Differential expression of HSP60 and exercise effects on its levels in Obese Diabetic and Non-Diabetic humans

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Background and Objectives

Obesity is a growing epidemic, characterized by low grade inflammation and impaired heat shock response(HSR). Physical activity is known to improve overall health by modulating metabolic stress pathways including HSR. Heat shock protein 60 (HSP60), a member of HSR, is a key protein in the crosstalk between cellular stress and inflammation. Interestingly, it can induce both pro-inflammatory and anti-inflammatory markers, however, the status of HSP60 in diabetes and obesity is unclear. We recently reported that HSP60 acting as a chaperonin for proteins in the mitochondrion, was increased in human obese. Its expression levels in obese diabetic are yet to be investigated. In this study, we assessed the expression levels of HSP60 in diabetic and nondiabetic obese (**Table 1**), along with its correlation with inflammatory and metabolic markers as well as its modulation by physical exercise.

Methods

Study Population, Exercise protocol and anthropometric measurements : Adult male and female subjects consisting of non-diabetic (138) and diabetic (92) obese were recruited in accordance with the Declaration of Helsinki .All eligible subjects were enrolled to a supervised exercise program and anthropometric measurements were taken at baseline and after 3 months of exercise.

Blood and tissue sampling: Venous peripheral blood and subcutaneous adipose tissue (SAT) biopsies were obtained at baseline and after 3 months.

Blood inflammatory and metabolic markers: Glucose and lipid profiles were measured on Siemens Dimension RXL chemistry analyzer. Insulin and High sensitivity CRP were determined using ELISA kit. Inflammatory and metabolic markers were assessed using Bioplex-200 system.

Immunohistochemistry(IHC) and Immunoflourescence (IF) :Formalin fixed paraffin embedded SAT sections were used and IHC quantification was done using Imagescope software. IF was done and analyzed with Zeiss LSM 710 confocal microscope.

Quantitative Real time PCR (qRT-PCR): Total RNA was extracted from frozen SAT using Rneasy Lipid Mini kit and cDNA was synthesized with High capacity cDNA reverse transcription kit.

Quantification of circulating proteins by ELISA: Immunoassay EIA kit was used for measuring plasma levels of HSP60 . Levels of plasma anti-HSP60 IgG/A/M were measured using ELISA kit.

Statistical Analysis: Statistical analyses were performed using SPSS software (v22.0; SPSS Inc., Chicago, IL, USA).

Results

- In this study,
- HSP6o expression was significantly decreased in SAT of diabetic obese humans compared to non–diabetic obese.(**Figure 1**)
 - This down regulation was concomitant with increased levels of IL6 and TNFα inflammatory markers in the SAT of diabetic lean and obese. (**Figure 2 & 3**)
 - There was no difference in the levels of circulating HSP6o and its auto-antibody between these two groups.
 - The 3-month exercise protocol significantly increased HSP6o expression levels in SAT of diabetic obese along with a decreased inflammation. In non-diabetic obese the same exercise protocol decreased both HSP6o and inflammatory markers TNF and IL6, suggesting a differential exercise effect in the two groups. (**Figure 1 & 2**)

Table 1: Physical, clinical, and biochemical characteristics of the subjects at baseline

