

**SERUM HSP27 AND DIABETIC COMPLICATIONS IN THE EURODIAB  
PROSPECTIVE COMPLICATIONS STUDY: A NOVEL CIRCULATING MARKER  
FOR DIABETIC NEUROPATHY**

Gabriella Gruden, MD, PhD<sup>1</sup>, Graziella Bruno, MD<sup>1</sup>, Nish Chaturvedi, MRCP<sup>2</sup>, Davina Burt, PhD<sup>1</sup>, Casper Schalkwijk, PhD<sup>3</sup>, Silvia Pinach, MSc<sup>1</sup>, Coen D. Stehouwer, MD, PhD<sup>3</sup>, Daniel R Witte, PhD<sup>4</sup>, John H. Fuller, FRCP<sup>4</sup>, Paolo Cavallo Perin, MD<sup>1</sup>: EURODIAB Prospective Complications Study Group.

**RUNNING TITLE: HSP27 and Type 1 Diabetes Complications**

<sup>1</sup>Department of Internal Medicine, University of Turin, Italy,

<sup>2</sup>National Heart & Lung Institute, Imperial College, London, UK

<sup>3</sup>Department of Internal Medicine, University Hospital Maastricht, the Netherlands

<sup>4</sup>Department of Epidemiology and Public-Health, Royal Free and University College London, Medical School, UK.

**Corresponding Author**

Gabriella Gruden  
Department of Internal Medicine  
University of Turin  
Corso AM Dogliotti 14  
Turin, 10126, Italy  
Tel: 0039 663 6448  
Fax: 0039 633 4751  
Email: gabriella.gruden@unito.it

Received for publication 04 January 2008 and accepted in revised for 28 March 2008.

*OBJECTIVE.* Heat shock protein 27 (HSP27) is a member of the small heat shock protein family of proteins. HSP27 expression is enhanced in target tissues of diabetic microvascular complications and changes in circulating HSP27 levels (sHSP27) have been reported in patients with macrovascular disease. We investigated whether sHSP27 levels were associated with micro- and macrovascular complications in type 1 diabetic patients.

*RESEARCH DESIGN AND METHODS.* A cross-sectional nested case-control study from the EURODIAB Prospective Complications Study of 531 type 1 diabetic patients was performed. Cases (n=363) were defined as those with one or more complications of diabetes; control subjects (n=168) were all those with no evidence of any complication. We measured sHSP27 levels and investigated their associations with diabetic complications.

*RESULTS.* Mean sHSP27 levels were significantly higher in cases with distal symmetrical polyneuropathy (DSP) than in controls, even after adjustment for age and AER (785.9 vs 574.7 pg/ml,  $p=0.03$ ). In logistic regression analysis, sHSP27 levels in the upper quartile were associated with a twofold increased odds ratio of DSP, independently of conventional risk factors, markers of inflammation, and AER (OR=2.41, 95% CI 1.11-5.24).

*CONCLUSION.* In this large cohort of type 1 diabetic subjects, we found an independent association between sHSP27 and DSP. This suggests that sHSP27 levels may be a novel marker for diabetic neuropathy.

**H**eat shock protein 27 (HSP27), a member of the small heat shock protein family of proteins, is a highly conserved peptide of approximately 27 kDa associated with cytoskeletal actin (1). In addition to its chaperone activity, HSP27 acts as a filament stabilizer under stress conditions, interferes with apoptotic pathways, and participates in cytoskeletal dynamics by controlling actin polymerization (2). Therefore, HSP27 plays an important role in both cytoprotection and cell motility.

Recent studies in experimental diabetes have shown HSP27 overexpression in the glomeruli (3), the dorsal root ganglia (4,5), the retina (6), and in the area adjacent to the atherosclerotic plaque (7), indicating HSP27 induction in target tissues of diabetic complications. HSP27 is also released into the circulation (8). A pilot study has shown reduced plasma HSP27 levels in patients with carotid stenosis (9), but in a more recent study HSP27 levels were increased in patients with acute coronary syndromes (7). No large study is, however, yet available on circulating HSP27 in vascular disease.

Type 1 diabetes is associated with a greatly increased risk of vascular complications, which cannot be completely accounted for by conventional risk factors. The aim of the present study was to assess whether high serum HSP27 (sHSP27) levels increased odds ratios of micro/macrovascular complications in a nested case-control sample of type 1 diabetic individuals from the EURODIAB Prospective Complications Study.

