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Are There Any Differences Between Stress Hormone Levels in Non-Stress Conditions and in Potentional Stress Overload (Heart Catheterisation) in Sows?

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Summary

In order to study a possible effect of mini-invasive heart intervention on a response of hypothalamo-pituitary-adrenal stress axis, we analyzed four stress markers (cortisol, cortisone, DHEA and DHEAS) in 25 sows using minimally invasive heart catheterisation as the stress factor. The marker levels were assessed in four periods of the experiment, (1) the baseline level on the day before intervention, (2) after the introduction of anesthesia, (3) after conducting tissue stimulation or ablation, and (4) after the end of the catheterisation. For statistical analyses we used the non-parametric Friedman test for four dependent samples (including all four stages of the operation) or three dependent samples (influence of operation only, baseline level was excluded). Statistically significant differences in both Friedman tests were found for cortisol and for cortisone. Significant differences for DHEA as well as for DHEAS were found for all tested stages but not for the effect of operation itself. We have concluded that cortisol levels are blunted by the influence of anesthesia after its administration, and therefore decrease back to the baseline at the end of the operation. The other markers (cortisone, DHEA and DHEAS) acted as balanced systems against the injurious stress effect.

Key words

Stress • Stress hormones • Heart catheterisation • Cortisol • Sow

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Introduction

In our presented study, the stress overload during minimally invasive heart catheterisation on animal, young sows, model was studied. Concentration of the adrenal cortex steroid (cortisol, cortisone, DHEA and DHEAS) was determined. The homeostasis of organism could be affecting by many stimuli (stressors), which could arise from many different origins, e.g. ecological (acute environmental changes, absence of nutrition or shelter, temperature variations), sociobiological (unstable social hierarchy), health (infection, injury, surgery), transport and many others – all these stressors could affected stress response during our experiment. Stressors trigger a stress response the organism prior to injurious effect (Schreiber 1985, Greenberg *et al.* 2002, Möstl and Palme 2002).

Cortisol and cortisone

Cortisol's molecular structure is lipophilic, allowing the unbound cortisol to freely enter the target

cells through the cell membrane into the cytoplasm where it is bound to specific receptors. The cortisol-receptor complex then enters the nucleus and identifies glucocorticoid response elements (GREs), special palindromic DNA sequences, binds to them, and then acts as a transcription modulator (Seckl 1997).

Cortisone is a steroid hormone also produced by the adrenal cortex. This hormone is characterized by its possession of a keto-group on C-11 (cortisol has hydroxyl group on C-11) and cannot be bound to cytoplasmatic receptors. Cortisone functions as a reserve pool of cortisol, providing more cortisol when needed (e.g. in stress response). Two isoenzymes of 11β-hydroxysteorid dehydrogenase (11β-HSD) act as important regulation factors for the conversion of cortisone to cortisol (11β-HSD1) and conversely cortisol to cortisone (11β-HSD2).

DHEA, DHEAS

Dehydroepiandrosterone (DHEA) and its sulphate (DHEAS) are steroid hormones originating mainly from the adrenal cortex. Both hormones have a positive effect on learning and memory. The plasma levels of DHEA and DHEAS change with aging: the highest levels are present during young adulthood, and from then on slowly and permanently decrease. These steroid hormones have neuroprotective effects and minimize degenerative changes (Aly *et al.* 2011). DHEA and DHEAS are positive modulators of *N*-methyl *D*-aspartate (NMDA) receptors (Bičíková *et al.* 2000) and negative modulators of the γ-aminobutyric acid ($GABA_A$) receptor (Gartside *et al.* 2010).

The aim of the presented study was to determine stress marker levels in each period of the experiment – we compared the level obtained at the breeding farm (under non-stress conditions) with levels obtained during the heart catheterisation experiment (potential stress events). Our second aim was to determine, if there are any differences in stress markers levels during minimally invasive heart intervention itself (the first blood collection was excluded).

We hope our findings could help improve elective cardiac procedures to minimise their effect on human patients or animal recipients.

