microvascular complications, which both will be demonstrated for some specific cytokines within this work. Although the manuscript will primarily focus on previously published work of the applicant, some yet unpublished data will also be shown, if appropriate. All data will be demonstrated within the respective scientific context.

2. Cytokines and type 2 diabetes mellitus

2.1 Inflammation and type 2 diabetes mellitus

As early as 1876, the first report on glucose lowering effects of the anti-inflammatory effects of salicic acid has been reported in the *Berliner Medizinische Wochenschrift* (9). Although this effect was not further evaluated until the early sixties, it is now becoming increasingly clear that T2DM is a manifestation of an ongoing acute-phase response, which is primarily characterized by alterations of the so called acute phase proteins such as C-reactive protein (CRP) (10, 11). Cross-sectional and prospective studies demonstrated increased concentrations of inflammatory markers, including CRP, serum amyloid-A and sialic acid in patients with T2DM (6, 7, 10-17). Elevated levels of IL-6, a major stimulator of acute-phase proteins, were demonstrated to be associated with an elevated risk of diabetes mellitus (7). However, it is well known, that cytokines operate as a network in stimulating the production of acute-phase proteins. Thus, the effects of IL-6 on CRP synthesis depend on an interaction with Interleukin-1ß (IL-1ß) (18). The acute-phase response in various artificial inflammatory models requires both IL-6 and IL-1ß as demonstrated in the respective knock-out mice models (19, 20). Recent work of the applicant extended this field with respect to the polycystic ovarian syndrome (PCOS), a disease being associated with

type 2 diabetes mellitus (21, 22). In a more direct approach a prospective nested case-control cohort designed to further investigate the role of specific cytokines in the development of type 2 diabetes mellitus, was analysed (6). Finally the role of a IL-6 promoter-polymorphism was addressed within the mentioned cohort (23).

2.1.1 Inflammation and polycystic ovarian syndrome (PCOS)

PCOS is among the most frequent endocrine disorders in women. The prevalence has been estimated to be between 5-10 % of reproductive-age women (24-27). A high proportion of patients with PCOS are obese and/or insulin resistant. There is considerable evidence that hyperinsulinemia may have a pathogenic role in hyperandrogenemia, one of the characteristics in women with PCOS (25, 26). Although powerful prospective studies do not exist, there is quite good evidence that women with PCOS have an increased risk for developing impaired glucose tolerance and type 2 diabetes mellitus (28-30). Therefore it is of obvious interest whether women with PCOS display features that link PCOS with subsequent diabetes mellitus. As mentioned above, the development of type 2 diabetes has been associated with a subclinical, chronic inflammation. We thus aimed to investigate whether such an inflammation is primarily associated with PCOS itself or whether it primarily reflects obesity and / or impaired glucose metabolism. In our cohort of 57 PCOS women and 20 healthy age-matched controls no significant differences in plasma IL-6 or CRP levels could be demonstrated after adjustment for markers of obesity. Comparably we found no differences of inflammatory markers between women with POCS and controls after stratification of the cohort at a Body mass index (BMI) of 25 kg/m^2.
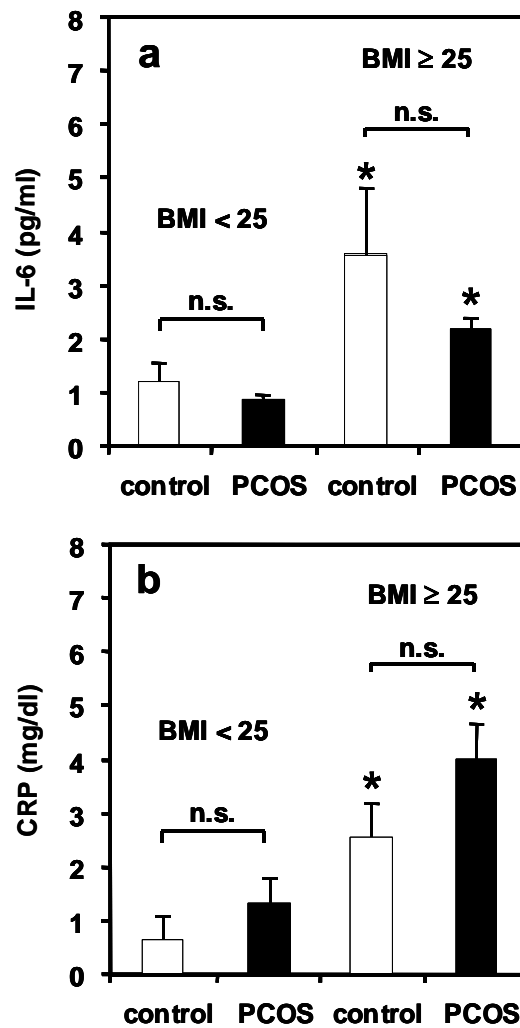
Figure 1: IL-6 and CRP plasma concentrations in patients with PCOS and age-matched healthy controls. * p<0.05 vs lean subjects.

Plasma IL-6 and CRP levels were significantly increased in both obese PCOS and obese control women as compared to their lean counterparts, and both parameters correlated with markers of obesity and insulin resistance in the PCOS cohort. Linear regression models revealed that BMI rather than insulin resistance as indicated by HOMA is the dominant parameter determining IL-6 and CRP values in PCOS, which is consistent with a recent report from a cohort of premenopausal women (31).

Thus our data do not support the hypothesis that PCOS per se activates low grade chronic inflammation thereby increasing a priori the risk for the development of type 2 diabetes mellitus. This interpretation is further suggested by our finding that none of the endocrine markers of PCOS correlated with plasma IL-6 or CRP concentrations. Our data support a recent report demonstrating comparable IL-6 levels in patients with PCOS and healthy controls (32). However, another previous study reported higher elevated CRP levels in a cohort of 17 PCOS women compared to 15 controls (33), which is in contrast to our findings.

The C-174G polymorphism within the IL-6 gene promoter region has been suggested to modify promoter activity in vitro (34) and has been associated in some but not all studies to increased IL-6 levels, insulin resistance, resting energy expenditure, obesity, and hyperandrogenism (32, 35-38). Thus we also aimed to investigate the role of this polymorphism in our cohort and speculated that it might affect IL-6 levels or any PCOS-associated endocrine abnormalities. However, neither IL-6 levels nor markers of obesity were linked to a certain genotype at IL-6-C-174G, which argues against a major impact of the C-174G IL-6 promoter polymorphism on IL-6 levels and obesity in PCOS patients. Although the heterozygous GC genotype was associated with lower androstendione and a tendency to lower testosterone levels rather than to hyperandrogenemia, this finding should not be overemphasised as this cohort was clearly to small to allow conclusive interpretation of these data. Certainly further studies are required to elucidate the precise role of the C-174G IL-6 promoter polymorphism on androgen production in PCOS.

We additionally investigated, whether treatment with metformin affected inflammatory markers. As expected, treatment of patients with PCOS resulted in a significant weight loss, reduction of total fat mass and decreased levels of total testosterone as reported previously (39). However, plasma IL-6 and CRP did not change significantly

although the decrease in CRP concentrations closely failed to be significant. Again, this does not support a direct link between hyperandrogenemia and low grade chronic inflammatory processes in PCOS, as the decrease in BMI alone completely explains this reduction of CRP. In contrast it is rather surprising that the described weight loss alone did not cause a significant reduction in IL-6 and CRP, although the extent of BMI and fat mass reduction might have been insufficient to cause a substantial decrease in the inflammatory parameters.

In summary, we demonstrated that plasma levels of IL-6 and CRP were not increased in women with PCOS, if the degree of obesity was taken into account (21). In our study, PCOS-related endocrine abnormalities did not correlate with any co-existing inflammatory situation and was unlikely to enhance the type 2 diabetes risk in these patients, at least via an inflammatory axis. Although Metformin treatment improved a subclinical inflammation, this effect was likely to be due to changes in obesity and/or metabolic alterations.

2.1.2 Inflammatory cytokines and diabetes risk

Various cross-sectional and prospective studies demonstrated an association of elevated inflammatory markers with type 2 diabetes. However, although net effects of inflammatory cytokines are likely to be more important than single cytokines, no study had been performed investigating various cytokines with respect to diabetes risk. We therefore designed a nested case-control study within the prospective population-based EPIC-Potsdam cohort of 27,548 men and women to evaluate the combined role of TNF-α, IL-1ß and IL-6 in the development of T2DM. The EPIC-Potsdam study is part of the multi-centre, population-based cohort study "European

Investigation into Cancer and Nutrition" (EPIC). In brief, 27,548 subjects, women aged 35 to 65, and men aged 40 to 65, were recruited from the general population. Baseline examinations included anthropometric measurements, blood sampling, a self-administered food frequency questionnaire, and a personal interview on life-style habits and medical history were conducted between 1994 and 1998. Follow-up questionnaires are being send to the study participants every two to three years to obtain information, among others, on current medication and newly developed diseases, including diabetes mellitus.

Case subjects were defined as those individuals, who were disease-free at baseline of the study and subsequently developed type 2 diabetes mellitus during the follow-up (mean 2.3 years). Cases were identified by self-report questionnaires and verified by recalls to the respective clinician, who needed to confirm the diagnosis. Only those individuals with a verified diagnosis were included into the analysis.

Characteristic	Cases (N=188)	Controls (N=377)	P-value
Age (y)	56 (7)	56 (7)	matching variable
BMI (kg/m^2)	30.7 (4.8)	26.7 (3.5)	< 0.0001
WHR	0.95 (0.09)	0.89 (0.09)	< 0.0001
Sports (h/week)	0.5 (1.1)	0.9 (1.6)	0.0100
Alcohol consumption (g/day)	18.5 (28.2)	16.1 (16.4)	0.4678
IL 1ß (pg/ml)	0.57 (0.93)	0.47 (0.79)	0.1959
IL 6 (pg/ml)	2.45 (1.80)	1.67 (1.59)	< 0.0001
TNF-alpha (pg/ml)	2.04 (1.51)	1.79 (1.28)	0.0094
CRP (µg/ml)	4.14 (5.1)	2.45 (4.38)	< 0.0001
Men (N, %)	111 (59%)	222 (59%)	matching variable
Current smokers (N, %)	36 (19%)	80 (21%)	0.5661
Less than high school education (N, %)	83 (44%)	142 (38%)	0.1383
Prevalence of hypertension (N, %)	148 (79%)	195(52%)	< 0.0001
Prevalence of hyperlipoproteinemia (N, %)	81 (43%)	120 (32%)	0.0085

Table 1: Baseline characteristics of individuals in the prospective nested case-control study (EPIC-Potsdam; n=563)

As expected, case subjects were more obese, exercised less (Table 1) and had higher HbA_{1c} levels compared to controls.

