

# Arterial stiffness and endothelial inflammation in prediabetes and newly diagnosed diabetes patients

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## ABSTRACT

**Objective:** There is a growing body of data supporting the association between diabetes and microcirculatory dysfunction. We aimed to study e-selectin levels, and their associations with serum markers of inflammation and arterial stiffness in prediabetes and newly diagnosed diabetes patients in this study. **Subjects and methods:** Sixty patients (25 females) with a newly established elevated fasting serum glucose [20 impaired fasting glucose (IFG), 20 impaired glucose tolerance (IGT), 20 newly diagnosed diabetes (T2DM)] and 17 healthy controls (13 females) were included in the study. Serum e-selectin and hs-CRP levels, and arterial stiffness parameters of the patients were studied. **Results:** Fasting serum glucose was the most important predictor of serum e-selectin levels. Pulse wave velocity and central aortic pressures were significantly higher in IFG, IGT and T2DM groups, compared to controls ( $p = 0.001$ ,  $< 0.001$ ,  $0.013$  and  $0.015$ ,  $0.002$ ,  $0.009$ , respectively). The mean arterial pressure did not show any significant association with serum e-selectin and hs-CRP levels ( $\beta$  coefficient:  $0.092$ ,  $p = 0.358$ ; and  $\beta$  coefficient:  $0.189$ ,  $p = 0.362$ , respectively). **Conclusion:** Prediabetes patients have increasing e-selectin levels through the diagnosis of T2DM. E-selectin is associated with serum glucose levels. Prediabetic and newly diagnosed diabetics have higher arterial stiffness measurements. Serum e-selectin may be a good marker of endothelial inflammation and dysfunction increasing in parallel with serum glucose levels, predicting future cardiovascular events. Arch Endocrinol Metab. 2015;59(5):407-13

## Keywords

E-selectin; arterial stiffness; inflammation; cardiovascular risk; hypertension

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## INTRODUCTION

*Diabetes mellitus* is characterized with increased serum glucose levels deriving as a result of disturbances in insulin production or sensitivity. The prevalence of type 2 *diabetes mellitus* (T2DM) is rising rapidly and its key feature, insulin resistance, results mainly from low physical activity and obesity. In fact, the disease do not show an abrupt onset like type 1 diabetes and patients acquire T2DM after a particular time. At first, minimal high fasting blood glucose levels indicate impaired fasting glucose (IFG). Then, rising glucose levels show increasing insulin resistance and failure to lower postmeal blood glucose indicating impaired glucose tolerance (IGT). The patient is accepted to have T2DM after a particular serum glucose level. This disease course represents a spectrum, so drawing clear lines between these stages may be inaccurate, and probably the endothelial inflammation and microcirculatory dysfunction starts with the first rises in blood glucose levels (1,2).

T2DM is associated to bad cardiovascular outcomes in time (3). Inasmuch that, diabetes has been accepted as a cardiovascular disease equivalent, meaning that these patients should be accepted as having atherosclerotic disease at the time of diagnosis. The blamed underlying factors of these risks are the mentioned endothelial inflammation and microcirculatory dysfunction (4). These patients will benefit much from any intervention in the diagnosis, follow-up, and therapy. Since about a decade, a growing number of studies have reported the association between various estimates of microvascular dysfunction in T2DM and IFG (5).

Selectin family are the molecules that generate the first adhesive stage contributing to the rolling of leukocytes into the vascular endothelium for the distribution of immune cells to the tissue of inflammation (6). Soluble isoforms of e-selectin is found in the supernatant of *in vitro* cultured endothelial cells after 24 hours of cytokine activation and are composed through a largely caspase-dependent shedding process (7,8). In healthy subjects, low levels of soluble e-selectin may be

found in serum, but the levels increase in patients with an inflammatory condition. Higher concentrations of this marker is derived mainly from microcirculatory endothelium, so increased e-selectin levels reflect further microvascular endothelial dysfunction and is associated with cardiovascular disease (9,10).

Arterial stiffness measurement has high cardiovascular predictive value, reproducibility, availability and cost effectiveness in determining the future cardiovascular risk (11). Increased arterial stiffness is a common indicator for the atherosclerotic involvement of the vascular system and is known to occur as a result of atherosclerotic risk factors, such as *diabetes mellitus*, smoking, hypertension, hypercholesterolemia, and aging (12). Increased arterial stiffness is also associated with coronary artery disease, cerebrovascular disease and peripheral arterial disease (13).

