

Relationship of Circulating Tumor Necrosis Factor Alpha (TNF- α) and Insulin Secretion and Resistance in Euglycaemic Dogs

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ABSTRACT

Background: Insulin resistance is a state that is characterized with reduced sensitivity of peripheral tissues to insulin. It can be related with increased level of tumor necrosis factor alpha (TNF- α) in dogs. Insulin resistance can be evaluated by homeostasis model assessment (HOMA-IR, HOMA- β). The aim of this study was to determine correlation of circulating TNF- α level with insulin production and insulin resistance indexes in euglycaemic dogs.

Materials, Methods & Results: Seventy dogs of normal body score were included in this study. After blood sampling levels of glucose, insulin and TNF- α were determined and indexes HOMA-IR and HOMA- β were calculated. Three groups in accordance to TNF- α levels were formed: the first-TNF- α 0-2.0 pg/mL, the second-TNF- α below median (2.1-17.0 pg/mL) and the third-TNF- α above median (17.1-51.8 pg/mL). Differences in insulin and glucose levels, HOMA-IR and HOMA- β were determined in all three groups. ANOVA and posthoc LSD analyses were used. Correlation between HOMA-IR and HOMA- β was determined. Linear regression between HOMA- β /HOMA-IR ratio and glucose concentration was calculated. SPSS statistical program was used (IBM). Highest insulin level was detected in the second group and the lowest was detected in the third group. The lowest glucose level was detected in the first group. The highest value of HOMA- β index was noted in the first group and it decreases with TNF- α increase. The highest HOMA-IR value was detected in the second group and the lowest was in the third group. Positive correlation was noted between HOMA-IR and HOMA- β . Significant linear correlation was noted between glucose levels in function of HOMA- β /HOMA-IR ($R^2 = 0.51-0.78$, $P = 0.0007$). The first group showed the minor change of glucose level ($b = 0.29$ mmol/L). In the third group the greatest change of glucose level in function of HOMA- β /HOMA-IR was noted ($b = 0.52$ mmol/L). In the third group the highest increase of glucose level followed by decrease in HOMA- β /HOMA-IR ratio was noted.

Discussion: Increase of TNF- α followed by increase of insulin was noted in the second group. That indicates reduced insulin action and compensatory increase in his concentrations in order to achieve the same effect. TNF- α induces reduction in expression of glucose transporter 4 that is insulin-regulating hormone. Serine phosphorylation of insulin receptor substrate-1 that is induced by TNF- α causes inhibition of insulin receptors. These actions cause insulin resistance and compensatory increase in insulin secretion. Increased tissue resistance is reflected in increased HOMA-IR index which is directly associated with insulin and glucose level. Increase of insulin value and HOMA-IR index were noted in second group. This indicates the influence of TNF- α on insulin resistance. The lowest insulin level was noted in the third group of dogs. Decrease in production and secretion leads to reduction in circulating insulin and can be evaluated by HOMA- β index. The highest value of this index was noted in first group and decreases with TNF- α increase. This can be related with apoptotic effect that TNF- α has on beta-pancreatic cells. Increase of HOMA- β index causes increase of HOMA-IR. This is indication of increased peripheral resistance. Compensatory mechanism for that state is increased insulin secretion. Glucose level will increase more during increase of HOMA-IR and decrease of HOMA- β . This indicates that TNF- α regulates glucose level directly and explains differences in glucose concentrations in dogs with different concentrations of TNF- α showed. Values of insulin resistance indexes, glucose and insulin were affected by circulating concentration of TNF- α . The most unfavorable change in glucose concentration based on insulin production and tissue resistance was founded in dogs with the highest circulating TNF- α concentration in blood.

Keywords: dogs, insulin resistance, HOMA, TNF- α .

