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MORPHOFUNCTIONAL CHANGES OF THE ADRENAL GLANDS IN EXPERIMENTAL METABOLIC SYNDROME: HISTOLOGICAL, MORPHOMETRIC AND HORMONAL ASSESSMENT

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Abstract. Metabolic syndrome (MS) is accompanied by profound endocrine disturbances, yet the structural basis of adrenal dysfunction in this condition remains insufficiently characterised. The aim of the study was to assess histological, morphometric and hormonal changes of the adrenal glands in diet-induced experimental MS and to determine the relationships between structural and functional indices. Forty male Wistar rats were divided into a control group (n=10, standard chow) and an experimental group (n=30) receiving a high-fat diet (60% fat) together with 10% fructose solution for 10 weeks. Adrenal sections were stained with haematoxylin–eosin and Van Gieson and analysed in ImageJ; serum cortisol, aldosterone and DHEA-S were measured by ELISA. Experimental animals developed obesity, hyperglycaemia, dyslipidaemia and arterial hypertension. The adrenal cortex thickened by 15.8% owing to widening of the zona glomerulosa (+30.2%) and zona fasciculata (+21.1%), whereas the zona reticularis narrowed by 9.0%. Fasciculata cells showed hypertrophy (+22.8%), massive lipid vacuolization and a decreased nucleo-cytoplasmic ratio; stromal connective tissue increased by 85.4% and capillary density decreased by 18.0%. Serum cortisol and aldosterone rose by 72.0% and 55.8%, respectively, while DHEA-S fell by 26.2%. Strong correlations were found between zona thickness and the corresponding hormone levels (r=0.55–0.71). The findings indicate that adrenal cortical remodelling is an integral component of MS pathogenesis.

Keywords: metabolic syndrome; adrenal gland; adrenal cortex; morphometry; cortisol; aldosterone; DHEA-S; high-fat diet; fructose; Wistar rats.

INTRODUCTION

Metabolic syndrome is a cluster of interrelated risk factors – abdominal obesity, insulin resistance, atherogenic dyslipidaemia, hyperglycaemia and arterial hypertension – that substantially increases the risk of type 2 diabetes and cardiovascular disease (Alberti et al., 2009). Its prevalence has reached epidemic proportions: roughly one quarter of the world's adult population is estimated to meet the diagnostic criteria, and the burden is rising rapidly in developing countries owing to changes in diet and physical activity (Saklayen, 2018). Uzbekistan is no exception, where

unbalanced nutrition, sedentary lifestyle and chronic stress favour the spread of the syndrome among young people.

The adrenal glands occupy a central place in maintaining metabolic homeostasis. Their cortex consists of three functionally distinct zones: the zona glomerulosa producing aldosterone, the zona fasciculata producing glucocorticoids and the zona reticularis producing adrenal androgens (Vinson, 2016). Clinical similarity between MS and hypercortisolism led to the hypothesis that a functional, subclinical overactivity of the hypothalamic–pituitary–adrenal (HPA) axis contributes to visceral fat accumulation, insulin resistance and hypertension (Anagnostis et al., 2009; Björntorp, 2001; Rosmond, 2005; Walker, 2006). In parallel, obesity is associated with elevated aldosterone levels, partly mediated by mineralocorticoid-releasing factors secreted by adipocytes (Ehrhart-Bornstein et al., 2003), and excess aldosterone promotes renal and cardiovascular injury (Briet & Schiffrin, 2010). Thus, a bidirectional adrenal–adipose "cross talk" has been proposed as one of the mechanisms linking obesity with its complications (Kargi & Iacobellis, 2014).

Despite abundant hormonal data, structural studies of the adrenal glands in MS are scarce and inconsistent. In mice fed a high-fat diet for 18 weeks, Swierczynska et al. (2015) observed expansion of the adrenal cortex together with increased corticosterone and aldosterone secretion, whereas Topal et al. (2019) reported an increase in adrenal weight and in the volume fraction of the zona reticularis after nine weeks of high-fat feeding. Combined high-fat and high-carbohydrate (fructose) diets reproduce the full human-like MS phenotype in rats more reliably than single-component diets (Panchal et al., 2011; Wong et al., 2016), but adrenal morphology has rarely been quantified in such models together with hormonal profiles.

The aim of the present study was to determine histological, morphometric and hormonal changes of the adrenal glands in experimental MS induced by a high-fat–high-fructose diet and to evaluate the correlation between structural and functional parameters.

