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# Relationship of Tumor Necrosis Factor Alpha Genotypes With Various Biochemical Parameters of Normal, Over-weight and Obese Human Subjects

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**Abstract.-** Tumor Necrosis Factor (TNF-a1pha) is expressed primarily in adipocytes and elevated levels of this cytokine have been associated with obesity. The purpose of this investigation was to test whether the TNF-a1pha -308 polymorphism were associated with insulin resistance or obesity related traits in non-diabetic and diabetic patients visiting Sheikh Zayed Hospital, Lahore, Fatima Hospital and Irfan Clinic in Sargodha. In non diabetic subjects the AA allele carriers, compared with homozygous G allele carriers had significantly lower (28%) triglyceride values and 15% higher HDL ya1ues, whereas other parameters tested 8lid not show any significant variation. In diabetic patients the AA allele carriers, compared with GG allele carriers, besides having 31% higher FBS and 26% higher creatinine, had 20% higher cholesterol and 34% higher trig1ycerides. The HDL values were 14% less, compared to GG allele carriers. In normal subjects (BMI 22.85±0.25 kg/m$^2$), the AA allele carriers showed 132%, 125%, 65% and 112% higher triglycerides, cholesterol and LDL values compared with GG allele carriers. The HDL and creatinine did not show any significant change. In the overweight subjects (BMI: 27.17±0.17 kg/m$^2$) all these values were lower than in AA allele carriers compared with GG allele carriers. The AA allele carries had FBS, triglycerides, cholesterol and LDL 28%, 48%, 14% and 14% lower than in the GG allele' carriers, respectively. In obese subjects, (BMI: 36.73±0.78 kg/m$^2$), however, the FBS, triglycerides, cholesterol and creatinine values were 5%, 8%, 7% and 14% higher in AA allele carries compared to GG allele carriers, respectively. The LDL content was 8% lower in AA allele carrier as compared with the respective GG allele carriers, It is concluded that replacement of G at -308 with A leads to reduced risk for cardiovascular disease in non-diabetic subject, whereas in diabetic patients this mutation-increases the risk of CVD. Using BMI as index of obesity, it was observed that obese subject with AA alleles had greater risk of CVD, as compared with those with GG alleles. Comparatively, however, this risk was much higher in AA allele carriers with BMI 22.85±0.25 kg/m$^2$ (normal subjects) than with their respectively GG carriers or those of obese subjects.

**Key words:** Obesity, body mass index, blood lipid profiles, TNFα.

## INTRODUCTION

The experimental animal models show that environmental factors may cause visceral obesity and its associated abnormalities in glucose, insulin and lipid metabolism, including hypertension, bringing the importance of non-genetic factors in the control of human health to the forefront (Rosmond, 2004). Elevated circulating cortisol has been associated with visceral obesity, atherosclerosis, raised cholesterol levels and increased incidence of type 2 diabetes mellitus (Rosmond, 2001; Mathews, 2002).

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Feinleib *et al.* (1977) indicated that familial aggregation for obesity was due to genetic factors rather than environment. Subsequently, in 1986, Stunkard used 1,974 monozygotic and 2,097 dizygotic twin pairs, and estimated a heritability value for weight of 0.78, which increased to 0.81 on completion of a 25 year follow up (Stunkard *et al.*, 1986). These values were similar to the heritability value of 0.80 for height that was estimated in the same study. In 1989, Hasstedt and colleagues suggested a recessive mode of inheritance for a phenotypic measure that is derived by calculating the ratio of subscapular skinfold thickness to the sum of subscapular and suprailiac skinfold thickness (Hasstedt *et al.*, 1989). Selby *et al.* (1988) determined heritability of 0.43 was after correction

for overall obesity as a measure of central body fat in the NHLBI Cohort. Several studies in different labs. Have shown that inter-individual variation in BMI was due to genetic effects (Pietialainen *et al.*, 1999; Koeppen-Schomerus *et al.*, 2001; Katzmarzyk *et al.*, 2000). Bouchard *et al.* (1990) overfed pairs of twins and found that, within twin pairs, weight-gain correlation was high (>70%), despite the fact that some twin pairs gained as little as 4.3kg and others as much as 13.3 kg. With the increasing scale of the obesity problem and the body of evidence outlined above for a strong genetic contribution to development of the disease, many groups have started to study the genetics of polygenic obesity to better understand the pathogenesis of the disease and highlight possible pharmacological targets.

The obesity gene map shows putative loci on all chromosomes except Y. More than 300 genes, markers and chromosomal regions have been associated or linked with human obesity phenotypes (Chagnon *et al.*, 2003). The presence, absence, or mutation of one or a combination of these genes may make the individuals more susceptible to obesity. Association and linkage studies have found some of the following genes to be associated with or linked to body fat or BMI: UCP, TNF-alpha, LPL, and DRD2 (Bouchard *et al.*, 1998). It has been suggested that most widely associated gene with common obesity is melanoacortin-4 receptor genes (Eckel, 2003).

The obesity associated genes can be divided into two broad categories (i) rare gene variants that have a strong influence; and (ii) common gene variants that have a weaker influence on obesity phenotypes. A few genes have variants that are powerful enough to cause obesity all by themselves. For instance, rare monogenic causes of obesity resulting from mutations in the leptin-hypothalamic feedback loop have been identified, including defects in leptin, the leptin receptors and the pro-opiomelanocortin (Chen and Garg, 1999).

The gene variants known to cause most cases of obesity are still not known. Recent studies have provided evidence for a role of Tumor necrosis factor-alpha (TNF-alpha) in obesity and insulin resistance (Hotamisligil *et al.*, 1993, 1994), indicating that changes in TNF-alpha metabolism may affect the onset of type 2 diabetes and play a role in the development of cardiovascular disorders (Pociot *et al.*, 1993).

TNA-alpha is a cytokine with a wide range of activities (Vassalli *et al.*, 1992; Beutler, 1992). It is produced primarily by monocytes/macrophages (Beutler, 1989), although significant amounts are also secreted by several other cell types including adipocytes (Hotamisligil *et al.*, 1995). TNF-alpha is synthesized as a 26 KDa membrane-bound protein (Pro TNF) that is cleaved by TNF converting enzymes to release the soluble 17 KDa TNF-alpha molecule (Black *et al.*, 1997; Moss *et al.*, 1997; Rosendahl *et al.*, 1997). The mature TNF-alpha protein can subsequently bind to one of its receptors, TNF receptor-1 (TNF-R1) or TNF receptor-2 (TNF-R2), which are expressed in most nucleated cells (Beutler and Van Huffel, 1994). Upon interaction of TNF-alpha with these receptors, a variety of responses are elicited that affect the regulation of a large number of genes (Beutler, 1992). The increased production of TNF-alpha is observed in obese rodents and has to be implicated a causative factor in obesity related insulin resistance and the pathogenesis of type 2 diabetes (Hotamisfigil *et al.*, 1995).