## **METHODS**

The EURODIAB Prospective Complications Study (PCS; 1997-1999) is a follow up of the EURODIAB IDDM Complications Study (1989-1991), which was designed to explore risk factors for diabetic complications in 3250 randomly selected people with type 1 diabetes, aged 15–60 years, attending 31 diabetic centres in 16 European countries (10,11).

A cross-sectional nested case-control study was designed at the 1997–1999 follow-up examination (12-15). The response rate at follow up examination was 57.8% (16). Cases were selected to have the greatest complication burden as possible to provide sufficient numbers for subgroup analyses. Thus, cases were all those with cardiovascular disease or proliferative retinopathy or micro-macroalbuminuria at follow-up. Control subjects were selected to be completely free of complications. This design allowed us to compare individuals with single or multiple complications with individuals free of complications, according to the study question, as efficiently as possible. Applying these criteria, this yielded 363 cases and 168 controls with full data on complications and samples available for analysis. The sample size provides power of 95% and of 80% ( $\alpha=0.05$ ), respectively, to detect a difference in log-HSP27 of at least one third of a standard deviation between all cases and controls and between cases with single complications and controls.

Patient evaluation for the presence of cardiovascular risk factors (hypertension, body mass index, waist to hip ratio, smoking, cholesterol, triglycerides, HbA<sub>1c</sub>) is described elsewhere (12-15). Retinopathy was graded according to the EURODIAB protocol (17). Albumin excretion rate (AER), assessed on two 24-h urine collections by immunoturbidimetric method, was categorised as normoalbuminuria (<20 µg/min), microalbuminuria (20-200 µg/min), and macroalbuminuria (≥200 µg/min). Cardiovascular disease (CVD) was defined as physician diagnosed myocardial infarction, angina, coronary artery bypass graft, or stroke and/or ischemic changes on centrally Minnesota-coded electrocardiogram. Distal symmetrical polyneuropathy (DSP) was diagnosed on the basis of: (i) presence of one or more neuropathic symptoms; (ii) absence of two or more ankle or knee reflexes; and (iii) abnormal vibration perception threshold,

measured by centrally calibrated biesthesiometers (Biomedical, Newbury, Ohio, USA) on the right big toe and on the right medial malleolus.

Soluble vascular cell adhesion molecule (sVCAM-1), soluble E-selectin (s-E-selectin), IL6, and TNF- $\alpha$  were measured by commercially available ELISA (R&D Systems, Oxon, UK); plasma levels of C-reactive protein (CRP) and Amadori-albumin by in-house ELISA (12-13). Plasma homocysteine was determined with an automated fluorescence polarization immunoassay on an Abbott IMx analyzer (Abbott Laboratories, IL, USA) (18). sHSP27 levels were measured by ELISA (Calbiochem San Diego, CA, USA). Briefly, 96-well plates were pre-coated with a mouse anti-human HSP27, used as capture antibody. Then, both HSP27 standards and samples, together with a rabbit polyclonal detection antibody specific for human HSP27, were simultaneously incubated in the pre-coated wells. After washing, a goat anti-rabbit IgG conjugated to horseradish peroxidase was added. Reaction was revealed by 3,3',5,5'-tetramethylbenzidine dihydrochloride substrate and stopped with sulphuric acid. The absorbance was read at 450 nm and HSP27 levels determined by comparing the absorbance of samples with the values obtained from the standard curve. The range of the assay was 20-1000 pg/ml and intra- and inter-assay coefficients of variations were 4.4% and 8.5-9% for both low (132 pg/ml) and high (993 pg/ml) range HSP27 levels. Samples were coded and tested blind.