In this study, we aimed to study e-selectin levels, serum markers of inflammation and their associations with arterial stiffness in prediabetes and newly diagnosed T2DM patients.

## SUBJECTS AND METHODS

### Patients

The study was conducted in a tertiary referral center. A total of 77 subjects from internal medicine and cardiology outpatient clinics were consecutively included in the study. Seventeen subjects were controls and they were entirely from healthy subjects coming to outpatient clinic for control reasons. Sixty were patients with a newly detected elevated fasting serum blood glucose level above the normal range, 100 mg/dL. Patients were stratified to have IFG, IGT or T2DM according to the American Diabetes Association (ADA) guidelines, as 100 mg/dL < [fasting serum glucose] < 126 mg/dL, 140 mg/l < [second hour serum glucose of a 75 g glucose loading test] < 200 mg/dL, and [two times fasting serum glucose > 126 mg/dL] or [serum glucose of a 2 hour 75 g glucose loading test > 200 mg/dL], respectively (14). Patients with evidence of any target organ damage from high blood pressure (BP), thyroid dysfunction, liver disease, renal failure, malignancy and other chronic diseases, inflammatory conditions, and those who were receiving any medications were excluded. The patients were asked to sit down for 10 minutes before the BP was recorded using a manual mercury sphygmomanometer. The mean

of two BP measurements was considered. The mean arterial blood pressure was calculated according to the following formula:  $MAP = DBP + ((SBP-DBP)/3)$ . A complete blood count, renal and hepatic function tests, electrolytes, serum lipid levels, and thyroid hormones of the patient and control groups were measured. The demographic parameters, antropometric measurements, blood pressures, medications and laboratory results of the participants were directly recorded in a Microsoft Excel 2010 file. All participants gave written informed consent. The study protocol was approved by The Research and Ethics Committees of Gulhane Military Medical Academy. A part of data was published elsewhere.

### Anthropometric measurements

Weight (in kg) and height (in cm) were measured and BMI was calculated as body weight/height<sup>2</sup> (kg/m<sup>2</sup>).

### Measurement of arterial stiffness

TensioMed measurement of arterial stiffness (TensioMed Ltd. Budapest-Hungary) device was used. Systolic and diastolic pressures, pulse wave velocity (PWV), augmentation index (AIX) and central aortic pressure (CAP) measurements were recorded.

### PWV, AIX and CAP analysis

TensioMed arteriography measurement device automatically reported the measurements. Measurements were made after 5 minutes rest of the patients, non smoking-or taking caffeinated beverages for at least the last 30 minutes, in the sitting position in a reserved quiet room away from external stimuli. The distance between the jugular notch and the symphysis pubis of the patients were measured and the data was recorded. During the measurement, brachial artery occlusion was made (only 8-20 seconds) and the blood flow was stopped as a part of the process. Then, the systolic pressure, diastolic pressure, mean arterial blood pressure, pulse rate, pulse pressure, PWV, AIX and CAP were taken from the device. The pressure waves were recorded along with the AIX, PWV and CAP values.

### Blood sample collection and analysis of parameters

All blood samples were drawn in the morning after at least 10 h of fasting. Routine laboratory tests and biochemistry including a full blood count, serum glucose, urea, creati-

nine, sodium, potassium, liver enzymes were performed during the outpatient visit. Serum samples for e-selectin were run in the same assay. These samples were promptly centrifuged, the plasma and serum were separated and stored at  $-80^{\circ}\text{C}$ . Fasting plasma glucose, total cholesterol (TC), triglyceride and high-density lipoprotein cholesterol (HDL-C) levels were measured by an enzymatic colorimetric method with an Olympus AU2700 auto analyzer using reagents from Olympus Diagnostics (GmbH, Hamburg, Germany). Low-density lipoprotein cholesterol (LDL-C) was calculated by Friedewald's Formula (15). ELISA kits were used according to the manufacturer's instructions (Usclife Science & Technology Co., Ltd, Wuhan, China) to determine e-selectin levels. We measured all samples in duplicates and used the mean values. hs-CRP was measured in serum by high sensitive C-reactive protein (hs-CRP) ELISA Kit (Cell Biolabs, Inc, San Diego, USA).