Several single-nucleotide polymorphisms have been identified in the human TNF-alpha gene promoter. The polymorphism at position -308 (TNF -308 G>A), which involves substitution of G for A, lead to a higher rate of TNF-alpha gene transcription than the wild-type genotype GG in *in-vitro* expression studies (Bayley *et al.*, 2001).

A number of groups have set out to determine whether the -308 polymorphism could affect transcription factor binding and hence influence TNF transcription and expression levels. Some studies have failed to show any functional difference between two allelic forms (Abraham and Kreoger, 1999). For instance, a study was conducted to test whether the TNF-alpha -308 polymorphism and TNF-alpha -238 polymorphism were associated with insulin resistance or obesity related traits in 424 subjects self referred to the Johns Hopkins Weight Management Center (JHWMC). They found no differences in allele frequencies of either polymorphism by obesity category in the JHWMC and a lean control group (Walston *et al.*, 1999).

Obesity is increasing in prevalence among

Punjabis and is associated with several adverse health problems, including type 2 diabetes, hyperlipidemia, and hypertension. The influence of obesity on the development of type 2 diabetes is complex and is likely due to an interaction of genetic, nutritional, and metabolic factors (Brownell and Wadden, 1992). Much attention has been focused on the identification of molecular pathways that contribute to the development of obesity and type 2 diabetes (Leibel *et al.,* 1995; Walston *et al.,* 1995; Silver *et al.,* 1997; Fernandez-Real *et al.,* 1997).

The aim of this study was to investigate the relationship between A/G variation at position 308 in the TNF-alpha promoter and the body mass index, lipid profile and fasting blood sugar concentration in Punjabis. One hundred sixty six subjects, 49 men and 117 women, were genotyped by Amplification Refractory Mutational System Polymerase Chain Reaction (ARMS-PCR). GG, GA, and AA allele carriers were compared with respect to body mass index, as well as fasting glucose, total cholesterol, triglycerides, high density lipoprotein, low density lipoprotein and creatinine concentrations.

## MATERIALS AND METHODS

*Subjects*

Patients, male and female, of all age groups, visiting Diabetes Clinic in Shaikh Zayed Hospital, Lahore and Fatima Hospital and Irfan Clinic in Sargodha were included in the study.

The patients/subjects who were over weight (BMI 25.0-29.9 kg/m$^2$) or obese ($\geq$30 kg/m$^2$) and had history of diabetes, and hypertension, and, had fasted for 8 to 12 hours were included in this study.

The patients/subjects, who were under medication, had with diabetic history and were hypertensive for more than 5 years, and were pregnant females, were not included in this study.

A total of 18 control (BMI 18.5-24.9kg/m$^2$), 60 overweight (BMI 25.0-29.9kg/m$^2$), and 88 obese patients comprising 49 males and 117 females were included. The control subjects with BMI between 20-24.9kg/m$^2$, ranged between 20-60 years, had no history of diabetes and were under no medication. The over weight subjects with BMI between 24-29 kg/m$^2$, ranged between 20-60 years, had diabetes for not more than 5 years, and were not under medication. The obese patients 20-60 years of ages with BMI $\geq$30 and diabetes for not more than 5 years, were not under medication.

*Blood samples*

About 5ml blood was drawn from above subjects with the help of 5ml syringe and this blood was added in a tube containing EDTA for separation of buffy coat. The tube was centrifuged at 4,000 rpm (806xg) for five minutes. The buffy coat was separated with great care from red cells, and was used for extraction of DNA.

*Isolation of genomic DNA*

DNA was extracted from 5 ml sample of blood by using Promega DNA Extraction Kit (Wizard DNA purification kit, Cat. # A 1125).

*Amplification refractory mutational system – Polymerase chain reaction*

ARMS – PCR was used to amplify and detect alleles for TNF-alpha (Perrey *et al.,* 1999). DNA was amplified in a 10 µl reaction. Final concentration of reagents was as follows:

| **Reagents** | **Final conc.** | **Amount add to 10µl** |
|---|---|---|
| 10 x PCR buffer | 1x | 1 µl |
| 25 mM $MgCl_2$ | 2 mM | 0.8 |
| 10 mM dNTPS | 0.2 mM | 0.5 µl |
| TNF (anti-sense) 100 pmol/µl | 10 pmol | 1 µ |
| TNF (anti-sense)/TNF (sense) | 20 pmol | 2 µl |
| Distilled water | | 2.5 µl |
| Taq polymerase (5 U/µl) | 1 U | 0.2 µl |
| DNA sample | | 2 µl |

Following primer sequences were used:

i) TNF-alpha 308 primer generic (antisense)
5'- TCT CGG TIT CTT CTC CAT CG-3'

ii) TNF-alpha 308 Primer G (Sense)
5'-ATA GGT TTT GAG GGG CAT GG-3'

iii) TNF-alpha 308 Primer A (Sense)

