biochemical parameters, significant but weak associations were found *(Table 3)* although no correlation to any genetic variant of the TNF-α gene investigated could be shown. In bivariate correlation analysis, greater age turned out to be a predictor for elevated plasma levels of circulating TNF-α protein ($P = 0.038$). Furthermore, increased TNF-α protein level, were associated with elevated levels of, the inflammatory marker uric acid ($p < 0.001$), parameters of the lipid metabolism, namely body mass index ($P = 0.01$), and HDL-cholesterol (negative correlation, $P = 0.005$), as well as two factors of the coagulation/fibrinolysis balance, namely plasminogen ($P = 0.013$), and fibrinogen ($P = 0.002$).

In multivariate analysis with stepwise regression, the effect of confounding factors was proven. Since a very strong interdependence between fibrinogen and plasminogen levels could be determined in the present study ($P < 0.001$) only one factor, namely plasminogen, was included in this model. When analyzing the complex interaction of all factors significantly correlated with TNF-α protein in this complex multivariate regression model,

Table 3
Significant data of the bivariate regression analysis of plasma levels of circulating TNF-α protein in association with age, body mass index and biochemical parameters

Variable	Bivariate correlation coefficient (R)	R^2	Adjusted R^2	SE	*P*-value
Age	0.151	0.023	0.017	0.725	0.038*
Uric acid	0.297	0.088	0.083	0.690	< 0.001*
Body mass index	0.189	0.036	0.03	0.726	0.01*
HDL-cholesterol	– 0.201	0.041	0.035	0.72	0.005*
Plasminogen	0.197	0.039	0.030	0.711	0.013*
Fibrinogen	0.251	0.063	0.058	0.707	0.002**

* For values distributed normally the Pearson correlation coefficient was assessed.
** For values not distributed normally, the Spearman correlation coefficient was assessed.

body mass index ($P = 0.009$), uric acid ($P = 0.026$) and age ($P = 0.037$) turned out to be the remaining independent factors predicting the plasma level of circulating TNF-α protein *(Table 4a and 4b)*. The effect of HDL-cholesterol and plasminogen on TNF-α protein expression was shown to be less prominent and therefore the two factors were excluded from this stepwise model (adjusted $R^2 = 0.117$).

DISCUSSION

Recent research has established that inflammation plays an important role in the development of a variety of complex diseases. In this clinical study, the interdependence of the important pro-inflammatory cytokine TNF-α and several clinical, biochemical and genetic risk factors was investigated.

Table 4
Stepwise multivariate regression analysis of plasma levels of circulating TNF-α protein (dependent variable) in association with biochemical factors

a) Summary of the three models evaluated in stepwise regression analysis

	Model (included variables)	R^2	Adjusted R^2	SE	*P*-value
1	Body mass index	0.071	0.065	0.708	0.001
2	Body mass index	0.108	0.096	0.696	< 0.001
	Uric acid		0.104		
3	Body mass index	0.135	0.117	0.688	< 0.001
	Uric acid				
	Age				

b) Detailed statistics regarding the variables (included and excluded) in model 3

	Standardized correlation coefficient	*P*-value	t-statistics
Variables included in model 3			
Body mass index	0.214	0.009	2.66
Uric acid	0.182	0.026	2.25
Age	0.166	0.037	2.1
Variables excluded in model 3			
HDL-cholesterol	– 0.146	0.092	
Plasminogen	0.107	0.189	

Firstly, the possible interrelation of the individual TNF-α gene and protein expression was investigated. It was shown that there was no direct relationship between TNF-α mRNA levels measured in monocytes and the plasma levels of circulating protein. Although monocytes are known to be very important secretors of TNF-α the plasma level of TNF-α protein seems to be additionally affected by further factors and/or influenced by TNF-α production of other cells or tissues. Furthermore, no correlation between any of the clinical and biochemical risk factors and TNF-α mRNA levels could be determined, suggesting that any metabolic regulation is presumably not at the transcriptional level, at least not in monocytes.

However, it is possible that an individual genetic predisposition could influence the TNF-α protein and/or mRNA expression *in vivo*. Since very inconsistent data were obtained about a possible regulation of the TNF-α gene and/or protein expression by the genetic background (for review see 27), the importance of genetic variants on TNF-α mRNA and protein levels was investigated. No correlation between the plasma level of TNF-α protein and any genetic variant investigated could be demonstrated. This lack of a direct relation between the TNF-α gene structure and protein level supports the idea of an important post-transcriptional regulation of TNF-α protein levels. However, at the transcriptional level, the AG-genotype of the c.-238G > a polymorphism turned out to be significantly associated with elevated TNF-α mRNA levels. This result is in contrast with data published by Kajzel *et al.*, 1998 [29] who showed in a transfection assay that this polymorphism has no functional relevance for transcriptional activation of TNF-α. However, the *in vivo* metabolic interactions in human are more complex and this might explain, in part, the differences detected. However, since this polymorphism is located within a sequence similar to the so-called Y-box, a regulatory motif typical of a promoter of MHC class II genes [30], it is conceivable that changes in this region may influence the transcription of TNF-α. In a mouse model, this Y-box of the TNF-α promoter was shown to influence transcriptional expression due to binding of an abundant nuclear factor [31].

