Letter

Tumour necrosis factor- α (TNF- α) polymorphisms –857C/A and –863C/A are associated with TNF- α secretion from human adipose tissue

To the Editor: Tumour necrosis factor- α (TNF- α) plays a part in insulin resistance and Type II (non-insulin-dependent) diabetes mellitus. TNF- α is produced primarily by monocytes/macrophages, although other cell types also secrete the protein. It has been shown that the expression of the *TNF- α* gene is more frequent in adipocytes of obese subjects compared with lean subjects and that this is accompanied by an increased secretion of the cytokine from adipose tissue.

There is evidence that *TNF- α* expression is genetically determined, as polymorphic sites linked to the *TNF- α* locus are associated with differences in cellular TNF- α secretion. Five common polymorphisms, –238G/A, –308G/A, –857C/A, –863C/A and –1031T/C have been described in the proximal promoter of the *TNF- α* gene. Three of these are indicated to affect the expression of the gene in vitro, the –863C/A [1], the –308G/A [2] and the –238G/A [3]. The –863A allele is also associated with reduced serum TNF- α concentrations in healthy middle-aged men whereas the –238G/A (Skoog et al., unpublished results) and –308G/A polymorphisms are not [1].

The –308A allele has been reported to be linked to raised body fat [4] and to be more frequent in obese than in lean men [5]. These observations also pertain to obese women [6]. On the other hand, it has recently been shown that the –308A allele is not associated with an increased body mass index (BMI) in an obese cohort [7]. The distribution of –238A [5,7] and –857A [5] alleles did not differ between obese and lean individuals. In contrast, the –863C/A polymorphism has hitherto not been studied in relation to obesity and it is not known whether this polymorphism influences the expression and secretion of TNF- α in adipose tissue. In this study, we investigated whether TNF- α secretion from human subcutaneous adipose tissue is related to the –238G/A, –308G/A, –857C/A, –863C/A and –1031T/C polymorphisms.

Study group I consisted of 24 non-obese (BMI < 30) healthy control subjects and study group II of 59 obese (BMI > 30; according to the classification of the World Health Organisation) subjects, who were referred to hospital for uncomplicated

obesity. General characteristics of the study groups are shown in the Table. All subjects were Caucasians of Scandinavian ancestry. The obese subjects had no other known disorders and were not on any regular medication. None had engaged in weight-reducing efforts during the preceding six-month period. The hospital ethics committee approved the study. All subjects gave informed consent to participate in the study.

Measurements of height, weight, waist to hip circumference ratio (WHR) and blood pressure, analyses of plasma insulin, glucose, cholesterol, triglycerides and HDL cholesterol and calculation of body fat content were done as described [6]. The TNF- α secretion from adipose tissue was investigated as described in detail previously [8]. Subcutaneous adipose tissue biopsies were obtained and fat cell size and number were measured as described [8]. Genotyping for the –238G/A, –308G/A, –857C/A, –863C/A and –1031T/C polymorphisms were done as described [1].

We hypothesised that the rare –863A allele would be related to lower TNF- α secretion from adipose tissue compared with the wild-type –863C allele, because the –863A allele is associated with lower transcriptional activity of the *TNF- α* gene in vitro and with reduced serum TNF- α levels [1]. As obesity is a multifactorial condition, we first examined a group of non-obese, non-related Swedish subjects (18 women and 6 men) and related genotypes to TNF- α secretion from adipose tissue. With this strategy we wanted to minimise obesity-related and confounding factors which may induce the expression of the *TNF- α* gene. The allele frequency was 0.77/0.23 for the –863C/A polymorphism, and the distribution of genotypes was in Hardy-Weinberg equilibrium. The secretion rate of TNF- α was about 3 times greater in non-obese subjects carrying the –863C allele ($p < 0.05$) (Table). A similar difference was observed when TNF- α secretion was expressed in relation to lipid weight of adipose tissue (1.2 ± 0.3 vs 0.4 ± 0.1 ng/g lipids) ($p < 0.05$). The influence of the polymorphism on TNF- α secretion remained statistically significant when values were corrected for age, gender and BMI. Thus, adipose tissue from non-obese subjects carrying the –863A allele secretes significantly less TNF- α than adipose tissue from non-obese subjects carrying the –863C allele whether expressed per cell or weight of tissue. These findings support the notion that the C to A substitution at position –863 represents a functional polymorphism, which leads to decreased *TNF- α* gene expression and thereby less production and secretion of the cytokine. Furthermore, the –857C/A polymorphism, which had an allele frequency of 0.95/0.05, affected the secretion of TNF- α in non-obese individuals. Carriers of the A-allele secreted about 3 times more TNF- α than carriers of the C-allele (Table). The same was true when TNF- α secretion was expressed in relation to lipid weight of adipose tissue (2.1 ± 3.0 vs 0.7 ± 0.1 ng/g lipids) ($p = 0.01$). It should be stressed, however, that this result is based on only 3 individuals carrying

