Chapter 29

Adiponectin: A Perspective Adipose Tissue Marker with Antiinflammatory and Antiaterogenic Potencial

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Additional information is available at the end of the chapter

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1. Introduction

Adipose tissue as a substantial part of the human body contains about 10 % of body mass. It serves both as a reservoir of the energy storage and the active endocrine tissue producing many proactive substances including adipokines. These molecules have many important metabolic effects [1]. Adiponectin is an adipose tissue-derived adipokine which circulates at relatively high concentrations in blood. It has protective role in the initiation and progression of atherosclerosis through its antiinflammatory and antiatherogenic effects. Adiponectin serum levels are decreased in obesity, type 2 diabetes, and patients with coronary artery disease, etc [2]. The level of circulating adiponectin correlates positively with HDL cholesterol, and negatively with inflammatory markers, markers of insulin resistance, triglyceride-rich lipoprotein particles, and other adipokines. Adiponectin disposes of protective actions on development of various obesity-linked diseases. The antiinflammatory properties may be the major component of its beneficial effects on cardiovascular and metabolic disorders including atherosclerosis and insulin resistance. In addition, adiponectin displays a direct biological activity through the induction of a classical pathway of complement activation.

2. Adiponectin and atherosclerosis

Human adiponectin is a protein containing 244 amino acids. It is produced by apM1 cDNA transcripts. Adiponectin consists of two structurally distinct domains and the C-terminal part is likely to be involved in protection against atherosclerosis (Figure 1).

As a member of the soluble collagen superfamily, adiponectin has a structural homology with collagen type VIII, X, complement C1q and tumor necrosis factor alpha family [2]. In

human plasma, adiponectin is present in a variety of heterogeneous isoforms, from large multimeric structures of high molecular weight to trimeric isoform. Monomeric one is present only in adipose tissue. The biological activity of various multimeric isoforms are not fully known yet, but it appears different isoforms have varying effects in different diseases. Although some studies have proposed that the ratio of high molecular weight (HMW) form to the other forms may serve as a better indicator of metabolic disorders, the majority of studies that have linked adiponectin to metabolic diseases have used assays for total adiponectin.

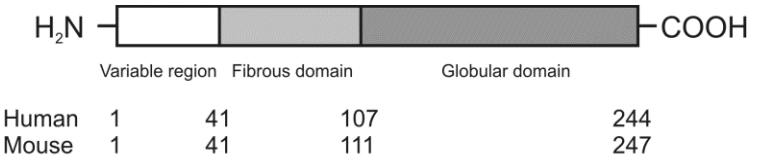

Figure 1. Schematic presentation of adiponectin structure (adapted from [2]).

Adiponectin gene is located on chromosome 3q27 and contains 3 exons and 2 introns. In 2003, DNA sequences encoding two receptors for adiponectin Adipo R1 and Adipo R2 were identified [3]. They are localized on chromosome 1 (1p36.13-q41) and 12 (12p13.31), respectively, with expression in most organs (AdipoR1 in skeletal muscle, AdipoR2 in the liver, in particular). Adiponectin gene is polymorphic, located in the region that contains susceptible loci for type 2 diabetes mellitus and metabolic syndrome. A number of single nucleotide polymorphisms (SNPs) and missense mutations were observed, especially in exons 2, 3 and the gene promoter.

Figure 2 schematically depicts some of the antiatherogenic properties of adiponectin towards different types of cells that have been established in experimental models. Adiponectin negatively regulates the expression of TNF alpha and C-reactive protein (CRP) in adipose tissue. On the contrary, its expression is negatively regulated by TNF alpha and interleukin 6 (IL 6). Adiponectin reduces expression of vascular and intracellular adhesion molecules (VCAM 1, ICAM 1), E-selectin, interleukin 8, and monocyte adhesion to human aortal endothelial cells after their stimulation with TNF alpha [4]. The proliferation and migration of smooth muscle cells induced by platelet growth factor (PDGF) is abolished or diminished by adiponectin action as inhibition of activation of nuclear factor kappa B in endothelial cells. This effect is partially mediated by its ability to support the action of cyclic adenosine monophosphate - proteinkinase A system (cAMP-PKA).

In endothelial cells, adiponectin inhibits the production of reactive oxygen species (ROS) induced by high levels of glucose via above mentioned the cAMP-PKA system. Adiponectin inhibits macrophage transformation to foam cells and reduces the intracellular content of cholesterol esters via suppression of expression of scavenger receptors, class A (SR-A). In these cells, adiponectin reduces lipopolysaccharides stimulated TNF alpha production. Recent clinical trials show a positive correlation of plasma levels of adiponectin and IL 10 [5]. In accordance with these findings, adiponectin has an antiatherogenic properties in mice

models. Adenovirus-mediated supplementation of adiponectin inhibits the formation of atherosclerotic lesions and reduces the levels of mRNA of SR-A, TNF alpha and VCAM 1 in the vascular wall [6]. It is interesting that in these models, adiponectin has no effect on glucose and lipid parameters. The authors conclude that adiponectin affects atherogenesis through a series of antiinflammatory effects on macrophages and vascular endothelium.

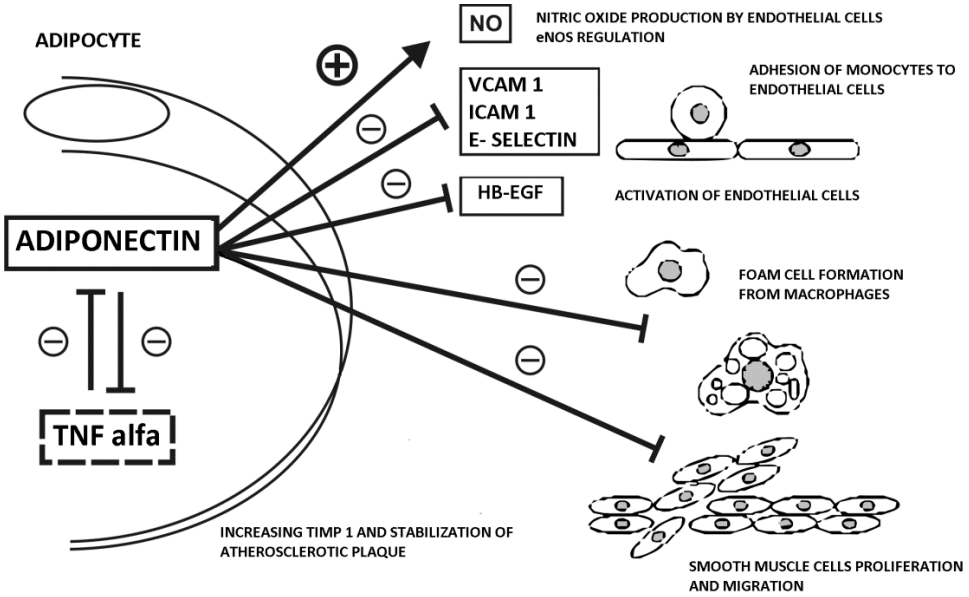

Figure 2. Some of protective actions of adiponectin (adapted from [2]).