#### *Statistical Analyses*

Variables distributed normally are presented as mean and standard deviation (SD), whereas variables with skewed distribution were analyzed after logarithmic transformation (triglycerides, AER, creatinine, CRP, IL-6, TNF- $\alpha$ , sVCAM, s-E-selectin, homocysteine, HSP27) and results presented as geometric means and interquartile range. Logistic regression analyses was employed to estimate the odds

ratios (ORs) of HSP27 for any complication (AER $\geq$ 20  $\mu$ g/min, retinopathy, neuropathy, CVD), independently of confounders and known risk factors. Both backward and forward strategies examining all explanatory variables were employed to select models. The likelihood ratio test was used to compare nested models examining the role of age, sex, diabetes duration, BMI, WHR, HbA<sub>1c</sub>, blood pressure, lipids, AER, CRP, IL-6, TNF- $\alpha$ , homocysteine, Amadori albumin, s-E-selectin, sVCAM, smoking. Analyses were hypothesis oriented and did not use stepwise regression (19). Variables were retained in the final model if they added significantly to the likelihood of models or to the estimated coefficients of predictors. In the light of the hypothesis of a different role of HSP27 in the pathogenesis of different complications, logistic regression models were also fitted separately for each complication. To assess pattern of odds ratio across increasing HSP27 values, they were categorized by the quartile distribution in controls. We tested for linear trends across quartiles by entering a single ordinal term into the models. When ORs in the lower quartiles of HSP27 were similar, they were aggregated as the reference category in the final analysis and compared with the upper quartile.

## **RESULTS**

The study population (n=531) had a mean age of 39.6 years, a diabetes duration of 21.5 years and an equal proportion of men and women. Those with vascular complications had a more adverse risk factor profile than control individuals (Table 1). Of the 363 cases, nephropathy was present in 206 (22.6% micro- and 34.3% macroalbuminuria), retinopathy in 292 (background 39.1% and proliferative 41.3%), DSP in 205 (56.5%), and autonomic neuropathy in 118 (27.6%). Most people, however, had more than one complication; indeed, 187 (51.5%) individuals had both AER $\geq$ 20  $\mu$ g/min and retinopathy; 128 (35.3%) had both AER $\geq$ 20  $\mu$ g/min and DSP;

123 (33.9%) had  $AER \geq 20$   $\mu\text{g}/\text{min}$ , DSP and retinopathy. CVD was present in 146 subjects (40.2%), all of them having also at least one microvascular complication, apart from 12 individuals who had CVD only.

HSP27 was measurable in all the 531 samples, with right skewed distribution of values (Table 1). sHSP27 levels were not significantly different in cases and controls even after adjustment for age (670.9  $\text{pg}/\text{ml}$  vs 548.8,  $p=0.08$ ). With respect to controls, however, we found significantly greater age-adjusted HSP27 levels in cases with DSP ( $p=0.002$ ) and in cases with micro-macroalbuminuria ( $p=0.03$ ). Although sHSP27 levels were also slightly higher in cases with retinopathy ( $p=0.06$ ), this was mainly due to the confounding association with AER as values became similar after further adjustment for AER ( $p=0.57$ ). On the contrary, the difference between cases with DSP and controls was significant even after further adjustment for AER (785.9 vs 574.7  $\text{pg}/\text{ml}$ ,  $p=0.03$ ). No difference was found between cases with CVD and controls.

We then performed logistic regression analyses to assess whether higher values of HSP27 conferred increased odds ratio of having any complication, independently of main risk factors. Models performed in all subjects and separately for each complication showed a tendency towards a negative confounding effect of both age and diabetes duration (increasing ORs from model 1 to model 2) and a positive confounding effect of  $\text{HbA}_{1c}$ , hypertension, smoking, and  $\text{TNF-}\alpha$  (decreasing ORs from models 2 to models 3). In the fully adjusted model, a significant linear trend of ORs across quartiles of HSP27 was evident for DSP ( $p=0.03$ ), whereas a significant linear trend for micro-macroalbuminuria and retinopathy was present exclusively in the age- and duration-adjusted model (model 2) (Table 2).

HSP27 values in the upper quartile ( $>1135$   $\text{pg}/\text{ml}$ ) conferred a 38% increased OR (95% CI 0.77-2.49) of any complications as compared to HSP27 values

in the lower quartiles ( $\leq 1135$   $\text{pg}/\text{ml}$ ). Final models, performed separately for each complication, showed that higher HSP27 values were associated with a more than twofold increased odd ratio of DSP, which was statistically significant (OR=2.45, 95% CI 1.20-5.03), even after further adjustment for AER values (OR=2.41, 95% CI 1.11-5.24). ORs for other complications were increased in the upper vs lower quartiles, but they did not reach statistical significance (Table 2). Study centre did not contribute significantly to the final model, nor did it modify estimated ORs.