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INTRODUCTION

Insulin resistance (IR) is a state of reduced sensitivity of peripheral tissues to circulating insulin. In order to improve insulin sensitivity in peripheral tissues organism requires higher insulin levels to regulate the same levels of glucose. In the state of resistance insulin levels can be normal, higher or reduced [4,19,34,32]. IR can be evaluated by indexes of insulin resistance [17,3]. Homeostasis model assessment (HOMA) is often used in clinical trials. This model evaluates the capacity of pancreatic beta cells to secrete insulin (HOMA- β) and values resistance of peripheral tissue to insulin (HOMA-IR) [29]. There are some limitations when HOMA is used on dog models [1] but it is performed from golden standard for evaluation of insulin IR so it's very significant [17].

IR is influenced by glycolipotoxic state, reactive oxygen species production, epigenetic factors etc. Increased levels of proinflammatory cytokines and chronic inflammation have significant influence on IR development [16]. Pathogenesis of this syndrome hasn't been fully understood, but it has been considered that mild inflammation has the crucial role. Experimental, clinical and epidemiological studies showed that proinflammatory cytokine levels, including tumor necrosis factor- α (TNF- α) are significantly higher in animals with IR and diabetes mellitus type II. This indicates crucial role of TNF- α in development of IR [2,5,9,21]. Many other experiments have been conducted in order to study the influence of TNF- α on IR in humans [3,10,14,18,23,32] and animals [11,12,27].

This study aims to examine correlation of circulating TNF- α with indexes of insulin resistance in euglycaemic dogs.

MATERIALS AND METHODS

Animals and laboratory analysis

Seventy dogs were included in this experiment. Research was conducted at Veterinary Clinic and Laboratory for pathophysiology, Department of Veterinary medicine (University of Novi Sad). Concentrations of TNF- α were determined and thereafter three groups were formed. First group: 50 dogs that have no detectable TNF- α concentrations (0-2.0 pg/mL). Second group: 10 dogs that have TNF- α concentrations below the median (2.1-17.0 pg/mL). Third group: 10 dogs that have TNF- α concentration above

the median (17.1-51.8 pg/mL). Blood samples were taken from *v. cephalica* in tubes that contained clot activator. Glucose concentration was determined by automatic biochemical analyzer Rayto Chemray 120 Vet¹. Glucose level was determined after its enzymatic oxidation in presence of glucose-oxidase. Obtained hydrogen peroxide from previous reaction further reacts with 4-amino-phenazine and phenol in presence of catalytic peroxidase. Red-purple color was obtained and its intensity was measured. Insulin concentration determination was conducted at endocrinological analyzer Tosoh AIA-360² using immunofluorescence method. Concentration of tumor necrosis factor alpha was determined simultaneously at all samples after their thawing. Canine TNF alpha ELISA³ kit was used. Color intensity was measured by ELISA Rayto 2100C microplate reader 450 nm. Optical densities were converted into pg/mL. Lower detection limit of this ELISA plate was 2 pg/mL.

HOMA-IR index was calculated according to the formula: $[\text{glucose (mmol/mL)} \times \text{insulin } (\mu\text{IU/mL})] / 22,5$ and HOMA- β : $20 \times \text{insulin } (\mu\text{IU/mL}) / \text{glucose (mmol/mL)} - 3.5$.

Statistical analysis

Differences in insulin concentration, glucose concentrations, HOMA-IR and HOMA- β were determined in all groups of dogs. ANOVA and posthoc LSD analyses were used. Correlation between HOMA-IR and HOMA- β was determined. Linear regression between value of quotient of these two indexes and glucose concentration was calculated in all three groups of animals. SPSS statistical program was used (IBM)⁴.

RESULTS

Insulin resistance indexes, insulin and glucose concentrations were significantly affected by concentration of circulating TNF- α concentration. Highest insulin concentration was detected in group of dogs with TNF- α concentration below the median, while lowest concentration of insulin was detected in group with TNF- α concentration above the median. Group of dogs with undetectable TNF- α was between these two groups (Figure 1).

The lowest glucose concentration was detected in first group and increases with TNF- α increase (Figure 2). The highest value of HOMA- β index was noted in group of dogs that expressed undetectable TNF- α

concentration and it decreases with TNF- α concentration increase (Figure 3).

The highest HOMA-IR value was detected in second group of dogs and the lowest was in the third group (Figure 4). Positive correlation was noted between HOMA-IR and HOMA- β (Figure 5).