A very important finding was observed in recent work describing the relationship of adiponectin-immune system. Adiponectin is able to bind to a number of target molecules, including the damaged endothelium and the surface of apoptotic cells. The significance of this phenomenon is not entirely clear. The study describes in vitro binding of purified C1q complement to recombinant adiponectin and dependence on calcium and magnesium ions. It was found that this binding stimulates the classical pathway of complement activation. Adiponectin does serve as an antiinflammatory factor, but may also induce biological activity through activation of complement. The authors hypotesize the binding of C1q leads to conformational changes in the adiponectin molecule, which induces the classical pathway of complement activation. Adiponectin may play an important role in immunity by its direct biological effect [7]. There is also evidence of adiponection accumulation on injured vascular arterial wall (but not in healthy one). This may lead to the hypothesis of the "consumption" of circulating adiponectin in patients with ischemic heart disease.

In some recent studies, adiponectin has a positive effect in endothelial homeostasis. It acts as a regulator of the enzyme endothelial nitric oxide synthase (eNOS), which is a key determinant of endothelial function and angiogenesis (the production of NO inhibits the

inflammatory response in the arteries), and also promotes phosphorylation of eNOS in endothelial cells, increases its expression and induces NO production after suppression of its activity caused by the effect of oxidized low-density lipoproteins (oxLDL) [8].

Adiponectin promotes cyclooxygenase 2 (COX-2) expression and prostaglandin E2 (PGE2) synthesis in cardiac cells. It also has an antiapoptotic properties in vitro, as in endothelial cells. In the heart tissue adiponectin thus acts as a regulator of cardiac damage through its antiinflammatory effect and as a factor preventing the reconstruction of cardiac tissue. In order to become a useful biomarker of cardiovascular risk, it is necessary to determine which of its isoforms exhibit cardioprotectivity, and to clarify mechanism of their action in various pathophysiological conditions [7].

There is an increasing number of papers on experimental models point to the fact that adiponectin plays an important protective role in the development of insulin resistance and diabetes. Severe insulin resistance was seen in adiponectin-deficient knockout mice (KO-AD) after administration of high fat and/or carbohydrates diets. Administration of adiponectin led to reduced hyperglycemia in the diabetic mice without affecting insulin levels. In another study, increased muscle fatty acid oxidation and reduction of plasma glucose, free fatty acids and triglycerides were observed. Studies on experimental animal models revealed the administration of adiponectin has a beneficial action against the development of obesity and atherosclerosis. It seems that adiponectin acts not only as a factor increasing insulin sensitivity, and the protective effect may result from its ability to suppress production of proinflammatory cytokine [4].

2.1. Adiponectin and its relationship to obesity and metabolic syndrome

There has been a growing evidence of significantly reduced levels of adiponectin in obese individuals compared to subjects with normal body mass index (BMI) [9]. An inverse relationship with BMI was observed in both men and women, as well as negative correlation of adiponectin with visceral fat accumulation. It is obvious that hypoadiponectinemia (levels typically less than 4 mg/l) is associated with the development of insulin resistance and type 2 diabetes mellitus, independently of BMI and metabolic syndrome. Low adiponectinemia are considered the independent risk factor for developing hypertension. Kern et al. measured adiponectin plasma concentrations and mRNA levels in adipose tissue in nondiabetic subjects with varying degree of obesity and IR. They found a strong correlation of these two parameters. The obese individuals had significantly lower plasma adiponectin. When BMI was less than 30 kg/m2, women had twice more the body fat than men, but adiponectin levels were higher on average of 65% than in men (14.2 mg/l vs. 8.6 mg/l). Individuals with the highest levels of mRNA secreted the lowest levels of TNF alpha in adipose tissue. The authors conclude that adiponectin expression in adipose tissue is highest in lean subjects and women, and correlates with higher index of insulin sensitivity and lower TNF alpha expression [9]. Another study found that expression of adiponectin mRNA in adipose tissue may reflect short-term energy changes in some obese subjects. Expression of adiponectin and insulin sensitivity may be influenced by genetic variations in the adiponectin gene in response to acute energy fluctuations [10].

Metabolic syndrome characterized by abdominal obesity, dyslipidemia, hypertension and hyperglycemia, is a general risk for the development of atherosclerotic vascular disease. The study with 661 Japanese individuals investigated possible application of adiponectin as a biomarker of the metabolic syndrome [11]. Its plasma levels negatively correlated with waist circumference, visceral fat, TGL concentration, glucose and fasting insulinemia, systolic and diastolic blood pressure, and positively with HDL cholesterol. With decreasing levels of adiponectin, on the contrary, the number of components of metabolic syndrome increased. Total of 52% men and of 38% women with levels below 4.0 mg/l met criteria for MS. The authors suppose hypoadiponectinemia is closely associated with the clinical phenotype and its measurement could be useful in the MS treatment. Saely et al. observed a group of patients undergoing coronary angiography. Low adiponectin levels were independently associated with both metabolic syndrome and angiographically confirmed coronary atherosclerosis [12]. The highest levels of adiponectin were seen in subjects without MS and heart disease (12.1 ± 8.3 mg/l), whereas the lowest levels in patients with MS and presence of heart disease (6.7 ± 3.8 mg/l). Another study then identified a link between serum adipokines and cholesterol metabolism in individuals with MS. In 58 subjects with impaired glucose tolerance or elevated fasting glucose and signs of MS the markers of cholesterol synthesis were measured (determined by the ratio of non-cholesterol sterols to cholesterol and dietary cholesterol portion), in relation to adipokines and ultrasensitive CRP (hsCRP). It was found that adiponectin, leptin and CRP were associated with cholesterol metabolites (variations) and the high ratio of cholesterol synthesis to its absorption is characterized by high levels of serum leptin and low adiponectin [13].

2.2. Adiponectin and its relationship to heart disease

The hypoadiponectinemia was also found in patients with angiographically documented coronary atherosclerosis or acute coronary syndrome. In men, plasma adiponectin significantly predicted the extent of coronary atherosclerosis [14]. A prospective study of patients with end-stage renal disease showed an inverse relationship between cardiovascular events and adiponectinemia. Higher adiponectin levels represent a low risk of myocardial infarction in healthy men individuals and moderately reduced risk of coronary heart artery disease in diabetic men patients [4]. In contrast, adiponectin concentrations did not correlate significantly with the risk of heart disease in American Indians or the British women. A large prospective study involving British men with heart disease combined with a meta-analysis of seven previously published studies found only a weak association of adiponectin with the disease [4]. This inconsistent data could be due to differences in study populations (ethnicity, gender, type of disease etc.). In any cases, it remains unclear whether hypoadiponectinemia is a reliable indicator of heart disease.