As shown in previous studies (7, 14), elevated levels of CRP were associated with an increased risk of T2DM after adjustment for age, sex, sex normalized waist-hip ratio, sporting activities, smoking status, alcohol consumption, educational attainment and HbA_{1c} (fully adjusted model) (OR 1.9; 95% CI 1.2 – 3.2). A subgroup analysis (HbA_{1c} < 5.8%) yielded comparable results to the analysis including all participants, which was performed to reduce a bias from individuals with prevalent diabetes at baseline. Thus, the risk of individuals with elevated CRP levels to develop T2DM was 2.1 (95% CI 1.2 – 3.7) in the fully adjusted model in this subgroup.

IL-6 baseline levels were higher among cases compared to controls. In the fully adjusted model, IL-6 was also found to be an independent predictor of T2DM (OR 2.57; 95% CI 1.24 – 5.47). Comparable results were found in those analyses restricted to subjects with a HbA_{1c} < 5.8% only.

In contrast to our findings with CRP and Interleukin-6, elevated levels of Tumor necrosis factor-α and Interleukin-1ß alone were not independently associated with future T2DM. While there was no difference with respect to Interleukin-1ß, elevated concentrations of TNF-α were observed in case subjects compared to controls. Although the risk of T2DM increased with increasing quartiles of TNF-α in the crude analysis, this effect completely vanished after adjustment for BMI or waist-hip-ratio (WHR). Again, analysis of participants with a HbA_{1c} < 5.8% yielded a similar picture as analysis of all participants.
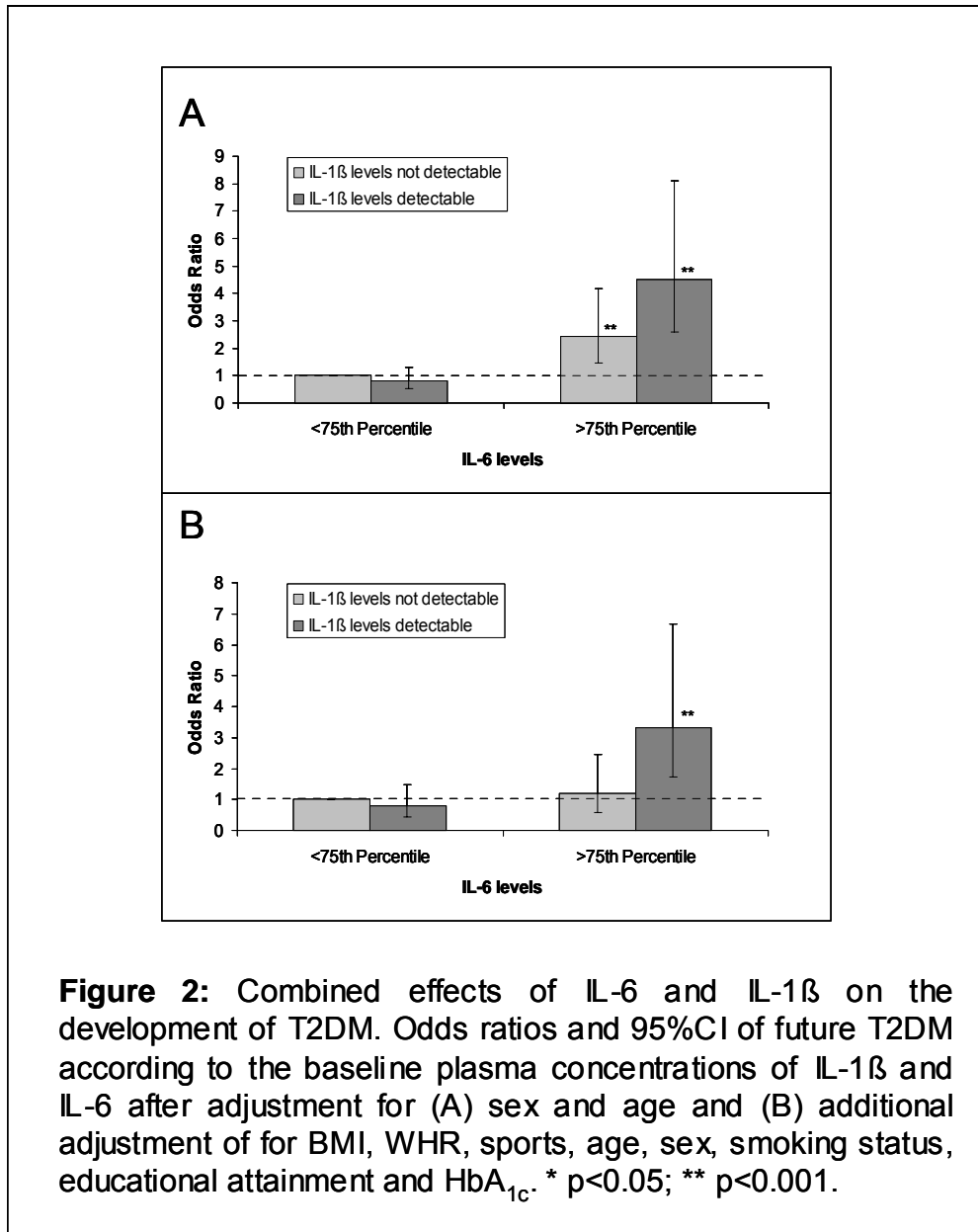
	Categories			
IL 1ß	1	2		
Median, category limits	0.1 (0.1 – 0-.1)	0.8 (0.1 – 5.5)		
Age and sex adjusted analysis (OR, 95% CI)	1.0 (reference)	1.2 (0.8 – 1.7)		
+ BMI adjusted analysis (OR, 95% CI)	1.0 (reference)	1.2 (0.8 – 1.8)		
Adjusted for all risk factors (OR, 95% CI)*	1.0 (reference)	1.2 (0.8 – 1.8)		
Adjusted for all risk factors and HbA1c (OR, 95% CI)	1.0 (reference)	1.2 (0.7 – 2.0)		
CRP	1	2		
Median, category limits	0.6 (0.1 – 1.5)	3.6 (1.5–39.7)		
Age and sex adjusted analysis (OR, 95% CI)	1.0 (reference)	3.5 (2.4 – 5.1)		
+ BMI adjusted analysis (OR, 95% CI)	1.0 (reference)	2.2 (1.5 – 3.3)		
Adjusted for all risk factors (OR, 95% CI)*	1.0 (reference)	1.9 (1.2 – 2.9)		
Adjusted for all risk factors and HbA1c (OR, 95% CI)	1.0 (reference)	1.9 (1.2 – 3.2)		
IL 6	1	2	3	4
Median, interquartile range	0.7 (0.1 – 0.9)	1.2 (0.9 – 1.5)	1.8 (1.5 – 2.2)	3.3 (2.2–10.8)
Age and sex adjusted analysis (OR, 95% CI)	1.0 (reference)	1.8 (1.0 – 3.3)	3.9 (2.2 – 7.0)	7.3 (4.2–13.3)
+ BMI adjusted analysis (OR, 95% CI)	1.0 (reference)	1.4 (0.7 – 2.6)	2.2 (1.2 – 4.1)	3.3 (1.8 – 6.2)
Adjusted for all risk factors (OR, 95% CI)*	1.0 (reference)	1.2 (0.6 – 2.4)	1.9 (1.0 – 3.7)	2.8 (1.4 – 5.3)
Adjusted for all risk factors and HbA1c (OR, 95% CI)	1.0 (reference)	1.1 (0.5 – 2.5)	1.7 (0.8 – 3.6)	2.6 (1.2 – 5.5)
TNF-α	1	2	3	4
Median, interquartile range	0.8 (0.4 – 1.1)	1.3 (1.1 – 1.6)	1.8 (1.6 – 2.3)	2.9 (2.3–15.2)
Age and sex adjusted analysis (OR, 95% CI)	1.0 (reference)	1.9 (1.1 – 3.1)	1.4 (0.8 – 2.4)	2.3 (1.4 – 3.9)
+ BMI adjusted analysis (OR, 95% CI)	1.0 (reference)	1.7 (0.9 – 2.9)	1.1 (0.6 – 1.9)	1.7 (1.0 – 3.0)
Adjusted for all risk factors (OR, 95% CI)*	1.0 (reference)	1.6 (0.9 – 2.9)	1.0 (0.6 - 1.9)	1.5 (0.8 – 2.8)
Adjusted for all risk factors and HbA1c (OR, 95% CI)	1.0 (reference)	1.9 (0.9 – 3.9)	1.1 (0.5 – 2.2)	1.6 (0.8 – 3.2)

Table 2: Odds ratios (OR) and 95% confidence intervals (95% CI) for the risk of Type 2 diabetes among middle-aged men and women, according to baseline plasma concentrations of Interleukin-1ß (IL-1ß), Interleukin-6 (IL 6), Tumor necrosis factor-alpha (TNF-α) and C-reactive protein (CRP). CRP and IL-1ß were dichotomised due to the relatively high number of undetectable values. *Adjusted for age, sex, BMI, WHR, sporting activities, smoking status, alcohol consumption and educational attainment.

Despite IL-1ß alone had no effect on diabetes risk, we found an interaction between IL-1ß and IL-6 with respect to diabetes risk. Participants with a combined elevation of IL-6 levels and detectable levels of IL-1ß were found to have an increased risk of future T2DM compared to the low level reference group in the fully adjusted model. In contrast, individuals with elevated levels of IL-6 but non-detectable levels of IL-1ß

had no significantly increased risk to develop T2DM compared to the low level reference group in the fully adjusted model (Figure 2).