Materials and Methods

For this study, serum concentrations of four adrenal activity markers (cortisol, cortisone, DHEA and DHEAS) were determined in sows undergoing elective heart catheterisation. Although it was only minimally invasive surgery, this provided a stress overload event. Marker levels were measured during four well defined periods of the experiment (details were published elsewhere (Skarlandtová *et al.* 2012) and described below) in order to evaluate any changes in their concentrations. Our aim was to determine if elective minimally invasive intervention has an influence on the hypothalamic-pituitary-adrenal (HPA) axis and consequently on the activity of the adrenal cortex.

The experiment was performed in accordance with Czech law and corresponding EU regulations and was approved by the Institutional Animal Care and Use Committee.

Animals

Twenty-five four month old sows (*Sus scrofa domestica*) were used in the experiment, from the crossbreed Landrace x Large White (details of breeding, housing, etc. of animals were described in our previous publication (Skarlandtová *et al.* 2012). The sows were in prepubertal age; therefore we can exclude the estrous cycle influence on the stress marker levels.

Experiment

The effect of heart catheterisation on stress marker levels in blood serum was tested. Changes in blood serum concentrations of cortisol, cortisone, DHEA and DHEAS were determined.

Heart catheterisation

Heart catheterisation was performed following the standard catheterization procedure (through arteria and vena femoralis using a 7-9F sheet). The catheterizations were carried out within the frame of electrophysiological projects, where cardiac stimulation or conducting tissue electrical radiofrequency ablation was performed. In all tested animals four markers were assessed in the blood serum: cortisol, cortisone, DHEA and DHEAS.

Anesthesia and medication

Stresnil (5 mg/kg), Atropin (0.05 mg/kg) and Narcetan (14 mg/kg) were used *via* intramuscular injection for pre-medication and sedation. An 18G or 20G IV cannula was inserted into the marginal ear vein to obtain intravenous (IV) access. Intravenous anesthetic introduction was initiated using a Propofol

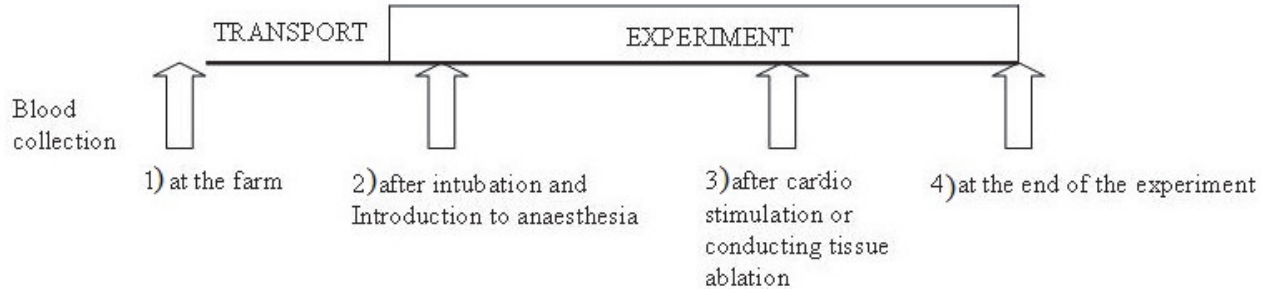

Fig. 1. The blood collection system. Arrows indicate the blood sampling in four defined parts of the experiment. For the first time, sows were blood sampled at the breeding farm, in non-stress conditions, after twenty-four hours were sows transported to the experimental lab, and then were blood sampled after intubation and introduction to anesthesia (second sample), after cardiostimulation or conducting tissue ablation and the last, at the end of the catheterisation.

(2 mg/kg) and Morphine (0.2 mg/kg) bolus. Intubation under direct laryngoscopic control was performed with 7 or 7.5 mm orotracheal tubes, depending on the size of the sow. Anesthesia was maintained with a Propofol (4 mg/kg/h) IV infusion, and as an analgesic, a morphine (0.2 mg/kg) IV bolus was administered every hour. Ventilation was sustained at an average volume of 8 to 10 ml/kg and respiratory rates 15 per minute.

During the IV anesthesia a continuous monitoring of mean arterial pressure (MAP), heartbeat rate (HR), O_2 saturation (SO_2) and exhalated capnometry (PCO_2) was observed on a multiparameter bed-side monitor.