2.3. Adiponection and inflammation

CRP is known to be an independent predictor of future risk for cardiovascular events and risk factor for developing MS. Its positive association with BMI is considered as a useful

biomarker for chronic inflammation linked to obesity. Plasma levels of CRP correlated negatively with adiponectin levels [15] which was confirmed by various studies. Since CRP mRNA in humans is expressed in adipose tissue, adiponectin can apparently participate in influencing CRP levels in plasma by regulation of its expression. The regulation of CRP synthesis in the liver is also influenced by proinflammatory adipokines IL 6 and TNF alpha. On the one side, adiponectin expression is regulated by proinflammatory cytokines, on the other side, adiponectin modulates the activity and the production of TNF alpha in different tissues. Several studies have found links between hypoadiponectinemia and elevated serum IL 6. So far, there is no evidence of a link between adiponectin and TNF alpha in plasma in humans. Nishida et al. describe the action of IL 6, adiponectin, CRP and metabolic syndrome in subclinical atherosclerosis [16]. The relative influence of these parameters on a group of healthy subjects was observed. In 714 men and 364 women aged 40 to 59 years, thickness of the intima-media complex (IMT), pulse blood flow velocity and components of MS were measured. IL 6 levels correlated with IMT parameter, while adiponectin correlated negatively with IMT only in men. Individuals with either high IL 6 or CRP, or low levels of adiponectin, had increased IMT in the presence of MS. Increasing number of MS components was expressed more strongly in women than in men. The authors speculate IL 6 and adiponectin are important risk factors for premature arterial alterations in men.

In another study, the relationship of adiponectin to markers of inflammation, atherogenic dyslipidemia and heart disease was investigated in patients with coronary artery disease [17]. Study participants were in a rehabilitation program to reduce the cardiovascular risk factors. After adjusting for age and sex, adiponectin was associated positively with HDL cholesterol and N- terminal propeptide of B natriuretic peptide (NT-proBNP), while the association was negative for triglycerides. In this study, no relationship was found with markers of inflammation. The same results were obtained after next adjustment for other parameters; BMI, alcohol intake, smoking, presence of diabetes and/or hypertension and lipid-lowering therapy, and fasting glucose. The authors conclude serum adiponectin is associated with the presence of the atherogenic dyslipidemia and NT-proBNP levels, but not with markers of systemic inflammation (IL 6, CRP) in patients with manifest coronary heart disease. Atherogenic dyslipidemia may be a link between adiponectin and progression of atherosclerosis. The role of systemic inflammation as part of the adiponectin-atherosclerosis relationship may decrease during the course of the disease, and could be more amplified in the earlier stage of disease development.

3. Adiponectin and gene polymorphisms

As mentioned above, the adiponectin gene is located on chromosome 3q27, containing 3 exons and 2 introns. This region also encopasses the susceptibile loci for type 2 diabetes and metabolic syndrome. The sequence polymorphism was found in the form of several single nucleotide polymorphisms (SNPs) and a number of missense mutations. Sequence analysis of the gene for adiponectin in Japanese and Caucasian populations found more than 10 SNPs, some of which are associated with BMI, metabolic syndrome, insulin sensitivity,

hyperglycemia, type 2 diabetes, levels of plasma adiponectin, etc. The results of studies, however, are inconsistent, providing conflicting results. In many cases, the haplotype analysis was performed from a combination of alleles of individual SNPs.

Kondo et al. analyzed a cohort of Japanese patients with type 2 diabetes and nondiabetic controls to detect mutations in the gene for adiponectin [18]. Four missense mutations in the globular domain (I+164T, R+112 C, H+241P, R+221S) were identified. The frequency of one mutation, the substitution of I+164T, was significantly higher in patients than in controls of comparable age and body weight. Mutation carriers had lower adiponectin concentrations in plasma and also showed the presence of a feature characteristic of the metabolic syndrome (hypertension, hyperlipidemia, diabetes, atherosclerosis). Hypoadiponectinemia was already evident at the same time in heterozygotes I+164 T mutation carriers and also in R +112 C, but this was the case of only 3 patients. The authors suggest I+164 T variant is associated with low adiponectin levels in plasma and type 2 diabetes mellitus.

Another study has examined the adiponectin gene locus as a candidate site for coronary artery disease [19]. 383 Japanese patients with angiographically confirmed disease and 318 individuals adjusted for age and BMI were the subjects of this study. Analyses of SNPs were performed using real time polymerase chain reaction (rtPCR) and restriction fragment length polymorphism (RFLP). In patients, the higher incidence of T+164 mutation and lower adiponectin levels in plasma were seen, independently of BMI. Subjects with the mutation showed a clinical phenotype of metabolic syndrome. According to the authors, the I+164T polymorphism is associated with metabolic syndrome and coronary artery disease in Japanese population.

Hara et al. examined the relationship between two SNPs located at exon 2 of adiponectin gene (T+45G and G+276T) and type 2 diabetes in the Japanese population [20]. Subjects with the GG genotype at position +45 or +276 had an increased risk of DM compared to TT genotypes. GG +276 homozygotes showed higher insulin resistance index and the presence of G allele at position 276 was characterized by lower levels of plasma adiponectin in subjects with higher BMI (GG: 10.4 mg/l, TT: 16.6 mg/l). The different results showed the study focused on the relationship between haplotypes of the adiponectin gene with obesity and other signs of metabolic syndrome in nondiabetic Caucasian population [21]. Both polymorphisms, T+45G and G+276T, separately significantly correlated with IR. The common haplotype was also closely associated with a number of components of metabolic syndrome. Homozygotes for middle-risk haplotype TG (i.e. individuals with +45 TT variant and +276 GG variant) had higher body weight, waist circumference, blood pressure, fasting glucose, insulin, cholesterol/ HDLcholesterol ratio and lower adiponectin levels, after adjustment for age, sex and body weight. However, in the second group (614 Caucasian individuals with type 2 DM) the risk haplotype was associated with increased body weight, not with DM. It is hypothesized the variability of the adiponectin gene is connected with obesity and other features of insulin resistance, but the risk haplotype is probably a marker of linkage disequilibrium with a polymorphism yet unidentified that directly affects the plasma levels of adiponectin and insulin sensitivity. Moreover, Fillipi et al. found no

association of SNP T+45G with insulin resistance [22]. The T+276 G polymorphism was associated with higher BMI, lower insulin and adiponectin, but, unlike previous study, in the TT genotype. In discussion the authors analyzed possible causes of these results and conclude the same mentioned above. There is the high probability of the existence of further SNPs or gene mutations, which is in linkage disequilibrium with SNP +276 and which determines its effect. Variations in the adiponectin gene and risk for subsequent type 2 diabetes in women has been of interest in the study of Hu et al. [23].

A prospective study focused on the determination of SNPs participation in the development of IR in French population found that variations in the adiponectin gene affects weight gain, body fat distribution and the development and the onset of hyperglycemia, as well as serum adiponectin [24]. At the start of a three-year study, the normoglycemic individuals with no signs of diabetes or impaired glucose tolerance were influenced mainly by two SNPs: G-11391A and T+45G.

An interesting work was published in 2006 in Clinical Chemistry by Hegener et al. [24]; the prospective study monitoring the risk of atherothrombotic disease in individuals with no signs of diabetes. Five SNPs in the gene for adiponectin were investigated in 600 Caucasian men with subsequent aterotrombotic events (myocardial infarction or stroke) and 600 controls. After adjustment for potential risk factors, regression analysis then revealed two variants with a decreased risk of stroke (C-11377G and G-11066A). This study has provided evidence of links of specific adiponectin gene variants with reduced risk of stroke.

3.1. Relationship between G+276T single nucleotide polymorphism of adiponectin gene and markers of insulin resistance in dyslipidemic patients

In many recent studies, the adiponectin gene has been proposed as a potential candidate gene for insulin resistance but only a few of them have confirmed this relationship. Insulin resistance is considered the key factor in the patogenesis of common disorders, such as atherosclerosis, metabolic syndrome and diabetes mellitus. The genetic backround is likely to be polygenic but the genes involved are mostly unknown.