MATERIALS AND METHODS

Animals. The study was performed on 40 healthy male Wistar rats (*Rattus norvegicus*) with an initial body weight of 180–200 g. The animals were kept under standard vivarium conditions (22±2 °C, relative humidity 60–70%, 12/12 h light–dark cycle) with free access to food and water. After a 7-day acclimatisation period the rats were randomly allocated to two groups: the control group (n=10) received standard laboratory chow and tap water; the experimental group (n=30) received a high-fat diet (60% of energy from fat, 20% protein, 20% carbohydrates) and 10% fructose solution instead of drinking water for 10 weeks, following established dietary models of MS (Buettner et al., 2007; Panchal et al., 2011).

Verification of the model. Body weight was recorded weekly. Systolic blood pressure was measured non-invasively by the tail-cuff method. At the end of the experiment, fasting blood glucose, total cholesterol (TC), triglycerides (TG), high- and low-density lipoprotein cholesterol (HDL-C, LDL-C) were determined by standard enzymatic colorimetric methods. MS was considered established when obesity, hyperglycaemia, dyslipidaemia and elevated blood pressure were present simultaneously; these changes were recorded from the 8th week of feeding.

Hormonal assay. Blood was collected in the morning (08:00–10:00) to minimise circadian variation. Serum cortisol, aldosterone and dehydroepiandrosterone sulphate (DHEA-S) were

measured by enzyme-linked immunosorbent assay (ELISA) using commercial kits validated for rat serum, according to the manufacturers' instructions.

Histology and morphometry. The animals were euthanised under ketamine–chloral hydrate anaesthesia. Both adrenal glands were excised, weighed, fixed in 10% neutral buffered formalin and embedded in paraffin. Serial sections 4–5 μm thick were cut through the central part of the gland and stained with haematoxylin and eosin (general structure) and by Van Gieson's method (connective tissue) according to standard protocols (Suvarna et al., 2019). Digital images were obtained with a light microscope and analysed in ImageJ (Schneider et al., 2012). The following indices were measured: capsule thickness; thickness of the whole cortex and of each zone; diameters of cells and nuclei; nucleo-cytoplasmic ratio (NCR, ratio of nuclear area to cytoplasmic area); proportion of vacuolized (lipid-laden) cells; capillary density (number of capillary profiles per mm²) and the area fraction of connective tissue. At least 50 cells and 10 fields of view per animal were analysed for each parameter by an investigator blinded to group allocation.

Statistics. Data were processed in Microsoft Excel and Statistica 12.0 and are presented as mean ± standard deviation (M±SD). Between-group differences were assessed with Student's unpaired t-test; Pearson's correlation coefficient (r) was calculated in the experimental group. Differences were considered significant at $p<0.05$.

Ethics. All procedures complied with international principles of humane treatment of laboratory animals and the ARRIVE recommendations (Kilkenny et al., 2010); measures were taken to minimise pain and stress, and each determination was repeated at least three times.

RESULTS AND DISCUSSION

General and metabolic parameters. By the end of the 10th week the experimental rats had developed a complete MS phenotype (Table 1). Their body weight exceeded that of the controls by 23.7%, and abdominal fat mass more than doubled. Fasting glucose increased by 58.3%, triglycerides almost doubled, LDL-C more than doubled while HDL-C decreased by one third; systolic blood pressure rose by 20.3%. The absolute adrenal mass increased from 28.4±2.1 to 38.6±3.0 mg (+35.9%; $p<0.001$), and the relative mass – from 9.1±0.7 to 10.0±0.8 mg/100 g body weight (+9.9%; $p<0.01$), indicating true adrenal enlargement rather than a mere reflection of body weight gain.

Table 1

Body weight and metabolic parameters in control and experimental rats (M±SD)

Parameter	Control (n=10)	MS (n=30)	Δ, %	p
Final body weight, g	312±18	386±24	+23.7	<0.001
Abdominal fat mass, g	6.8±0.9	14.2±1.8	+108.8	<0.001
Fasting glucose, mmol/L	4.8±0.4	7.6±0.7	+58.3	<0.001
Total cholesterol, mmol/L	1.62±0.18	2.48±0.26	+53.1	<0.001
Triglycerides, mmol/L	0.94±0.12	1.86±0.21	+97.9	<0.001

HDL-C, mmol/L	0.86±0.08	0.58±0.07	−32.6	<0.001
LDL-C, mmol/L	0.48±0.07	1.12±0.15	+133.3	<0.001
Systolic BP, mmHg	118±6	142±8	+20.3	<0.001

Hormonal profile. Serum cortisol in the experimental group reached 148.6±15.8 nmol/L versus 86.4±9.2 nmol/L in the controls (+72.0%; p<0.001), and aldosterone – 486±52 versus 312±34 pmol/L (+55.8%; p<0.001). In contrast, DHEA-S decreased from 0.42±0.05 to 0.31±0.04 µmol/L (−26.2%; p<0.001) (Fig. 1). The divergent shifts – activation of glucocorticoid and mineralocorticoid output with suppression of the androgenic one – indicate a redistribution of steroidogenesis rather than uniform adrenal hyperfunction.