Figure 2: Combined effects of IL-6 and IL-1ß on the development of T2DM. Odds ratios and 95%CI of future T2DM according to the baseline plasma concentrations of IL-1ß and IL-6 after adjustment for (A) sex and age and (B) additional adjustment of for BMI, WHR, sports, age, sex, smoking status, educational attainment and HbA_{1c}. * $p<0.05$; ** $p<0.001$.

This interaction was confirmed by inclusion of a multiplicative interaction term within the respective model, which was significant between detectable IL-1ß and elevated IL-6 (Table 3). In a crude analysis, those individuals with a combined elevation of IL-6 and TNF-α or with TNF-α and IL-1ß had a substantially increased risk compared to

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individuals with elevated levels of IL-6 alone or compared to the low level reference group. However, as described for TNF-α alone, this effect did not remain significant in the fully adjusted model (Table 3).

After adjustment for age, sex, BMI, WHR, sporting activities, HbA1c, smoking status, alcohol consumption and educational attainment	OR (95% CI)
A	
Reference (TNF-α low/ IL-1ß undetectable)	1
TNF-alpha (high)	0.89 (0.45 – 1.73)
IL-1ß (undetectable)	0.95 (0.54 – 1.67)
Interaction term TNF*IL-1ß (high/detectable)	2.51 (0.84 – 7.57)
B	
Reference (TNF-α low/ IL-6 low)	1
TNF-alpha (high)	1.32 (0.68 – 2.52)
IL-6 (high)	2.15 (1.12 – 4.11)
Interaction term TNF*IL-6 (both high)	0.7 (0.23 – 2.16)
C	
Reference (IL-6 low/ IL-1ß undetectable)	1
IL-1ß (undetectable)	0.8 (0.43 – 1.44)
IL-6 (high)	1.14 (0.55 – 2.32)
Interaction term IL-1ß*IL-6 (detectable/high)	3.31 (1.14 – 9.87)

Table 3: Investigation of interaction between Tumor necrosis factor-alpha (TNF-α) and Interleukin-1ß (IL-1ß) (A), TNF-α and Interleukin-6 (IL-6) (B) or IL-1ß and IL-6 (C) on the diabetes risk, respectively. EPIC-Potsdam Study, Germany, N=565. Individuals were dichotomised according to cytokine plasma levels. IL-1ß remained dichotomised as previously described (detectable vs. undetectable levels), while IL-6 and TNF-α were dichotomised by using the 75th percentile as cut-off point.

Our data support the concept that a chronic inflammation participates in the development of type 2 diabetes mellitus. The results, that markers of inflammation predict T2DM, were in good agreement with previous studies, as demonstrated in Table 4.

Adjustment for	Schmidt et al 1999 BMI-WHR	Pradhan et al 2001 BMI, Life-Hist	Festa et al 2002 Demog	Freeman et al 2002 Multi	Spranger et al 2003 Multi
Leukocytes	1.5	*	*	*	*
CRP	*	4.2	1.47	1.33	1.9
Sialic acid	2.8	*	*	*	*
IL-6	*	2.3	*	*	2.6
PAI-1	*	*	2.02	*	*

Table 4: Odds ratios of inflammatory markers in various prospective cohorts with respect to T2DM.

As a major outcome, our study suggested that a complex cytokine pattern rather than single cytokines affect the development of T2DM. It is well known that cytokines are members of rather large and complex signaling networks (18-20). Various mechanisms may mediate those effects of combined elevations of different cytokines. Thus, increased levels of very low density lipoprotein (VLDL) and decreased high density lipoprotein (HDL) can be induced by direct action of both, IL-6 and IL-1ß, on the liver to produce the characteristic dyslipidaemia of the metabolic syndrome (11). Combined elevation of IL-6 and IL-1ß dramatically increased the expression of the acute-phase proteins compared to the effect of each cytokine alone (18). Another potential molecular mechanism, how inflammation may be involved in the pathogenesis of T2DM has been elucidated in recent studies showing that sensitising of insulin signaling by salicylates is induced via inhibition of the activity of IκB kinase-ß (IKK-ß) (40-42). IL-1ß is well known to activate the IKK-ß and might thereby induce insulin resistance. However, a recent study mentioned that inhibition

of the activity of IKK-ß may not be of that outstanding importance as initially suggested (43). In contrast effects of salicylates on the JNK-pathway have been demonstrated and indeed, inhibition of this pathway appears to improve insulin sensitivity (44).Thus beneficial effects of salicylates may act not exclusively via inhibition of the activity of IκB Kinase. A better understanding of the underlying molecular mechanisms of the here described findings might ultimately offer additional therapeutic approaches, which may be based on anti-inflammatory effects.

In summary, we have shown that a combined elevation of IL-1ß and IL-6 increases the risk of type 2 diabetes mellitus (6). In more general terms, our data support the concept, that a subclinical chronic inflammation is involved in the development of type 2 diabetes mellitus. We demonstrated that a specific pattern of cytokines is associated with an increased risk of T2DM, rather than isolated elevation of the respective cytokines.

2.1.3 IL-6 −174 G/C as a genetic polymorphism affecting diabetes risk

As mentioned above we and others have demonstrated that IL-6 is associated with an increased risk of type 2 diabetes (6, 7). IL-6 gene transcription was found to be affected in vitro by the C-174G polymorphism within the IL-6 promoter (34). However, data about the effects of this polymorphism on IL-6 levels in humans are controversial. While one study reported elevated IL-6 levels in individuals with CC genotype (35), another study found no differences (36) and two studies even described lower IL-6 levels in subjects with CC genotype. Interestingly, the IL-6 C-174G polymorphism was found to be associated with insulin resistance (37, 38) and

energy expenditure (37), but the results of these two groups were again partially contradictory.

We made use of the above mentioned prospective nested case control study and investigated whether the IL-6 C-174G polymorphism affected diabetes risk.

As a major finding we demonstrated that the C-174G polymorphism within the IL-6 promoter affected the correlation between BMI and IL-6 levels. The slope of the correlation between BMI and IL-6 was steeper for individuals with CC genotype compared to those with GG genotype. The correlation of individuals being heterozygous was basically between the two homozygous variants, suggesting a dose-response relationship.

Given these data, we next analysed whether the C-174G polymorphism modified the association between BMI and the risk of T2DM. Indeed, obesity was associated with a higher diabetes risk (more than 5 times higher in our study cohort) among those subjects with the CC genotype than among those with the remaining genotypes. This conclusion was also valid in the fully adjusted model and after additional inclusion of various environmental factors such as nutrient intake (protein intake, fat intake, carbohydrate intake, alcohol consumption) or drug use (ACE-inhibitors and other antihypertensive drugs and statins and other lipid lowering drugs as well as corticosteroids and antiphlogistic drugs).

Effect modification between a genotype and an environmental factor is a scientifically tempting concept and might reflect reality more appropriate than simple comparison of individuals with different genotypes. Here we demonstrated effect modification of the BMI-dependent diabetes risk by the C-174G polymorphism within the IL-6 promoter. The statistical analyses indicated that for the CC genotype a high BMI is associated with a higher risk of T2DM than for the remaining genotypes (Table 5).

Variable	Odds ratio	95% CI	p-value
BMI $\geq$ 28 kg/m^2	3.32	1.35 – 8.39	0.01
GC or CC at C-174G	1.15	0.53 – 2.59	0.73
CC at C-174G	0.18	0.03 – 0.69	0.029
BMI $\geq$ 28 kg/m^2 by GC or CC	0.89	0.29 – 2.63	0.83
BMI $\geq$ 28 kg/m^2 by CC	6.01	1.25 – 45.00	0.042

Table 5: BMI was dichotomised at 28 kg/m^2. Variables for genotypes were GC or CC at C-174G (GC or CC =1, else 0) and CC at C-174G (CC =1, else 0). Interaction terms describe multiplicative interaction between dichotomised BMI variable and the variables for the genotypes.

Given the effect modification between C-174G polymorphism and BMI and especially the different correlations between BMI and IL-6 levels depending on the genotype, it appears mandatory to re-analyse other reports on that issue taking into account the BMI more properly. Indeed, the described interaction might help to explain some of the controversial results of previous studies. For example, one study found GG genotype more common in diabetics versus non-diabetics (45) and apparently differences in the degree of obesity might underlie this result. Berthier and co-workers (46) described recently the G allele at C-174G more common in lean subjects. This result fits well to the finding of a lower basal metabolic rate in case of CC genotype (37) possibly leading to body weight gain. In our study focusing on the development of T2DM, BMI was not different between the C-174G genotypes.
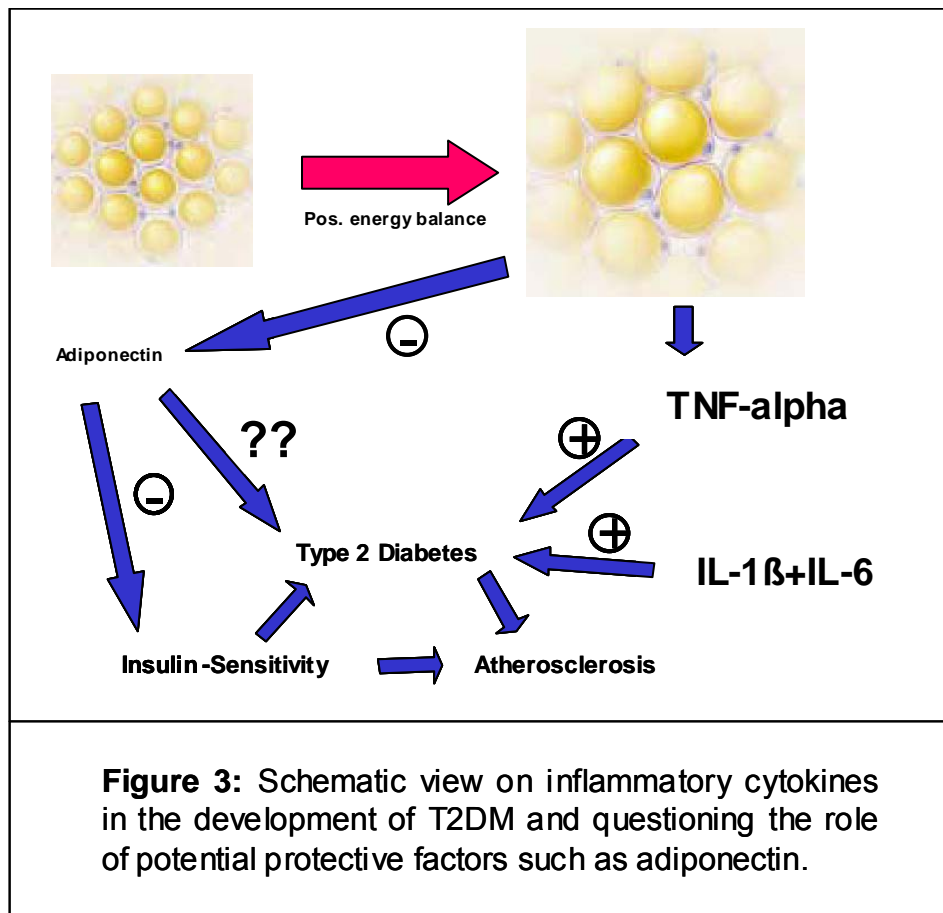
In summary, we demonstrated that the IL-6 C-174G polymorphism modifies the BMI-dependent diabetes risk (23). This effect might be based on different correlations between BMI and IL-6, depending on the IL-6 C-174G polymorphism. In more general terms we claim that it is mandatory for genetic studies to take environmental factors into account.