In our work, we have studied the possible relationship between single nucleotide polymorphism G+276T and IR markers, including lipid and lipoprotein profiles and adiponectin plasma levels in 355 dyslipidemic patients and their first-degree relatives.

3.2. Subjects

The group consisted of 355 patients attending Lipid Center of 3rd Medical Clinic, Faculty Hospital Olomouc, and their first-degree relatives. Patients had the first examination between January 2004 and January 2006. All patients were examinated by a physician and the family history were collected and medical history with physical examinations were performed. All individuals were tested for secondary hyperlipidemia, especially on the presence of diabetes mellitus, hypothyroidism, hepatic and renal failure and nephrotic syndrome. Violation of the following criteria led to exclusion from the study: hypolipidemic

treatment in the previous 6 weeks, the presence of secondary hyperlipidemia, acute infection, acute cardiovascular or cerebrovascular attack within the past 3 months, cardiac disease (NYHA III and IV). Participiants were also divided into three groups. Group G1 included the presence of individuals with clinically manifest atherosclerosis, the group G2 individuals with dyslipidemia defined by Sniderman [25] (apolipoprotein B > 1.2 g/l and/or triglycerides > 1.5 mmol/l) but without clinical signs of the presence of atherosclerosis. Group 3 consisted of healthy individuals with the apolipoprotein B < 1.2 g/l and triglycerides < 1.5 mmol/l. The participants signed informed consent before taking a blood sample for DNA testing. The study was approved by the Ethical Committee of the Faculty of Medicine, Faculty Hospital Olomouc.

3.3. Materials and methods

Venous blood for biochemical tests were collected after 12-hour fasting. Total cholesterol, HDL cholesterol and triglycerides were determined enzymatically using an analyzer Modular SWA (Roche, Switzerland), as well as other routine biochemical analyses. LDL cholesterol was calculated using the Friedewald equation for specimens with TG < 4.5 mmol/l (available for 242 subjects). Concentrations of apolipoproteins AI and B were determined by immunoturbidimetric method, as well as C-reactive protein levels, established by highly sensitive method (all Roche, Switzerland). Insulin was determined by IRMA (Immunotech, France). HOMA parameter (homeostatic model) was calculated from the formula: fasting glucose x fasting insulin / 22.5. C-peptide and proinsulin were determined by commercially available kits (Immunotech, France, DRG Instruments GmbH, Germany, respectively). Serum levels of soluble adhesion molecules ICAM 1 and VCAM 1 were analyzed by immunoenzymatic technique (Immunotech, France). Adiponectin determination was performed by the ELISA method (BioVendor, Czech Republic). The following markers of endothelial dysfunction were examined: plasminogen activator inhibitor-1 (PAI-1) and tissue plasminogen activator (tPA), both determined by ELISA methods (Technoclone, Vienna, Austria). Concentrations of adhesion molecules, insulin, proinsulin, C-peptide and adiponectin were measured on samples frozen at - 80 ° C until analysis.

G+276T adiponectin gene SNP was detected by real time polymerase chain reaction with fluorescent hybridization probes (FRET) on the Light Cycler instrument, v.2.0 (Roche), according Fillipi et al [22]. Genotyping was performed after the isolation of DNA from peripheral blood samples using phenol method [26]. DNA isolates were then stored at - 20 °C until analysis. The primer and probe synthesis was made at the in TibMolbiol (Germany). The sequence of oligonucleotides for the detection of SNP +276 G> T were as follows:

Primers:

5'- GGC CTC TTT CAT CAC AGA CC -3'

5'- AGA TGC AGC AAA GCC AAA GT -3'

Probes:

5'- AAG CTT TGC TTT CTC CCT GTG TCT A--FL

5'- LCRed640- GCC TTA GTT AAT AAT GAA TGC CTT—PH

Individual genotypes were determined by melting curve analysis after the amplification process. The fluorescence signal was converted and delivered to the graph as the dependency of negative fluorescence change with temperature (y axis) on temperature (x axis). As the result, creation of the characteristic peaks representing the melting temperature of the product and allow to distinguish the genotypes GG, GT and TT was performed. Example of analysis is shown in Figure 2.

3.4. Statistical analysis

Quantitative data were expressed as a mean ± standard deviation. Parameters with abnormal distribution were logarithmically transformed before statistical analysis. Differences between genotypes in continuous variables were determined by using ANOVA after adjustment for age, gender and waist circumference (SPSS 12.0 statistical package, SPSS Inc., USA). Furthermore, the calculation of frequency of alleles (G and T) and genotypes (GG, GT and TT) in individual groups and subgroups were performed.

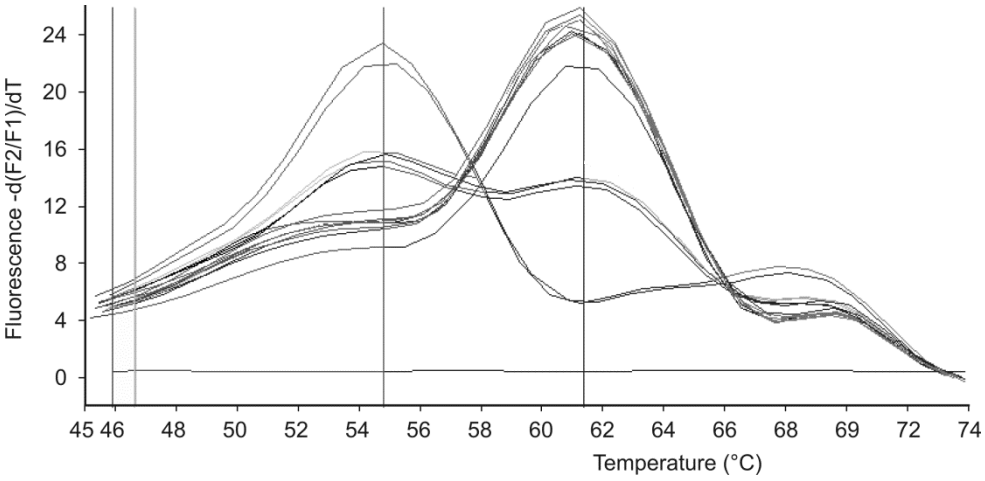

Figure 3. An example of the melting curve analyses for G+276T polymorphism of adiponectine gene. (melting temperature for T and G alelles: $T_{m(T)} = 54.8 \pm 1.5$ ºC, $T_{m(G)} = 61.3 \pm 1.5$ ºC).

