

Testosterone does not influence CD40 expression in the human umbilical vein endothelial cells (HUVECs) in high glucose medium

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Introduction

- ▣ **Cardiovascular disease (CVD) is the leading cause of mortality in developed countries**
- ▣ **CVD significantly contributes to morbidity (nearly 30%): potential life years lost, common cause of premature death, labor force (economic costs), family life (deterioration of the quality of life)**

Types of Cardiovascular Disease

- ▣ **Coronary heart disease (CHD, ischemic heart disease, heart attack, myocardial infarction, angina pectoris)**
- ▣ **Cerebrovascular disease (stroke, TIA, transient ischemic attack)**
- ▣ **Hypertensive heart disease**
- ▣ **Peripheral vascular disease**
- ▣ **Heart failure**
- ▣ **Rheumatic heart disease (streptococcal infection)**
- ▣ **Congenital heart disease**
- ▣ **Cardiomyopathies**

Classification of Risk Factors of CHD

Major modifiable risk factors

- High blood pressure
- Abnormal blood lipids
- Tobacco use
- Physical inactivity
- Obesity
- Unhealthy diet
- Diabetes mellitus

Other modifiable risk factors

- Low socioeconomic status
- Mental ill health (depression)
- Psychosocial stress
- Heavy alcohol use
- Use of certain medication
- Lipoprotein(a)

Non-modifiable risk factors

- Age
- Heredity or family history
- Gender
- Ethnicity or race

"Novel" risk factors

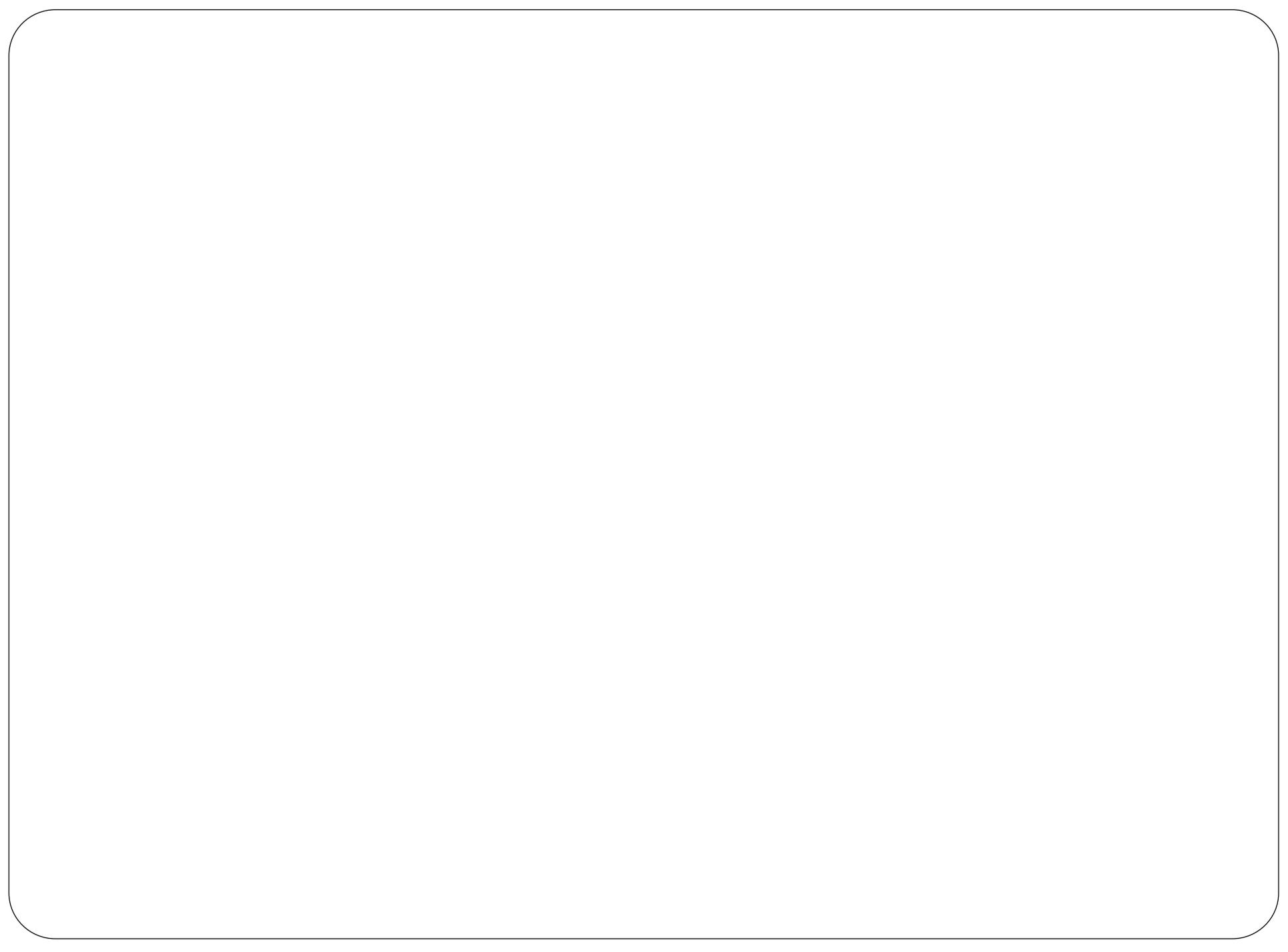
- Excess homocysteine in blood
- Inflammatory markers (C-reactive protein)
- Abnormal blood coagulation (elevated blood levels of fibrinogen)