Blood collection

Blood was collected from the jugular vein during each of the four defined periods of the experiment. The first (1) was collected at the farm, in non-stress domestic conditions (control sample, baseline stress marker levels), other samples were collected 10 min after the presumed stress situation: the second sample (2) 10 min after intubation and the introduction to anesthesia, the third sample (3) 10 min after cardio stimulation or conducting tissue ablation and the last (4) at the end of the intervention, before the animal was sacrificed. Blood samples (10 ml) were collected in 10 ml serum Vacutainer system tubes (BD Vacutainer, SSt II Advance), and after a 30 min incubation at room temperature were centrifuged (2000 x g) for 15 min and then serum was stored at –20 °C until later analysis. The whole experiment can be divided into two sections: the first period (non-stress conditions, baseline reference sample) and the second, surgery section (the second, third and fourth periods of the experiment) – see Figure 1.

Laboratory analyses

Cortisol and cortisone were measured using the method published elsewhere (Šimůnková *et al.* 2008). In brief, the serum samples were twice extracted and then the hormones separated using a high performance liquid chromatography (HPLC) system from Dionex Softron (Germering, Germany). HPLC separation was carried out with reverse phase EC 250/4 NUCLEOSIL® 100-5 C18 column (MACHEREY-NAGEL, Düren, Germany), and to avoid possible column contamination the Phenomenex SecurityGuard system with cartridge C18 (Phenomenex, Torrance, CA) was used. Merck (Darmstadt, Germany) solvents were used as the mobile phase for HPLC. Cortisol and cortisone concentrations in the serum were determined according to a calibration curve using UV/VIS detection.

Dehydroepiandrosterone (DHEA) and its sulfate (DHEAS) were measured by commercial radioimmunoassay and immunoradiometric kits (Immunotech; BeckmanCoulter, Czech Republic). Intra-assay and inter-assay coefficients of variation (CVs) for DHEA were found below or equal to 7.9 % and 11.9 %. Intra- and inter-assay CVs for DHEAS were below or equal to 7.4 % and 10.6 %, respectively. The analytical parameters corresponded to those stated by the manufacturer.

Statistics

As our data did not have a standard Gaussian distribution, non-parametric statistical methods were used to analyse the differences in stress marker levels within the experiment. A Friedman test was designed for four dependent samples to cover all four periods of the experiment (see above).

Another Friedman test was calculated for three dependent samples (excluding the first period of the experiment) to determine the influence of only the surgical procedures on stress marker levels.

Results

Elementary statistical data was calculated for the measured markers (cortisol, cortisone, DHEA and DHEAS) for each period of the experiment (1-4; see Fig. 1). A wide variance in measured data was demonstrated, indicating substantial inter-individual differences in the assessed marker levels; for illustration, Box and Whisker plots (Figs 2-5) are shown for each marker. Medians (for exclusion outlier values) were noted and discussed, and the results shown separately for each marker.

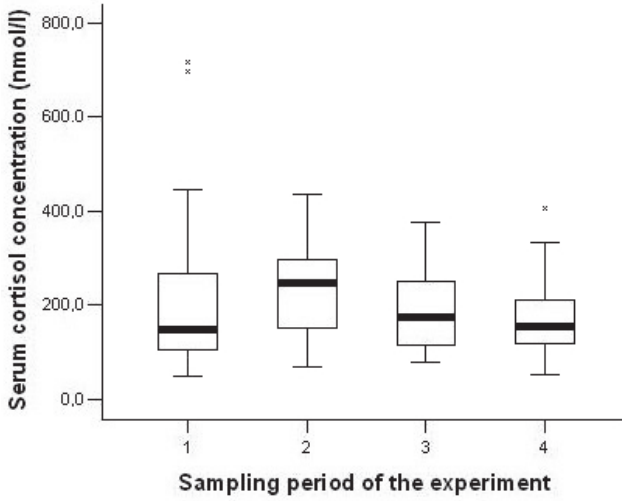

Fig. 2. Box and whisker plot: Serum cortisol concentrations during the four sampling periods of the experiment. There are 50 % of measured values of cortisol concentration in the box, the median is marked as a bold line in the box, the whiskers are 25 % measured values, and the stars are outlier values.