3.5. Results

In Table 1 the clinical and laboratory characteristics of the groups of dyslipidemic patients divided according to genotypes at position +276 of the gene for adiponectin are shown. Table 2 presents the results of laboratory parameters that differed significantly between each of groups determined by genotype at position +276. The data are adjusted for age, gender and waist circumference. The results show that the GG genotype carriers had significantly higher levels of total cholesterol (GG: 6.54 ± 1.74 mmol/l, GT: 6.18 ± 1.45 mmol/l, TT: 6.25 ± 1.64 mmol/l, $p < 0.05$) and LDL cholesterol (GG: 4.12 ± 1.49 mmol/l, GT: 3.78 ± 1.31 mmol/l, TT: 3.70 ± 1.34 mmol/l, $p < 0.05$) than T allele carriers. In heterozygotes, however, the presence of T allele at position +276 was associated with higher concentrations of PAI-1 (GG: 71.50 ± 41.0 μg/l, GT: 81.0 ± 38.7 μg/l, TT: 70.14 ± 44.4 μg/l, $p < 0.05$). We did not find any significant association with other markers of IR, such as BMI, blood glucose, insulin, or serum adiponectin. Table 3 depicts the frequencies of genotypes and alleles at position +276, Table 4 then presents the distribution of genotypes in groups according to triglyceride levels (cut-off value of TGL = 1.5 mmol/l).

	GG	GT	TT
Number	188	144	23
BMI (kg/m^2)	26 ± 4	26 ± 4	26 ± 5
Systolic blood pressure (mmHg)	130 ± 18	132 ± 18	129 ± 14
Diastolic blood pressure (mmHg)	80 ± 10	83 ± 9	80 ± 7
Total cholesterol (mmol/l) *	6.47 ± 1.73	6.18 ± 1.44	6.26 ± 1.64
Triglycerides (mmol/l)	2.38 ± 2.97	2.43 ± 2.23	2.30 ± 3.44
HDL cholesterol (mmol/l)	1.47 ± 0.44	1.44 ± 0.44	1.46 ± 0.39
LDL cholesterol (mmol/l) *	4.07 ± 1.47	3.79 ± 1.31	3.70 ± 1.34
Apolipoprotein AI (g/l)	1.55 ± 0.30	1.57 ± 0.34	1.60 ± 0.29
Apolipoprotein B (g/l)	1.21 ± 0.39	1.15 ± 0.32	1.15 ± 0.32
hsCRP (mg/l)	1.99 ± 1.94	2.20 ± 1.93	2.02 ± 1.69
tPA (μg/l)	4.08 ± 4.81	4.31 ± 4.46	4.03 ± 3.89
PAI-I (μg/l) *	69.7 ± 40.7	79.9 ± 39.0	72.3 ± 44.4
VCAM 1(μg/l)	808 ± 247	823 ± 287	743 ± 184
ICAM 1 (μg/l)	563 ± 140	592 ± 165	585 ± 209
Fasting glucose (mmol/l)	5.09 ± 0.91	5.25 ± 1.23	4.82 ± 0.68
Insulin (U/l)	8.33 ± 5.55	7.99 ± 4.84	7.77 ± 4.54
HOMA $_{IR}$	1.93 ± 1.45	1.92 ± 1.33	1.84 ± 1.18
C- peptide (μg/l)	2.38 ± 1.25	2.40 ± 1.12	2.41 ± 1.30
Adiponectin (mg/l)	12.9 ± 7.6	13.0 ± 7.0	12.0 ± 5.7

* GG vs. GT+TT, $p < 0.05$

Table 1. Clinical and laboratory characteristics according to adiponectin genotypes at position +276 (G+276T).

	GG	GT	TT	p
Number	188	144	23	
Total cholesterol (mmol/l)	6.54 ± 1.74	6.18 ± 1.45	6.25 ± 1.64	< 0.05
LDL cholesterol (mmol/l)*	4.12 ± 1.49	3.78 ± 1.31	3.70 ± 1.34	< 0.05
PAI-I (μg/l)	71.5 ± 41.0	81.0 ± 38.7	70.14 ± 44.4	< 0.05

*only 242 patients included

Table 2. Laboratory characteristics according to adiponectin genotypes at position +276 (G+276T) with significant differences between groups (GG vs. GT+TT, after adjustment for sex, age and BMI).

APM1 G+276T	Patients (n = 355)
Genotype	
GG	188 (53 %)
GT	144 (41 %)
TT	23 (6 %)
Allele	
G	520 (73 %)
T	190 (27 %)

Table 3. Genotype and allele frequencies for G+276T polymorphism in dyslipidemic patients.

Genotype	GG	GT	TT
TG ≤ 1.5 (n = 225)	119 (53 %)	94 (42 %)	12 (5 %)
TG > 1.5 (n = 148)	85 (57 %)	52 (35 %)	11 (8 %)

Chi-square 1.981, p = 0.37

Table 4. Genotype frequencies for G+276T polymorphism in dyslipidemic patients according to level of triglycerides (mmol/l).

3.6. Discussion

Insulin resistance is considered the key factor in the pathogenesis of complex diseases such as atherosclerosis, metabolic syndrome and diabetes mellitus. Genetic background IR is probably multifactorial but the participating genes are largely unknown.

In this study, the relationship of polymorphism G+276T of adiponectin gene and markers of insulin resistance was investigated. We found an association between genotype GT and one marker of IR, PAI-I. However, we found no association with serum adiponectin, insulin, HOMA and BMI. Our work did not confirm the preliminary findings from 2005, where the relationship between the adhesion molecules ICAM 1 and TT genotype was observed [27].

Possible association between SNPs and dyslipidemic phenotypes defined by Sniderman classification, based on serum TGL and apo B, was not seen. We found no linkage (data not specified), even in a situation where the only criterion was TGL alone. The genotype distribution in this case was comparable in both groups.

As shown in Table 1, GG genotype was associated with higher levels of total cholesterol and LDL cholesterol compared with GT and TT genotypes. This was found in our previous study as well [27].

Table 3 displays the fact that distribution of genotypes at position 276 is comparable with those published in previous works [20, 21, 22].

3.7. Conclusions

In summary, our study found only a weak association of adiponectin gene SNP G+276T with IR markers. The relationship of GG genotype and selected quantitative lipid parameters were confirmed, in accordance with several studies. Based on some recent literature we suggest the gene variant G+276T may be marker of one or more haplotypes containing a causal polymorphism determining IR or diabetes mellitus. Differences among populations on the linkage disequilibrium structure may result in association on the disease haplotype with different SNP alleles in different population. More studies will be necessary to perform for evaluation of the influence of G+276T SNP on insulin resistance.

4. Adiponectin and its relationship to endothelial dysfunction

In vitro experiments revealed the physiological concentrations of adiponectin inhibited TNF alpha induced expression of VCAM 1 and ICAM 1 on the endothelium and exhibited other antiatherogenic effects. In 2008 Vaverková et al. published a study concerning the relationship between adiponectin and serum concentrations of soluble adhesive molecules VCAM 1 and ICAM 1 as well as with markers of insulin resistance and inflammation in patients with cardiovascular disease and in dyslipidemic patients at high risk of cardiovascular disease [28].

The aim of the study was to evaluate the relationship of adiponectin to soluble forms of vascular cell adhesion molecule 1 (VCAM 1) and intercellular cell adhesion molecule 1 (ICAM 1) in patients with cardiovascular disease or dyslipidemia.

The data from experimental research in animals support the hypothesis of antiaterogenic properties of adiponectin. Adiponectin accumulates in the arterial wall of injured arteries [29]. In adenovirus-treated animals the increase of adiponectin significantly reduced progression of atherosclerotic lesions [6]. In vitro experiments revealed the fact that physiological concentrations of adiponectin inhibited TNF alfa induced expression of VCAM 1 and ICAM 1 on the endothelium [29] and exhibited other antiatherogenic effects.

