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SHORT COMMUNICATION

Association between indices of body mass and antibody titres to heat-shock protein-60, -65 and -70 in healthy Caucasians

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We have previously shown that antibody titres to several heat-shock proteins (Hsps) are elevated in dyslipidaemic patients and subjects with established vascular disease. Obesity is known to be associated with raised serum inflammatory markers suggesting a state of heightened immune activation. Hence, we have investigated the association between indices of obesity and several Hsp antibody titres in healthy subjects. Subjects ($n=170$) were recruited from among employees at the University of Surrey and the Royal Surrey County Hospital, Guildford, UK. Of these subjects, 35 were obese with a body mass index (BMI) $\geqslant 30\,kg/m^2$ (19 male and 16 female subjects), 58 were overweight with $30 > BMI \geqslant 25\,kg/m^2$ (36 male and 22 female subjects) and 77 were of a normal weight with $BMI < 25\,kg/m^2$ (31 male and 46 female subjects). Overall, obese subjects had significantly higher plasma anti-Hsp-60 ($P<0.001$), anti-Hsp-65 ($P<0.05$) and anti-Hsp-70 ($P<0.05$) compared with overweight and normal weight subjects.
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Keywords: BMI; heat-shock proteins; antibody titres

Introduction

Cells respond to environmental stresses by expressing several proteins of the heat-shock protein (Hsp) family.[1,2] The Hsps are involved in the renaturation of proteins damaged during exposure to these environmental stressors; however, they may themselves be altered in the process, becoming antigenic.[1,3] Clinical studies have reported positive associations between plasma antibody titres to Hsps and extent of cardiovascular disease.[4–6] However, it is unclear at which stage during atherogenesis these antibodies develop and what factors influence their appearance. Obesity is a known coronary risk factor associated with a heightened inflammatory state.[7] We therefore wished to investigate the association between indices of obesity and Hsp antibody titres in individuals without clinically overt coronary disease.

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Materials and methods

One hundred and seventy subjects without overt coronary disease were recruited from among employees at the University of Surrey and the Royal Surrey County Hospital, Guildford, UK. Subjects on any medication were excluded from the study.

Informed written consent was obtained from each subject, and ethics approval was obtained from the University of Surrey Advisory Committee on Ethics.

All subjects were measured for height, waist and hip circumference (in centimetres) and weighed in kilograms using a stand-on Bio Impedance Analyzer (BIA) (Tanita-305 body fat analyzer, Tanita Corp., Tokyo, Japan). The latter was also used to estimate percent body fat. Body mass index (BMI) was calculated by the formula: BMI = weight (kg)/height (m^2).

We have already shown that the precision of this BIA was good (CV was $<1\%$), but sensitive to physiological factors, such as exercise and food intake with an effect of up to 2.6% on the % body fat estimate. Therefore, the BIA was used under constant conditions, fasted, before exercise and the same device was used in all subjects.[8]

A fasting lipid profile, comprising total cholesterol, triglycerides and high-density lipoprotein-cholesterol (HDL-cholesterol), was determined for each patient. Low-density lipoprotein-cholesterol was calculated using the Friedewald formula,[9] except for patients with serum triacylglycerol >4.0 mmol/l. Lipids, high-sensitivity C-reactive protein (hs-CRP) and glucose were measured on a Bayer Advia 1650 analyzer (Bayer, Newbury, UK).

Plasma Hsp antibody titres were measured using in-house enzyme-linked immunosorbent assays as previously described.[10]

Statistical analyses were carried out using the statistical package Minitab Release 13 (Minitab Inc., 3081 Enterprise Drive, State College, PA 16081-3008, USA).

Multiple-regression analysis was used to investigate the relationship between the anti-Hsp titres and the hs-CRP concentration and individual factors including age, sex, indices of obesity, lipid profile, fasting blood glucose and blood pressure.