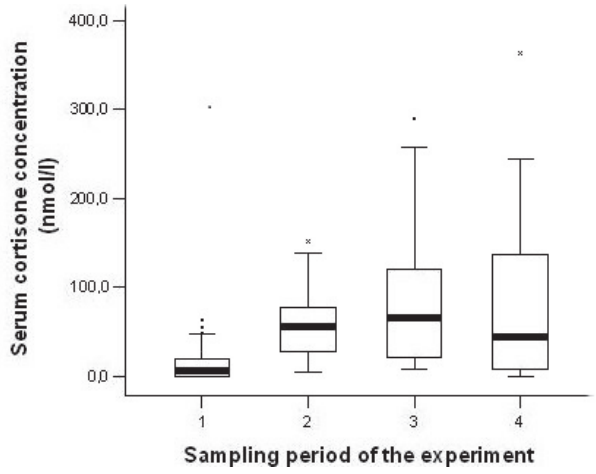

Fig. 3. Box and whisker plot: Serum cortisone concentrations during the four sampling periods of the experiment. There are 50 % of measured values of cortisone concentration in the box, the median is marked as a bold line in the box, the whiskers are 25 % measured values, and the stars are outlier values.

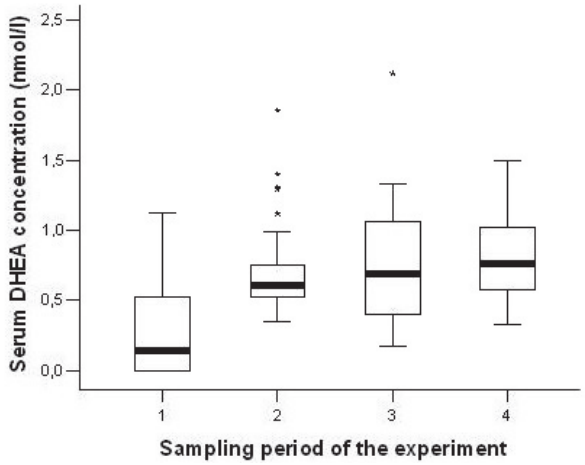

Fig. 4. Box and whisker plot: Serum DHEA concentrations during the four sampling periods of the experiment. There are 50 % of measured values of DHEA concentration in the box, the median is marked as a bold line in the box, the whiskers are 25 % measured values, and the stars are outlier values.

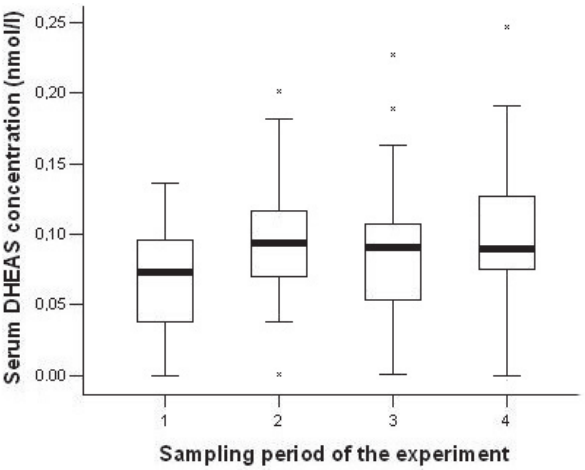

Fig. 5. Box and whisker plot: Serum DHEAS concentrations during the four sampling periods of the experiment. There are 50 % of measured values of DHEAS concentration in the box, the median is marked as a bold line in the box, the whiskers are 25 % measured values, and the stars are outlier values.

Cortisol

As we expected, the serum cortisol concentration was the lowest (148.35 nmol/l) in the non-stress sample at the farm. Therefore we can regard the samples from the first period of the experiment as the baseline level. The cortisol concentration then increased to the highest level (246.41 nmol/l) during the second period of the experiment (after the introduction anesthesia). During the third and fourth periods of the experiment, cortisol levels decreased to concentrations close to the baseline (175.42 nmol/l and 154.30 nmol/l respectively).

The statistics (Friedman test) were significant ($p<0.05$) in both tests – for all four sampled periods of the

experiment, and for the three stages excluding the first one. This suggests that the surgery section itself also had an important influence on the levels of cortisol.

Cortisone

Similar to the cortisol median levels, the concentration of cortisone was the lowest during the first period of the experiment (6.19 nmol/l). Levels increased during the second and third periods (the highest level was recorded during the third sampling time; 66.12 nmol/l). The cortisone level then decreased at the end of the intervention, but was still seven times higher than the baseline (44.70 nmol/l). Statistics for all periods of the experiment were significant ($p<0.001$), and, if we exclude the first stage of the experiment, statistics were $p<0.05$. This means that the cortisone levels statistically changed in all periods of the experiment, and also for the three stages of heart catheterisation when viewed separately from the non-stress (baseline) period.