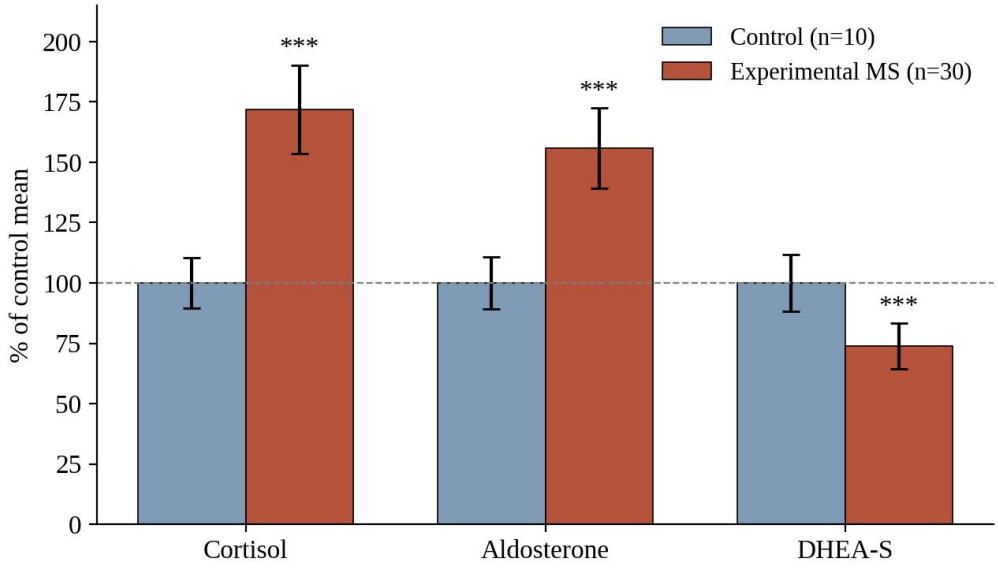


Figure 1. Serum adrenal hormones in experimental MS expressed as percentage of control mean (M±SD); *** – p<0.001 vs control

Histological findings. In control animals the adrenal gland had a typical structure: a thin fibrous capsule, a narrow zona glomerulosa composed of arched clusters of small cells, radially oriented cords of the zona fasciculata with moderately vacuolated cytoplasm and a compact zona reticularis bordering the medulla. In the experimental group the capsule was thickened and loosened, the zona glomerulosa was widened, and its cells were enlarged with lighter cytoplasm. The zona fasciculata showed the most pronounced changes: the cells were hypertrophied, the cytoplasm was overloaded with lipid vacuoles ("spongiocytes"), some nuclei were displaced to the periphery, and sinusoidal capillaries were dilated and congested. Foci of cellular dystrophy and binucleated cells were frequently found. The zona reticularis was narrowed, with signs of nuclear pyknosis and cytoplasmic eosinophilia. Van Gieson staining revealed thickening of the capsule and of the perisinusoidal collagen fibres.

Morphometric data. Quantitative analysis confirmed the visual impressions (Table 2). The total cortical thickness increased by 15.8%, mainly at the expense of the zona glomerulosa (+30.2%) and the zona fasciculata (+21.1%), while the zona reticularis became 9.0% thinner. The diameter of

fasciculata cells increased by 22.8%, nuclear diameter – by 11.3%; however, cytoplasmic growth outpaced nuclear growth, so the NCR decreased by 19.5%. The share of vacuolized cells rose from 34.2% to 58.6%. The connective tissue fraction almost doubled, and capillary density in the zona fasciculata fell by 18.0%. In the medulla, chromaffin cells were moderately enlarged (+12.5%).

