Original Article

Stress Perception and (GT)n Repeat Polymorphism in Haem Oxygenase 1 Promoter Are Both Risk Factors in Development of Eating Disorders

(haem oxygenase 1 / eating disorders / stress / anorexia / gene environment interactions)

L. ŠLACHTOVÁ[1], D. KAMINSKÁ[2], M. CHVÁL[3], L. KRÁLÍK[1], P. MARTÁSEK[1], H. PAPEŽOVÁ[2]

[1]Department of Paediatrics and Adolescent Medicine, [2]Department of Psychiatry, First Faculty of Medicine, Charles University in Prague and General University Hospital in Prague, Czech Republic
[3]Institute for Research and Development of Education, Faculty of Education, Charles University in Prague, Czech Republic

Abstract. Haem oxygenase 1 (HO-1) plays a pivotal role in metabolic stress protecting cells in dependence on reactive oxygen species. This study investigated a potential gene environment interaction between the (GT)n repeat *HO1* polymorphism and the stress perception in patients with eating disorder and in controls. Stress perception and (GT)n polymorphism were measured in 127 patients with eating disorders and in 78 healthy controls using Stress and Coping Inventory and genotyping. Based on the inventory, overall, specific and weighted stress scores were defined. Clinical stress score was generated according to the patient's history and interviews. According to our hypothesis, 1) all stress scores describing subjective stress perception were significantly higher in patients compared to controls ($P \leq 0.001$; $P \leq 0.002$; $P \leq 0.001$), 2) the L/L genotype of GT promoter repeats (L > 25 GT repeats, S < 25 GT repeats) in the patients was associated with higher overall ($P \leq 0.001$), specific ($P \leq 0.010$) and weighted stress score ($P \leq 0.005$) compared to the L/S variant, and 3) Pearson's correlation of clinical versus objective stress scores showed not very tight relationship (0.198; 0.287; 0.224, respectively). We assume potential risk of the L allele of *HO1* promoter polymorphism for the stress response and contribution of the subjective stress perception together with the L/L genotype to the development of eating disorder. Decreased *HO1* expression in the presence of L/L genotype plus more intensive stress perception in the patients can lead to secondary stress, with increasing severity of the symptoms and aggravation of the disease.

Received August 6, 2013. Accepted August 14, 2013.

This study was supported by grants IGA NT 14094/3 and RVO-VFN64165/2012 from the Ministry of Health of the Czech Republic, Research programme MSM 0021620849 (MSMT), PRVOUK P24/LF1/3 and P26/LF1/4, UNCE 204011 from Charles University in Prague, Czech Republic.

Corresponding author: Pavel Martásek, Department of Paediatrics and Adolescent Medicine, First Faculty of Medicine, Charles University in Prague and General University Hospital in Prague, Ke Karlovu 2, 128 08 Prague 2, Czech Republic. E-mail: pavel.martasek@gmail.com

Abbreviations: AN – anorexia nervosa, BDNF – brain-derived neurotrophic factor, BMI – body mass index, BN – bulimia nervosa, CSS – Clinical Stress Score, ED – eating disorders, EDE-Q – Eating Disorder Examination Questionnaire, HO-1 – haem oxygenase 1, OSS – Objective Stress Score, SCI – Stress and Coping Inventory, WSS – Weighted Stress Score.

Introduction

Restricted and insufficient food intake results in malnutrition with the lack of vital nutrients including vitamins and minerals (ascorbic acid, retinol, α-tocopherol, selenium, zinc, etc.). Altered balance between oxidants and antioxidants results in oxidative stress, which has been described in anorexia nervosa, obesity, or type 2 diabetes (Chen et al., 2002; Agnello et al., 2012; Faienza et al., 2012). Disturbed feeding behaviour has serious consequences on the cell and complex metabolism affecting energy metabolism, thermogenesis or hormone secretion. Nutritive factors can also influence gene expression. For example, an animal model of anorexia exhibits disturbed oxidative phosphorylation via down-regulating assembly factor of complex I Ndufaf1 (Lindfors et al., 2011). Another very important up- and down-regulated gene that responds sensitively to metabolic stress is haem oxygenase 1 (*HO1*, Abraham and Kappas, 2008).

Eating disorders (ED) including anorexia nervosa (AN), bulimia nervosa (BN) and unspecified eating disorder are multifactorial diseases with a clear genetic

component. As in other complex disorders the pathophysiology remains unknown. EDs are very heterogeneous diseases and the endophenotype approach with detailed focus on the clinical subgroups and their characteristics provides more opportunities for ED therapy. We used this approach to investigate the influence of stress perception and (GT)n repeat polymorphism in haem oxygenase 1 in ED patients.