5'-AAT AGG TIT TGA GGG GCA TGA-3'
PCR Product size = 184 bp

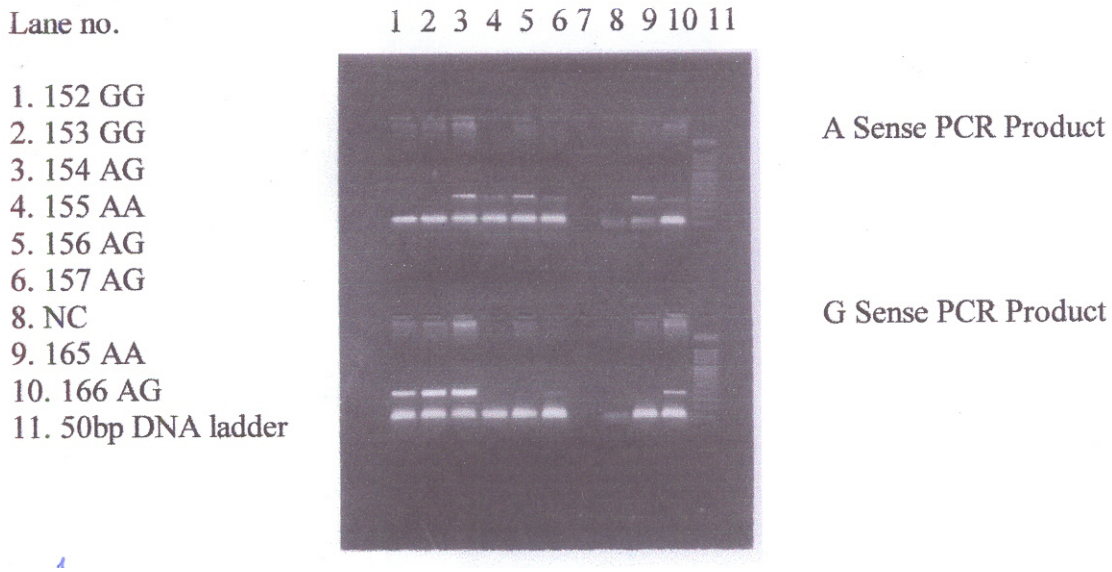


Fig. 1. Gel electrophoretic pattern of the ARMS-PCR product. In ARMS-PCR two different sense primers (A sense and G sense) are used which differ at their 3' terminal residues and are specific to either the wild type DNA sequence or the mutated sequence at given base. The A sense and G sense PCR products were run in different wells. If the sample is homozygous mutant or homozygous wild type amplification will only occur in one well and can be' observed on gel as one band and if the sample is heterozygous, amplification can be seen in both wells as two bands on the gel. Figure 1 shows 11 lanes in two gels (top and bottom). The top gel has A sense PCR product, whereas bottom gel has G sense PCR product. On the basis of these bands the genotype of -308 region of a gene can be interpreted. The lanes 1 and 2 show bands in G-sense gel which only contain G-sense primer. Its genotype is therefore GG. In contrast lanes 4 and 9 show bands in A-sense gel with A sense primer. Its genotype is hence AA. Lanes 3, 5, 6 and 10 show bands both in A sense and G-sense primers containing sample wells have genotype AG. Lane 11 shows bands of ladder of 184 bp.

PCR was performed on master thermal cycler (Eppendorf) using the following protocol: Hold 94°C for 5 minutes (3 step PCR), 94°C for 30 seconds, 55.3°C for 30 seconds, 72°C for 30 seconds, hold 72°C for 10 minutes, hold 4°C forever. At least 35 cycles were required to achieve acceptable levels of amplification.

The product was monitored by electrophoresis on a 2% agarose containing 10 mg/ml ethidium bromide against a 184bp ladder.

*Analysis of genotype*

Hardy Weinberg analysis was done for genotyping results of ARMS-PCR (Strickberger, 2001).

Figure 1 shows gel electrophoretic pattern of the ARMS-PCR product. The basis of interpretation of genotypes has been described in the legend of Figure 1.

*Statistical analysis*

Two factorial design ANOVA was used to compare non-diabetic and diabetic patients with their respective age groups (Sokal and Rohlf, 1984).

## RESULTS

*Obesity related blood biochemical parameters*

Blood samples of diabetic and non-diabetic patients were divided into three allelic groups, *viz.*, GG allele carriers, GA allele carriers, and AA allele carriers (Fig. 1). The different biochemical parameters used in this study were then compared and the differences statistically analyzed. The Table I shows comparison of BMI, FBS, triglycerides, cholesterol, HDL, LDL, and creatinine in diabetic and non-diabetic patients of different allelic groups.

All these parameters have been discussed individually below.

**Table I.- Effect of diabetes on obesity related blood biochemical parameters in the three different genotypes.**

| Parameters | Obese group | GG[1] | GA | AA |
|---|---|---|---|---|
| BMI (kg/m$^2$) | Non-diabetic | 30.7±1.5[2] (n=17) | 32.9±1.1 (n=73) | 101.5±6.98 (n=17) |
| | Diabetic | 30.4±0.99 (n=17) | 31±.1.2 (n=37) | 31.3±3.7 (n=6) |
| | Mean | 30.55±0.08 (n=34) | 31.95±0.81 (n=110) | 32.7±1.5 (n=23) |
| FBS (mg/100 ml) | Non-diabetic | 101.5±6.98* (n=17) | 99.97±3.8* (n=73) | 96±8.5[a*] (n=17) |
| | Diabetic | 156.6±16.4 (n=10) | 176.9±14.3 (n=30) | 205.8±45.4 (n=6 |
| | Mean | 129.05±8.97 (n=27) | 138.43±6.05 (n=103) | 150.9±17.7 (n=23) |
| Triglycerides (mg/100 ml) | Non-diabetic | 182±27.09 (n=17) | 159±9.7 (n=73) | 131±12.4 (n=13) |
| | Diabetic | 179±20.3 (n=17) | 185±14.4 (n=37) | 241±70.3[*a] (n=6) |
| | Mean | 180±16.6 (n=34) | 172±8.07 (n=110) | 186±25.46 (n=19) |
| Cholesterol (mg/100 ml) | Non-diabetic | 161.7±8.29 (n=17) | 166.5±5.7 (n=73) | 156.8±11.75 (n=13) |
| | Diabetic | 158±15.38 (n=17) | 169.2±7.1 (n=37) | 190.2±17.3[*a] (n=6) |
| | Mean | 159.85±10.09 (n=34) | 167.85±8.0 (n=110) | 173.5±10.09 (n=19) |
| HLD (mg/100 ml) | Non-diabetic | 33±2.25 (n=17) | 33.56±1.15 (n=73) | 38.15±2.2 (n=13) |
| | Diabetic | 39±5.6 (n=17) | 32.27±2.5 (n=37) | 33.33±4.5 (n=6) |
| | Mean | 36±3.08 (n=34) | 33.915±1.13 (n=110) | 35.74±2.05 (n=19) |
| LDL (mg/100 ml) | Non-diabetic | 94.35±7.2 (n=17) | 101.6±4.8 (n=73) | 95.38±10.5 (n=13 |
| | Diabetic | 102.6±10.9 (n=17) | 105.4±5.7 (n=37) | 104.7±20.3 (n=6) |
| | Mean | 98.475±6.5 (n=34) | 103.5±3.68 (n=110) | 100.04±9.3 (n=19) |
| Creatinine (mg/100 ml) | Non-diabetic | 0.96±0.04 (n=7) | 0.96±0.02 (n=73) | 1.06±0.0 (n=13) |
| | Diabetic | 0.78±0.07* (n=17) | 0.89±0.04* (n=37) | 0.97±0.4 (n=6) |
| | Mean | 0.87±0.05 (n=34) | 0.9±0.02 (n=110) | 0.9±0. (n=19) |

[1]Subjects homozygous for G and A allele were grouped as GG and AA, whereas the subjects heterozygous were grouped as GA.
[2]Mean±SEM, student's `t' test used to compare non-diabetic subjects with diabetic patients; *P<0.05;
GG, GA, and AA allele carriers compared within each row. GG allele carriers had been compared with GA< and AA allele carriers [a,b]P<0.05

**Normal values:** Total cholesterol: 200 mg/dl; Triglycerides: 150 mg/dl; HDL-High density lipid: Men, 41.0-58.7 mg/dl, Women, 48.5-75.0 mg/dl; LDL-cholesterol: >150 ml/dl; Creatinine: Men, 0.6-1.1 mg/dl; Women, 0.5-0.9 mg/dl; Glucose: Plasma/Serum (Fasting) 95-105 mg/dl.