## DISCUSSION

In this cross-sectional sample of type 1 diabetic patients from the EURODIAB Prospective Complications Study, we have provided the first evidence of an independent association between sHSP27 levels and DSP.

Mean sHSP27 levels were significantly higher in cases with DSP than in controls, even after adjustment for age and AER. Furthermore, in logistic regression analysis higher circulating HSP27 levels conferred a twofold increased odds ratio of DSP, independently of conventional risk factors, markers of inflammation, and AER. The lack of circulating markers for DSP represents an important limit of clinical research in this field; therefore, our findings may be of potential clinical relevance. Availability of a surrogate marker of DSP, which can be easily and non-invasively obtained, may facilitate diagnosis, measurement of progression, and assessment of therapeutic interventions.

The rise in circulating HSP27 expression in patients with DSP may result from neuronal overexpression. Consistently with this hypothesis, studies in experimental diabetes have shown HSP27 induction in the sensory neurons of the dorsal root ganglia (4,5). Intracellular HSP27, a key survival factor for neurons, plays an important role in

axonal regeneration (20) and mutations of the *HSPB1* gene encoding for HSP27 cause inherited distal peripheral neuropathies, such as hereditary distal motor neuropathy and Charcot-Marie-Tooth disease type 2 (21). The mechanism of HSP27 neuroprotection is unclear, but preservation of the cytoskeletal stability and both chaperone-like and anti-apoptotic activities have been implicated (22). In diabetic patients with DSP overexpression of HSP27 may, thus, be aimed to counteract the neurological damage caused by the diabetic milieu. On the other hand, HSP27 release can also contribute to the neuronal damage as anti-HSP27 autoantibodies, which are produced in response to extracellular HSP27 exposure, can induce neuronal apoptosis (23).

There are certain limitations to our study. Firstly, this is a cross-sectional study and this restricts our ability to assess temporal relationships between sHSP27 levels and microvascular complications and to identify causal biological mechanisms underlying this association. However, no data on HSP27 in large groups of type 1 diabetes patients exist; therefore this study may serve as a reasonable starting point to explore the role of this molecule in type 1 diabetes. Secondly, the number of controls was lower than the overall number of cases, thus reducing the power of analyses; comparisons between controls and cases with single complications allowed a more favorable case/control ratio, but multiple comparisons within the same case-control study base might have caused significant results due to chance. Thirdly, although serum samples were adequately stored, the possibility of protein degradation cannot be excluded; however, random misclassification would have biased downward our estimates, without affecting significant associations. Unlike previous studies, a key strength here is the ability to account for confounding by other risk factors and complications, and the large sample size provides sufficient power for these analyses. In addition, our patients were

from a representative sample of people with type 1 diabetes across Europe, and our results, therefore, are likely to be generalisable.

In conclusion, this is the first study measuring sHSP27 in a large group of subjects and our results provide evidence that sHSP27 levels are independently associated with DSP in type 1 diabetic patients. Further studies are required to determine causal relationships and elucidate underlying mechanisms.

#### **ACKNOWLEDGMENTS**

This work was supported by the “Compagnia di San Paolo”, the Piedmont Region, and the University of Turin.