2.2   Adiponectin and type 2 diabetes mellitus

Interestingly a relatively high number of individuals do never experience T2DM despite being obese or living a sedentary lifestyle (47). This apparently is somewhat surprising given the fact that obesity and reduced physical activity are major risk factors for T2DM. Therefore yet unknown factors appear to protect individuals against development of T2DM.

Some years ago, an adipose-specific protein, adiponectin, has been discovered (48-51). Adiponectin is exclusively expressed in white adipose tissue and has been shown to have a physiological role in the mediation of insulin sensitivity and to inhibit the central inflammatory NFkappaB pathway (52-54). Various studies have shown reduced adiponectin levels in patients with obesity or type 2 diabetes (55, 56). Adiponectin-knock-out mice displayed a mild or no insulin resistance and glucose intolerance (57, 58). However, substitution of adiponectin substantially improved insulin sensitivity in various animal models (53). A potential mechanism might be the activation of the central "fuel sensor" AMP-Kinase (AMPK) (59, 60). Decreased levels of adiponectin were shown to precede the onset of disease in an animal model of diabetes (61). Recent studies from Eckels lab suggested that adiponectin might also protect beta-cells from cytokine induced cytotoxicity (62). Therefore, adiponectin has insulin sensitising and anti-inflammatory properties and might directly protect the beta cell, thereby preserving insulin secretion. In addition, a diabetes-susceptibility locus has been mapped to human chromosome 3q27, where the adiponectin gene is located (63, 64). Various polymorphisms within the adiponectin gene have been more or less closely linked to T2DM, obesity or insulin resistance (65-68). Making things even more complicated, the expression of adiponectin receptors also appears

to be regulated and linked to the metabolic situation (69-71). Thus, both genetic and functional data make adiponectin a highly interesting candidate to be involved in the pathogenesis of type 2 diabetes, as depicted in figure 3.

Figure 3: Schematic view on inflammatory cytokines in the development of T2DM and questioning the role of potential protective factors such as adiponectin.

However, no clear data were available in human states of disease, especially the prospective value of adiponectin had not been investigated in large population studies until recently. We thus analysed the relationship between circulating adiponectin and insulin sensitivity in women with PCOS, patients with an elevated diabetes risk. In addition we made use of the above described nested case control study thereby investigating whether adiponectin independently affects diabetes risk in apparently healthy individuals.

2.2.1 Adiponectin and polycystic ovarian syndrome

Various recent studies analysed the relationship between adiponectin, obesity and insulin sensitivity in women with PCOS (72-74). These studies suggested that low adiponectin levels are basically driven by the degree of obesity. However, in non-PCOS individuals, adiponectin is also rather closely linked to insulin resistance (75-78). As in PCOS patients the degree of insulin resistance is not necessarily and entirely explained by the degree of obesity and the precise interaction between adiponectin, insulin resistance, and obesity was still to be defined in PCOS women. In addition, PCOS-associated hyperandrogenemia may further modulate plasma adiponectin (79), which could provide a potential mechanism whereby PCOS-related hyperandrogenemia enhances the susceptibility of PCOS women to the insulin resistance syndrome and its long-term complications.

We thus measured plasma adiponectin and parameters of obesity, insulin resistance and hyperandrogenism in sixty-two women with PCOS and compared them to thirty-five healthy female controls. Nine insulin resistant PCOS women were treated with metformin for six months and the effects on body weight, insulin resistance and hyperandrogenemia were correlated to changes in circulating plasma adiponectin.
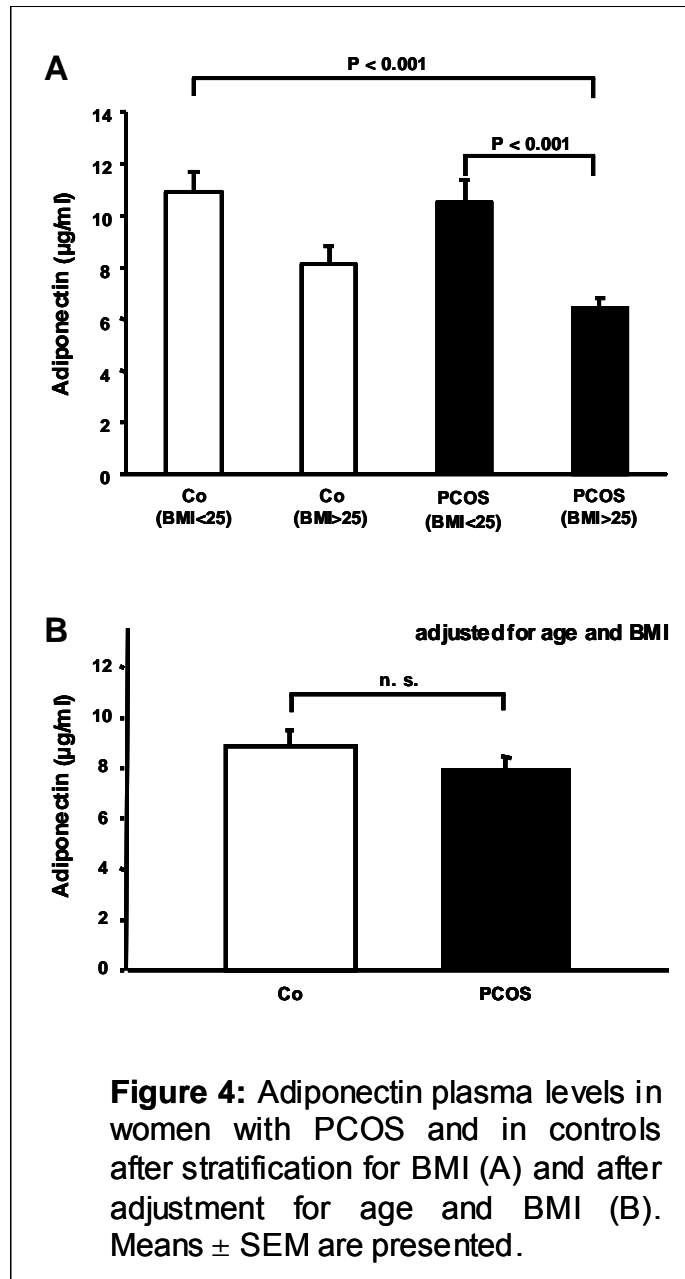
The characteristics of the PCOS patients and the healthy controls are summarized in Table 6. In women suffering from PCOS testosterone, androstendione, 17-OH-progesterone and the LH/FSH ratio were significantly increased and they had a higher BMI and were more insulin resistant according to fasting insulin concentrations and HOMA analysis than the healthy controls. Plasma adiponectin concentrations averaged to 7.6 ± 0.5 µg/ml (n = 62) in patients with PCOS which was significantly lower than in controls (9.8 ± 0.6 µg/ml; n = 35; p = 0.006).