*Body mass index (BMI)*

The average BMI of GG allele carriers was 30.55±0.88 kg/m$^2$, 31.95±0.81 kg/m$^2$ in GG and GA allele carriers and 32.7±1.6 kg/m$^2$ in AA allele carriers. The diabetic patients have their BMI varying between 30.4±0.99 to 31.3±3.7 kg/m$^2$ and non-diabetic patients have their BMI varying between 30.7±1.5 to 34.1±1.6 kg/m$^2$, irrespective of the allelic polymorphism. In diabetic patients the BMI of GG allele carriers was 30.4±0.99 kg/m$^2$, whereas in GA allele carriers and AA allele carriers it was respectively 2% and 3%, more than that of GG allele carriers. In the case of non-diabetic subjects the BMI was 7% and 11% more in the GA and AA allele carriers, when compared with its respective non-diabetic GG allele carriers.

The BMI of diabetic patients was 1%, 6%, and 9% lower in GG, GA, and AA allele carriers; when compared with their respective non diabetic GG, GA and AA allele carriers.

*Fasting blood sugar (FBS)*

The average FBS of GG, GA, and AA allele carriers was 129.05±8.97, 138.435±6.05 and 151±17.7 mg/dl. The diabetic patients had their FBS concentration varying between 156.6±16.4 to

205.8±45.4 mg/dl, and the non-diabetic patients had their FBS concentrations varying between 96±8.5 mg/dl to 101.5±6.98 mg/dl, irrespective of the allelic polymorphism.

In diabetic patients the FBS concentration of the GG allele carriers was 156.6±16.4 mg/dl, whereas the GA and AA allelic. carriers had FBS 13% and 31% higher, respectively when compared with the GG allele carriers. In non-diabetic patients the FBS concentration of the GG allele carriers was 101.5±6.98 mg/dl, whereas the GA allele carriers and AA allele carriers had 2% and 5% lower values, respectively, when compared with their respective non-diabetic GG allele carriers.

The FBS in diabetic patients was 35%, 43% and 53% higher in GG, GA and AA allele carriers, respectively; when compared with their respective non-diabetic groups.

*Triglycerides*

The average triglycerides concentrations in GG, GA, and AA allele carriers were 180±16.6, 172±8.07, and 186±25.46 mg/dl, respectively. The diabetic patients had their triglyceride concentrations varying between 179±20.3 to 241±70.3 mg/dl and the non-diabetic patients had their triglyceride concentrations varying between 131±12.4 to 182±27.09 mg/dl, irrespective of the allelic polymorphism.

In the diabetic patients the triglycerides concentration of GG allele carries was 179±20.3 mg/dl, but it was 3% and 35% more, respectively, in GA and AA allele carries, when compared with the GG allele carries. In the case of non-diabetic patients the triglyceride concentration of GG allele carries was 182 mg/dl, whereas it was 13% and 28% lower in GA and AA allele carries, respectively, when compared with their respective non-diabetic GG allele carries. In the non diabetic groups the triglyceride concentration of GA and AA allele carriers showed 14% and 46% lower values, respectively, whereas the GG allele carries had 2% more; when compared with their counterparts in the diabetic group.

*Cholesterol*

The average cholesterol of GG, GA, and AA allele carriers was 160±8.6, 168±8.0 and 174±10.09 mg/dl, respectively. The diabetic patients had their cholesterol concentration varying between 158±15.38 to 190.2±17.3 mg/dl; irrespective of the allelic polymorphism.

In diabetic patients the total cholesterol concentration of the GG allele carriers was 158±15.38 mg/dl, which was 7% and 20% more in GA and AA allele carriers; respectively when compared with the diabetic GG allele carriers. In the non-diabetic subjects the cholesterol concentration of GG allele carrier was 162±8.29 mg/dl, which was 3% more in GA and 3% low in AA allele carrier; when compared with its respective non-diabetic GG allele carriers.

The cholesterol concentrations of diabetic patients with GA allele carrier were 2% and 18% higher respectively, whereas it was 2% lower in GG allele carriers; when compared with their respective non-diabetic GA, AA and GG allele carriers.

*High density lipoproteins (HDL)*

The average HDL concentration in GG, GA and AA allele carriers was 36±3.08, 34±1.13 and 36±2.05 mg/dl, respectively. The diabetic patients had their HDL concentrations varying between 32.27±2.5 to 39±5.6 mg/dl and non-diabetic patients had their HDL concentration varying between 33±4.5 to 38.15±2.2 mg/dl; irrespective of the allelic polymorphism.

In diabetic patients the HDL concentration of GG allele carriers was 39±5.6mg/dl, whereas it was 17% and 15% low in GA and AA allele carriers, respectively, compared with GG allele carriers. In the case of non-diabetic patients the HDL concentration of GG allele carriers was 33±2.25 mg/dl, whereas GA and AA allele carriers had 8% and 16% more, when compared with their respective non-diabetic GG allele carriers. The HDL concentration of non-diabetic patients with GA and AA allele carriers, increased 10% and 14% respectively, whereas this concentration was 15% lower in non-diabetic GG allele carriers, when compared with their respective diabetic GA, AA and GG allele carriers.

*Low density lipoprotein (LDL)*

The average LDL concentration of GG, GA and AA allele carriers was 98.4±6.5, 104±3.68 and

100±9.3 mg/dl. The diabetic patients had their LDL concentration varying between 103±10.9 to 105±20.3 mg/dl and the non-diabetic patients had their LDL concentration varying between 94.35±7.2 to 102±4.8 mg/dl, irrespective of the allelic polymorphism.

In diabetic patients the LDL concentration of the GG allele carriers was 103±10.9 mg/dl, which was respectively 3% and 2% higher in GA and AA allele carriers; when compared with the respective GG allele carriers. In non-diabetic patients the LDL concentration of the GG allele carriers was 94.35±7.2 mg/dl which was 8% and 1% higher in GA and AA allele carriers respectively; when compared with the respective non-diabetic GG allele carriers.

The LDL concentration of diabetic patients showed 8, 4 and 9% higher values in GG, GA and AA allele carriers respectively; when compared with their respective non diabetic GG, GA and AA allele carriers.