HO-1

Haem oxygenase 1 (HO-1 or HMOX-1), the key enzyme of haem degradation, catalyses the oxidative cleavage of haem into biliverdin, Fe^{2+} and CO. Subsequent rapid conversion of biliverdin to bilirubin by biliverdin reductase makes HO-1 the only rate-limiting enzyme of bilirubin formation (Tenhunen et al., 1968). Besides the role of the enzyme in catalysing haem degradation, the indirect effect of its reaction products (CO, biliverdin, bilirubin) are of great importance in maintaining cell homeostasis and regulating cell metabolism (Shibahara et al., 1987). Expressed in many tissues and up-regulated by multiple stimuli, HO-1 is involved in numerous anti-oxidant, anti-proliferative and anti-inflammatory processes with the same aim: to protect the cell against oxidative stress (Tenhunen et al., 1969; Poss and Tonegawa, 1997; Exner et al., 2004, 2006; Abraham and Kappas, 2008; Grochot-Przeczek et al., 2012).

The highly conserved *HO1* gene is strongly expressed especially in the liver and spleen. Constitutive expression of HO-1 provides a fast and effective tool in regulating signal cascades via HO-1 reaction products; the signalling molecule, CO, is involved in anti-inflammatory pathways via modulation of MAPK, JNK, transcription factor AP-1 or nuclear factor NF-κB (Otterbein et al., 2000; Megias et al., 2007, 2009). Besides the stimulated expression of *HO1* in response to oxidative stress, transcription of the gene is modulated via genetic variants. So far, three functional polymorphisms in the *HO1* gene have been identified: (GT)n variable tandem repeat polymorphism and two single-nucleotide polymorphisms, G-1135A and T-413A, with the main interest focused on GT repeats (Hirai et al., 2003).

(GT)n repeat polymorphism

The variable tandem repeat polymorphism in the promoter of the *HO1* gene is based on different numbers of consecutive GT nucleotides. It is located approximately 250 bp upstream from the transcription start site. The size of the GT repeats varies from 12 to 40. *In vitro* studies demonstrate reduced transcriptional activity of the *HO1* gene in cell lines with a larger size of (GT)n repeats (L allele) compared to shorter variants (S allele). This leads to an impaired induction of HO-1 exposed to reactive oxygen species and a decreased response to oxidative stress (Yamada et al., 2000; Chen et al., 2002; Taha, et al., 2010). Several classifications according to the allelic size are used, with the most frequent alleles of 23 and 30 GT repeats (Yamada et al., 2000; Baan et al., 2004; Schillinger et al., 2004).

Stress perception and its inheritance

Recent research has supported the role of lifetime severe stress and trauma in the development, maintenance and relapses of eating disorders (Johnson et al., 2002). The stress contributes not only to the development of ED, mainly their specific symptoms (bulimic symptoms and drive for thinness), but also to subsequent stress generation, negative life stressors and depressive symptoms (Alda et al., 1989; Bodell et al., 2012). Negative stressors, anxiety or depression have been shown to have high heritability. Therefore, a gene-environment (G x E) approach is now being applied in many studies (Wade et al., 2009). In addition to the shared environmental influence of behavioural patterns there are many inherited traits resulting from the altered function of the genes of interest (e.g., serotonergic, dopaminergic, opioidergic) (Trace et al., 2013). AN or BN are accompanied by distorted body self-perception, anxiety, fear of gaining weight combined with restricting or sometimes lifetime adverse events. Taken together with the lack of nutrients and metabolic consequences of the stress itself, secondary stress is generated. Genetic factors may play both, protective and non-protective roles in the stress response, depending on the presence of functional polymorphisms or otherwise influenced gene expression or epigenetic modifications resulting from gene-environment interactions (Nagata et al., 2000; Abraham et al., 2007; Sachs-Ericsson et al., 2012).

Material and Methods

Ethics and Samples

A total of 127 patients and 78 control samples were analysed. All patients and controls were Caucasian females from the Czech Republic. The study was approved by the Ethics Committee of the First Faculty of Medicine, Charles University in Prague and General University Hospital in Prague. All probands agreed with their participation in the study and signed the informed consent. The control group was recruited from healthy female students and young healthy woman working in hospital matched by age, sex and socio-economical status. All patients and controls answered the inventories and gave their DNA samples anonymously.

Measures

The Stress and Coping Inventory (SCI) was designed for health promotion, as an educational instrument and a research tool, and its reliability and validation were carried out by Holmes and Rahe (Holmes and Rahe, 1967; Rahe et al., 2000). SCI Questionnaires for Youth and Adults with 49 questions were answered by patients during the first two weeks of their hospitalization. The same questionnaires were answered by healthy controls. SCI data were evaluated via the models of stress scores. Objective Stress Score (OSS) was calculated as a cumulative sum of positive answers (adverse event present =

1, absent = 0) to all SCI items (with the maximal value of 49 points). Specific Stress Score (SSS) was counted as a sum of selected items known as the risk factors for ED specific items; event present = .1, absent = 0. These items (1, 2, 4, 5, 8, 12, 13, 15, 18, 21, 25, 26, 27, 29, 34, 39, 44, 45, 49) are known to play important roles in the onset or relapse of ED (Pavlova, 2010). Finally, Weighted Stress Score (WSS) was evaluated according to Rahe, giving each item different value (e.g. personal injury or illness 53 points, change in social activities 18 points, etc.) (Holmes and Rahe, 1967; Rahe et al., 2000).