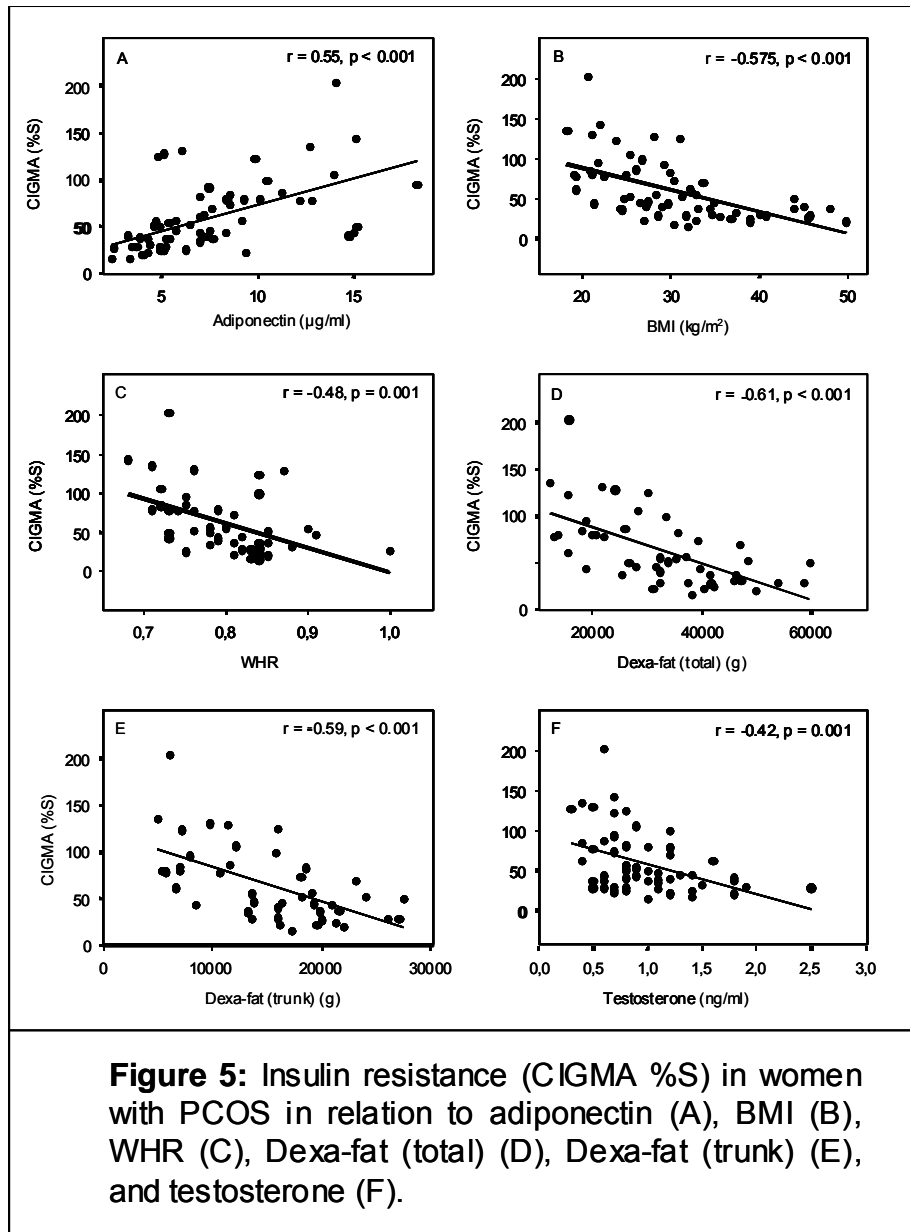|	Mean ± SEM		
Variable	controls (n = 35)	PCOS (n = 62)	P-value
Age (yr)	30.4 ± 1.0	28.9 ± 0.6	0.240
BMI (kg/m^2)	25.1 ± 1.0	30.6 ± 0.9	0.001
WHR	0.78 ± 0.01	0.80 ± 0.01	0.058
Fasting glucose (mmol/l)	5.0 ± 0.1	4.4 ± 0.1	0.001
Fasting insulin (pmol/l)	56.3 ± 5.2	88.7 ± 7.1	< 0.001
HOMA (%S)	111 ± 6	86 ± 7	0.001
LH/FSH	0.99 ± 0.08	2.00 ± 0.30	0.001
E_2 (pg/ml)	48.4 ± 6.6	68.4 ± 11.9	0.687
Progesterone (ng/ml)	0.74 ± 0.09	1.39 ± 0.40	0.107
Testosterone (ng/ml)	0.49 ± 0.04	0.96 ± 0.05	< 0.001
SHBG (nmol/l)	77.5 ± 8.6	60.8 ± 6.3	0.068
Androstendione (ng/ml)	1.29 ± 0.89	2.37 ± 0.11	< 0.001
17-OH-Progesterone (ng/ml)	0.49 ± 0.05	0.84 ± 0.08	0.001
DHEAS (µg/ml)	2.49 ± 0.27	2.83 ± 0.18	0.124

Table 6: Clinical and endocrine features of PCOS patients and healthy controls. Mean ± SEM and p-value of non-parametric Mann-Whitney U test are indicated.

As the degree of obesity is known to affect adiponectin levels, we initially stratified the control and PCOS women according to WHO criteria (BMI < 25 kg/m^2 or ≥ 25 kg/m^2).

As expected, the adiponectin concentrations were lower in the obese groups, however, there were no differences between lean or obese controls and PCOS patients, respectively (Figure 4A). In line with these results, adiponectin levels were not different between controls and women with PCOS after adjustment for age and BMI (Figure 4B). We thus confirmed some recent reports, that PCOS per se is not associated with low levels of adiponectin (72, 73), suggesting that adiponectin is not directly involved in the pathogenesis of PCOS.

Figure 4: Adiponectin plasma levels in women with PCOS and in controls after stratification for BMI (A) and after adjustment for age and BMI (B). Means ± SEM are presented.

Since adiponectin is known to influence insulin sensitivity, we next aimed to investigate the parameters that determine insulin sensitivity in women with PCOS, and we tested whether circulating adiponectin could be involved. The major findings of simple correlation analysis is depicted in Figure 5.

Figure 5: Insulin resistance (CIGMA %S) in women with PCOS in relation to adiponectin (A), BMI (B), WHR (C), Dexa-fat (total) (D), Dexa-fat (trunk) (E), and testosterone (F).

To further investigate whether adiponectin indeed independently affects insulin resistance, a linear regression analysis was performed. As shown in Table 7 age, various markers of obesity such as BMI, total or truncal fat-mass, and adiponectin were independently associated with CIGMA %S. The models presented explained about 60 % of the variability of insulin resistance in PCOS. According to these models about 18 % of insulin resistance could be attributed to circulating plasma adiponectin.

A	Standardized Beta	Correlation	Standardized Beta * Correlation*100 (%)	p-value
Age	0.22	0.252	5.5	0.02
Adiponectin	0.321	0.586	18.8	0.003
BMI	-0.413	-0.61	25.2	<0.001
Testosterone	-0.183	-0.414	7.5	0.058
R^2 of complete model			57	

B	Standardized Beta	Correlation	Standardized Beta * Correlation*100 (%)	p-value
Age	0.273	0.301	8.2	0.011
Adiponectin	0.302	0.574	17.3	0.011
DEXA fat (total)	-0.462	-0.631	29.2	<0.001
Testosterone	-0.116	-0.416	4.8	0.294
R^2 of complete model			59.5	

C	Standardized Beta	Correlation	Standardized Beta * Correlation*100 (%)	p-value
Age	0.267	0.316	8.4	0.022
Adiponectin	0.314	0.562	17.7	0.014
DEXA fat (trunk)	-0.396	-0.589	23.3	<0.001
Testosterone	-0.148	-0.442	6.5	0.222
R^2 of complete model			56	

Table 7: Linear regression analysis in women with PCOS using lnCIGMA as the dependent variable and including BMI (A), Dexa-fat (total) for general obesity (B) or Dexa-fat (trunk) representing abdominal obesity (C).

Our results were in line with various reports in non-PCOS individuals demonstrating an independent and significant association between adiponectin and insulin resistance (76, 78, 80). Thus, although obesity is a driving force for circulating adiponectin concentrations, the relationship between adiponectin and insulin sensitivity does not simply reflect obesity-associated insulin resistance. This is of

special interest with respect to reports indicating that insulin resistance in PCOS women is not entirely explained by the degree of obesity (74) and that additional factors are involved. We proposed with our data that adiponectin might be such a factor dissecting obesity and insulin resistance in women with PCOS.

Metformin is currently in widespread clinical use for treatment of PCOS-related symptoms (81). Nine obese and insulin resistant PCOS women were treated with metformin. Given an alpha- and beta-error of 5%, we should have been able to detect at least a 23% difference with respect to adiponectin. After metformin treatment for 6 months the women lost weight and hyperandrogenemia had improved. Insulin sensitivity slightly increased, although changes in HOMA %S and CIGMA %S closely failed to be significant. In contrast, adiponectin values did not change during the treatment period (Figure 6).

Figure 6: Influence of metformin treatment on adiponectin plasma levels in nine women with PCOS.
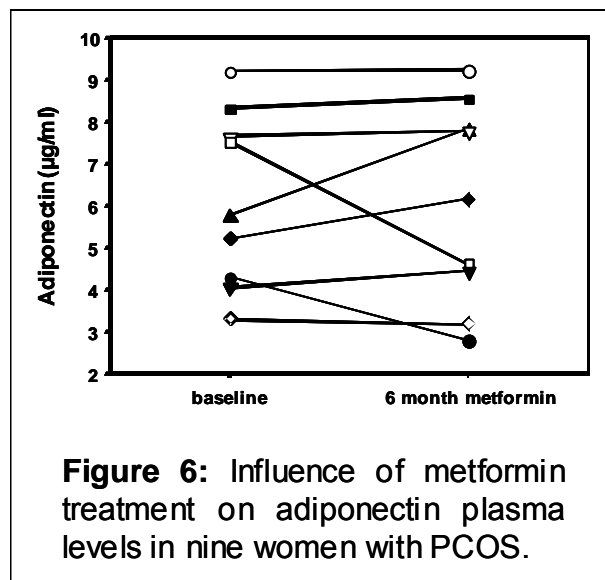
This is consistent with data in type 2 diabetics (82), showing that metformin treatment does not appear to exert additional or favourable effects on plasma adiponectin in PCOS. However, our results about metformin and adiponectin are based on a non-

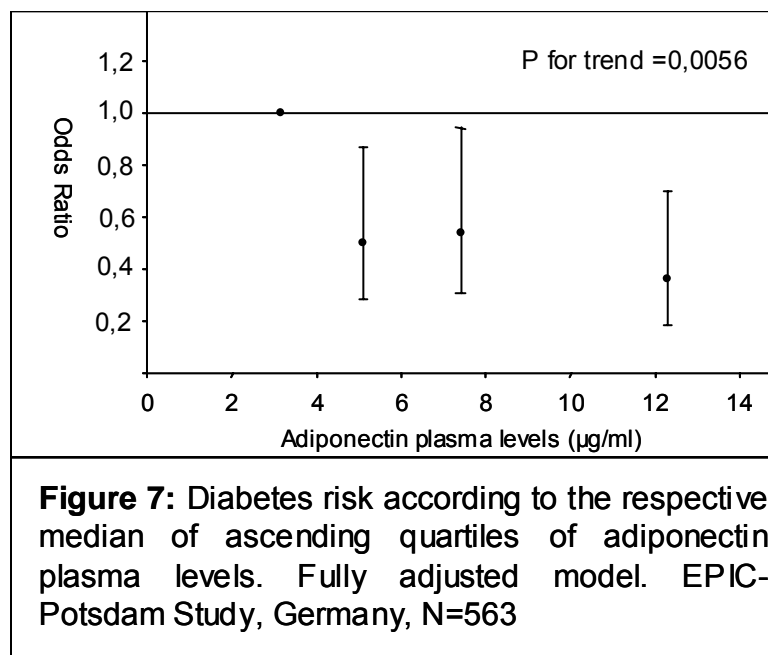controlled study with a small number of participants and needs to be confirmed in larger cohorts.