*Creatinine*

The average creatinine of GG, GA and AA allele carriers were 0.87±0.05, 0.9±0.02 and, 0.98±0.04 mg/dl, respectively. The diabetic patients had their creatinine concentration varying between 0.78±0.07 mg/dl to 0.97±0.06 and the non-diabetic patients had their creatinine concentration varying between 0.96±0.04 to 1.009±0.05 mg/dl; irrespective of the allelic polymorphism.

In the diabetic patients the creatinine concentration of GG allele carriers was 0.779±0.07 mg/dl, whereas it was 13% and 25% more in GA and AA allele carriers, respectively when compared with diabetic GG allele carriers. In non-diabetic patients the creatinine concentration of GG allele carriers was 0.96±0.04 mg/dl, which was 2% and 5% higher in the GA and AA allele carriers, when compared with non-diabetic allele carriers.

The creatinine concentration of GG, GA and AA allele carriers from amongst non-diabetics was 23% 10% and 4%, respectively, higher when compared with their respective diabetic counterparts.

*Relationship of TNFα genotypes with biochemical parameters*

The three allelic groups (GG, GA, AA) have been divided into three BMI groups: Group 1 labeled as normal (BMI: 18.5 - 24.9 kg/m$^2$, Group 2 labeled as overweight (BMI: 25 - 29.9 kg/m$^2$, Group 3 labeled as obese (BMI: $\geq$30 kg/m$^2$). Different biochemical parameters used in this study were then compared and the differences statistically analyzed.

Table II shows comparison of BMI, F8S, triglycerides, cholesterol HDL, LDL and creatinine in the subjects of three allelic groups of different BMIs. All these parameters have been discussed individually below.

*Body mass index*

The average BMIs of GG, GA, and AA allele carriers were 28±0.88, 29.3±0.8 and 29.3±1.58 kg/m$^2$. In GG allele carriers, the overweight and obese subjects had BMI 17% and 48% greater than that of normal subjects. In GA as well as AA allele carriers the BMI in overweight and obese subjects showed 17% and 65% higher values, respectively, compared to normal GA allele carriers.

*Fasting blood sugar*

The average FBS of GG, GA and AA allele carriers were 121.33±8.97, 122.3±6.1 and 165±17.7 mg/dl, respectively. The FBS in normal group varied between 118 to 278 mg/dl, whereas in overweight subjects it varied between 87±6.28 to 124±10.67 mg/dl. The FBS in normal AA allele carriers showed 131 % higher values compared with normal GG allele carriers. Amongst overweight subjects the FBS concentration was 28% lower in AA allele carriers compared to GG allele carriers. The overweight and obese AA allele carriers had, FBS concentration 69% and 53% respectively, lower compared with normal AA allele carriers.

*Triglycerides*

The average triglyceride concentration of GG, GA and AA allele carriers was 86.67±16.67, 165.33±8.2, and 160±25.4 mg/dl. The normal subjects had their triglycerides concentration varying between 83±13 and 187±48 mg/dl, whereas it varied between 106±14.86 to 204±32.38 mg/dl in overweight, and between 172±11.55 to 187±37.8 mg/dl in obese patients.

The normal GG allele carriers had

triglycerides 83±13 mg/dl, which showed 92 and 125% higher values, respectively, in normal GA and AA allele carriers, compared with normal GG allele carriers. The overweight GG allele carriers had

**Table II.- Relationship of different genotypes on the various biochemical parameters on normal, over-weight and obese subjects.**

| Parameters | Subjects | GG[1] | GA | AA |
|---|---|---|---|---|
| BMI (kg/m$^2$) | Normal | 23±0.00[2] (n=2) | 23±0.33 (n=12) | 23±0.5 (n=2) |
| | Over-weight | 27±0.35[a] (n=14) | 27±0.19[a] (n=40) | 27±0.7[a] (n=5) |
| | Obese | 34±0.98[a] (n=18) | 38±1.12[a] (n=57) | 38±1.3[a] (n=12) |
| | Mean | 28±0.88 (n=34) | 29.3±0.8 (n=109) | 29.3±1.58 (n=19) |
| FBS (mg/100 ml) | Normal | 120±0.00 (n=2) | 118±24.2 (n=12) | 278±0.00 (n=2) |
| | Over-weight | 120±12.79 (n=14) | 124±10.67 (n=40) | 87±6.28[a] (n=5) |
| | Obese | 124±13.54 (n=18) | 125±7.95 (n=57) | 130±22.07[a] (n=18) |
| | Mean | 121.33±8.97 (n=34) | 122.3±6.1 (n=109) | 165±17.7 (n=19) |
| Triglycerides (mg/100 ml) | Normal | 83±13 (n=2) | 160±26.3[a] (n=12) | 187±48[a] (n=2) |
| | Over-weight | 204±32.38[a] (n=14) | 164±13.2 (n=40) | 106±14.86[a] (n=5) |
| | Obese | 173±17.4[a] (n=18) | 172±11.55 (n=57) | 187±37.8 (n=12) |
| | Mean | 86.67±16.67 (n=34) | 165.33±8.2 (n=109) | 160±25.4 (n=19) |
| Cholesterol (mg/100 ml) | Normal | 118±14.5 (n=2) | 156±10.0 (n=12) | 198±51 (n=2) |
| | Over-weight | 165±12.69[a] (n=14) | 171±8.5 (n=40) | 142±9.45[a] (n=14) |
| | Obese | 161±12.7[a] (n=18) | 168±5.95 (n=57) | 172±13.04 (n=12) |
| | Mean | 148±8.6 (n=34) | 165±1.17 (n=109) | 170.67±10.09 (n=19) |
| HLD (mg/100 ml) | Normal | 36±5.5 (n=2) | 32±2.79 (n=12) | 31±8.5 (n=2) |
| | Over-weight | 32±3.0 (n=14) | 35±2.49 (n=40) | 40±2.2[a] (n=5) |
| | Obese | 39±5.4 (n=18) | 35±1.19 (n=57) | 36±2.85 (n=12) |
| | Mean | 35.67±3.08 (n=34) | 34±1.15 (n=109) | 35.67±2.04 (n=34) |
| LDL (mg/100 ml) | Normal | 68±6 (n=2) | 93±906 (n=12) | 144±59 (n=2) |
| | Over-weight | 97±10.47 (n=14) | 106±6.8 (n=40) | 83±8.5[a] (n=5) |
| | Obese | 103±9.0[a] (n=18) | 103±4.98 (n=57) | 97±11[a] (n=12) |
| | Mean | 89.33±6.48 (n=34) | 100.67±3.7 (n=109) | 108±9.3 (n=19) |
| Creatinine (mg/100 ml) | Normal | 0.93±0.07 (n=2) | 0.89±0.05 (n=12) | 0.98±0.21 (n=2) |
| | Over-weight | 0.90±0.07 (n=14) | 0.098±0.04 (n=40) | 1.08±0.06 (n=5) |
| | Obese | 0.84±0.06 (n=18) | 0.9±0.02 (n=57) | 0.96±0.04 (n=12) |
| | Mean | 0.89±0.04 (n=34) | 0.92±0.02 (n=109) | 1.007±0.04 (n=19) |