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Table 1. General characteristics of the study groups and TNF- α secretion according to *TNF- α* genotype

	Group I: non-obese	Group II: obese	<i>p</i> value
<i>n</i>	24	59	
Age (years)	43.5 $\pm$ 2.1	41.9 $\pm$ 1.5	0.55
Male/Female	6/18	11/48	0.52
BMI (kg/m ²)	24.6 $\pm$ 0.5	40.0 $\pm$ 0.7	< 0.0001
pl-Insulin (pmol/l)	6.8 $\pm$ 0.7 (<i>n</i> = 17)	17.3 $\pm$ 1.7 (<i>n</i> = 39)	< 0.001
pl-Glucose (mmol/l)	4.8 $\pm$ 0.1 (<i>n</i> = 22)	5.5 $\pm$ 0.1 (<i>n</i> = 57)	< 0.001
pl-Cholesterol (mmol)	5.3 $\pm$ 0.2 (<i>n</i> = 22)	5.7 $\pm$ 0.1 (<i>n</i> = 58)	0.19
pl-HDL-cholesterol (mmol/l)	1.5 $\pm$ 0.1 (<i>n</i> = 22)	1.2 $\pm$ 0.04 (<i>n</i> = 50)	< 0.001
pl-Triglyceride (mmol/l)	1.1 $\pm$ 0.1 (<i>n</i> = 22)	1.7 $\pm$ 0.1 (<i>n</i> = 58)	< 0.01
Fat cell volume (pl)	578 $\pm$ 33	860 $\pm$ 19	< 0.0001
TNF- α secretion (ng/10 ⁷ cells/2 h)			
-238 G/G	5.7 $\pm$ 1.4 (21)	11.4 $\pm$ 1.6 (51)	
G/A and A/A	5.4 $\pm$ 3.7 (2)	13.7 $\pm$ 5.6 (7)	
-308 G/G	6.1 $\pm$ 1.6 (19)	10.3 $\pm$ 1.6 (42)	
G/A and A/A	3.2 $\pm$ 1.4 (5)	14.5 $\pm$ 3.3 (17)	
-857 C/C	4.3 $\pm$ 0.9 (21)	11.1 $\pm$ 1.5 (54)	
C/A and A/A	13.9 $\pm$ 7.3 (3)**	15.8 $\pm$ 5.7 (5)	
-863 C/C	7.4 $\pm$ 1.8 (15)	10.9 $\pm$ 1.9 (36)	
C/A and A/A	2.3 $\pm$ 1.1 (9)*	12.6 $\pm$ 2.5 (23)	
-1031 T/T	7.3 $\pm$ 1.9 (14)	10.4 $\pm$ 2.0 (28)	
T/C and C/C	2.9 $\pm$ 1.2 (10)	12.5 $\pm$ 2.2 (31)	

pl, fasting plasma. * *p* < 0.05 when compared with non-obese C/C; ** *p* = 0.01 when compared with non-obese C/C. Values are means $\pm$ SEM. Statistical significance was determined using a Student's unpaired *t* test

the A-allele. In contrast, the limited power of our study notwithstanding, neither of the other three polymorphisms, -238G/A, -308G/A or -1031T/C, significantly affected the secretion of TNF- α .

We then assessed whether the same effect of the -863A and -857A alleles on the secretion of TNF- α was present in adipose tissue from obese individuals. As shown in the Table, the secretion of TNF- α did not differ between carriers and non-carriers of the A-allele of either polymorphism. The reason why the polymorphisms do not modulate TNF- α secretion from adipose tissue in obese subjects is not clear. Transcription factors binding outside of the polymorphic sites in the *TNF- α* promoter could be the main regulators causing the enhanced promoter activity associated with obesity. Furthermore, obesity is a multifactorial condition caused by multiple genes, a range of environmental influences and gene-environment interactions. Obesity, in itself, might have a much stronger influence on TNF- α secretion than the investigated polymorphisms. There were no differences in BMI, WHR, age or gender distribution according to *TNF- α* genotype in either of the study groups.

In conclusion, genetic variability in the *TNF- α* gene promoter is linked to altered TNF- α secretion from human adipose tissue.

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