- For many decades the endothelium was viewed simply as a semipermeable barrier between blood and interstitium.
- However, recently, it has been demonstrated that the endothelium has an enormous range of vital homeostatic functions that contribute to the normal function of the cardiovascular system.
- Endothelial dysfunction and activation play a necessary role in the development of CVD

- In endothelial activation, there are changes in the protein expression (phenotype) of the endothelial cell, such as the increase expression of ICAM-1, VCAM-1, and selectin-E.
- Those adhesion molecules will stimulate inappropriate platelet adhesion to endothelium and followed by platelet aggregation and formation of thrombus.

- Cluster of differentiation-40 molecule (CD40) is a receptor on the cell surface that is included in TNF family. CD40 was firstly identified and known to affect B lymphocytes
- Its ligand (CD40 ligand or CD40L) is identified on CD4⁺ T lymphocytes.
- Interactions CD40-CD40L plays an important role in T cell-dependent B cell activation.

- It is now known that CD40 is widely expressed on monocytes, dendritic cells, epithelial cells, endothelial cells.
- CD40L is also expressed on mast cells, basophils, eosinophils, B lymphocytes, natural killer cells, monocytes, macrophages, vascular smooth muscle cells, endothelial cells, and platelets.
- Ligation of CD40 on endothelial cells by platelets CD40L will stimulate endothelial cells and lead to the increase of ICAM-1 expression (Li et al., 2009).



- Endothelium directly has a face to face with blood, so that changes in internal milieu could affect endothelial cells homeostasis.
- Under normal condition blood glucose concentration is within 3.8 to 5.6 mM, but in conditions of disturbed homeostasis, blood glucose concentration can increase to >10 mM (hyperglycemia).

- High glucose environment can interfere with the metabolism and endothelial cell homeostasis (Popov, 2010).
- It was reported that high glucose also increases expression of adhesion molecules and endothelial pro-inflammatory cytokines (Basta et al., 2007).

Research question

- Whether CD40 expression in endothelial cells exposed to testosterone and high glucose is higher than CD40 expression in endothelial cells exposed to high glucose without testosterone?

Aims

- To compare the CD40 expression in endothelial cells exposed to testosterone and high glucose with CD40 expression in endothelial cells exposed to high glucose without testosterone.

Materials and Methods

- Study design
 - In vitro
 - Experimental study
 - Randomized
 - Post test only measurement
 - with control group

Study sample

- Endothelial cells derived from human umbilical cord
- In partu mother:
 - No hypertension, preeclampsia, infection, autoimmune
 - Delivered aterm baby , with birth weight 2500-3400g, good APGAR score, and not twin babies
 - Normal placenta and umbilical cord