## REFERENCES

1. Welsh MJ, Gaestel M. Small heat-shock protein family: function in health and disease. *Ann N Y Acad Sci.* 1998; 851:28-35.
2. Arrigo AP. The cellular "networking" of mammalian Hsp27 and its functions in the control of protein folding, redox state and apoptosis. *Adv Exp Med Biol.* 2007; 594:14-26.
3. Dunlop ME, Muggli EE. Small heat shock protein alteration provides a mechanism to reduce mesangial cell contractility in diabetes and oxidative stress. *Kidney Int.* 2000; 57:464-75.
4. Kamiya H, Zhangm W, Sima AA. Apoptotic stress is counterbalanced by survival elements preventing programmed cell death of dorsal root ganglions in subacute type 1 diabetic BB/Wor rats. *Diabetes.* 2005; 54:3288-95.
5. Zochodne DW, Verge VM, Cheng C, Sun H, Johnston J. Does diabetes target ganglion neurones? Progressive sensory neurone involvement in long-term experimental diabetes. *Brain.* 2001; 124:2319-34.
6. Jousen AM, Huang S, Poulaki V, Camphausen K, Beecken WD, Kirchhof B, Adamis AP. In vivo retinal gene expression in early diabetes. *Invest Ophthalmol Vis Sci.* 2001; 42:3047-57.
7. Park HK, Park EC, Bae SW, Park MY, Kim SW, Yoo HS, Tudev M, Ko YH, Choi YH, Kim S, Kim DI, Kim YW, Lee BB, Yoon JB, Park JE. Expression of heat shock protein 27 in human atherosclerotic plaques and increased plasma level of heat shock protein 27 in patients with acute coronary syndrome. *Circulation.* 2006; 114:886-93.
8. De AK, Roach SE. Detection of the soluble heat shock protein 27 (hsp27) in human serum by an ELISA. *J Immunoassay Immunochem.* 2004; 25:159-70.
9. Martin-Ventura JL, Duran MC, Blanco-Colio LM, Meilhac O, Leclercq A, Michel JB, Jensen ON, Hernandez-Merida S, Tunon J, Vivanco F, Egido J. Identification by a differential proteomic approach of heat shock protein 27 as a potential marker of atherosclerosis. *Circulation.* 2004; 110:2216-9.
10. The EURODIAB IDDM Complications Study Group: Microvascular and acute complications in IDDM patients: the EURODIAB IDDM Complications Study. *Diabetologia.* 1994; 3: 278-85.
11. Chaturvedi N, Sjoelie AK, Porta M, Aldington SJ, Fuller JH, Songini M, Kohner EM; EURODIAB Prospective Complications Study. Markers of insulin resistance are strong risk factors for retinopathy incidence in type 1 diabetes. *Diabetes Care.* 2001; 24: 284-9.
12. Chaturvedi N, Schalkwijk CG, Abrahamian H, Fuller JH, Stehouwer CD; EURODIAB Prospective Complications Study Group. Circulating and urinary transforming growth factor beta1, Amadori albumin, and complications of type 1 diabetes: the EURODIAB prospective complications study. *Diabetes Care.* 2002; 25:2320-7.
13. Schram MT, Chaturvedi N, Schalkwijk C, Giorgino F, Ebeling P, Fuller JH, Stehouwer CD; EURODIAB Prospective Complications Study. Vascular risk factors and markers of endothelial function as determinants of inflammatory markers in type 1 diabetes: the EURODIAB Prospective Complications Study. *Diabetes Care.* 2003; 26: 2165-73.
14. Schram MT, Schalkwijk CG, Bootsma AH, Fuller JH, Chaturvedi N, Stehouwer CD; EURODIAB Prospective Complications Study Group. Advanced glycation end

- products are associated with pulse pressure in type 1 diabetes: the EURODIAB Prospective Complications Study. *Hypertension*. 2005; 46:232-7.
15. Schram MT, Chaturvedi N, Schalkwijk CG, Fuller JH, Stehouwer CD; EURODIAB Prospective Complications Study Group. Markers of inflammation are cross-sectionally associated with microvascular complications and cardiovascular disease in type 1 diabetes: the EURODIAB Prospective Complications Study. *Diabetologia*. 2005; 48:370-8.
  16. Giunti S, Bruno G, Lillaz E, Gruden G, Lolli V, Chaturvedi N, Fuller JH, Veglio M, Cavallo-Perin P; EURODIAB IDDM Complications Study Group. Incidence and risk factors of prolonged QTc interval in type 1 diabetes: the EURODIAB Prospective Complications Study. *Diabetes Care*. 2007; 30:2057-63.
  17. Aldington SJ, Kohner EM, Meuer S, Klein R, Sjolie A-K, the EURODIAB IDDM Complications Study Group: Methodology for retinal photography and assessment of diabetic retinopathy: the EURODIAB IDDM Complications Study. *Diabetologia* 1995; 38:437-444.
  18. Shipchandler MT, Moore EG. Rapid, fully automated measurement of plasma homocyst(e)ine with the Abbott IMx analyzer. *Clin Chem* 1995; 41: 991-4.
  19. Rothman KJ, Greenland S, Modern epidemiology. 2nd ed. Lippincott Williams & Wilkins, Philadelphia, 1998.
  20. Muchowski PJ, Wacker JL. Modulation of neurodegeneration by molecular chaperones. *Nat Rev Neurosci*. 2005; 6:11-22.
  21. Evgrafov OV, Mersiyanova I, Irobi J, Van Den Bosch L, Dierick I, Leung CL, Schagina O, Verpoorten N, Van Impe K, Fedotov V, Dadali E, Auer-Grumbach M, Windpassinger C, Wagner K, Mitrovic Z, Hilton-Jones D, Talbot K, Martin JJ, Vasserman N, Tverskaya S, Polyakov A, Liem RK, Gettemans J, Robberecht W, De Jonghe P, Timmerman V. Mutant small heat-shock protein 27 causes axonal Charcot-Marie-Tooth disease and distal hereditary motor neuropathy. *Nat Genet*. 2004; 36:602-6.
  22. Dierick I, Irobi J, De Jonghe P, Timmerman V. Small heat shock proteins in inherited peripheral neuropathies. *Ann Med*. 2005; 37:413-22.
  23. Tezel G, Wax MB. The mechanisms of hsp27 antibody-mediated apoptosis in retinal neuronal cells. *J Neurosci*. 2000; 20:3552-62.