### Statistical analysis

Statistical analysis was carried out using IBM SPSS Statistics for Windows, Version 22.0. Armonk, NY: IBM Corp. Independent samples *t* test, Chi-square test, Mann-Whitney U statistical analysis, Pearson-rho correlation, univariate analysis and linear regression analysis tests were used. Quantitative variables were expressed as mean  $\pm$  standard deviation. The Kolmogorov-Smirnov and Shapiro Wilkins tests were used to determine the distribution characteristics of variables and Levene's test was used to determine the equality of variance. Differences between groups were studied for significance by independent samples *t*-test as appropriate. Categorical variables were compared by Chi-square test. Pearson and Spearman correlation analysis were used to evaluate the relationship between variables. Univariate analysis was used to adjust the effects of covariates on variables. Results of the analysis were expressed as percent for qualitative variables and mean  $\pm$  standard deviation for continuous variables. A two-sided  $p < 0.05$  was considered significant.

## RESULTS

### General characteristics of the patients

A total of 60 patients with a newly established elevated fasting serum glucose (25 females) and 17 healthy controls (13 females) were included in the study. The mean ages were  $50.4 \pm 12.1$  and  $40.2 \pm 15.1$  years, respectively, for patient and control groups ( $p = 0.007$ ). Of patients, the mean age was  $49.5 \pm 16.1$  years in males ( $n = 35$ ) and

$51.6 \pm 13.7$  years in females ( $n = 25$ ) ( $p = 0.592$ ). The mean BMI's of the male and female patients were  $27.2 \pm 4.7$  and  $28.9 \pm 4.5$ , respectively ( $p = 0.158$ ). There was no significant difference in terms of age and BMI between male and female patients. The patient group were divided into three groups according to ADA criteria, considering the fasting serum glucose measurements, as IFG, IGT and newly diagnosed T2DM groups (Table 1). The IFG and IGT groups were not age and BMI-matched to the control group, but T2DM group was the same as control group in terms of these parameters. There were differences in terms of blood pressure measurements between groups ( $p < 0.001$ ). The T2DM group did not match in terms of sex to the control group ( $p = 0.001$ ).

### Arterial stiffness measurements

PWV and CAP measurements were significantly higher in IFG, IGT and T2DM groups compared to controls (Table 1). Aix measurements were similar in all groups. There was only a weak negative correlation between e-selectin levels and Aix in the patient group (Pearson,  $r = -0.304$ ,  $p = 0.021$ ).

### Laboratory findings

The laboratory findings are shown in table 1. There was no statistically significant difference in terms of LDL, total cholesterol, LDL, creatinine levels between groups. Fasting serum glucose levels were 88.8, 112.1, 115.8, and 223.8 mg/dL, respectively, for controls, IFG, IGT and T2DM groups ( $p < 0.001$ , for each groups). The mean e-selectin levels were significantly higher in IGT, IFG and T2DM groups compared to controls. In regression analysis, e-selectin levels were significantly associated with fasting serum glucose, hs-CRP levels, sex, BMI and age (Table 2). Fasting serum glucose was the most important predictor of e-selectin levels. The mean arterial pressure did not show any significant association with serum e-selectin levels and was excluded by the regression analysis ( $\beta$  coefficient: 0.092,  $p = 0.358$ ). Also, the mean arterial pressure did not have any influence on hs-CRP levels ( $\beta$  coefficient: 0.189,  $p = 0.362$ ). So, the intergroup blood pressure differences were disregarded. Charts showing the association between e-selectin levels, fasting serum glucose and hs-CRP levels in the entire group are given in figures 1 and 2. After adjustment for age, BMI, sex, and fasting blood glucose, e-selectin levels were increasing through the initiation of T2DM and are shown in figure 3.