Next, the Clinical Stress Score (CSS) was established as the objective stress measure which resulted from complex clinical evaluation by an independent psychiatrist, trained in ED, trauma research and treatment. CSS was based on the observations on the ward, clinical interviews during the hospitalization, and evaluation of the present and past case history; all information available was considered (from the patient's outpatient and inpatient documentation). The life adverse events were rated absent = 0, present = 1, severe and/or repeated = trauma 2.

Clinical assessment

The study cohort consisted of female patients with primary DSM-IV diagnoses of AN or BN, successively hospitalized at the Specialized Unit for Eating Disorders in General University Hospital in Prague in the years 2006–2013. Patients with ED and comorbid primary diagnosis of alcohol abuse or personality disorders were excluded from the study.

Eating pathology was assessed with the 36-item Eating Disorder Examination Questionnaire (EDE-Q), semi-structured interview that monitors the severity of specific eating-related pathology over 28 days before assessment. It contains a Global Scale, which is the average of the four subscales: Shape Concern, Weight Concern, Eating Concern and Restraint. This measure has been demonstrated to have acceptable reliability and validity (Rizvi, et al., 2000; Berg, et al., 2012; Kelly et al., 2012). The diagnoses were confirmed by two experienced psychiatrists specialized in ED.

Average age was 25 ± 7.1 years in patients with ED and 26 ± 5.1 years in controls. Average body mass index (BMI in kg/m^2) was 15.4 ± 2.7 in AN patients; 21.7 ± 3.1 in BN patients and 19.9 ± 3.9 in controls. Clinical data from the patients with ED were collected during their six- to eight-week hospitalization at the Specialized Unit for Eating Disorders, with a comprehensive specialized ED programme (re-alimentation, regime, psychotherapy and rehabilitation).

Genotyping and statistical analyses

Genetic analyses were performed after the DNA extraction using a standard desalting method. *HO1* promoter polymorphisms of GT repeats were genotyped according to Král et al. (2011). For genotype distribution in the *HO1* gene, we classified the cases according to long (L ≥ 25 GT repeats) and short alleles (S < 25 GT repeats). Statistical analyses were performed with SPSS software version 17.0 (SPSS Inc., Chicago, Ill). To estimate the differences of subjective stress perception among the groups – patients compared to controls – *t*-test was used. The same *t*-test was used for the comparison of stress scores in L/L and L/S genotypes. Correlation of the objective and subjective stress indexes was evaluated with Pearson's correlation.

Results

Stress indexes

Subjective perception of stressful events in patients and controls was described using cumulative stress scores OSS (Overall), SSS (Specific) and WSS (Weighted). Subjective evaluation of the presence of stressful life events in patients according to SCI was significantly higher compared to controls ($P \leq 0.001$ for OSS; $P \leq 0.002$ for SSS and $P \leq 0.001$ for WSS), see Table 1. The average of Weighted Stress Scores for subjective stress perception was 471 points in patients compared to 359 points in the controls (see Fig. 1 for details).

G x E interaction of (GT)n repeats and stress perception

Association of the *HO1* promoter polymorphism and subjective stress perception was tested in 127 patients and 78 controls (*t*-test). The distribution of *HO1* genotypes in the patients and controls did not deviate from Hardy-Weinberg equilibrium (Wellek et al., 2010). Also, the frequency of the minor allele S in both groups of patients and controls was higher than 0.05 cut-off for rare alleles. The genotype frequencies found in our cohort are consistent with the population data from other Caucasians (Denschlag et al., 2004; Rueda et al., 2007; Weis et al. 2012; Gregorek et al., 2013). To analyse the subjective stress perception and its score in particular genotypes, S/S genotype carriers were excluded involving 11 patients and eight controls. Even when the rare incidence of S/S genotype was in accordance with the epidemiological data, the group size was not sufficient to be included into further statistical analyses.