In a second part of this clinical investigation, possible interactions between clinical and biochemical factors and plasma levels of TNF-α protein were evaluated.

In agreement with previous clinical studies [23, 32], a significant but weak correlation between uric acid, as a marker of inflammation, and TNF-α protein level was demonstrated in the present investigation. Uric acid was shown to be elevated in patients with congestive heart failure [32] and sepsis [33], and may therefore confirm the role of inflammatory markers in the development of complex diseases. However, no associations between TNF-α protein and other markers of inflammation including leucocytes or C-reactive protein were detectable.

Since a strong direct effect of TNF-α on lipid metabolism has been suggested by previous studies [21, 22, 34], the possible interactions between TNF-α levels and lipid parameters were investigated *in vivo* in this clinical study. In agreement with other investigations [21, 22], a significant but weak correlation between TNF-α protein and obesity (positive), body mass index (positive),

and HDL-cholesterol (negative) could be demonstrated in bivariate analysis.

Changes in lipid metabolism were shown to be associated with a variety of inflammatory disorders. On the one hand, it was shown that inflammation reduces the concentration of HDL-cholesterol in diseases such as sepsis [35] and atherosclerosis [36, 37]. On the other hand, a dose-dependent reduction of TNF-α expression following HDL-cholesterol administration was shown, suggesting anti-inflammatory properties of HDL-cholesterol [38]. Therefore, the association between TNF-α protein levels and HDL-cholesterol could suggest that the increase in the inflammatory cytokine is followed by an unbalanced change in lipid metabolism and *vice versa*. Other lipid parameters including triglycerides, which were reported to be correlated to TNF-α [22, 39], turned out not to be associated with TNF-α protein levels in our study. Surprisingly, in contrast to other studies [4, 5] no correlation between plasma levels of TNF-α protein and diabetes mellitus was shown. However, this result may be due to the small number of 29 patients suffering from diabetes mellitus that were included in our study.

Furthermore, a significant but again weak association between plasma levels of circulating TNF-α protein and two factors of the coagulation/fibrinolysis balance, plasminogen and fibrinogen, could be demonstrated *in vivo* in bivariate analysis. This result was in accordance with previous studies, which showed that the administration of TNF-α was associated with a changed expression pattern of factors of the coagulation/fibrinolytic system [40-42]. Our data suggest that there could be a direct relationship between the human fibrinolytic system and TNF-α levels in plasma, not only in response to TNF-α administration. Since fibrinolytic factors, leading, for example, to a reduced degradation rate of fibrin clots, may be involved in the development of sepsis [43] and the earliest stages of atherosclerotic plaque formation [44], their association with TNF-α may reflect a co-action of the inflammatory and fibrinolytic system in complex diseases.

However, in the stepwise multivariate regression model, body mass index, uric acid and age turned out to be the strongest predictors of plasma levels of circulating TNF-α protein in this clinical study. It is established that aging is associated with increased inflammatory activity reflected, for example, by elevated levels of circulating TNF-α [19, 20, 45]. However, no inter-dependence between age and all other factors shown to be associated with TNF-α protein levels in the present study was determined. Nevertheless, our results suggest that age-related activation of the immune response, indicated by an increase in TNF-α protein levels may reflect age-associated changes in overall health status, possibly leading along with other factors, to the development of complex diseases.

The bivariate and multivariate correlations measured were proven to be statistically significant but very weak in our study. This may be due to possible interactions of other, so far unnoticed, factors and TNF-α protein expression *in vivo*. In order to evaluate further factors that potentially interact with TNF-α expression, larger, population-based studies should be performed.

In summary, the plasma level of the pro-inflammatory cytokine TNF-α, as a sensitive marker of inflammation and immunity, was shown to be associated with a complex network of several clinical and biochemical factors involved in different metabolic processes. However, in the present clinical study, only the body mass index, as a predictor of an unbalanced lipid status, uric acid, as an inflammatory marker, and age were shown to be strong, independent indicators of an elevated TNF-α plasma level.

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