We have investigated the relationship between adiponectin and serum concentrations of VCAM 1 and ICAM 1 as well as with markers of insulin resistance and inflammation in patients with cardiovascular disease and in dyslipidemic patients at high risk of CVD.

4.1. Subjects

264 patients of Lipid Center at Faculty Hospital Olomouc were included in the study. All patients were examined by a physician and the following information were obtained:

medical history, physical examination and NYHA classification. Subjects were tested for secondary hyperlipidemia. Patients were divided into three groups, those with the presence of clinically manifest atherosclerosis (G1), those with dyslipidemia defined according to Sniderman, but without clinically manifest atherosclerosis (G2), and healthy individuals (G3).

4.2. Results

The characteristics of the three subgroups of the studied cohort are shown in Table 5. Participants with CVD (G1) had comparable lipid, lipoprotein and apolipoprotein profile to the dyslipidemic subjects without CVD (G2) but were more insulin resistant. These differences persisted after adjustment for age, sex and BMI. The G1 had also the highest soluble ICAM 1, the difference in VCAM 1 was not statistically significant. Subjects with dyslipidemia (G2) had significantly lower adiponectin levels and higher levels of ICAM 1 compared with G3. Lower adiponectin levels in patients with CVD did not reach statistical significance, possibly due to a small number of patients. Adiponectin correlated with many lipid and nonlipid markers of insulin resistance. Adiponectin did not correlate with ICAM 1, but there was a strong positive association of adiponectin with VCAM 1. While ICAM 1 and VCAM 1 were strongly intercorrelated, they showed different association pattern with other risk factors. ICAM 1 correlated strongly with many markers of insulin resistance and hsCRP, while VCAM 1 were negatively associated with apo AI and apo B, and positively with adiponectin. Association of adiponectin with VCAM 1 was most prominent in group G1 and G2, but was not significant with G3. Results of multiple backward stepwise regression analysis confirmed these observations. Adiponectin levels were independently positively associated with sex (higher in women), HDL cholesterol and VCAM 1, and negatively with hsCRP. In multiple stewise regression analysis with VCAM 1 as the dependent variable, VCAM 1 was independently associated with ICAM 1 ($p < 0.0001$), adiponectin ($p < 0.0001$), HDL cholesterol ($p = 0.0208$) and triglycerides ($p = 0.0091$). On the other hand, ICAM 1 was independentely associated with VCAM 1 ($p < 0.0001$), atherogenic index ($p < 0.0001$), hsCRP ($p = 0.0001$) and HOMA ($p = 0.0307$). (More detailed results are given in lit. [28].)

4.3. Discussion

Our study confirms the previously described correlations of adiponectin with many lipid and nonlipid markers of IR as well as its relationships with HDL cholesterol, sex and hsCRP [30, 31, 32]. The unexpected finding was the significant independent positive association of adiponectin with VCAM 1 but not with ICAM 1 serum concentrations in patients with or at risk for CVD. Their expression results in adhesion of circulating leukocytes to the endothelial cells and their subsequent transendothelial migration- an important step in initiation and progression of atherosclerosis. VCAM 1 and ICAM 1 have different expression pattern and probably different roles in atherogenesis [33]. Soluble forms of these molecules can be measured in peripheral circulation. The origins of circulating soluble cell adhesion molecules are not entirely clear, but they may derive from shedding or proteolytic cleavage from endothelial cell.

	G1 (CVD+, DLP+/-)	G2 (CVD-, DLP+)	G3 (CVD-, DLP-)
Number	29 (M 18/F 11)	173 (M 97/F 76)	62 (M 19/ F 43)
Age (years)	60.0 ± 9.1	44.9 ± 13.8	36.4 ± 14.5
BMI (kg/m^2)	27.5 ± 3.7	26.3 ± 5.7	23.6 ± 4.3
Waist (cm)	92.3 ± 13.1	88.4 ± 11.4	77.4 ± 10.8
Systolic blood pressure (mm Hg)	143 ± 15	131 ± 17	120 ± 13
Diastolic blood pressure (mm Hg)	86.3 ± 9	83 ± 8	75.6 ± 9.8
Total cholesterol (mmol/l)	6.8 ± 1.2	6.7 ± 1.4	4.7 ± 0.7
Triglycerides (mmol/l)	3.0 ± 2.1	2.7 ± 2.3	0.9 ± 0.2
AIP: log (TGL/HDLchol)	0.29 ± 0.38	0.24 ± 1.16	-0.2 ± 0.2
HDL cholesterol (mmol/l)	1.32 ± 0.43	1.34 ± 0.37	1.56 ± 0.36
LDL cholesterol (mmol/l)	4.2 ± 1.1	4.3 ± 1.3	2.8 ± 0.6
Apolipoprotein AI (g/l)	1.52 ± 0.28	1.51 ± 0.29	1.60 ± 0.30
Apolipoprotein B (g/l)	1.29 ± 0.3	1.33 ± 0.33	0.84 ± 0.17
hsCRP (mg/l)	3.4 ± 4.9	2.68 ± 3.6	2.69 ± 5.6
VCAM 1(μg/l)	885 ± 261	800 ± 285	860 ± 265
ICAM 1 (μg/l)	673 ± 202	601 ± 164	538 ± 114
Fasting glucose (mmol/l)	5.8 ± 1.8	5.1 ± 0.8	4.8 ± 0.6
Insulin (mIU/l)	8.8 ± 4.8	8.9 ± 5.3	6.6 ± 3.4
HOMA IR	2.3 ± 1.6	2.1 ± 1.3	1.4 ± 0.8
C- peptide (μg/l)	3.2 ± 1.2	2.6 ± 1.0	1.9 ± 0.7
Proinsulin (mIU/l)	17.0 ± 8.0	15.6 ± 9.9	11.3 ± 5.2
Adiponectin (mg/l)	15.5 ± 8.0	12.3 ± 6.6	16.1 ± 6.8

Table 5. The demographic, clinical and laboratory characteristics of the study population

The expression pattern of adhesion molecules may explain why VCAM 1 is a marker of increased risk for future coronary events only in patients with atherosclerosis [34]. Patients with stable CAD have moderately increased and in several studies even normal levels of soluble VCAM 1 in comparison with healthy controls. The highest level of VCAM 1 was noted in patients with acute myocardial infarction [35]. In another study, VCAM 1 was a useful marker for predicting future ischemic events in the 6 months after presentation with unstable angina pectoris or nonQ myocardial infarction [36]. In our cohort, levels of VCAM 1 in the CVD patients were not significantly higher than in controls. This is in agreement with several other works.

4.4. Conclusions

Many studies, including experiments in vitro, animal models and studies in human, have shown that adiponectin has antiatherogenic and antiinflammatory properties. Low

adiponectin levels were found in patients with CAD independently of other risk factors. Therefore, the finding of positive and independent association of adiponectin with the marker of endothelial dysfunction VCAM 1 was suprising. This positive association was present both in patients with CVD and dyslipidemic subjects without CVD, but it was not significant in healthy subjects without dyslipidemia. We hypothesize that adiponectin, which accumulates in the arterial wall only in place of endothelial injury and atherosclerotic plaques (that is the same places where VCAM 1 is expressed) may be involved in shedding of ectodomains of VCAM 1 from endothelial surface. This may represent a mechanism by which VCAM 1 effects on the cell surface can be downregulated. In this way, adiponectin could protect vascular wall from adhesion of leukocytes and thus from progression of atherosclerosis.