	Obese non-diabetic (n = 138)	Obese diabetic (n = 92)	p value
Physical and clinical characteristics			
Age (years)	45.76 ± 9.91	48.28 ± 8.17	0.16
Gender (M/F)	71/67	49/43	0.44
BMI (kg/m²)	33.82 ± 3.47	33.55 ± 2.94	0.59
PBF (%)	38.20 ± 5.35	37.02 ± 4.95	0.54
Waist (cm)	106.02 ± 12.00	108.88 ± 8.75	0.053
Hip (cm)	115.06 ± 12.64	113.96 ± 12.40	0.97
Resting HR (beats/min)	76.67 ± 11.01	86.01 ± 11.68	<0.0001
SBP (mmHg)	120.92 ± 13.04	121.88 ± 11.92	0.64
DBP (mmHg)	78.13 ± 8.58	78.70 ± 6.16	0.28
VO ₂ , max (ml/kg/min)	17.57 ± 4.70	15.89 ± 4.10	0.04
WBC1o	6.65 ± 1.89	7.54 ± 1.91	0.0003
Metabolic markers			
Cholesterol (mmol/l)	5.21 ± 0.89	5.05 ± 1.16	0.22
HDL (mmol/l)	1.22 ± 0.36	1.11 ± 0.33	0.027
LDL (mmol/l)	3.35 ± 0.85	3.16 ± 1.24	0.09
TG (mmol/l)	1.45 ± 0.95	2.10 ± 1.74	0.0002
Glucose (mmol/l)	5.43 ± 0.47	9.82 ± 3.85	<0.0001
HbA1c (%)	5.76 ± 0.49	8.70 ± 1.93	<0.0001
Insulin (ng/ml)	3.82 ± 1.94	4.22 ± 2.04	0.26
HOMA-IR	0.96 ± 0.55	1.60 ± 0.88	<0.0001
C-peptide (µg/ml)	1.88 ± 1.13	1.87 ± 1.25	0.91
Glucagon (ng/ml)	0.16 ± 0.04	0.18 ± 0.05	0.11
GIP (ng/ml)	0.76 ± 0.55	0.92 ± 0.61	0.13
GLP-1 (ng/ml)	0.27 ± 0.05	0.29 ± 0.04	0.002
Leptin (ng/ml)	9.10 ± 6.25	8.66 ± 3.99	0.74
Inflammatory markers			
IL-1b (pg/ml)	8.29 ± 3.28	7.82 ± 1.94	0.96
IL-6 (pg/ml)	17.30 ± 6.24	17.16 ± 4.87	0.71
IL-1o (pg/ml)	28.47 ± 20.05	33.02 ± 27.87	0.50
IP-1o (pg/ml)	571 ± 418	689 ± 617	0.54
TNF-α (pg/ml)	127 ± 40	123 ± 31	0.65
hsCRP (µg/ml)	5.38 ± 3.91	6.36 ± 4.52	0.24

Figure 1: HSP6o expression and modulation by exercise in the subcutaneous adipose tissue (SAT) of obese subjects with diabetes

(A) Immunohistochemical analysis of HSP6o expression in SAT from obese without (ND) and with diabetes (D) before and after a 3-month physical exercise intervention (n=10 for each group). (B) mRNA levels were measured by quantitative real time PCR using SAT from obese ND and obese D (n=10 for each group) and normalized using GAPDH. (C) Representative confocal immunofluorescence images illustrating HSP6o expression and localization in SAT from obese people without and with diabetes, before and after exercise (n=3 for each group). *denotes p < 0.05 between the diabetes and non-diabetes groups, and # denotes p < 0.05 between before and after exercise.

Figure 2: Increased inflammation and its modulation by exercise in the subcutaneous adipose tissue (SAT) of obese subjects with diabetes.

Immunohistochemical analysis of (A) IL6 and (B) TNF-α expression in SAT from obese people without (ND) and with diabetes (D) before and after 3 months of physical exercise (n=10 for each group). Data are presented as fold changes in the diabetes group compared to the non-diabetes group. * denotes p < 0.05 between the diabetes and non-diabetes groups , and # denotes p < 0.05 between before and after exercise.

Figure 3: Expression of HSP6o, IL6, and TNF-α in the subcutaneous adipose tissue (SAT) of lean subjects with and without diabetes

Representative confocal immunofluorescence images illustrating HSP6o (A), IL6 (B), and TNF-α (C) expression and localization in SAT from lean people with and without diabetes (n=3 for each group). Quantification of the staining in SAT slides was performed as mentioned in Methods.

Discussion & Conclusions

The role of the HSP's in the progression of obesity and diabetes is not yet fully elucidated. Our current study shows reduction in HSP6o expression in diabetes whereas exercise increase its expression along with reduced inflammation. Interestingly, the effects of physical exercise on HSP6o levels in diabetic and non-diabetic obese were opposite. This might reflect a specific adaptive response to exercise depending on the metabolic stress status. Therefore, enhancing HSR defense system through exercise might have a beneficial impact on future approaches to diabetes prevention and treatment.

References:

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