**Table 1: Baseline characteristics of the 531 subjects with type 1 diabetes of the EURODIAB Prospective Complication Study.**

	Case subjects	Control subjects	P
<b>N</b>	363	168	
<b>Age (years)</b>	41.4 ± 10.5	35.7 ± 7.7	<0.0001
<b>Diabetes duration (years)</b>	24.4 ± 9.3	15.4 ± 6.7	<0.0001
<b>Males (%)</b>	52.3%	48.8%	0.45
<b>BMI (kg/m<sup>2</sup>)</b>	24.9 ± 3.5	23.6 ± 2.5	<0.0001
<b>WHR</b>	0.89 ± 0.12	0.89 ± 0.17	0.64
<b>HbA<sub>1c</sub> (%)</b>	8.9 ± 1.6	7.7 ± 1.2	<0.0001
<b>Systolic blood pressure (mmHg)</b>	127.0 ± 21.7	114.9 ± 13.1	<0.0001
<b>Diastolic blood pressure (mmHg)</b>	75.8 ± 11.7	73.7 ± 10.6	0.04
<b>Hypertension (%)</b>	54.6%	13.8%	<0.0001
<b>Total cholesterol (mmol/l)</b>	5.46 ± 1.18	4.91 ± 1.08	<0.0001
<b>LDL-cholesterol (mmol/l)</b>	3.60 ± 1.11	3.06 ± 0.97	<0.0001
<b>HDL-cholesterol (mmol/l)</b>	1.61 ± 0.44	1.67 ± 0.42	0.14
<b>Triglycerides (mmol/l)</b>	1.21 (0.83-1.58)	0.84 (0.66-1.09)	<0.0001
<b>AER µg/min</b>	51.0 (7.3-347.6)	6.4 (4.5-9.2)	<0.0001
<b>CRP (mg/l)</b>	1.23 (0.52-2.88)	0.75 (0.36-1.69)	<0.0001
<b>IL6 (pg/ml)</b>	2.48 (1.34-3.91)	1.71 (1.06-2.50)	<0.0001
<b>TNF-α (pg/ml)</b>	3.22 (2.34-4.29)	2.14 (1.67-2.78)	<0.0001
<b>Homocysteine (µmol/l)</b>	7.7 (5.7-9.6)	6.8 (5.6-81)	0.002
<b>Amadori albumin (U/ml)</b>	47.0 ± 13.5	42.2 ± 12.3	0.0001
<b>E-selectin (ng/ml)</b>	33 (26-44)	29 (22-38)	0.0001
<b>sVCAM (ng/ml)</b>	412 (340-500)	368 (516-420)	<0.0001
<b>HSP27 (pg/ml)</b>	658.0 (286.4-1315.0)	567.6 (250.0-1136.0)	0.18

Data are expressed as mean ± SD, percentage or geometric mean (25<sup>th</sup>, 75<sup>th</sup> centile) for log-transformed data.