In summary, circulating adiponectin is independently associated with the degree of insulin resistance in PCOS (22). Our observations support the concept that low adiponectin may contribute to the development and/or maintenance of insulin resistance. Although our study did not directly address this question, our results were suggestive that adiponectin may provide a link to the risk of type 2 diabetes mellitus, which was further investigated in the study described in the next paragraph.

2.2.2 Adiponectin as a predictor of type 2 diabetes mellitus

As mentioned above, a huge amount of data suggested that adiponectin is an excellent candidate to affect diabetes risk. With respect to prospective data, a recent study demonstrated in an animal model of type 2 diabetes, that decreased levels of adiponectin precede the onset of disease (61). Vice versa, it was tempting to speculate that high adiponectin levels might be able to prevent onset of T2DM. We therefore made use of the above in more detail described prospective, nested case-control study based on the EPIC-Potsdam cohort and assessed whether baseline plasma levels of adiponectin independently modified the risk of type 2 diabetes in these individuals.

Indeed the mean adiponectin concentration was lower in individuals with incident T2DM compared to controls (p<0.0001) and increasing concentrations of adiponectin were associated with a lower risk of subsequent T2DM. With increasing quartiles of adiponectin plasma levels, the ORs (95% CI) of developing T2DM were 1.0 (reference), 0.6 (0.4-0.9) , 0.4 (0.3-0.7) and 0.2 (0.1-0.4) in the age and sex adjusted

analysis. Although the effects were slightly modulated in the fully adjusted model, persistent positive effects of adiponectin were observed after adjustment for sex, age, BMI, WHR, sports, smoking, education, alcohol consumption and HbA$_{1c}$ (Figure 7). The ORs (95% CI) for subsequent T2DM were 1.0 (reference), 0.5 (0.3-0.99), 0.5 (0.3-0.99) and 0.3 (0.2-0.7) for increasing quartiles of adiponectin plasma levels in the fully adjusted model.

Figure 7: Diabetes risk according to the respective median of ascending quartiles of adiponectin plasma levels. Fully adjusted model. EPIC-Potsdam Study, Germany, N=563

We additionally included adiponectin levels as a continuous variable instead of adiponectin quartiles in the respective statistical models. Again, we found a significant association between adiponectin plasma levels and risk of T2DM. OR for the continuous variable (in µg/ml) was 0.90 (95%CI 0.84-0.97; p=0.0068) in the fully adjusted model.

As individuals with undiagnosed prevalent T2DM at baseline might have biased our results we additionally performed analyses restricted to subjects with a HbA$_{1c}$ < 6.0% only. Results of this subgroup (HbA$_{1c}$ < 6%) were comparable to analyses in all

individuals. Odds ratios for crude, BMI/WHR-adjusted and a model with adjustment for all significant covariates including HbA$_{1c}$ are demonstrated in Table 8.

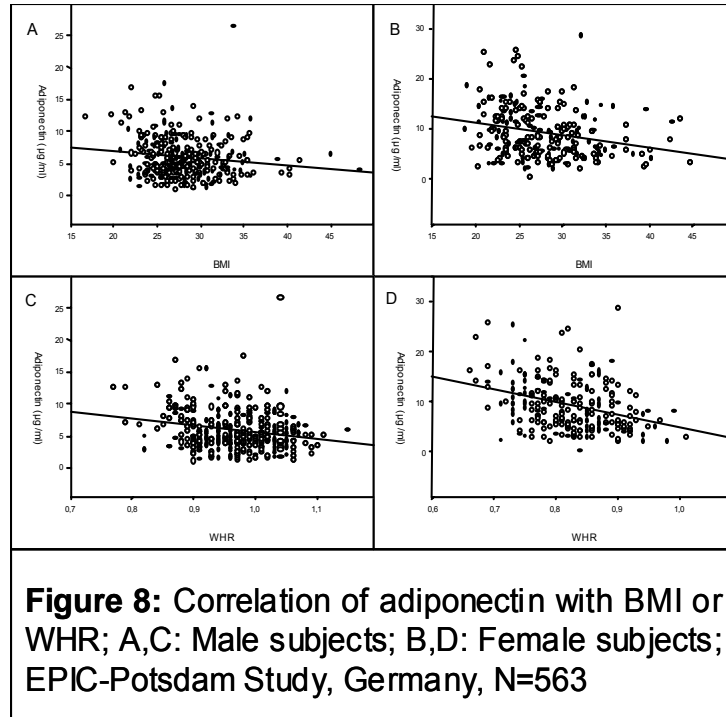|	Quartile			
Adiponectin	1	2	3	4
Median, interquartile range (µg/ml)	3.37 (0.21 – 4.49)	5.26 (4.51 – 6.4)	8.06 (6.43 – 9.74)	12.92 (9.74 – 28.72)
Age and sex adjusted analysis (OR, 95% CI)	1.00 (reference)	0.81 (0.45 – 1.48) (p=0.49)	0.54 (0.29 – 1.02) (p=0.056)	0.28 (0.13 – 0.58) (p=0.001)
+ BMI and WHR adjusted analysis (OR, 95% CI)	1.00 (reference)	0.7 (0.36 – 1.33) (p=0.27)	0.59 (0.3 – 1.17) (p=0.13)	0.42 (0.19 – 0.94) (p=0.035)
Adjusted for all risk factors** (OR, 95% CI)	1.00 (reference)	0.67 (0.34 – 1.35) (p=0.26)	0.56 (0.27 – 1.15) (p=0.12)	0.36 (0.16 – 0.86) (p=0.021)

Table 8: Analysis restricted to individuals with a baseline HbA$_{1c}$ below 6% (N=462). Odds ratios (OR) and 95% confidence intervals (95% CI) for the risk of Type 2 diabetes among apparently healthy men and women, according to baseline plasma concentrations of adiponectin. (**) Adjusted for age, sex, BMI, WHR, sporting activities smoking status, educational attainment, alcohol consumption and HbA$_{1c}$.

The risk estimate after inclusion of adiponectin as a continuous variable was also basically unchanged in analysis restricted to participants with a HbA$_{1c}$ below 6% at baseline compared to analysis including all individuals showing a risk reduction of 8.1% per µg/ml (p=0.021).

The plasma levels of adiponectin were inversely associated with anthropometric parameters of obesity (BMI: r=-0.196; p<0.0001; WHR: r=-0.455; p<0.0001) (Figure 8).

There was a moderate inverse correlation of adiponectin levels with chronic blood glucose control measured by HbA$_{1c}$ (r=-0.133; p=0.002), while we found no correlation with the inflammatory marker C-reactive Protein (r=-0.052; p=0.217). Sex stratification yielded a slightly attenuated picture. Women showed a higher inverse correlation between WHR (r=-0.341), BMI (r=-0.243), HbA$_{1c}$ (r=-152) and CRP (r=-
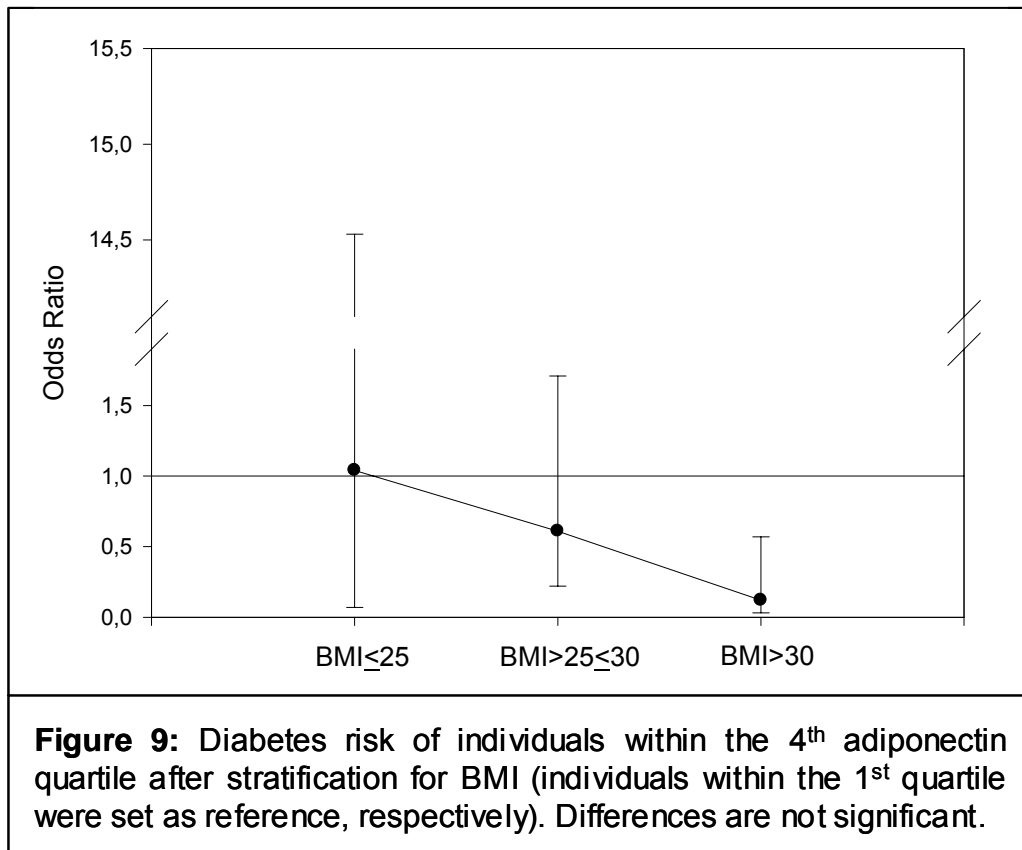
0.244) and adiponectin compared to men (r: -0.204; -0.134; -0.70 and −0.094, respectively). (Figure 8)

Figure 8: Correlation of adiponectin with BMI or WHR; A,C: Male subjects; B,D: Female subjects; EPIC-Potsdam Study, Germany, N=563

To asses whether the effects of high adiponectin plasma levels depend on the degree of obesity, we calculated OR after BMI stratification. Notably high levels of adiponectin resulted in a stronger risk reduction in participants with a BMI greater than 30 kg/m^2 (4th vs. 1st quartile; OR 0.1; 95%CI 0.02-0.57) compared to individuals with a BMI between 25 and 30 kg/m^2 (4th vs. 1st quartile; OR 0.6; 95%CI 0.2-1.7) and to individuals below 25 kg/m^2 (4th vs. 1st quartile; OR 1.04; 95%CI 0.1-14.5) in the fully adjusted model (Figure 9). However, these differences failed to be statistically significant and analyses with interaction terms were not indicative of a significant interaction between BMI and adiponectin plasma levels within our models.