5. Adiponectin and dyslipidemia: Relationship of adiponectin, fibroblast growth factor 21 and adipocyte fatty acid binding protein levels to dyslipidemic phenotypes – Pilot study

5.1. Background

Adipose tissue is an important place of many metabolic and inflammatory processes. Adipokines are considered to be the mediators of these pathways.

Adiponectin (ADP, AdipoQ, apM1, GBP28) is a "favourable" adipokine of fat tissue circulating at relatively high concentrations in human plasma. Adiponectin has the protective effects in early stages and during progression of atherosclerosis probably by its antiinflammatory and antiatherogenic actions.

Fibroblast growth factor 21 (FGF 21) is also a "favourable" cytokine of adipose tissue considered as a new metabolic regulator of non insulin dependent glucose transport in cells [37]. Systematic administration of FGF 21 decreases plasma levels both of glucose and triglycerides, and leads to improving of lipoprotein profiles in genetic compromised FGF transgenic mice and primates [38]. Increased levels of FGF 21 and a negative correlation with HDL and adiponectin were found in patients with metabolic syndrome [39].

Adipocyte fatty acid binding protein (A-FABP) is a „unfavourable" adipokine, probably a new marker and/or predictor of metabolic syndrome [40]. A-FABP is a dominant cytoplasmic protein of mature adipocytes and a regulator of lipid and glucose metabolism, present also in macrophages of fat tissue. Its expression is induced by oxidated LDL [41]. Higher levels of A-FABP are associated with increased fasting glucose, triglycerides, insulin BMI and waist circumference, and decreased HDL in patients with metabolic syndrome. Inhibition of A-FABP action is associated with reversion of atherosclerosis (improving of diabetic and lipoprotein parameters).

The aim of our study was to evaluate the relationship between adiponectin, FGF 21 and A-FABP levels and dyslipidemic phenotypes defined on the basis of concentrations of triglycerides and apolipoprotein B [25].

5.2. Subjects, material and methods

119 pacients of Lipid Center at Faculty Hospital Olomouc were included on the pilot scheme. Routine serum biochemical parameters were analyzed on Modular SWA (Roche, Switzerland) in the day of blood collection. Levels of ADP, FGF 21 and A-FABP were determinated by imunochemical Elisa methods (BioVendor, Czech Republic). The analytical characteristics from data sheets were verificated according to laboratory protocol for all procedures.

119 individuals were divided into four dyslipidemic phenotypes (DLP) according to Sniderman classification- see Table 6.

	TGL (mmol/l)	Apo B (g/l)
DLP1	< 1.5	< 1.2
DLP2	≥ 1.5	< 1.2
DLP3	< 1.5	≥ 1.2
DLP4	≥ 1.5	≥ 1.2

Table 6. Classification of dyslipidemic phenotypes

5.3. Results

Basic clinical characteristics are shown in Table 7. Concentrations of adipokines and other biochemical parameters are given in Table 8.

	Number (n)	F/M	Age (y)	Waist (cm)	SBP (mg Hg)	DBP (mm Hg)	Smoking (n)	Manifestation of ATS
DLP1	32	16/16	41 ± 10.0	85 ± 9.3	129 ± 12	77 ± 9	4	3
DLP2	38	20/18	47.1 ± 10.1	96 ± 12	130 ± 19	78 ± 11	7	3
DLP3	13	3/10	47.8 ± 10.5	88 ± 8.0	125 ± 18	75 ± 7	2	0
DLP4	36	22/15	49.9 ± 10.7	92 ± 9.0	126 ± 15	75 ± 9	9	4

Table 7. Basic clinical characteristics of DLP groups

	ADP (mg/l)	FGF 21 (ng/l)	A-FABP (ug/l)	CHOL (mmol/l)	TGL (mmol/l)	HDLchol (mmol/l)	LDLchol (mmol/l)	Apo AI (g/l)	Apo B (g/l)	GLU (mmol/l)	BMI (kg/m^2)
DLP1	10.6 ± 6.0	186 ± 100	22.5 ± 10.3	5.68 ± 0.8	1.04 ± 0.26	1.74 ± 0.41	3.48 ± 0.75	1.74 ± 0.41	0.91 ± 0.16	5.16 ± 0.66	25.9 ± 5.2
DLP2	8.0 ± 5.1	333 ± 360*	33.9 ± 29.0*	6.23 ± 1.92	5.29 ± 8.0	1.22 ± 0.31*	3.14 ± 0.93	1.51 ± 0.30	0.98 ± 0.14	5.57 ± 1.19*	28.3 ± 4.7*
DLP3	8.6 ± 4.9	165 ± 104	14.4 ± 4.6	7.47 ± 1.14	1.11 ± 0.2	1.45 ± 0.33	5.52 ± 1.27	1.51 ± 0.25	1.41 ± 0.25	5.03 ± 0.52	25.2 ± 3.1
DLP4	9.0 ± 5.9	384 ± 347**	29.2 ± 18.4**	8.43 ± 2.0	3.5 ± 2.2	1.27 ± 0.45**	5.59 ± 2.0	1.50 ± 0.43	1.62 ± 0.39	5.36 ± 1.27***	27.2 ± 5.0**

Differences between groups were analyzed with ANOVA. Parameters with skewed distribution (TGL, ADP, FGF 21, A-FABP) were log transformed to normalize their distributions before statistical analyses.

* DLP2 vs. DLP1 and DLP3, $p < 0.01$, ** DLP4 vs. DLP1 and DLP3, $p < 0.01$, *** DLP4 vs. DLP1 and DLP3, $p < 0.05$

Table 8. Adipokines and other biochemical parameters in connection with DLP

The highest levels of ADP were observed in DLP1 (no significance). Suprisingly, there was seen no negative association between adiponectin levels and DLP2 (DLP4). FGF 21 and A-FABP were significantly increased in the groups with the most important atherogenic potential (DLP2, DLP4). These two parameters correlated with higher levels of triglycerides, fasting glucose, BMI and lower HDL cholesterol, both in DLP2 and DLP4.

5.4. Conclusions

No association was found between ADP levels and other adipokines in DLP groups in our study. There was the correlation between FGF 21 and A-FABP in groups with TGL > 1.5 mmol/l. Increased levels of both parameters were associated with increased glucose, BMI and decreased HDL cholesterol levels (in accordance with lit.[40]). The increase of FGF 21 concentrations are probably due to the compensatory response to higher A-FABP that is considered the predictor of metabolic syndrome. In individuals with MS, the determination of A-FABP could be considered as a parameter with the independent metabolic effects [42]. The clinical potential especially of A-FABP in diagnostics and prediction of metabolic syndrome should be continue to observe.