**Table 2: Odds ratios for diabetes complications by HSP27 values in the nested case-control study within the EURODIAB Prospective Complication Study**

	<b>OR</b>	<b>OR*</b>	<b>OR**</b>
<b>All complications</b>			
<b>logHSP27</b>	1.11(0.95-1.31)	1.14 (0.95-1.36)	1.15 (0.93-1.43)
<b>&lt; 250.0</b>	1.00	1.00	1.00
<b>250.0-507.1</b>	1.22 (0.72-2.07)	1.22 (0.66-2.23)	0.94 (0.45-1.97)
<b>507.2-1135.0</b>	1.13 (0.67-1.92)	1.42 (0.78-2.60)	0.92 (0.44-1.95)
<b>&gt;1135.0</b>	1.42 (0.85-2.39)	1.55 (0.85-2.82)	1.31 (0.64-2.73)
<i>p for trend</i>	<i>0.24</i>	<i>0.13</i>	<i>0.47</i>
<b>DSP</b>			
<b>logHSP27</b>	1.24 (1.04-1.49)	1.32 (1.06-1.63)	1.53 (1.16-2.02)
<b>HSP 27 (pg/ml) &lt; 250.0</b>	1.00	1.00	1.00
<b>250.0-507.1</b>	1.36 (0.74-2.50)	1.13 (0.54-2.39)	0.65 (0.24-1.74)
<b>507.2-1135.0</b>	1.39 (0.76-2.54)	1.84 (0.89-3.82)	1.19 (0.46-3.10)
<b>&gt;1135.0</b>	1.94 (1.08-3.50)	2.13 (1.04-4.33)	2.27 (0.90-5.75)
<i>p for trend</i>	<i>0.03</i>	<i>0.016</i>	<i>0.03</i>
<b>Micro-macroalbuminuria</b>			
<b>logHSP27</b>	1.19 (1.00-1.42)	1.21 (0.98-1.41)	1.24 (0.92-1.68)
<b>HSP 27 (pg/ml) &lt; 250.0</b>	1.00	1.00	1.00
<b>250.0-507.1</b>	1.20 (0.66-2.18)	1.22 (0.58-2.55)	0.88 (0.31-2.52)
<b>507.2-1135.0</b>	1.31 (0.72-2.37)	2.10 (1.02-4.32)	1.10 (0.38-3.16)
<b>&gt;1135.0</b>	1.68 (0.94-2.99)	1.92 (0.95-3.89)	1.56 (0.58-4.20)
<i>p for trend</i>	<i>0.08</i>	<i>0.03</i>	<i>0.31</i>
<b>Retinopathy</b>			
<b>logHSP27</b>	1.13 (0.96-1.34)	1.15 (0.94-1.41)	1.16 (0.90-1.51)
<b>HSP 27 (pg/ml) &lt; 250.0</b>	1.00	1.00	1.00
<b>250.0-507.1</b>	1.15 (0.66-1.99)	1.21 (0.61-2.40)	0.98 (0.41-2.38)
<b>507.2-1135.0</b>	1.23 (0.71-2.13)	1.94 (0.99-3.82)	1.35 (0.55-3.29)
<b>&gt;1135.0</b>	1.48 (0.87-2.54)	1.69 (0.86-3.31)	1.39 (0.58-3.34)
<i>p for trend</i>	<i>0.15</i>	<i>0.06</i>	<i>0.34</i>
<b>CVD</b>			
<b>logHSP27</b>	1.13 (0.93-1.37)	1.18 (0.94-1.49)	1.10 (0.83-1.46)
<b>HSP 27 (pg/ml) &lt; 250.0</b>	1.00	1.00	1.00
<b>250.0-507.1</b>	1.43 (0.75-2.72)	1.86 (0.84-4.14)	1.22 (0.47-3.15)
<b>507.2-1135.0</b>	1.25 (0.65-2.41)	1.83 (0.82-4.08)	1.09 (0.41-2.86)
<b>&gt;1135.0</b>	1.54 (0.81-2.91)	1.91 (0.86-4.24)	1.23 (0.48-3.16)
<i>p for trend</i>	<i>0.28</i>	<i>0.16</i>	<i>0.74</i>

\* adjusted for age and diabetes duration;

\*\* adjusted for age, diabetes duration, hypertension, HbA<sub>1c</sub>, smoking and log-TNF $\alpha$