Significant differences among patients with the genotype L/L compared to L/S were found in the Overall Stress Score ($P \leq 0.001$), Specific Stress Score ($P \leq 0.01$)

Table 1. Subjective stress perception differences between ED patients and controls

		N	Mean	t	P value
Overall Stress Score	controls	78	10.03	-3.726	0.001
	patients	127	13.72		
Special Stress Score	controls	78	3.45	-3.07	0.002
	patients	127	4.71		
Weighted Stress Score	controls	78	359	-3.318	0.001
	patients	127	471		

Degrees of freedom are the same for all scores, df = 203

Table 2. Subjective stress perception in L/L and L/S genotypes in the patients

		N	Mean	t	P value
Overall Stress Score	L/S	57	11.18	-3.372	0.001
	L/L	59	15.66		
Special Stress Score	L/S	57	3.86	-2.622	0.010
	L/L	59	5.37		
Weighted Stress Score	L/S	57	398	-2.852	0.005
	L/L	59	527		

Degrees of freedom are the same for all scores, df = 114

Table 3. Subjective stress perception in L/L and L/S genotypes in the mixed cohort

		N	Mean	t	P value
Overall Stress Score	L/S	87	10.37	-3.372	0.002
	L/L	99	13.62		
Special Stress Score	L/S	87	3.53	-2.622	0.005
	L/L	99	4.73		
Weighted Stress Score	L/S	87	364	-2.852	0.002
	L/L	99	470		

Degrees of freedom are the same for all scores, df = 184

and Weighted Stress Score ($P \leq 0.005$), with higher value of stress scores in patients with the L/L genotype compared to L/S genotype, see Table 2. Detailed values of WSS are shown in Fig. 1. Moreover, the analysis of the grouped cohort of patients and controls (N = 186) showed the same tendency. The combined cohort showed a significant increase of all Subjective Stress Scores in the carriers of L/L genotype compared to L/S (OSS $P \leq 0.002$; SSS $P \leq 0.005$ and WSS $P \leq 0.002$; see Table 3). In summary, the L/L genotype of the *HO1* gene was found significantly associated with higher values of objective stress perception both in the control and in the patient groups (Fig. 1).

Subjective versus objective stress perception

Subjective stress perception expressed by OSS, SSS and WSS was compared with the Clinical Stress Score. This analysis was performed only in the patients (N = 127). Pearson's correlation of subjective (OSS, SSS, WSS) and objective stress (CSS) did not reveal a tight relationship between the subjective and objective stress scores (.198* for OSS; .287* for SSS and .224* for WSS; *P value for all cases ≤ 0.025), see Table 4. This represents the validity of all three indexes on one side and their different focus on the other.

Table 4. Objective versus subjective stress perception

	OSS	SSS	WSS
CSS Pearson's Corr.	0.198	0.287	0.224
P value	0.025	0.001	0.010

Stress scores: CSS – Clinical, OSS – Overall, SSS – ED Specialized, WSS – Weighted

Discussion

Development of ED can be interpreted as a maladaptive defence reaction against a perceived threat or trauma (relational, physical, sexual stress or trauma). In ED, physical and psychological stress is closely related (in malnutrition, cognition or concentration). Stress perception or sensitivity to stress, the very important factors in ED pathology, are influenced just by the coincidence of physical and psychical factors, often interacting in the gene environment interactions.

In our study we focused on stress perception described via SCI (Rahe et al., 2000). Several stress scores have been developed including Overall, Specific, Weighted (subjective stress perception) and Clinical Stress Score (objective stress perception). All scores describing the subjective stress perception regardless of their different methodology showed significantly in-

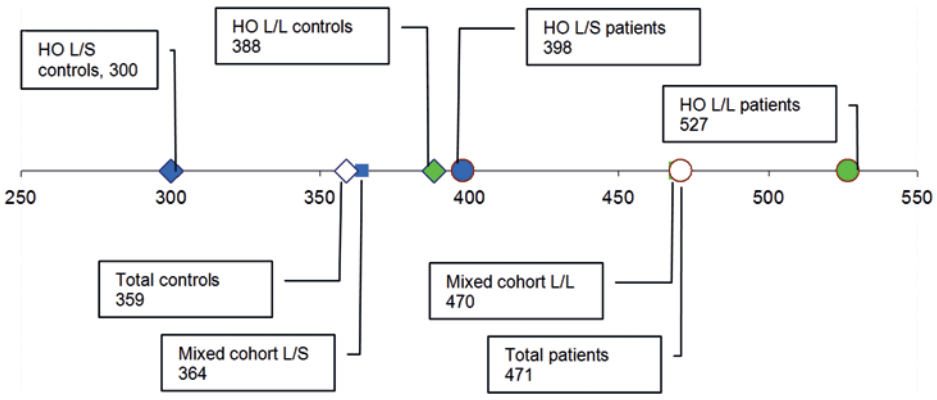

Fig. 1. Values of objective WSS according to *HO1* genotypes in the patients and controls are shown on the horizontal axis. Legend of the point distribution on the axis: rhombus – controls, square – mixed cohort of patients and controls, circle – patients; blue – L/S genotype, green – L/L genotype, white – all genotypes mixed.
The lowest stress score was observed in the "more protective" L/S genotype in the controls, followed by controls with the L/L genotype. A higher stress score was found in patients with the L/S genotype and the highest value in L/L patients. The Weighted Stress Score was measured according to Rahe (Holmes and Rahe, 1967; Rahe et al., 2000).