Study flow chart

- Collection of umbilical cord



- Isolation of endothelial cells



- HUVEC Primary culture



- Subculture



- Treatment with high glucose & testosterone

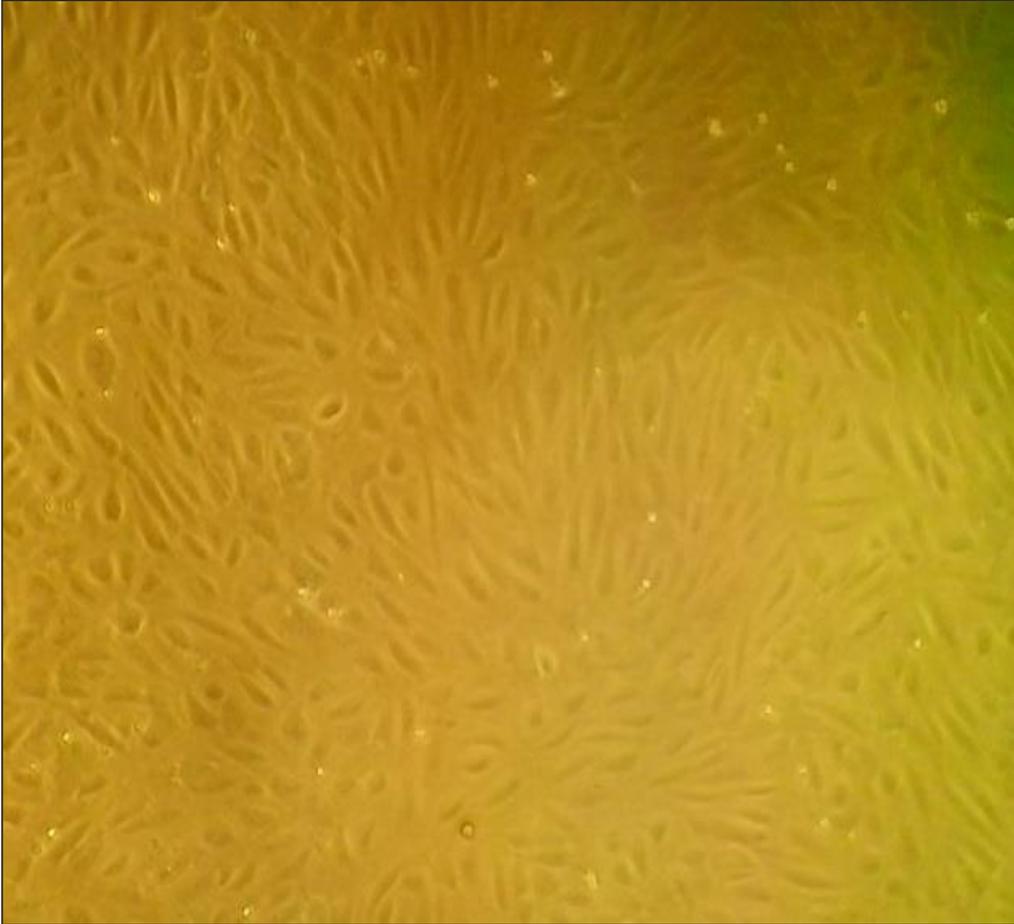
Variables

- Independent variables
 - Testosterone in 3 doses: 1 nM, 10 nM, 100 nM
- Dependent variable
 - Concentration of CD40 in lysate of endothelial cells (measured using ELISA)
- Controlled variables
 - Endothelial cells count: 1.7×10^4 cells/ group
 - High glucose medium: 22.4 nM