DHEA

The median concentration levels for DHEA were the lowest at the beginning of the experiment (farm sample, baseline level) (0.15 nmol/l), then increased, with the highest levels being found at the end of the catheterisation (0.76 nmol/l). For all four tested periods of the experiment (including the first, baseline level) the p value was 0.001, however, for catheterisation alone $p>0.05$. DHEA levels rapidly increased after the first section of the experiment, then during the surgery section the levels were very close. This suggests that surgery itself did not affect the levels of DHEA.

DHEAS

Overall, the median levels of DHEAS did not differ much throughout the procedure, but the lowest concentrations were measured during the first period (0.07 nmol/l), and then slightly increased during the surgical part of the experiment (0.09 nmol/l in the second, third and the fourth part of the experiment). The Friedman test for all periods of the experiment gave a p value that was below the significance level ($p<0.05$). When only the surgery section of the experiment was tested (excluding the first period), there were no significant changes ($p>0.05$).

Discussion

Our study was focused on the HPA (hypothalamo-pituitary-adrenal) axis activity, but not on that of the SAM (sympatho-adreno-medullar) axis. The first and the most important reason was the fact that catecholamines (unlike glucocorticoids) are released in a few seconds following the stress stimulus, and the design of our experiment technically disallowed the blood collection in this very short time. Moreover it is unable to assess catecholamines and glucocorticoids (and other tested markers) in the same blood sample and two blood collections at every stage of the experiment could cause two problems: 1. the volume of the collected blood and 2. possible affection of the second sample results by the preceding blood collection.

Basic levels of cortisol in our group of experimental animals differ from some data reported previously (e.g. Perremans *et al.* 2001). The difference can be explained by many factors: different environmental conditions of live, procedure of cannulation, different genetic groups of animals, and big interindividual differences among individual animals. This was one of reasons why we had to measure our own basic levels of examined hormones and did not rely on literature data.

Anesthesia influence on adrenal hormones secretion

Anesthesia is used to minimize the traumatic effect of surgical procedures. It is well known that anesthesia blunts stress response through the suppression of the secretion of stress hormones (catecholamines and glucocortioids). There are divergent types of anesthesia with different suppression rates of stress response – balanced (inhalation) and total intravenous anesthesia (TIVA). Many studies have found lower stress marker levels in TIVA (a combination of Propofol and an opioid as an analgesic component) compared to balanced anesthesia (a combination of inhalation gases, e.g. Sevoflurane, Isoflurane, Enflurane, etc. and an opioid) (Schricker *et al.* 2000, Ledowski *et al.* 2005, Ihn *et al.* 2009, Kostopanagiotou *et al*. 2010, Marana *et al.* 2010). TIVA, consisting of a combination of Propofol and Morphine, was used in our experiment (for details see *Anesthesia and medication*). Propofol is lipophilic weak acid with voltage-gated ion L-calcium channels in heart influence. It improves decreased sympatic activity alpha and beta adrenergic receptors (Krzych *et al.* 2009). Combination of Propofol and Morphine (or other opioid) decreasing catecholamines and glucocorticoid released to blood (Fragen *et al.* 1987, Schricker *et al.* 1999, 2000, Ihn *et al.* 2009).

The difference between TIVA using Propofol, and inhalation anesthetics using various anesthetic gases, is that the Propofol/opioid combination suppresses HPA activity at each level. That is, the suppression of the production of the corticotrophin releasing hormone (CRH), adrenocorticotropin hormone (ACTH) and the adrenal hormones (glucocorticoids and DHEA). This could be caused by a synergic Propofol-opioid reaction on the hypothalamic receptors that suppress noxious afferent stimuli and then suppress CRH release (due to the increase in the GABA receptor inhibitor concentration) (Kostopanagiotou *et al*. 2010, Marana *et al.* 2010).