Significant linear correlation was noted between change in glucose concentrations in function of HOMA- β /HOMA-IR ($R^2=0.51-0.78$, $P=0.0007$). Change of glycaemia in function of HOMA- β /HOMA-IR in all three groups was showed in regression ana-

lyze- group of dogs with undetectable TNF- α showed the minor change of glucose concentrations ($b=0.29$ mmol/L).

In the group with TNF- α above the median greatest change of glucose concentration in function of HOMA- β /HOMA-IR was noted ($b=0.52$ mmol/L). Second group was between them ($b=0.29$ mmol/L). In group of dogs with highest TNF- α concentration highest increase of glucose concentration followed by decrease in HOMA- β /HOMA-IR ratio was noted (Figure 6).

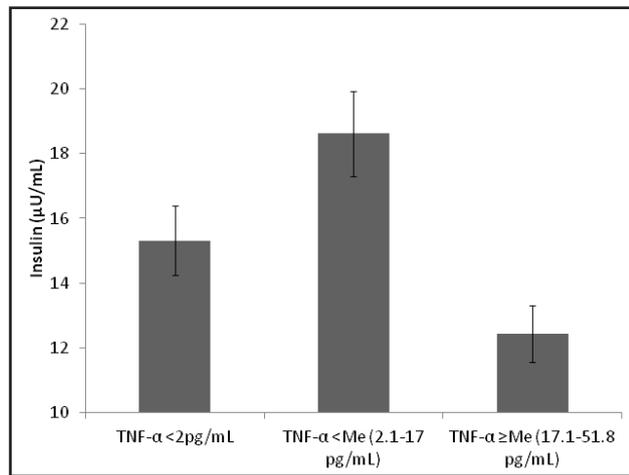


Figure 1. Insulin concentrations according to TNF- α concentration.

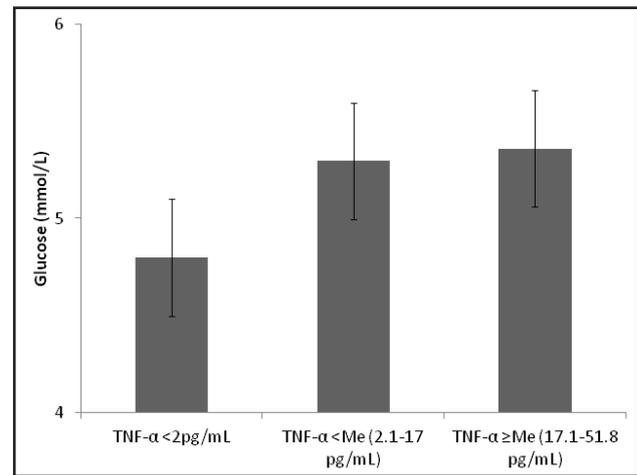


Figure 2. Glucose concentrations according to TNF- α concentration.

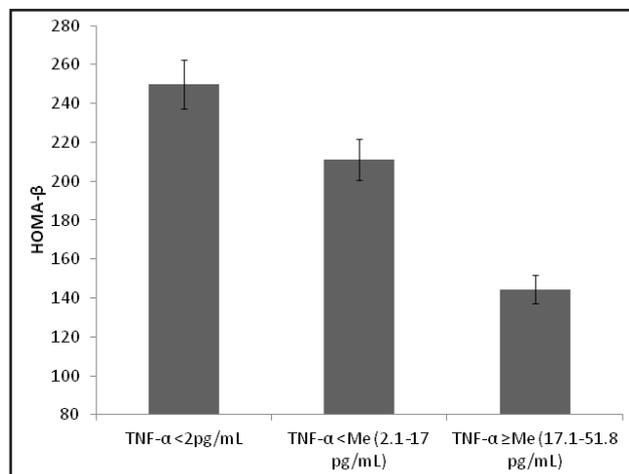


Figure 3. HOMA- β index value according to TNF- α concentration.