[1]Subjects homozygous for G and A allele were grouped as GG and AA, whereas the subjects heterozygous were grouped as GA.
[2]Mean±SEM, student's `t' test used to compare non-diabetic subjects with diabetic patients; *P<0.05;
GG, GA, and AA allele carriers compared within each row. GG allele carriers had been compared with GA< and AA allele carriers [a,b]P<0.05
**Normal values:** Total cholesterol: 200 mg/dl; Triglycerides: 150 mg/dl; HDL-High density lipid: Men, 41.0-58.7 mg/dl, Women, 48.5-75.0 mg/dl; LDL-cholesterol: >150 ml/dl; Creatinine: Men, 0.6-1.1 mg/dl; Women, 0.5-0.9 mg/dl; Glucose: Plasma/Serum (Fasting) 95-105 mg/dl.

triglyceride concentration 204±32.38 mg/dl, whereas in overweight GA and AA allele carriers it was, respectively, 20% and 48% lower compared with their GG allele carriers. The triglyceride concentration of overweight and obese GG allele carriers was higher by 145% and 108%, respectively, compared with their normal GG allele carriers. The overweight AA allele carriers had triglyceride concentration values 43% lower than the normal AA allele carriers.

*Total cholesterol*

The average cholesterol level of GG, GA and AA allele carriers was 148±8.6, 165±1.17 and 170.67±10.09 mg/dl, respectively. The normal subjects had cholesterol concentration varying between 118±14.5 to 198±51 mg/dl, and obese patients varying between 161±12.7 to 172±13.04 mg/dl. The total cholesterol concentration in overweight and obese GG allele carriers was 40% and 36%. respectively. higher than the normal GG allele carriers.

Total cholesterol concentration in overweight and obese AA allele carriers showed, 28% and 15%. respectively. lower values than the normal AA allele carriers.

The total cholesterol concentration in normal GG allele carriers was 118±14.5 mg/dl whereas GA and AA allele carriers showed 32% and 68% higher values.

*HDL cholesterol*

GG, GA and AA allele carriers had an average of 35.6±3.08, 34±1.15 and 35.67±2.04 mg/dl of HDL cholesterol, respectively. The overweight patients had their HDL-cholesterol concentration varying between 32±3.0 to 40±2.2 mg/dl. and the obese patients had their HDL-cholesterol concentration varying between 35±1.19 to 39±5.4 mg/dl. The overweight AA allele carriers had higher HDL-cholesterol concentration compared to AA allele carriers of normal and overweight GG allele carriers.

*LDL cholesterol*

GG, GA and AA allele carriers had 89.33±6.48, 100.67±3.7 and 108±9.3 mg/dl of LDL-cholesterol, respectively. The normal subjects had LDL-cholesterol concentration varying between 68±6 to 144±59 mg/dl. Whereas, overweight and obese subjects had these between 83±8.5 to 106±6.8 mg/dl and 97±11 to 103±9 mg/dl respectively.

The normal GG allele carriers had LDL-cholesterol concentration 68±5 mg/dl. Whereas, GA and AA allele carriers had 37% and 112% higher values respectively, compared to normal GG allele carriers. The overweight and obese GG allele carriers had 42.6% and 51.47% higher values, when compared with its respective normal GG allele carriers. The overweight and obese AA allele carriers showed 42% and 33% lower values of LDL-cholesterol compared with respective normal AA allele carriers.

*Creatinine*

The GG, GA and AA allele carriers had creatinine on the average. 0.89±0.04, 0.92±0.02 and 1.00±0.04 mg/dl, respectively. No significant difference was observed in creatinine value of any group.

## DISCUSSION

Effect of diabetes on the various biochemical parameters of 166 individual population with three different genotypes was studied. The average BMIs was not different across these three allele carrier groups *i.e.* GG, GA and AA of non-diabetic and diabetic populations. Their BMI fall in the category of highly obese individuals. The striking feature was observed in case of diabetic population. FBS, TG, total cholesterol and creatinine concentrations were extremely increased and HDL-cholesterol concentration is decreased in diabetic AA allele carriers, when compared with diabetic. GA and GG allele carriers. A more recent study (Hoffstedt *et al.*, 2000) revealed that M variant at position -308 in the promoter region of the TNF-x gene could be an important genetic factor for the excessive fat accumulation in women. We similarly observed the considerable increase in fasting blood sugar. triglyceride. total-cholesterol and creatinine concentrations and decrease in HDL-cholesterol concentration mainly in the group of the obese A allele carriers, but no relationship of the A allele with fat accumulation and obesity was found. Wybranska *et al.* (2003) also observed the considerable increase in insulin resistance in the group of the obese A allele female carriers, but no relationship of the A allele with obesity was found (Maclean *et al.*, 2000).

Association of TNF-alpha gene with obesity was studied. Our data indicated that the TNF -308 variant allele correlated with increased BMI or influenced traits related to obesity. The similar

conclusion was obtained by Walston *et al.* (1999), while working on association of tumor necrosis factor alpha -238 and .308 polymorphism with traits related to obesity and insulin resistance. This does not support the conclusion of a previous study that found an association between the -308 allele and increased insulin resistance (Femandez-Real *et al.*, 1997).

TNF-alpha is expressed primarily in adipocytes and elevated levels of this cytokine have been associated with obesity. The purpose of this investigation was to test whether the TNF-alpha -308 polymorphism were associated with insulin resistance or obesity related traits in non-diabetic and diabetic patients visiting Sheikh laid Hospital, Lahore, Fatima Hospital and Irfan Clinic in Sargodha.

In non diabetic subjects the AA allele carriers, compared with homozygous G allele carriers had significantly lower (28%) triglyceride values and 15% higher HDL values, whereas other parameters tested did not show any significant variation. In diabetic patients the AA allele carriers, compared with GG allele carriers, besides having 31% higher FBS and 26% higher creatinine, had 20% higher cholesterol and 34% higher triglycerides. The HDL values were 14% less, compared to GG allele carriers.