creased subjective stress perception in the patients compared to controls (see Table 1). This was consistent with our hypothesis assuming that ED patients are more sensitive to stress than healthy controls. Higher sensitivity to stress is accompanied with malnutrition, exhaustion, neuro-endocrinological changes or psychological factors such as high demands on one's own body, restraining, perfectionism and other stress-related behavioural factors (Kyrou and Tsigos, 2007, 2009; Hague et al., 2013; Patterson and Abizaid, 2013). Malnutrition can have, and in ED patients often has, fatal consequences. We can also speculate that long-term stress may have impact on the brain function. Recently, reduction in the 5-HT1A receptor binding potential has been shown in long-term occupational stress (Blix et al., 2013). Stressful life events resilience is also associated with the Val66Met polymorphism in brain-derived neurotrophic factor (BDNF) or Val158Met in the catechol-O-methyltransferase gene. Val66Met in BDNF has also been studied in ED, but with inconsistent results (Trace et al, 2013).

Because of the importance of G x E interactions in ED pathogenesis, we investigated the *HO1* promoter repeat polymorphism and its association with subjective stress perception in patients with ED an in controls. HO-1 is a crucial enzyme whose expression is strongly regulated in response to stress stimuli. Decreased expression of the *HO1* gene with L allele compared to the S allele has been demonstrated in *in vitro* studies (Yamada et al., 2000; Chen et al., 2002; Taha et al., 2010). To our knowledge, no data investigating the *HO1* gene polymorphism and stress response or eating disorders are available. In this study we hypothesize that the combined effect of *HO1* (GT)n repeat polymorphism and subjective stress perception may play a significant role in the development of eating disorders or in their stress response. We assume that carrying the L/L genotype with the lowest level of HO-1 expression results in less efficient protection against metabolic stress. Several studies describe the HO-1 impact on certain metabolic disorders including obesity, type 2 diabetes, hepatic injury or hormonal regulation (Bakken et al., 1972; Abraham et al., 2007; Eipel et al., 2007; Abraham et al., 2008).

Our data suggest a significantly increased tendency of stress perception in subjects with the "non-protective" L/L genotype and its contribution to ED pathology (Table 2, Fig. 1). For genotype environment interactions of *HO1* and subjective stress perception the evaluated weighted stress score according to Rahe was used as a representative (Fig. 1) (Holmes and Rahe, 1967; Rahe et al., 2000). Very similar results of overall and specific stress scores according to the *HO1* genotype were obtained (Table 2).

The same association of subjective stress perception and *HO1* (GT)n repeat polymorphism was documented in a mixed cohort of patients and controls with interesting results. Even in the mixed cohort, a significant association of the L/L genotype with higher subjective stress perception was observed. This confirms the solid association of the L/L genotype with subjective stress perception in all three scores (Table 3). The importance of stress perception and *HO1* repeat polymorphism in ED pathology is summarized in Fig. 1. First, the L/L genotype is associated with higher stress perception in the patients and also in the mixed cohort. Second, the controls always show a lower stress score than ED patients. Taken together, the lowest stress score was observed in the suspected more protective L/S genotype in the controls (300 points), followed by 388 points in the controls with L/L genotype, next in the patients with L/S (398) and finally 527 points in L/L patients.

Another aim of the study was to compare the subjective and objective stress perception, which included very detailed clinical observations and careful history. Lifetime exposure to stressful events (here represented by objective stress perception or clinical stress score) and subjective stress perception, even when each describes a different perspective, are both important factors in ED pathology. Stress perception and response in ED are tightly related to hormonal balance, as it has been demonstrated in animal models (Heiman et al., 1997; Hague et al., 2013; Patterson et al., 2013). In addition, several studies report stressful life events heritability (Plomin et al., 1990; Kendler et al., 2007; Power et al., 2013). The correlation of objective and subjective stress scores confirmed the hypothesis that the patients with ED have altered perception of one's own body or stress (Cash and Deagle, 1997; Skrzypek et al., 2001; Stice et al., 2002; Keizer et al., 2013; Madsen et al., 2013). For the subjective perception stress scores, the highest level of consistency with the clinical score was shown by ED Specific Stress Score (SSS = 0.287; WSS = 0.224; OSS = 0.198). The Specific Stress Score has been established by ED specialized psychiatrists based on the clinical relevance of the items from Rahe's SCI.

In conclusion, the current study showed that the objective stress perception together with (GT)n promoter repeat polymorphism in the *HO1* gene influence ED pathology or stress-related disorders. Considering the limited size of our cohort, further studies are needed to confirm the G x E interactions of stress perception and the *HO1* gene.