**Table 1.** Comparisons of the IFG, IGT and newly diagnosed T2DM groups to controls

Parameter/Study group	Control	IFG	p	IGT	p	T2DM	p
n	17	20	0.742*	20	0.742*	20	0.742*
Age (yrs)	40.24 ± 12.05	49.45 ± 9.68	0.017	53.25 ± 12.88	0.003	48.45 ± 20.82	0.145
Sex (F) (%)	13 (76)	9 (45)	0.053*	12 (60)	0.239*	4 (20)	0.001*
BMI (kg/m <sup>2</sup> )	25.28 ± 3.67	28.93 ± 3.51	0.004	28.71 ± 4.62	0.016	26.12 ± 5.33	0.581
Smokers (n) (%)	4 (24)	8 (40)	0.373*	9 (45)	0.264*	8 (40)	0.373*
Systolic blood pressure (mmHg)	105.29 ± 11.92	134.15 ± 19.9	< 0.001	135.15 ± 16.89	< 0.001	136.45 ± 12.57	< 0.001
Diastolic blood pressure (mmHg)	64.41 ± 7.26	81.1 ± 10.13	< 0.001	77.9 ± 7.66	< 0.001	78.85 ± 11.3	< 0.001
MAP (mmHg)	78.04 ± 8.15	98.78 ± 12.99	< 0.001	96.98 ± 9.48	< 0.001	98.05 ± 10.78	< 0.001
PR (beats/min)	72.17 ± 10.85	74.95 ± 10.32	0.482	77.9 ± 10.36	0.155	77.25 ± 16.84	0.308
Pulse wave velocity (m/sec)	7.12 ± 1.15	8.71 ± 1.18	0.001	9.59 ± 1.89	< 0.001	8.83 ± 2.4	0.013
Central aortic pressure (mmHg)	108.99 ± 16.48	126.02 ± 19.38	0.015	131.55 ± 19.89	0.002	126.53 ± 16.88	0.009
Augmentation index (%)	18.68 ± 15.12	24.04 ± 17.29	0.372	27.53 ± 14.65	0.119	19.31 ± 19.73	0.922
E-selectin levels (ng/mL)	43.76 ± 18.51	73.58 ± 47.51	0.016	81.15 ± 50.41	0.005	139.84 ± 75.91	< 0.001
Serum hs-CRP (mg/dL)	1.28 ± 0.99	2.98 ± 2.45	0.009	4.5 ± 4.32	0.004	3.11 ± 3.85	0.053
Fasting serum glucose (mg/dL)	89.59 ± 8.1	112.1 ± 6.98	< 0.001	115.85 ± 16.22	< 0.001	223.85 ± 148.44	0.001
Total cholesterol (mg/dL)	193.82 ± 30.43	209.12 ± 40.41	0.222	196.6 ± 46.26	0.828	218.17 ± 44.84	0.069
LDL (mg/dL)	116.94 ± 24.3	121.88 ± 39.27	0.67	120.65 ± 36.62	0.715	131.17 ± 40.85	0.218
Serum triglyceride (mg/dL)	104 ± 55	179 ± 121	0.029	175 ± 122	0.034	217 ± 109	0.001
Serum HDL (mg/dL)	56 ± 10	50 ± 16	0.286	47 ± 14	0.046	46 ± 11	0.018

The comparisons of the demographic parameters, anthropometric measurements, arterial stiffness parameters and laboratory results of the impaired fasting glucose (IFG), impaired glucose tolerance (IGT) and newly diagnosed T2DM groups to controls, indicated by p values. F: female, BMI: body mass index, MAP: mean arterial pressure, PR: pulse rate, hs-CRP: high sensitive CRP, LDL: low density lipoprotein cholesterol, HDL: high density lipoprotein cholesterol. The *italic* p values refer to significant group differences compared to controls. \* Chi-square test, otherwise independent samples t test.

**Table 2.** Parameters associated to e-selectin levels in patients with a newly detected elevated serum glucose

Parameter	β	p
Fasting serum glucose (mg/dL)	.456	0.000
hs-CRP (mg/dL)	.216	0.023
Sex (M:1)	.342	0.000
BMI (kg/m <sup>2</sup> )	.304	0.002
Age (yrs)	-.255	0.005

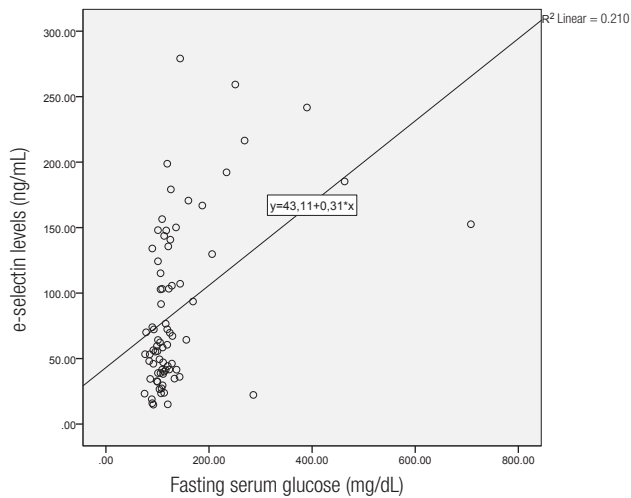
hs-CRP: high sensitive C-reactive protein; M: male; BMI: body mass index; β: standardized coefficient; p: the level of significance.