Women were found to have a higher mean adiponectin concentrations than men (p<0.001). To investigate effect modification by sex, we determined ORs stratified by

sex stratification, which yielded slightly stronger risk estimates with increasing quartiles of adiponectin for women compared to men (data not shown).

Figure 9: Diabetes risk of individuals within the 4th adiponectin quartile after stratification for BMI (individuals within the 1st quartile were set as reference, respectively). Differences are not significant.

Our study was the first prospective analysis linking adiponectin plasma levels to the risk of type 2 diabetes in apparently healthy individuals. We found a risk reduction to develop type 2 diabetes of 9.9% per unit (µg/ml) of circulating adiponectin levels (range: 2 - 27 µg/ml). Previous cross-sectional studies found decreased levels of adiponectin in individuals with obesity or existing type 2 diabetes (55, 56). Because our cohort was based on medical reports of diabetes, undetected diabetes at study entry might have biased our results. To minimize this effect and to exclude a significant influence of chronic blood glucose control, we included HbA_{1c} into the fully adjusted model. Despite adjustment for different markers of obesity and control for

chronic blood glucose metabolism, the risk estimates in the fully adjusted model were comparable to those of the crude analysis. To further test the robustness of our results we performed separate analyses among individuals with a baseline HbA_{1c} below 6% only. Among participants who provided fasting blood samples 96.2% of those individuals with a HbA_{1c} below 6% had a fasting plasma glucose below 7mmol/l, demonstrating that the percentage of individuals with prevalent diabetes was very small within this subgroup. Although the statistical power was diminished by the reduced number of individuals within that analyses, the risk estimates for adiponectin plasma levels remained basically unchanged and significant. These data strongly suggest that low adiponectin levels are not only cross-sectionally related to T2DM but are indeed associated with future T2DM.

This concept has been confirmed by a considerable number of other studies (65, 83-86). Although the effects were not significant in our cohort, the more pronounced risk reduction of high adiponectin plasma levels in obese subjects is noteworthy. The data suggest that the inverse relation between adiponectin and the risk of T2DM may be stronger especially in obese individuals. Why a considerable number of obese individuals still have high adiponectin concentrations, despite the relatively strong inverse correlation of adiponectin with BMI and WHR, is unclear. It is tempting, especially against the background of a linkage in the region of the adiponectin gene with T2DM (63, 64), to speculate that genetic polymorphisms might be involved.

The phenomenon that women had higher adiponectin levels compared to men is explained at least in part by the lower WHR of women in our study cohort. In addition, women were found to have a stronger inverse correlation between markers of obesity such as WHR and adiponectin plasma levels compared to men, suggesting a potential influence of other yet unknown parameters (56). Some data suggest that oestrogen might influence the expression of adiponectin (P. Scherer, Albert Einstein

College of Medicine, New York, personal communication). In the control group we found slightly higher adiponectin levels in women with hormone replacement therapy compared to those without, while an opposite effect was identified in women with incident diabetes. However, both effects were small and not significant. Therefore, it is likely that other yet unknown factors participate to the sex dependent differences of adiponectin plasma concentrations. Recent data suggest that differences of total adiponectin levels are at least partially explained by differences of single adiponectin oligomers (87-89).

Adiponectin is thought to exhibit several characteristics that imply a fundamental role in development of type 2 diabetes. Beside the insulin-sensitising effects, adiponectin has been shown to exhibit anti-inflammatory properties (54). However, although adiponectin plasma levels were correlated with markers of obesity, as shown previously (76, 90), we found no correlation with circulating levels of the inflammatory marker C-reactive Protein (Table 9).

	Adiponectin	Interleukin-1ß	Interleukin-6	TNF-alpha
Interleukin-1ß	-0.033 p=0.43-	-		
Interleukin-6	**-0.109 p=0.01**	**0.089 p=0.036**	-	
TNF-alpha	-0.063 p=0.136	0.005 p=0.9	**0.177 p<0.001**	-
CRP	-0.049 p=0.244	0.066 p=0.12	**0.508 p<0.001**	**0.135 p=0.001**
Table 9: Correlation between adiponectin (µg/ml) and the inflammatory markers IL-1ß (pg/ml), IL-6 (pg/ml), TNF-alpha (pg/ml) and CRP (µg/ml); EPIC-Potsdam (n=563)				

In addition the risk estimates of adiponectin were basically unchanged after inclusion of CRP into the fully adjusted model (data not shown). Thus, in our study the effects of adiponectin appeared to be independent from the ongoing subclinical inflammation preceding the onset of type 2 diabetes (7, 10).

Most cross-sectional studies described moderate correlations between adiponectin and inflammatory markers, suggesting that expression of these factors might at least partially affect the other (91). Another prospective study in Pima Indians investigating the combined effect of adiponectin and inflammatory markers on diabetes risk has been published recently (92). In slight contrast to our results this study yielded moderate correlations between CRP and IL-6 with adiponectin, what we found in women only. However, comparable to our study, other inflammatory proteins were not correlated with adiponectin (sE-selectin, TNF-α, sICAM-1, sVCAM-1, vWF). In agreement with our study, there was no modification of the adiponecin effects by any of the inflammatory marker in this study (92).

Pathophysiologically the model of a cross-talk between inflammation and adiponectin is tempting and may still exist on a local basis. The initial report about putative anti-inflammatory properties of adiponectin demonstrated inhibition of the NfkappaB pathway in endothelial cells (54). However, this effect appears to be cell specific, as another study recently demonstrated that addition of adiponectin to C2C12 myocytes or myotubes led to activation of NFkappaB transcription factor (93). An in vitro study described that IL-6 suppresses adiponectin expression in 3T3-L1-adipocytes, although this effect was detectable in ng/ml concentrations, which is about 1000-fold higher than circulating levels of IL-6 (5). Nevertheless, local concentrations of IL-6 in adipose tissue may be much higher than those in the circulation, thus still leaving the possibility that these results are physiologically relevant. Engeli and co-authors found in a recent study an inverse correlation between IL6 and adiponectin gene
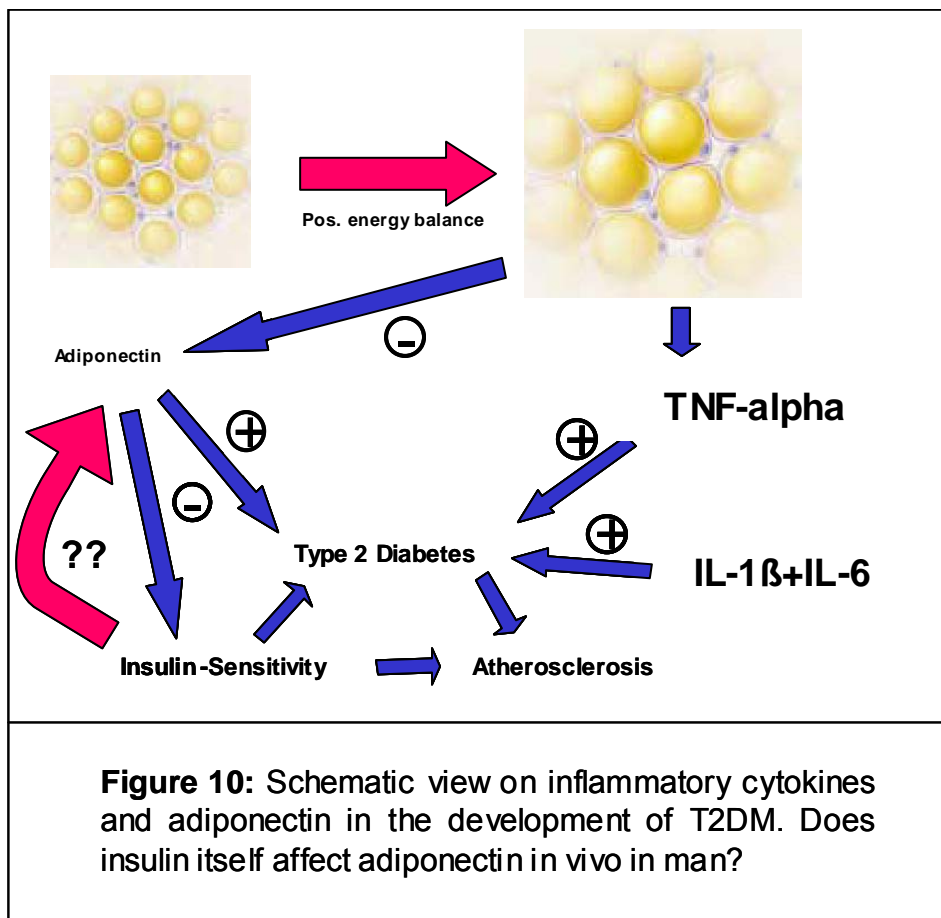
expression in subcutaneous adipose tissue samples of obese women (94). These data together with the in vitro data tentatively suggest that these factors might interact on a local basis in the adipose tissue rather than in the circulation. However, whether such local interplay does indeed exist, has to be investigated in future studies.