Many authors (e.g. Van Hemelrijck *et al.* 1995, Han *et al.* 2012, Offinger *et al.* 2012) found decreased cortisol levels 45 min after the introduction of anesthesia, which corresponds with our results. Stress steroid levels were lower in comparison to non-stress ones (e.g. Fragen *et al.* 1987, Schricker *et al.* 1999, 2000, Ihn *et al.* 2009). In our experiment, the results for cortisol concentration corresponded to referenced authors, with the highest levels determined in the second period of the experiment, i.e. a very short time after the introduction to anesthesia, then during the third and fourth periods the levels decreased approximately back to the baseline concentration. In comparison, cortisone, DHEA and DHEAS concentrations increased during the second period of the experiment and were higher compared with the baseline levels. We attribute this to the balance stress reaction of these steroids.

Surgical procedure

In this experiment the minimally invasive surgical procedure of heart catheterization was used. A number of previous studies found no difference in elevated cortisol levels between invasive open surgery and minimally invasive procedures in pigs (Mansour *et al.* 1992, Bessler *et al.* 1994, Burpee *et al.* 2002, Margulis *et al.* 2005, Matsumoto *et al.* 2005, Duchene *et al.* 2008). These findings could mean that even minimal intervention can cause a rise in cortisol levels. However, stress response to surgery could be modulated by some other parameters, e.g. type of anesthesia (mentioned above), handling, etc. Contrastingly, experimental measurements in human patients have found some differences in stress response for the same type of the laparoscopic intervention using a slightly different surgical approach (Han *et al.* 2012).

Our findings suggest that the most stressful stage of the heart catheterization is during its very beginning. This fact should be considered in invasive animal experiments but it would be very difficult to transfer this experience to human cardiac interventions particularly because of standard sedative premedication of human patients.

Cortisone

Cortisone represents cortisol's reserve pool when more cortisol is needed (as for instance in the stress response during our experiment) (Vogeser *et al.* 2003). Serum cortisone concentration in our experiment was at the lowest level during the first period of the experiment, then it increased to the highest levels during the third period, and during the fourth period it slightly decreased. This corresponds with the cortisol level, which was found to be at the highest concentration after exposure to anesthesia and then it decreased. We assume that the higher levels of cortisone were caused as a result of the higher activity of the 11β-HSD2 isoenzyme (the crucial regulation factor of cortisol/cortisone levels), in this way balancing the action of the cortisol.

DHEA, DHEAS

As previously noted, the steroid hormones DHEA and DHEAS are produced in the adrenal cortex, and to a lesser degree, in the gonads or placenta. They can also be produced in the central nervous system, as neurosteroids, which are characterized by their rapid non-genomic effect on receptor complexes (e.g. NMDA and $GABA_A$). In our experiment the circulating concentration of these steroids was measured. DHEAS is the main circulating form and acts as a reserve pool of DHEA. The concentration of these steroids is higher during stress events, and during such periods there is also a rise in cortisol concentration. This suggests that they react to higher CRH and ACTH levels (Nieschlag *et al.* 1973), and therefore they could be considered as a HPA axis activity marker (e.g. Goodyer *et al.* 2001, Maninger *et al.* 2010) DHEA and DHEAS have an anti-glucocorticoid effect; they act as "antistress" steroids and minimize negative glucocorticoid effects (Kimonides *et al.* 1998, 1999, Charney *et al.* 2004, Morgan *et al.* 2004, Maninger *et al.* 2009). In our results, these steroids are released in higher concentrations during the second part of the experiment. Our research therefore supports the findings of previous authors in demonstrating that these steroids balanced the stress reaction in our catheterized sows.

Conclusions

In our experiment cortisol, cortisone and DHEA(S) levels were determined during four stages of the heart catheterisation of young sows. We separately calculated Friedman tests for each marker during the four defined periods of the experiment; these tests were statistically significant for all markers. The lowest concentration levels for all markers were measured during the baseline, non-stress conditions. Therefore, we can conclude that the conditions at sows' home farm are non-stressful. From the baseline, the serum concentrations of all markers then increased to their highest levels; for cortisol during the second period, for cortisone during the third, and for DHEA(S) during the final part of the experiment. We can assume that the adrenal secretion of cortisol occurs in response to the most stressful conditions before the exposure to intravenous anesthesia, and then the anesthesia minimizes the stress response. The other hormone markers (cortisone, DHEA, DHEAS) act as a balanced system against the traumatic effects of stress.

Conflict of Interest

There is no conflict of interest.

Acknowledgements

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