## DISCUSSION

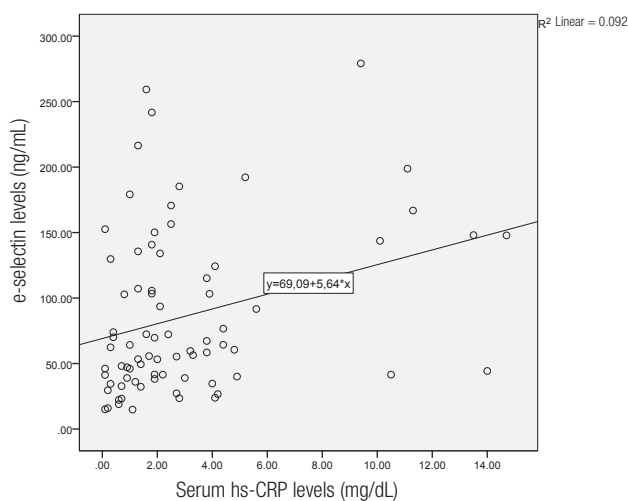
In this study, it has been found that e-selectin levels increase in prediabetes patients, consistently with rising glucose levels, through the diagnosis of T2DM. The fasting glucose levels are the most powerful predictor of e-selectin levels. Also, a significant association exists with serum hs-CRP levels. These data provide an evidence that diabetes candidates have an increasing endothelial dysfunction (ED) and inflammation with rising blood glucose levels, through the diagnosis of T2DM. ED is a mostly recognized part of macrovascular and

microvascular disease complications in diabetes (16). ED may also precede diabetes onset and may promote insulin resistance and glucose dysregulation leading to diabetes, but there is still controversy about the exact role of ED in patients with prediabetes and diabetes (17,18).

ED and atherosclerosis play an important role in the pathogenesis of end organ damage. The endothelial layer has many functions, such as homeostasis, vascular permeability, vasoregulation, angiogenesis and inflammatory response (19). It is widely accepted that ED is the atherosclerosis precursor. Previously, flow-mediated dilatation has been investigated as a gold standard indicator of endothelial damage and dysfunction (20). Lately, investigators focused on non-invasive methods in defining ED and atherosclerosis, such as arterial stiffness measurements and biomarkers. Arterial stiffness has high cardiovascular predictive value, reproducibility, cost effectiveness and availability in many healthcare facilities and it is suggested as an additional test for the evaluation of hypertensive patients according to the current guidelines (11).

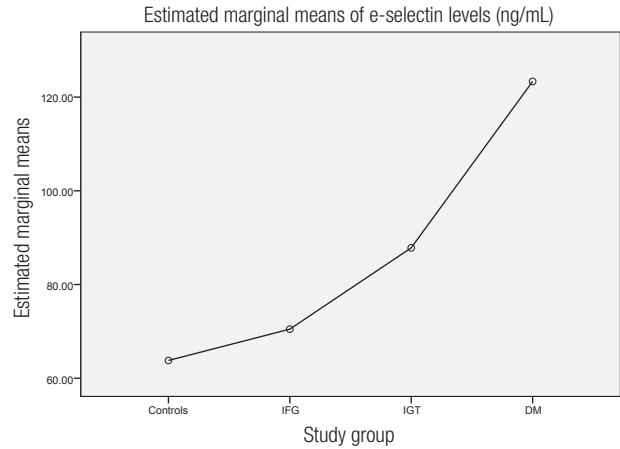


**Figure 1.** Chart showing the association between fasting serum glucose and e-selectin levels in the entire group.



**Figure 2.** Chart showing the association between serum e-selectin levels and hs-CRP in the entire group.

E-selectin is also known as CD62E or endothelial leukocyte adhesion molecule-1 (ELAM1). It is a type I membrane protein belonging to the surface molecules of the selectin family (CD62P and CD62L). Selectins are C type cell surface lectins playing a role in leukocyte adhesion to the endothelium of the vessel wall. CD62E (E-selectin) is an endothelial cell specific selectin expressed on cytokine induced endothelial cells, but only after a proinflammatory cytokine led activation (8,10). TNFalpha, IL1, and some bacterial components like lipopolysaccharides trigger the transcription of CD62E in an NFkB dependent signal cascade. CD62E is associated with endothelium of the blood vessel in diffe-