Results

Among the 170 subjects, 35 were obese with a BMI $\geqslant$ 30 kg/m^2 (19 male and 16 female subjects), 58 were overweight with 30 > BMI $\geqslant$ 25 kg/m^2 (36 male and 22 female subjects), and 77 were of normal weight with BMI < 25 kg/m^2 (31 male and 46 female subjects). Obese, overweight and normal weight subjects did not differ with respect to age, gender and smoking status ($P>0.05$). However, obese subjects had significantly higher measures of adiposity (BMI, % body fat and waist to hip ratio), higher systolic and diastolic blood pressure, higher serum triglycerides, hs-CRP and fasting blood glucose and lower HDL-cholesterol than non-obese subjects (Table 1).

Plasma antibody titres to Hsp-60, -65 and -70 did not differ significantly between male and female subjects ($P>0.05$). Nor did titres differ significantly between subjects who were smokers and those who were non-smokers ($P>0.05$).

Little of the variation in the antibody titres to any of the Hsps, or serum hs-CRP concentrations could be explained by the best-fitting models derived from stepwise multiple linear regressions: 4.3% of the variation in anti-Hsp-60 titres was explained by waist circumstance, 3% of the variation in anti-Hsp-65 titres was explained by systolic blood pressure, 4% of the variation in anti-Hsp-70 titres was explained by HDL-cholesterol concentrations, systolic blood pressure and age, and BMI accounted for approximately 17.8% of the variation in serum CRP concentrations.

There were strong correlations between individual antibody titres to Hsp-60, -65 and -70 ($P<0.001$).

Overall, obese subjects had significantly higher antibody titres to Hsp-60 ($P<0.001$), Hsp-65 ($P<0.05$) and Hsp-70 ($P<0.05$) compared with overweight and normal weight subjects (Table 2). Male obese subjects had significantly higher plasma antibody titres to Hsp-60 ($P<0.001$) and Hsp-70 ($P<0.05$) compared to non-obese subjects (Table 2). Female obese subjects had significantly higher anti-Hsp-60 titres ($P<0.05$), with differences that were borderline for anti-Hsp-65 ($P=0.07$), but not significant for anti-Hsp-70 titres compared to normal weight subjects (Table 2).

Table 1 Clinical and biochemical characterisation of obese, overweight and normal weight subjects

Group	*Obese*	*Overweight*	*Normal weight*
Number of subjects	35	58	77
Mean age (years)	51.3±2.1	48.5±1.8	48.7±1.6
Male:female ratio	19/16 (56%)	36/22 (50%)	31/46 (%)
Smoking habit			
Current no. (%)	6 (17)	8 (14)	17 (22)
Former no. (%)	10 (26)	12 (15)	13 (16)
BMI (kg/m^2)	34.0±0.6***	27.2±0.2	22.3±0.2
% body fat	31.2±0.8***	26.6±0.6	26.6±0.6
Waist:hip ratio	0.97±0.02***	0.89±0.01	0.83±0.01
Systolic blood pressure (mm Hg)	141.2±3.5***	127.9±1.9	123.4±1.9
Diastolic blood pressure (mm Hg)	83.3±1.8***	76.5±1.0	73.8±1.0
Total cholesterol (mmol/l)	5.7±0.2*	5.6±0.1	5.2±0.1
HDL-cholesterol (mmol/l)	1.4±0.1**	1.6±0.1	1.7±0.1
Triglycerides (mmol/l)	1.7 (1.3–2.3)***	1.2 (0.9–1.5)	1.0 (0.8–1.2)
Fasting blood glucose (mmol/l)	5.5±0.2**	5.2±0.1	4.9±0.1
Calculated 10 years Coronary risk (%)	10.4±1.3***	6.5±0.6	5.5±0.5
Hs-CRP (mg/l)	1.9 (1.1–6.0)***	0.8 (0.3–1.9)	0.4 (0.1–1.4)

Abbreviations: BMI, body mass index; HDL-cholesterol, high-density lipoprotein cholesterol; Hs-CRP, high-sensitivity C-reactive protein. Values are expressed as mean±s.e.m., or median and interquartile range. Categorical data were compared by Fisher's exact tests. Between-group comparisons were assessed by Kruskal–Wallis for non-normal distribution data (serum triglycerides and hs-CRP) and by one-way ANOVA for normally distributed data; *$P<0.05$, **$P<0.01$, ***$P<0.001$. The manifestations of coronary risk include angina pectoris, myocardial infarction (MI) and sudden cardiac death.