References

Abraham, N. G., Brunner, E. J., Eriksson, J. W., Robertson, R. P. (2007) Metabolic syndrome: psychosocial, neuroendocrine, and classical risk factors in type 2 diabetes. *Ann. NY Acad. Sci*. **1113**, 256-275.

Abraham, N. G., Kappas, A. (2008) Pharmacological and clinical aspects of heme oxygenase. *Pharmacol. Rev.* **60**, 79-127.

Agnello, E., Malfi, G., Constantintino A. M., Massarenti, P., Pugliese, M., Fortunati, N., Catalano, M. G., Palmo, A. (2012) Tumour necrosis factor α and oxidative stress as maintaining factors in the evolution of anorexia nervosa. *Eat. Weight Disord.* **17**, 194-199.

Alda, M., Dvorakova, M., Zvolsky, P., Papezova, H., Posmurova, M. (1989) Genetic aspects in chronic schizophrenia. Morbidity risks and contributory factors, *Schizophr. Res.* **2**, 339-344.

Baan, C., Peeters, A., Lemos, F., Uitterlinden, A., Doxiadis, I., Claas, F., Ijzermans, J., Roodnat, J. M., Weimar, W. (2004) Fundamental role for HO-1 in the self-protection of renal allografts. *Am. J. Transplant*. **4**, 811-818.

Bakken, A. F., Thaler, M. M., Schmid, R. (1972) Metabolic regulation of heme catabolism and bilirubin production. I. Hormonal control of hepatic heme oxygenase activity. *J. Clin. Invest.* **51**, 530-536.

Berg, K., Peterson, C., Frazier, P, Crow, S. (2012) Psychometric evaluation of the eating disorder examination and eating disorder examination-questionnaire: a systematic review of the literature. *Int. J. Eat. Dis*.**45**, 428-438.

Bodell, L. P., Hames, J. L., Holm-Denoma, J. M., Smith, A. R., Gordon, K. H., Joiner, T. E. (2012) Does the stress generation hypothesis apply to eating disorders?: An examination of stress generation in eating, depressive, and anxiety symptoms. *J. Affect. Dis*. **142**, 139-142.

Blix, E., Perski, A., Berglund, H., Savic, I. (2013) Long-term occupational stress is associated with regional reduction in brain tissue volumes. *PLoS One* **6**, e64065.

Cash T. F., Deagle E. A (1997) The nature of extent of body-image disturbances in anorexia and bulimia nervosa: a meta-analysis. *Int. J. Eat. Disord.* **22**, 107-125.

Chen, Y. H., Lin, S. J., Lin, M. W., Tsai, H. L., Kuo, S. S., Chen, J. W., Charng, M. J., Wu, T. C., Chen, L. C., Ding, Y. A., Pan, W. H., Jou, Y. S., Chau, L. Y. (2002) Microsatellite polymorphism in promoter of heme oxygenase-1 gene is associated with susceptibility to coronary artery disease in type 2 diabetic patients. *Hum. Genet.* **111**, 1-8.

Denschlag, D., Marculescu, R., Unfried, G., Hefler, L. A., Exner, M., Hashemi, A., Riener, E. K., Keck, C., Tempfer, C. B., Wagner, O. (2004) The size of a microsatellite polymorphism of the haem oxygenase 1 gene is associated with idiopathic recurrent miscarriage. *Mol. Hum. Reprod*. **10**, 211-214.

Eipel, C., Eisold, M., Schuett, H., Vollmar, B. (2007) Inhibition of heme oxygenase-1 protects against tissue injury in carbon tetrachloride exposed livers. *J. Surg. Res.* **139**, 113-120.

Exner, M., Minar, E., Wagner, O., Schillinger, M. (2004) The role of heme oxygenase-1 promoter polymorphisms in human disease. *Free Radic. Biol. Med.* **37**, 1097-1104.

Exner, M., Raith, M., Holzer, G., Gmeiner, B., Wagner, O., Kapiotis, S. (2006) Anti-inflammatory mechanisms of the Tibetan herbal preparation Padma 28 in the vessel wall. *Forsch. Komplem. Med.* **13,** 13-17.

Faienza, M. F., Francavilla, R., Goffredo, R., Ventura, A., Marzano, F., Panzarino, G., Marinelli, G., Cavallo, L., Di Bitonto, G. (2012) Oxidative stress in obesity and metabolic syndrome in children and adolescents. *Horm. Res. Paed*. **78**, 158-164.

Gregorek, A. C., Gornik, K. C., Polancec, D. S., Dabelic, S. (2013) GT microsatellite repeats in the heme oxygenase-1 gene promoter associated with abdominal aortic aneurysm in Croatian patients. *Biochem. Genet.* **51**, 482-492.

Grochot-Przeczek, A., Dulak, J., Jozkowicz, A. (2012) Haem oxygenase-1: non-canonical roles in physiology and pathology. *Clin. Sci.* **122**, 93-103.