Covariates appearing in the model are evaluated at the following values: Age = 47,8553, Sex (M) = .5132, BMI = 27,3552, Fasting serum glucose (mg/dL) = 137,4079.

**Figure 3.** The chart representing mean e-selectin levels in controls, impaired fasting glucose (IFG), impaired glucose tolerance (IGT) and newly diagnosed T2DM groups after adjustment for serum glucose, BMI, age and sex.

rent inflammatory situations. Previously published data have demonstrated that e-selectin is a proatherogenic and proinflammatory cytokine associated with insulin resistance, obesity, and cardiovascular disease (21). In a previous study, it has been suggested that circulating e-selectin concentration may be a biomarker for indicating subsequent development of metabolic diseases and in particular cardiovascular diseases from a healthy state (22). Higher levels of endothelial inflammation may also be associated to the chronic complications of *diabetes mellitus*. In a recent study by Sasongko and cols. it has been mentioned that higher CRP levels, but not markers of endothelial function, may be related to more severe diabetic retinopathy suggesting that inflammatory processes are involved in severe diabetic retinopathy (23). Our findings indicate that prediabetes patients have higher e-selectin levels as their serum glucose rise, and it looks as they get highest with the diabetes diagnosis. This is compatible with a hypothesis of rising endothelial inflammation with progression to *diabetes mellitus*.

In fact, hypertension is a condition with endothelial dysfunction and inflammation and this has been documented in previous studies (24,25). It may be expected that the rising blood pressure levels in our prediabetes and diabetes groups are responsible from the increase in e-selectin levels. But the regression analysis did not reveal a significant association between e-selectin levels; arterial blood pressure was excluded in terms of any significant effect on e-selectin levels by regression

analysis; so we could not show any association between blood pressure and e-selectin levels, which is a good marker to show endothelial inflammation. This may perhaps be due to any “covering effect” of diabetes, leading to higher levels of endothelial inflammation than hypertension, so the effect of increase in blood pressure on e-selectin levels is lower than the increase in blood glucose in this patient group. Also, there may be factors influencing e-selectin levels in diabetics rather than endothelial inflammation and this may be another mechanism to make these results.

Our study did not find any significant association between e-selectin levels and arterial stiffness parameters except Aix measurements. In fact, higher Aix measurements represent higher cardiovascular risk of these patients, and we may expect a positive correlation between these two parameters. This still carries a controversy. However, in a study by Llauro and cols., impaired endothelial function was not associated with arterial stiffness in adults with type 1 diabetes, and they suggested that endothelial dysfunction occurs earlier than arterial stiffness in type 1 diabetes (26). This may also be due to our previously called thesis, the covering effect, than blood glucose levels. Further studies are needed to reveal these associations.

Our study has some limitations. First, our study sample is relatively small. Secondly, we had difficulties in matching groups in terms of demographic and some laboratory parameters, so had difficulties in discussing the results. Thirdly, the differences in blood pressure measurements may be a challenge, but we neglected the effect of blood pressure on regression analysis. Fourth, about the newly diagnosed diabetic term, the patients have come to our outpatient clinics for control reasons or examination for other disease or conditions. We do not exactly know how long they are diabetic, or have IFG or IGT. This may have caused some disturbances in our observations.

## CONCLUSION

Our results show that prediabetes patients have increasing e-selectin levels, which is a marker showing endothelial inflammation, through the diagnosis of T2DM. e-selectin levels are significantly correlated with fasting serum glucose levels. Prediabetic and newly diagnosed diabetics have higher arterial stiffness measurements. E-selectin and hs-CRP levels are not influenced by blood pressure and arterial stiffness parameters. These

results suggest that e-selectin may be a good marker of endothelial inflammation and dysfunction increasing in parallel with serum glucose levels in prediabetes and newly diagnosed diabetes patients.

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