Data analysis

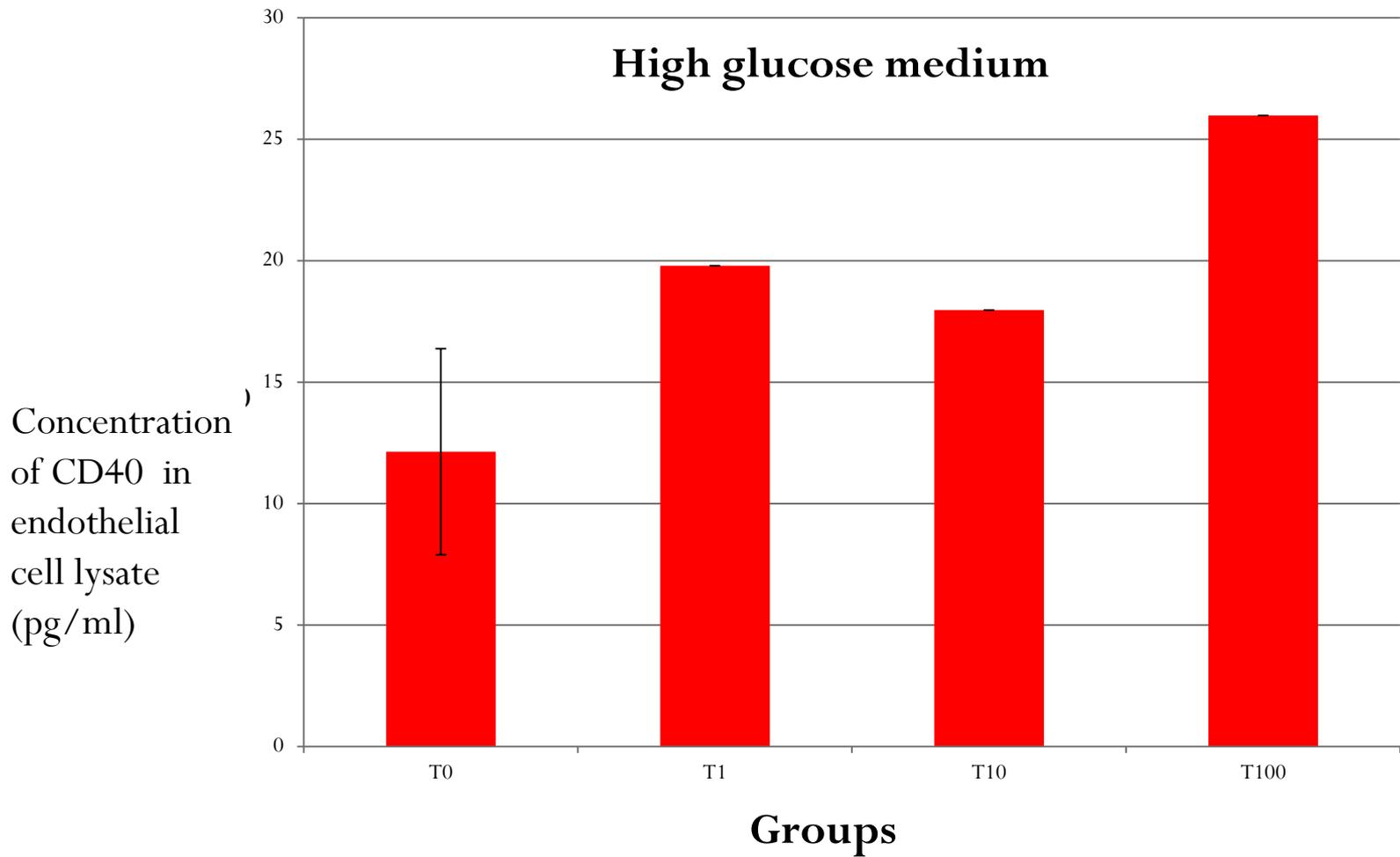
- Statistical test to test hypothesis
 - Independent t-test (multiple)
 - One-way ANOVA

Results



Subculture
of HUVEC

Results



Statistical analysis

- Multiple t-test
 - In high glucose medium, endothelial CD40 expression without testosterone (12.14 ± 4.24 pg/ml) was not significantly different to those with:
 - 1 nM testosterone (19.8 pg/ml; $p = 0.38$)
 - 10 nM testosterone (17.96 pg/ml; $p = 0.467$)
 - 100 nM testosterone (25.98 pg/ml; $p = 0.229$)
- One-way ANOVA
 - In high glucose medium, incremental doses of testosterone did not significantly influence endothelial CD40 expression ($p = 0.428$).

Discussion

- Eventhough exposure of testosterone had been observed to increase the expression of CD40 in HUVEC under condition of high glucose, but, however, the statistical analysis through multiple t-test and one-way ANOVA did not support this observation.
- Kelly (2013) mentioned that testosterone may inhibit the expression of CD40 via estrogen receptor (ER mechanism)

- Geraldes et al. (2006) showed that the increase expression of CD40 in the endothelial cells (pig) induced by IFN- γ was inhibited by estradiol, which in turn reserved by giving antagonist of ER expression.
- Given the contradictory results of our study and previous ones, these may reveal that testosterone has dual action to CD40 expression. Whether it inhibits or stimulates CD40 expression may related with which mechanism it works, i.e. through androgen receptors (AR) or estrogen receptors (ER), which is related with the condition of environment (for example: hyperglycemia)

Conclusion

- Testosterone does not influence the expression of CD40 in endothelial cells under condition of high glucose.