In normal subjects (BMI 22.85±0.25 kg/m$^2$), the AA allele carriers showed 132%, 125%, 65% and 112% higher triglycerides, cholesterol and LDL values compared with GG allele carriers. The HDL and creatinine did not show any significant change. In the overweight subjects (BMI: 27.17±0.17 kg/m$^2$) all these values were lower than in AA allele carriers compared with GG allele carriers., The AA allele carries had FBS, triglycerides, cholesterol and LDL 28%, 48%, 14% and 14% lower than in the GG allele carriers, respectively. In obese subjects, (BMI: 36.7±30.78 kg/m$^2$), however, the FBS, triglycerides, cholesterol and creatinine values were 5%, 8%, 7% and 14% higher in AA allele carries compared to GG allele carriers, respectively. The LDL content was 8% lower in AA allele carrier as compared with the respective GG allele carriers.

It is concluded that replacement of G at -308 with A leads to reduced risk for cardiovascular disease in non-diabetic subject, whereas in diabetic patients this mutation increases the risk of CVD. Using BMI as index of obesity, it was observed that obese subject with AA alleles had greater risk of CVD, as compared with those with GG alleles. Comparatively, however, this risk was much higher in AA allele carriers with BMI 22.85±0.25 kg/m$^2$ (normal subjects) than with their respective GG carriers or those of obese subjects.

## REFERENCES

ABRAHAM, L.J. AND KREOGER, K.M., 1999. Impact of the -308 TNF promoter polymorphism on the transcription regulation of the TNF gene: relevance t disease. *J. Leukoc. Biol*., **66**: 562-566.

ALLISION, D.B., FAITH, M.S. AND NATHAN, J.S., 1996. Rich's values for human obesity. *Int. J. Obes. Relat. Metab. Disord*., **20**: 990-999.

BAYLEY, J.P., de ROOIJ, H., VAN DEN ELSEN, P.J., HUIZINGA, T.W. AND VERWEIJ, C.L., 2001. Functional analysis of linker-scan mutants spanning the -376, -308, -244 and -238 polymorphic sites of the TNF-alpha promoter. *Cytokine*, **14**: 316-323.

BEUTLER, B., 1989. Cachectin in tissue injury, shock, and related states. *Grit. Care Clin*, **5**: 353-367.

BEUTLER, B., 1992. Application of transcriptional and post-transcriptional reporter constructs to the analysis of tumor necrosis factor gene regulation. *Am. J. med. Sci*., **303**: 129-133.

BEUTLER, B. AND VAN HUFFEL, C., 1994. An evolutionary and functional approach to the TNF receptor/ligant family. *Ann. N.Y. Acad. Sci*., **730**: 118-133.

BLACK, R.A., RAUCH, C.T., KOZLOSKY, C.J., PESCHON, J.J., SLACK, J.L., WOLFSON, M.F., CASTNER, B.J., STOCKING, K.L., REDDY, P., SRINIVASAM, S., NELSN, N., BOIANI, N., SCHOOLEY, K.A., GERHART, M., DAVIS, R., FITZNER, J.N., JOHNSON, R.S., PAXTON, R.J., MARCH, C.J. AND CERRETTI, D.P., 1997. A metalloproteinase disintegrin that releases tumour-necrosis factor-alpha from cells. *Nature*, **385**: 729-733.

BOUCHARD, C., PREUSSE, L. AND RAO, D., 1998. The genetics of human obesity. In: *Handbook of obesity* (eds. G. Bray, C. Bouchard and W. James), pp. 157-190. Marcel Dekker, New York.

BOUCHARD, C., TREMBLAY, A., DESPRES, T.P., NADEAU, A., LUPIEN, P.J., THERIAULT, G., DUSSAULT, J., MORJANI, S., PINAULT. S. AND FOURNIER, G., 1990. The response to long term overfeeding in identical twins. *N. Engl. J. Med*., **322**: 1477-1482.

BROWNELL, K.D. AND WADDEN, T.A., 1992. Etiology and treatment of obesity: understanding a serious prevalent an refractory disorder. *J. Consult. Clin. Psychol.*, **60**:

505-517.

CHAGNON, Y.C., RANKINEN, T., SNYDER, E.E., WEISNAGEL, S.J., PERUSSE, L. AND BOUCHARD, C., 2003. The human obesity gene map: the 2002 update. *Obes. Res.*, **11**: 313-367.

CHEN, D. AND GARG, A., 1999. Monogenetic disorders of obesity and body fat distribution. *J. Lipid Res*., **40**: 1735-1746.

ECKEL, R., 2003. Obesity: a disease or a physiological adaptation for survival? In: *Obesity: mechanisms and clinical management* (ed. R. Eckel), pp.3-30. Lippincott Williams and Williams, Philadelphia.

FAITH, M.S., KELLER, K.L., JOHNSON, S.L., PIETROBELL, A., MATZ, P.E., MUST, S., JORGE, M.A., COOPERBERG, J., HEYMSFIELD. S.B. AND ALLISON, D.B., 2004. Familial aggregation of energy intake in children. *Am. J. clin. Nutr*., **79**: 844-850.

FEINLEIB, M., GARRISON, R.J., FABSITZ, R., CHRISTIAN, J.C., HRUBEC, Z., BORHANI, N.O., KANNEL, W.B., ROSENMAN, R., SCHWARTZ, J.T. AND WANGER, J.O., 1977. The NHLBI twin study of cardiovascular disease risk factor: methodology and summary of results. *Am. J. Epidemiol*., **106**: 284-285.

FEMANDEZ-REAL, J.M., GUTIERREZ, C., RICART, W., CASAMITJNA, R., FERNANDEZ-CASTAMER, M., VENDRELL, J., RICHARD, C. AND SOLER, J., 1997. The TNF-alpha gene NcoI polymorphism influences the relationship among insulin resistance, percent body fat, and increased serum leptin levels. *Diabetes*, **46**: 1468-1472.

HAINER, V., STUNKARD, A., KUNESOVA, M., PARIZKOVA, J., STICH, V. AND ALLISON, D.B., 2001. A twin study of weight loss and metabolic efficiency. *Int. J. Obes. Relat. Metab. Disord*., **25**: 533-573.

HASSTEDT, S.J., RAMIREZ, M.E., KUDIA, H. AND WILLIAMS, R.R., 1989. Recessive inheritance of a relative fat pattern. *Am. J. Hum. Genet*., **45**: 917-925.

HOFFSTEDT, J., ERIKSSON, P., HELLSTROM, L., ROSSNER, S., RYDEN, M. AND ARNER, P., 2000. Excessive fat accumulation is associated with the TNF alpha-308 G/A promoter polymorphism in women but not in men. *Diabetologia*, **43**: 117-120.

HOTAMISLIGIL, G.S., ARNER, P., CARO, J.F., ATKINSON, R.L. AND SPIEGELIMAN, B.M., 1995. Increased adipose tissue expression of tumor necrosis factor – in human obesity and insulin resistance. *J. clin. Invest*., **95**: 2409-2415.