In summary, elevated levels of adiponectin were found to be a powerful, independent and protective determinant against incident type 2 diabetes mellitus (95). The effects appear to be independent of its anti-inflammatory properties and may be more pronounced in obese individuals. Our data imply, that adiponectin may be a valuable adjunctive method for discrimination between individuals already at higher risk to develop type 2 diabetes according to their anthropometric characteristics. This might finally help to establish successful and cost-effective prevention programs against T2DM. These observations, combined with emerging functional and genetic evidence, support the concept, that adiponectin has a central role in the development of type 2 diabetes.

2.2.3 Insulin modulates circulating Adiponectin

Initial results of adiponectin knock out mice suggested that loss of adiponectin might induce insulin resistance (57, 58). However, changes were relatively small and a very recent study did not find hyperinsulinemia in these animals at all (96). Interestingly, elegant in vitro studies provided strong evidence that insulin itself inhibits the expression of adiponectin (97). Thus, hyperinsulinemia may decrease adiponectin expression and thereby facilitate vascular disease. However, although there was a cross-sectional correlation between insulin and adiponectin plasma concentrations,

the exact regulatory mechanism of adiponectin expression in vivo was unclear. There was especially in vivo in man still uncertainty, whether adiponectin influences insulin levels or, vice versa, whether insulin influences adiponectin expression (Figure 10). We therefore investigated the influence of a isolated short-term elevation of insulin on circulating adiponectin levels.
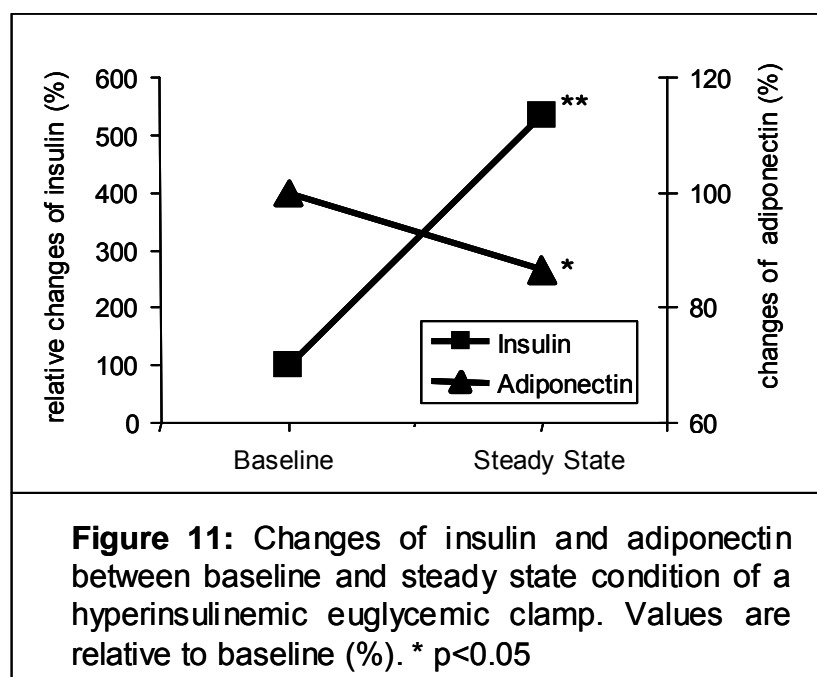

Figure 10: Schematic view on inflammatory cytokines and adiponectin in the development of T2DM. Does insulin itself affect adiponectin in vivo in man?

Following a 10 hour overnight fast, five male and healthy volunteers (age 34.8±8,6 yr, body mass index 24.7±3.7, waist hip ratio 0.88±0.06) were investigated. Hyperinsulinemic euglycemic clamp was performed for at least two hours using 40 mIU/m^2/min human insulin and a variable infusion of 10 % glucose. Capillary glucose concentration was maintained between 4.0 and 4.9 mmol/l. Blood samples were collected prior to the test and at least two hours after starting the test during steady
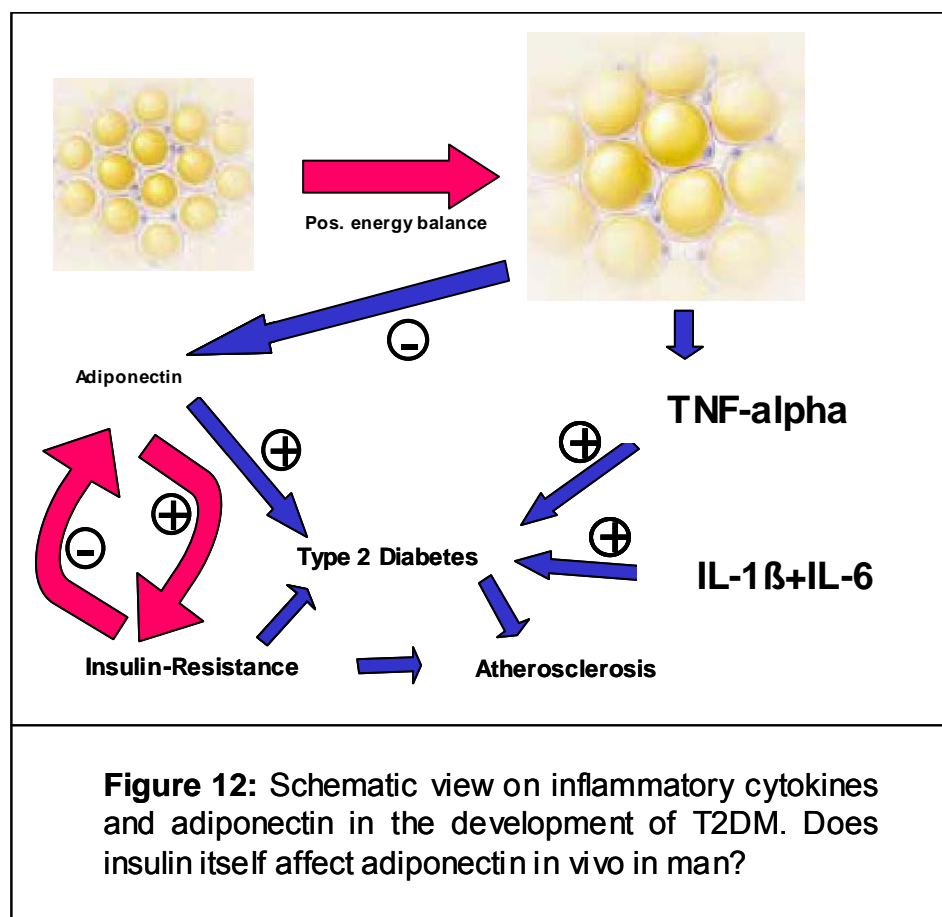
state condition. As expected, insulin significantly increased from 85.0±33.2 (at baseline) to 482.8±64.4pmol/l (under steady state conditions) (p<0.01). Indeed, this artificially induced hyperinsulinemia achieved during the steady state level of the hyperinsulinemic euglycemic clamp caused a more than 10% decrease of adiponectin (Figure 11). Circulating adiponectin plasma levels were 30.4±5µg/ml at baseline and 26.7±3.5µg/ml under steady state conditions (p<0.05).

We thus demonstrated that isolated elevation of insulin results in a reduction of circulating adiponectin plasma levels in vivo in man. These results of hyperinsulinemia induced reduction of adiponectin levels have been shown concurrently by another recently published trial (98).

Figure 11: Changes of insulin and adiponectin between baseline and steady state condition of a hyperinsulinemic euglycemic clamp. Values are relative to baseline (%). * p<0.05

Taking the above mentioned data into account we assume, that part of the negative effects of hyperinsulinemia on the risk of vascular disease seen in the metabolic syndrome and type 2 diabetes might be due to a decrease of adiponectin levels. The duration of hyperinsulinemia was short in our study and the insulin levels during

steady state were only slightly higher than that of insulin resistant individuals. Thus the observed effect is likely to be relevant under physiological conditions. Since in vitro data suggest a time course dependent decrease of adiponectin by insulin (20% reduction after a 2 hour stimulation and an about 60 % reduction after 8 hours) (97), one would expect in vivo an even more pronounced effect, if hyperinsulinemia is maintained for a longer period of time. Taking into account the dose dependent effects described by Paschke´s group (97), maintenance of higher insulin levels might result in even stronger effects.

Figure 12: Schematic view on inflammatory cytokines and adiponectin in the development of T2DM. Does insulin itself affect adiponectin in vivo in man?

In summary, we demonstrated that elevation of insulin caused a decrease of adiponectin plasma levels in man (99). Given the above mentioned data, that treatment with adiponectin improves insulin sensitivity at least in animal models, it is

tempting to speculate that there may exist a vicious circle between adiponectin and insulin resistance (Figure 12). Low adiponectin levels might thus contribute to insulin resistance to some extend, the resulting hyperinsulinemia further lowers adiponectin levels. However, this appears to be relevant only up to a certain threshold, as adiponectin KO-mice do not display an extensive degree of insulin resistance. Still this effect may contribute to the increased cardiovascular risk of insulin resistant individuals.

3.   Cytokines and diabetic retinopathy

3.1   Introduction

Recent estimates suggest about sixteen million individuals having diabetes mellitus, 50% of them being unaware of their disease (100). A considerable amount of patients present with vascular complications already at time of diagnosis (101). Diabetes mellitus is associated with various functional and morphological vascular alterations, some of them leading to severe complications of the eye, kidney, nerves and the heart.

The most frequent vascular complication of type 1 diabetes is diabetic retinopathy. Together with macula degeneration, it is the major cause of blindness in industrial nations (102).

Loss of pericytes is an early event in diabetic retinopathy and appears to be related to the angiopoetin system (103, 104). Clinically microaneurysms are among the early signs of diabetic retinopathy. Microaneurysms for themselves appear to have no apparent clinical impact, except the fact that they are predictive markers of the