Table 2

Morphometric parameters of the adrenal gland in experimental MS (M±SD)

Parameter	**Control (n=10)**	**MS (n=30)**	**Δ, %**	**p**
Capsule thickness, µm	18.2±1.6	24.6±2.2	+35.2	<0.001
Total cortex thickness, µm	862±41	998±52	+15.8	<0.001
Zona glomerulosa, µm	86±7	112±9	+30.2	<0.001
Zona fasciculata, µm	598±32	724±41	+21.1	<0.001
Zona reticularis, µm	178±14	162±13	−9.0	<0.01
Fasciculata cell diameter, µm	18.4±1.2	22.6±1.5	+22.8	<0.001
Fasciculata nuclear diameter, µm	6.2±0.4	6.9±0.5	+11.3	<0.001
NCR (zona fasciculata)	0.128±0.011	0.103±0.009	−19.5	<0.001
Vacuolized cells, %	34.2±3.8	58.6±5.1	+71.3	<0.001
Capillary density, per mm²	412±28	338±24	−18.0	<0.001
Connective tissue fraction, %	4.8±0.6	8.9±1.1	+85.4	<0.001
Chromaffin cell diameter, µm	16.8±1.1	18.9±1.3	+12.5	<0.001

Structure–function relationships. Correlation analysis in the experimental group showed that the morphometric changes of each zone were closely linked with the secretion of its hormone (Table 3). The strongest relationship was found between the thickness of the zona fasciculata and serum cortisol (r=0.71), which in turn correlated with fasting glucose (r=0.62). Capillary density was inversely related to cortisol, and the connective tissue fraction was associated with triglyceride levels.

Table 3

Correlations between morphological and functional indices in the experimental group (n=30)

Pair of indices	**r**	**p**
Zona fasciculata thickness – cortisol	0.71	<0.001
Fasciculata cell diameter – cortisol	0.66	<0.001
Zona glomerulosa thickness – aldosterone	0.64	<0.001

Cortisol – fasting glucose	0.62	<0.001
Zona glomerulosa thickness – systolic BP	0.57	<0.001
Zona reticularis thickness – DHEA-S	0.55	<0.01
Connective tissue fraction – triglycerides	0.52	<0.01
Capillary density – cortisol	−0.48	<0.01

Discussion. Our data show that diet-induced MS is accompanied by zone-specific remodelling of the adrenal cortex. Hypertrophy and lipid overload of the zona fasciculata together with elevated glucocorticoid levels agree with the concept of functional hypercortisolism in MS (Anagnostis et al., 2009; Walker, 2006) and with experimental findings of cortical expansion and increased glucocorticoid output in obese rodents (Swierczynska et al., 2015). The accumulation of lipid vacuoles reflects increased uptake of lipoprotein cholesterol – the substrate of steroidogenesis – under conditions of dyslipidaemia, while the decrease in NCR indicates that cell enlargement is driven mainly by cytoplasmic expansion. Since glucocorticoids promote visceral adiposity and hepatic gluconeogenesis (Björntorp, 2001), the observed cortisol–glucose correlation supports a vicious circle in which adrenal activation aggravates the metabolic disorder (Fig. 3).

Widening of the zona glomerulosa and the rise in aldosterone are consistent with the known hyperaldosteronism of obesity, which is partly mediated by adipocyte-derived secretagogues (Ehrhart-Bornstein et al., 2003; Kargi & Iacobellis, 2014). The correlation of glomerulosa thickness with systolic pressure suggests that this mechanism contributes to hypertension in our model, in line with the deleterious renal and vascular effects of aldosterone (Briet & Schiffrin, 2010). Narrowing of the zona reticularis with decreased DHEA-S differs from the data of Topal et al. (2019) obtained in mice after a shorter, fat-only diet; the discrepancy may reflect species differences, the duration of exposure and the additional effect of fructose (Tappy & Lê, 2010). Capsular thickening, stromal fibrosis and capillary rarefaction suggest that prolonged functional strain leads to microcirculatory insufficiency, which may eventually limit adaptive reserves of the gland.

A limitation of the study is that corticosterone is the principal glucocorticoid in rats and adrenal 17α-hydroxylase activity in this species is low; therefore cortisol and DHEA-S values should be regarded as integral indices of glucocorticoid and androgenic activity, and parallel determination of corticosterone and ACTH is advisable in further work.