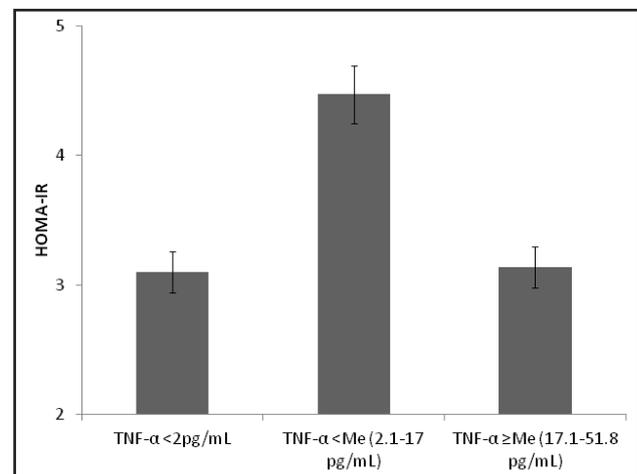


Figure 4. HOMA-IR values according to TNF- α concentration.

DISCUSSION

Increase of TNF- α followed by increase of insulin concentration was noted in the second group of animals that expressed TNF- α concentrations below the median. That indicates reduced insulin action and compensatory increase in his concentrations in order to achieve the same effect. This cytokine has a crucial role in development

of insulin resistance [2]. TNF- α induces reduction in expression of glucose transporter 4 (GLUT 4) which is insulin-regulating hormone in adipocytes, skeletal muscles and cardiac myocytes. Serine phosphorylation of insulin receptor substrate-1 (IRS-1) that is induced by TNF- α causes inhibition of insulin receptors and reduces activity of phosphatidylinositol-3 kinase. These actions cause insulin resistance and compensatory increase in insulin secretion.

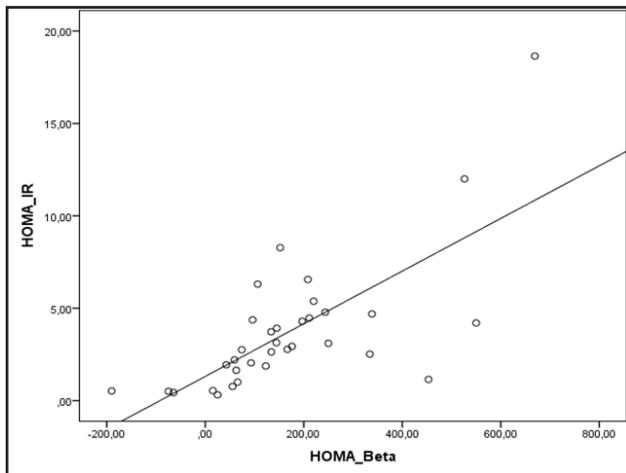


Figure 5. Correlation matrix of HOMA- β and HOMA-IR.

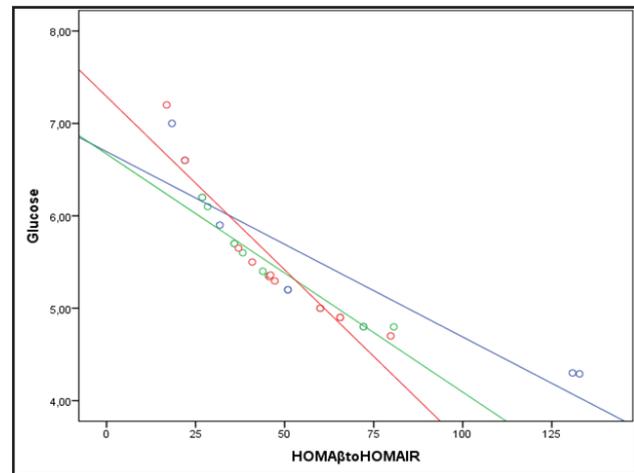


Figure 6. Correlation of HOMA- β /HOMA-IR ratio and glucose concentrations in experimental groups of dogs. Red line: TNF- α > Me; Green line: TNF- α < Me; Blue line: TNF- α undetectable concentration.