Table 2 Association between BMI and plasma antibody titres to Hsps (absorbance unit) in healthy subjects

	Obese	*Overweight*	*Normal weight*
Males			
Number of subjects	19	36	31
Anti-Hsp-60	0.42 (0.27–0.65)***	0.23 (0.17–0.31)	0.21 (0.16–0.32)
Anti-Hsp-65	0.48 (0.27–0.86)	0.33 (0.21–0.47)	0.35 (0.23–0.57)
Anti-Hsp-70	0.29 (0.16–0.49)*	0.18 (0.13–0.25)	0.20 (0.14–0.35)
Females			
Number of subjects	16	22	46
Anti-Hsp-60	0.31 (0.26–0.67)*	0.22 (0.11–0.31)	0.24 (0.15–0.28)
Anti-Hsp-65	0.32 (0.25–0.72)	0.36 (0.24–0.49)	0.26 (0.20–0.53)
Anti-Hsp-70	0.23 (0.15–0.39)	0.18 (0.15–0.31)	0.21 (0.12–0.27)
Males and females			
Number of subjects	35	58	77
Anti-Hsp-60	0.41 (0.28–0.65)***	0.22 (0.16–0.29)	0.22 (0.15–0.30)
Anti-Hsp-65	0.40 (0.25–0.72)*	0.33 (0.24–0.49)	0.30 (0.20–0.53)
Anti-Hsp-70	0.26 (0.16–0.47)*	0.19 (0.14–0.26)	0.20 (0.13–0.29)

Abbreviations: BMI, body mass index; Hsps, heat-shock proteins. Values are expressed as median and interquartile range. Between-group comparisons were assessed by Kruskal–Wallis as they are non-normal distribution data; *$P<0.05$, ***$P<0.001$.

There were significant correlations between antibody titres to Hsp-60 and CRP with indices of obesity including waist circumference ($r=0.20$, $P=0.006$; $r=0.31$, $P=0.0001$), waist to hip ratio ($r=0.18$, $P=0.01$; $r=0.17$, $P=0.02$) and BMI ($r=0.15$, $P=0.035$; $r=0.42$, $P=0.0001$), respectively. However, there were no significant correlations between antibody titres to Hsp-65 and Hsp-70 with waist, WHR or BMI.

Discussion

Atherosclerosis is now thought to be a chronic inflammatory condition and is associated with autoimmune responses directed against several antigens, including Hsps.[11] In the present study, we found that antibody titres to Hsp-60, -65 and -70 are raised in subjects who are obese, but free of clinically evident coronary disease.

Antibody titres to Hsp-60, Hsp-65 and Hsp-70 were not associated with gender or age.

There were strong correlations between antibody titres to Hsp-60, -65 and -70 ($P=0.001$ for each paired correlation). This may be due to the close antigenic homologies between Hsp-60, -65 and -70,[12,13] or due to a coordinated antibody response owing to a heightened state of immune responsiveness in the obese subjects.

The reported association between antibody titres to the Hsps and atherosclerosis is inconsistent.[14–17] It has been reported that anti-Hsp-70 titres are not elevated in subjects with severe coronary atherosclerosis, whereas titres to Hsp-60 and -65 were significantly raised in these patients.[15,17] It has also been reported that patients with type 2 diabetes mellitus have significantly higher anti-Hsp titres compared with non-diabetic controls.[18] Current evidence suggests that antibody titres to Hsps are elevated in patients with vascular disease, and that this is related to the severity of atherosclerosis.[19] In the current study, we have shown anti-Hsps are raised in obesity, in the absence of clinically evident coronary disease, but that they are not significantly raised in overweight subjects.

Conclusions

The high antibody titres to Hsp-60, -65 and -70 in obese subjects without established coronary disease may be related to a heightened state of immunoactivation associated with obesity, and the strong correlation between antibody titres to Hsp-60, -65 and -70 supports this possibility. It appears that the elevated Hsp antibody titres develop relatively early in the atherogenic process, although a prospective study would be required to confirm this.

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