Hague, Z., Akbar, N., Yasmin, F., Haleem, M. A., Haleem, D. J. (2013) Inhibition of immobilization stress-induced anorexia, behavioral deficits, and plasma corticosterone secretion by injected leptin in rats. *Stress* **16**, 353-362.

Heiman, M. L., Ahima, R. S., Craft, L. S., Schoner, B., Stephens, T. W., Flier, J. S. (1997) Leptin inhibition of the hypothalamic-pituitary-adrenal axis in response to stress. *Endocrinology* **138**, 3859-3863.

Hirai, H., Kubo, H., Yamaya, M., Nakayama, K., Numasaki, M., Kobayashi, S., Suzuki, S., Shibahara, S., Sasaki, H. (2003) Microsatellite polymorphism in heme oxygenase-1 gene promoter is associated with susceptibility to oxidant-induced apoptosis in lymphoblastoid cell lines. *Blood* **102**, 1619-1621.

Holmes, T., Rahe, R. (1967) The social readjustment rating scale. *J. Psychosom. Res.* **11**, 213-218.

Johnson, J. G., Cohen, P., Kasen, S., Brook, J. S. (2002) Childhood adversities associated with risk for eating disorders or weight problems during adolescence or early adulthood. *Am. J. Psych.* **159**, 394-400.

Keizer, A., Smeets, M. A., Dijkerman, H. C., Uzunbajakau, S. A., van Elburg, A., Postma, A. (2013) Too fat to fit through the door: first evidence for disturbed body-scaled action in anorexia nervosa during locomotion. *PLoS One* **8**, e64602

Kelly, N. R., Cotter, E. W., Mazzeo, S. E. (2012) Eating disorder examination questionnaire (EDE-Q): norms for black women. *Eat. Behav.* **13**, 429-432.

Král, A., Kovárník, T., Kralik, L., Skalická, H., Horák, J., Mintz, G. S., Uhrová, J., Sonka, M., Wahle, A., Downe, R., Aschermann, M., Martásek, P., Linhart, A. (2011) Genetic variants in haem oxygenase-1 and endothelial nitric oxide synthase influence the extent and evolution of coronary artery atherosclerosis. *Folia Biol. (Praha)* **57**, 182-190.

Kyrou, I., Tsigos, C. (2007) Stress mechanisms and metabolic complications. *Horm. Metab. Res.* **39**, 430-438.

Kyrou, I., Tsigos, C. (2009) Stress hormones: physiological stress and regulation of metabolism. *Curr. Opin. Pharmacol.* **9**, 787-793.

Lindfors, C., Nilsson, I. A., Garcia-Roves P. M., Zuberi, A. R., Karimi, M., Donahue, L. R., Roopenian, D. C., Mulder, J., Uhlen, M., Ekström, T. J., Davisson, M. T., Hökfelt, T. G., Schalling, M., Johansen, J. E. (2011) Hypothalamic mitochondrial dysfunction associated with anorexia in the anx/anx mouse. *Proc. Natl. Acad. Sci. USA* **108**, 18108-18113.

Madsen, S. K., Bohon, C., Feusner, J. D. (2013) Visual processing in anorexia nervosa and body dysmorphic disorder: Similarities, differences, and future research directions. *J. Psychiatr. Res.* **47,** 1483-1491.

Megias, J., Busserolles, J., Alcaraz, M. (2007) The carbon monoxide-releasing molecule CORM-2 inhibits the inflammatory response induced by cytokines in Caco-2 cells. *Br. J. Pharm.* **150**, 977-986.

Megias, J., Guillen, M. I., Clerigues, V., Rojo, A. I., Cuadrado, A., Castejon, M. A., Gomar, F., Alcaraz, M. J. (2009) Heme oxygenase-1 induction modulates microsomal prostaglandin E synthase-1 expression and prostaglandin E(2) pro-

duction in osteoarthritic chondrocytes. *Biochem. Pharm.* **77**, 1806-1813.

Nagata, T., Matsuyama, M., Kiriike, N., Iketani, T., Oshima, J. (2000) Stress coping strategy in Japanese patients with eating disorders. Relationship with bulimic and impulsive behaviors. *J. Ner. Ment. Dis*. **188**, 280-286.

Otterbein, L., Bach, F. H., Alam, J. Soares, M., Tao Lu, H., Wysk, M., Davis, R. J., Flavell, R. A.,Choi, A. M. (2000) Carbon monoxide has anti-inflammatory effects involving the mitogen-activated protein kinase pathway. *Nat. Med.* **6**, 422-428.

Patterson, Z. R., Abizaid, A. (2013) Stress induced obesity: lessons from rodent models of stress. *Front. Neurosci*. **7**, 1-20.