Stanley *et al.* [25] and Dong *et al.* [8] showed impaired insulin action on animal models and/or cell cultures that contained TNF- α receptors compared to animal models and/or cell cultures that lacked TNF- α receptors. That indicates the great importance of TNF- α in development of insulin resistance. Increase in HOMA-IR index was noted in our second group of animals (TNF- α below the median). This index is directly dependent of glucose and insulin concentration, and increase in this index suggests greater peripheral tissue resistance to circulating insulin. Values of HOMA-IR have been studied in patients suffering from rheumatoid arthritis, and found significant positive correlation between HOMA-IR and TNF- α concentration [24]. Our results are in accordance with theirs. Blocking the TNF- α activity they significantly reduced HOMA-IR index and increased sensitivity of peripheral tissues to insulin. Gayet *et al.* [11] in their study on dogs have been concluded that obese dogs have significantly higher TNF- α concentration that is related with increase in insulin concentration and development of insulin resistance. Our results are in accordance with previous research.

The lowest insulin concentration was noted in third group of dogs. Decrease in production and secretion leads to reduction in circulating insulin. That is represented in reduced HOMA- β insulin resistance index. HOMA- β shows secretory capacity of beta-pancreatic cells. The highest value of this index was noted in first group of dogs and decreases with TNF- α increase. So, the lowest value of HOMA- β was noted in the third group of dogs. This indicates

reduced secretory capacity of beta-pancreatic cells during TNF- α concentration increase. Reduced secretion can be caused by apoptosis of cells induced by TNF- α . Studies that observed human patients and animal models with diabetes mellitus type I (DM1) have been investigating apoptotic capacity of TNF- α [28,31]. Similarities have been observed in human patients with diabetes mellitus type II (DM2) but pathophysiologic mechanisms in these two states are different. Initial phase of DM2 is characterized by normal or increased insulin production that should compensate insulin resistance in peripheral tissue. Further progression of disease and many factors, including cytokines can cause inflammation and damage of pancreatic islands. Inflammation and destruction of beta- cells is reflected in their reduced secretory capacity and reduced mass [6]. Increase in free fatty acids (FFA) concentration contributes to progression of that process. Free fatty acids express lipo-toxic effects on beta-pancreatic cells [6,22]. Cytokine action and combined cytokines and lipids action cause activation of reactive oxygen species (ROS) that can induce activation of caspases, reduction of beta-pancreatic cells secretion and progression of their apoptosis [31]. Pancreatic cells of rats reduce their secretory capability after treatment of interleukin 1-beta (IL-1 β) [28]. Apoptotic effect was even more pronounced when cells have been treated with combination of IL-1 β and TNF- α . Long term exposure of beta-pancreatic cells to TNF- α leads to their reduced secretory capacity [34]. Reduced HOMA- β value was noted in second group compared to the first group

in our research. But the lowest HOMA- β value was noted in the third group of dogs with the highest concentrations of TNF- α . This result can indicate progressive reduction of beta-pancreatic cells function caused by TNF- α and other factors.

Increase of HOMA- β index causes increase of HOMA-IR [Figure 6]. This is indication of increased peripheral insulin resistance and compensatory mechanism for that state is increased insulin secretion in order to maintain normoglycaemia. That is in accordance with general concepts of insulin resistance. HOMA-IR increases while HOMA- β decreases during insulin resistance and diabetes. That represents their inverse effects [23]. Glucose concentration will increase more during increase of HOMA-IR and decrease of HOMA- β . Dogs with highest TNF- α concentration represent this. Application of inhibitors and antibodies against TNF- α cause improvement in glucose concentrations and decrease risk of diabetes development [25,33]. This proposed that TNF- α regulates glucose concentrations directly and explains differences in glucose concentrations in dogs with different concentrations of TNF- α showed in our results.

CONCLUSION

Based on everything listed it can be concluded that circulating TNF- α can affect all aspects of insulin resistance- insulin production and resistance in peripheral tissues in euglycaemic dogs. A change in glucose concentration in function of insulin production and peripheral resistance ratio is the most unfavorable in dogs with high concentrations of circulating TNF- α in blood. That confirms that this cytokine can control glycaemia by its own independently of degree of insulin resistance in dogs.

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