HOTAMISLIGIL, G.S., MURRAY, D.L., CHOY, L.N. AND SPIEGELMAN, B.M., 1994. Tumor necrosis factor alpha inhibits signaling from the insulin receptor. *Proc. natl. Acad. Sci. USA*, **91**: 4854-4858.

HOTAMISLIGIL, G.S., SHARGILL., N.S. AND SPIEGELMAN, B., 1993. Adipose expression of tumour necrosis factor-alpha: direct role in obesity-linked insulin resistance. *Science*, **259**: 87-89.

KATZMARZYK, P.T., MALINA, R.M., PERUSSE, L., RICE, T., PROVINCE, M.A., RAO, D.C. AND BOUCHARD, C., 2000. Familial resemblance in fatness and fat distribution. *Am. J. Hum. Genet.*, **12**: 395-404.

KOEPPEN-SCHOMERUS, G., WARDLE, J. AND PLOMIN, R.A., 2001. Genetic analysis of weight and overweight in 4 years old twin pairs. *Int. J. Obes. Relat. Metab. Disord*., **25**: 838-844.

LEIBEL, R.L., ROSENBAUM, M. AND HIRSCH, J., 1995. Changes in energy expenditure resulting from altered body weight. *N. Engl. J. Med*., **332**: 621-628.

MacLEAN, P.S., BOWER, J.F., VADLAMUDI, S., GREEN, T. AND BARAKAT, H.A., 2000. Lipoprotein subpopulation distribution in lean, obese, and type 2 diabetic women: a comparison of African and white Americans. *Obes. Res*., **8**: 62-70.

MATHEWS, S.G., 2002. Early programming of the hypothalamo-pituitary-adrenal axis. *Trends Endocrinol. Metab*., **13**: 373-380.

MOSS, M.L., JIN, S.L.C., BECHERER, J.D., BICKETT, D.M., BURKHART, W., CHEN, W.J., HASSLER, D., LEESNITNER, M.T., McGEEHAM, G., MILLA, M., MOYER, M., ROCQUE, W., SEATON, T., SCHONEN, F., WARNER, J. AND WILLARD, D., 1997. Structural features and biochemical properties of TNF-alpha converting enzyme (TACE). *J. Neuroimmunol*., **72**: 127-129.

PERREY, C., TURNER, S.J., PRAVICA, V., HOWELL, W.M. AND MUTCHINSON, I.V. 1999. ARMS-PCR methodologies to determine IL-10, TNF-alpha and TGF-I gene polymorphism. *Transpl. Immunol.*, **7**: 127-128.

PIETIALAINEN, K.H., KAPRIO, J., RISSANEN, A., WINTER, T., RIMPELA, A., VIKEN, R.J. AND ROSE, R.J., 1999. Distribution and heritability of BMI in finish adolescents aged 16 years and 17 years: a study of 4884 twins and 2509 singletons. *Int. J. Obes. Relat. Metab. Disord*., **23**: 107-115.

POCIOT, F., WILSON, A.G., NERUP, J. AND DUFF, G.W., 1993. No independent association between tumor necrosis factor-alpha promoter region polymorphism and insulin-dependent diabetes mellitus. *Eur. J. Immunol*., **23**: 3050-3053.

ROSENDAHL, M.S., KO, S.C., LONG, D.L., BREWER, M.T., ROSENZWEIG, B., HELD, E., ANDERSON, L., RYDE, S.M., MORELAND, J., MEYERS, M.A., KOHNO, T., LYONS, D. AND LICHEUSTEIN, H.S., 1997. Identification and characterization of a pro-tumor necrosis factor-alpha-processing enzyme from ADAM family of zinc metalloproteases. *J. biol. Chem*., **39**: 24588-24593.

ROSMOND, R., 2001. Visceral obesity and the metabolic syndrome. In: *The international textbook of obesity* (ed. P. Bjorntorp), pp. 339-350. John Wiley and Sons,

Chichester.

ROSMOND, R., 2004. Aetiology of obesity: a striving after wind. *Obes. Rev*., **5**: 177-187.

SELBY, J.V., NEWMAN, B., QUESENBERRY, Jr., C.P., FABSITZ, R.R., KING, M.C. AND MEANEY, F.J., 1988. Evidence of genetic influence on central body fat in middle-aged twins. *Hum. Biol*., **61**: 179-194.

SILVER, K., WALSTON, J., CHUNG, W.K., YAO, F., PARIKH, V.V., ANDERSON, R., CHESKIN, L.J., ELAHI, D., MULLER, D., LEIBEL, R.L. AND SHULDINER, A.R., 1997. The $Gln^{223}$ Arg and $Lys^{656}$ Asn polymorphisms in the human leptin receptor do not associate with traits related to obesity. *Diabetes*, **46**: 1898-1900.

SOKAL, R.R. AND ROHLF, F.J., 1984. Introduction a la bioestadistia serie de Biologia Fundamental. In: (ed. S.A. Reverte), pp. 90-126. Espana.

STRICKBERGER, M.W., 2001. Genetics. Prentice Hall of India, Pvt. Ltd.

STUNKARD, A.J., FOCH, T.T. AND HURBECZ, Z., 1986. A twin study of human obesity. *J. Am. med. Assoc*., **256**: 51-54.

VASSALLI, P., GRAU, G.E. AND PIGUET, P.F., 1992. TNF in autoimmune diseases, graft-versus-host reactions, and pulmonary fibrosis. *Immunol. Ser*., **56**: 409-430.

WALSTON, J., SILVER, K., BOGARDUS, C., KNOWLER, W.C., CELI, F.S., AUSTIN, S., MANNING, B., STROSBERG, A.D., STERN, M.P. AND RABEN, N., 1995. Time of onset of non-insulin-dependent diabetes mellitus and genetic variation in the B3-adrenergic-receptor gene. *N. Engl. J. Med*., **333**: 343-347.

WALSTON, J., SEIBERT, M., YEN, C.J, CHESKIN, L.J. AND ANDERSON, R.E., 1999. Tumor necrosis factor-alpha-238 and -308 polymorphisms do not addociate with traits related to obesity and insulin resistance. *Diabetes*, **24**: 2096-2098.

WYBRANSKA, L., MALCZEWSKA, M., NIEDBAT, S., NASKALSKI, J. AND DEMBINSKA-KIEC, A., 2003. The TNF-alpha NcoI polymorphism at position -308 of the promoters influences insulin resistance, and increases serum triglycerides after postprandial lipaemia in familiar obesity. *Clin. Chem. Lab. Med.*, **4**: 501-510.

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