6. Adiponectin in members of families with familial combined hyperlipidemia

Familial combined hyperlipidemia (FCH) is the most common genetic hyperlipidemia which affects 1.0% to 2.0% of the population. The lipids and lipoprotein levels are, however, only moderately elevated and do not fully explain the increased risk of cardiovascular disease. The aim of the study of Karásek et al. [43] was to evaluate plasma levels of adiponectin in asymptomatic, nonsmoking members of families with FCH. We also investigated the association between adiponectin and selected risk factors of atherosclerosis and markers of insulin resistance and chronic inflammation. Furthermore, we investigated the relationship between adiponectin and the intima-media thickness of the CCA (IMT), a recognized morphologic marker of early atherosclerosis.

6.1. Subjects and methods

The study was carried out with 82 members of 29 FCH families. A family with FCH was defined by a proband exhibiting plasma cholesterol and triglycerides concentrations above 90th percetile, adjusted for age and sex, based on data from the Czech population. At least one first-degree relative of the proband should have plasma cholesterol and/or triglycerides above 90th percentile, adjusted for age and sex, or level of apo B more than 1.25 g/l. Secondary hyperlipidemia was excluded by additional testing. Other exclusion criteria were a history of clinically manifest atherosclerosis, heart failure, cerebrovascular ischemic disease, peripheral vascular disease, smoking, hypolipidemic therapy in the previous 8 weeks, hormone therapy with estrogens and acute infection or trauma. Members of FCH were divided into 2 groups: HL (hyperlipidemic members of FCH families, i.e. probands

and their hyperlipidemic first-degree relatives) and NL (normolipidemic first-degree relatives). The control groups, C-HL and C-NL, were sex and age matched to groups. Control groups consisted of healthy individuals with a negative family history of hyperlipidemia and early manifestation of atherosclerosis. Nobody was treated for hypertension.

Laboratory parameters were analyzed by routine methods described above. Ultrasound scanning was performed with a 10 MHz linear array transducer (Hewlett-Packard, Image Point, M2410A). All measurements were performed with the subjects in a supine position. Three video records were made of common carotid artery (CCA). IMT measurements were processed off-line using software Image-Pro Plus (v. 4.0, Media-Cybernetics, Silver Spring). The average of the IMT of 3 frozen images of both sides was chosen as the outcome variable. Subjects with an atherosclerotic plaque in the evaluated region were not included in the study. The measurement of IMT was made without knowledge of laboratory results.

6.2. Results

In comparison to sex and age matched controls, HL subjects had significantly higher diastolic blood pressure (DBP), BMI, insulin resistance and elevated levels of C-peptide and proinsulin. They had higher IMT, hsCRP and ICAM 1 as well. By definition, the FCH subjects showed higher plasma cholesterol and triglycerides concentrations compared with controls and normolipidemic relatives. They had a more atherogenic lipid and lipoprotein profile as reflected by increased LDL cholesterol and apo B concentrations. Normolipidemic relatives had significantly higher DBP, TGL and proinsulin concentrations compared with their sex and age matched controls. There was no difference in other measured anthropometric and biochemical parameters.

Compared with healthy controls, HL subjects had lower levels of adiponectin (13.02 ± 4.58 mg/l vs. 16.19 ± 5.39 mg/l, $p < 0.05$). In the NL relatives, there was no significant differences in adiponectin (15.77 ± 2.95 mg/l vs. 16.53 ± 4.26 mg/l). In all FCH families, a significant negative correlation was found between adiponectin and TGL ($r = - 0.35$, $p < 0.01$), proinsulin ($r = - 0.26$, $p < 0.05$), hsCRP ($r = - 0.24$, $p < 0.05$), BMI ($r = - 0.27$, $p < 0.05$) and waist circumference ($r = -0.32$, $p < 0.01$). Levels of adiponectin did not correlate with IMT, in members of FCH families or in controls. By using regression model in HL subjects, levels of adiponectin were predicted by apo B ($p < 0.05$) and hsCRP ($p < 0.05$). (More detailed results are given in lit. [43].)

6.3. Discussion and conclusions

This study reported decreased adiponectin levels in asmyptomatic hyperlipidemic members of FCH families. There was no difference in serum adiponectin levels between their first-degree normolipidemic relatives and healthy controls. A negative correlation between adiponectin and markers of insulin resistance, chronic inflammation and visceral obesity was found in FCH families. The results were consistent with previous findings and support

an insulin-sensitizing effect of adiponectin. In hyperlipidemic individuals, the levels of plasma adiponectin were predicted by apolipoprotein B and high sensitive CRP, independent of insuline resistance and visceral obesity. Authors conclude low adiponectin levels are associated with proinflammatory status and insulin resistance, and could partially explain the increased risk of coronary heart disease, even if the lipids and lipoprotein levels are only moderately elevated.

The study did not confirm any correlation between adiponectin levels and IMT, a marker of subclinical atherosclerosis, in FCH subjects. Publications regarding the relationship between these parameters are not entirely consistent. Similar results were observed in other work published by Karásek et al [44] where IMT proved to correlate with age, lipid parameters, markers of insulin resistance and that of visceral obesity and blood pressure. These parameters seem to be risk factors instead of adiponectin. The lack of correlation between adiponectin and IMT does not argue for adiponectin as an independent predictor for next cardiovascular events in clinically asymptomatic, dyslipidemic individuals.

7. Conclusion of chapter

Adiponectin is another promising parameter of the metabolic syndrome, atherosclerosis and associated syndromes. Its effect should be studied in many other situations. Nowadays, its determination in plasma provides valuable information, for example in patients with angiographically documented coronary artery disease, even if not all studies confirm this relationship. We can rely on the fact that its levels show no or very little circadian variability, its concentration is independent of fasting, it has low intraindividual variability, it is present in high concentrations in plasma and its levels can be influenced by diet, lifestyle or medication. Probably the most effective way to increase adiponectin levels in plasma and thus to reduce cardiovascular risk in obese individuals is a reduction in body weight. Beneficial effect of thiazolidinediones are also used to treat patients with type 2 diabetes to increase adiponectin production and plasma levels.

On the other hand, although adiponectin is associated with many of the traditional cardiovascular risk factors and further evidence has shown that hypoadiponectinemia is associated with atherosclerotic cardiovascular events such as myocardial infarction and brain infarction [45, 46], recent epidemiologic studies have shown contradictory results. Some of them revealed that hyperadiponectinemia rather than hypoadiponectinemia is associated with liver cirrhosis, rheumatoid arthritis, inflammatory bowel disease and systemic lupus erythematosus, all of which are conditions predisposed to wasting . Release of adiponectin from fat tissue is increased under conditions of malnutrition and plasma adiponectin concentration rises in the inflammatory state. Therefore, adiponectin can act as a mirror reflecting the degree of systemic wasting, and thus can predict death [47].

We can speculate about the real impact of high adiponectin levels on atherosclerosis: are they protective of harmful? In healthy subjects without clinically important signs of atherosclerosis, adiponectin has the protective effects especially due to its tissue-insulin senzitizing action. However, in individuals with advanced atherosclerosis and/or

inflammatory disease, the positive association of adiponectin levels with markers of endothelial dysfunction/hemostasis (VCAM 1, but also with thrombomodulin and von Willebrand factor) could explain the increased total and cardiovascular mortality and the one associated with high adiponectin levels. It could be also the case of other populations, such as elderly people, patients with heart failure, patients with chronic kidney diseases, patients with type 1 diabetes mellitus etc. In recent studies, adiponectin effects should be evaluated in these populations.

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