Pavlova, B. (2010) Psychosocial risk factors in eating disorders (2010) In: Papežová, H. *Spectrum of Eating Disorders,* pp.35-36. Grada Publishing, ISBN 978-80-247-2425-6, Prague, Czech Republic. (in Czech)

Plomin, R., Lichtenstein, P., Pedersen, N. L., McClearn, G. E., Nesselroade, J. R. (1990) Genetic influence on life events during the last half of the life span. *Psychol. Aging* **5***,* 25-30.

Poss, K. D., Tonegawa, S. (1997) Heme oxygenase 1 is required for mammalian iron reutilization. *Proc. Natl. Acad. Sci. USA* **94**, 10919-10924.

Rahe, R., Veach, T. L., Tolles, R. L., Murakami, K. (2000) The stress and coping inventory: an educational and research instrument. *Stress Med.* **16**, 199-208.

Rizvi, S. I., Peterson, C., Crow, S., Agras, W. (2000) Test-retest reliability of the eating disorder examination. *Int. J. Eat. Dis.* **28**, 311-316.

Rueda, B., Oliver, J., Robledo, G., Lopez-Nevot, M.A., Balsa, A., Pascual-Salcedo, D., Gonzalez-Gay, M.A., Gonzalez-Escribano, M.F., Martin, J. (2007) HO-1 promoter polymorphism associated with rheumatoid arthritis. *Arthr. Rheum.* **56**, 3953-3958.

Sachs-Ericsson, N., Keel, P. K., Holland, L., Selby, E. A., Verona, E., Cougle, J. R., Palmer, E. (2012) Parental disorders, childhood abuse, and binge eating in a large community sample. *Int. J. Eat. Dis.* **45**, 316-325.

Schillinger, M., Exner, M., Minar, E., Mlekusch, W., Mullner, M., Mannhalter, C., Bach, F. H., Wagner, O. (2004) Heme oxygenase-1 genotype and restenosis after balloon angioplasty: a novel vascular protective factor. *J. Am. Coll. Cardiol.* **43**, 950-957.

Shibahara, S., Muller, R. M., Taguchi, H. (1987) Transcriptional control of rat heme oxygenase by heat shock. *J. Biol. Chem.* **262**, 12889-12892.

Skrzypek, S., Wehmeier, P. M., Remschmidt, H. (2001) Body image assessment using body size estimation in recent studies on anorexia nervosa. *Eur. Child Adolesc. Psych.* **10**, 215-221.

Stice, E., Shaw, H. E. (2002) Role of body dissatisfaction in the onset and maintenance of eating pathology: a synthesis of research findings *J. Psychosom. Res.* **53**, 985-993.

Taha, H., Skrzypek, K., Guevara, I., Nigisch, A., Mustafa, S., Grochot-Przeczek, A., Ferdek, P., Was, H., Kotlinowski, J., Kozakowska, M., Balcerczyk, A., Muchova, L., Vitek, L., Weigel, G., Dulak, J., Jozkowicz, A. (2010) Role of heme oxygenase-1 in human endothelial cells: lesson from the promoter allelic variants. *Arterioscler. Thromb. Vasc. Biol.* **30**, 1634-1641.

Tenhunen, R., Marver, H. S., Schmid, R. (1968) The enzymatic conversion of heme to bilirubin by microsomal heme oxygenase. *Proc. Natl. Acad. Sci. USA* **61**, 748-755.

Tenhunen, R., Marver, H. S., Schmid, R. (1969) Microsomal heme oxygenase. Characterization of the enzyme. *J. Biol. Chem.* **244**, 6388-6394.

Trace, S. E., Baker, J. H., Peñas-Lledó, E., Bulik, C. M. (2013) The genetics of eating disorders. *Annu. Rev. Clin. Psychol.* **9**, 589-620.

Wade, T. D., Treloar, S. A., Heath, A. C., Martin, N. G. (2009) An examination of the overlap between genetic and environmental risk factors for intentional weight loss and overeating. *Int. J. Eat. Dis.* **42**, 492-497.

Weis, S., Jesinghaus, M., Kovacs, P., Schleinitz, D., Schober, R., Ruffert, C., Herms, M., Wittenburg, H., Stumvoll, M., Bluher, M., Grutzmann, R., Schulz, H. U., Keim, V., Mossner, J., Bugert, P., Witt, H., Drenth, J. P., Krohn, K., Rosendahl, J. (2012) Genetic analyses of heme oxygenase 1 (HMOX1) in different forms of pancreatitis. *PLoS One*, **7**, e37981.

Wellek, S., Goddard, K. A, Ziegler, A. (2010) A confidence-limit-based approach to the assessment of Hardy-Weinberg equilibrium. *Biom. J.* **52**, 253–270.

Yamada, N., Yamaya, M., Okinaga, S., Nakayama, K., Sekizawa, K., Shibahara, S., Sasaki, H. (2000) Microsatellite polymorphism in the heme oxygenase-1 gene promoter is associated with susceptibility to emphysema. *Am. J. Hum. Genet.* **66**, 187-195.