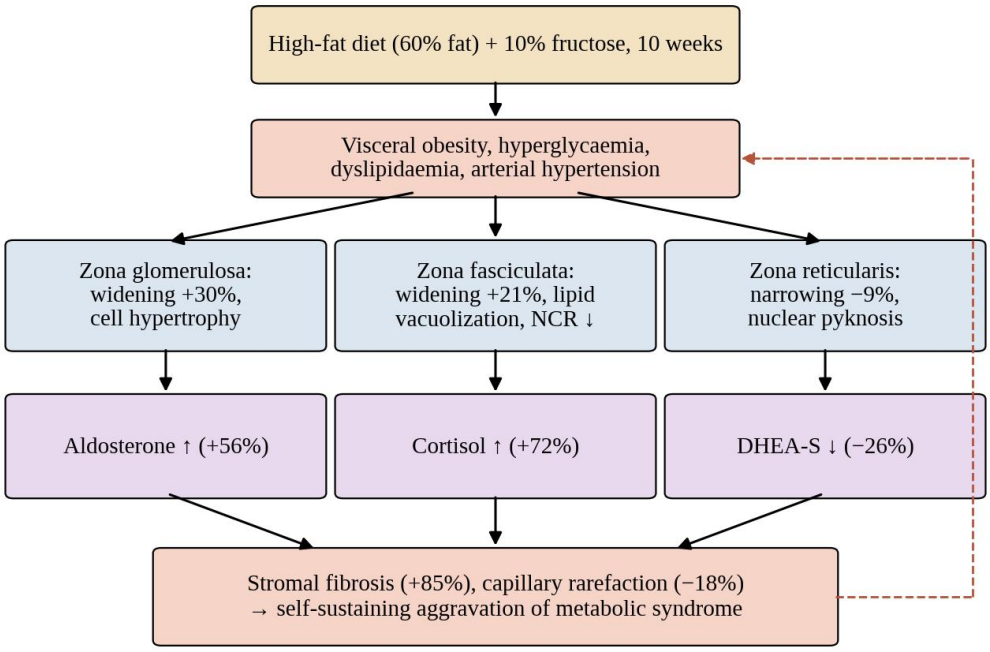


Figure 2. Proposed scheme of adrenal remodelling and hormonal dysregulation in experimental MS (dashed line – feedback aggravation)

CONCLUSION

1. A 10-week high-fat–high-fructose diet in Wistar rats reproduces metabolic syndrome with obesity, hyperglycaemia, dyslipidaemia and arterial hypertension and leads to true enlargement of the adrenal glands.

2. Adrenal remodelling in MS is zone-specific: hypertrophy of the zona glomerulosa and zona fasciculata with lipid vacuolization and decreased NCR, atrophy of the zona reticularis, capsular and stromal fibrosis and capillary rarefaction.

3. Structural changes are accompanied by increased cortisol (+72.0%) and aldosterone (+55.8%) and decreased DHEA-S (−26.2%), and correlate closely with these hormonal shifts (r=0.55–0.71), which confirms the involvement of the adrenal cortex in the pathogenesis of MS. The morphometric indices obtained may serve as morphological criteria of adrenal involvement in MS and as reference points for evaluating corrective therapy.

REFERENCES

1. Alberti, K. G. M. M., Eckel, R. H., Grundy, S. M., Zimmet, P. Z., Cleeman, J. I., Donato, K. A., Fruchart, J.-C., James, W. P. T., Loria, C. M., & Smith, S. C., Jr. (2009). Harmonizing the metabolic syndrome: A joint interim statement. *Circulation, 120*(16), 1640–1645. https://doi.org/10.1161/CIRCULATIONAHA.109.192644
2. Anagnostis, P., Athyros, V. G., Tziomalos, K., Karagiannis, A., & Mikhailidis, D. P. (2009). The pathogenetic role of cortisol in the metabolic syndrome: A hypothesis. *The Journal of Clinical Endocrinology & Metabolism, 94*(8), 2692–2701. https://doi.org/10.1210/jc.2009-0370

3. Björntorp, P. (2001). Do stress reactions cause abdominal obesity and comorbidities? *Obesity Reviews, 2*(2), 73–86. https://doi.org/10.1046/j.1467-789x.2001.00027.x
4. Briet, M., & Schiffrin, E. L. (2010). Aldosterone: Effects on the kidney and cardiovascular system. *Nature Reviews Nephrology, 6*(5), 261–273. https://doi.org/10.1038/nrneph.2010.30
5. Buettner, R., Schölmerich, J., & Bollheimer, L. C. (2007). High-fat diets: Modeling the metabolic disorders of human obesity in rodents. *Obesity, 15*(4), 798–808. https://doi.org/10.1038/oby.2007.608
6. Ehrhart-Bornstein, M., Lamounier-Zepter, V., Schraven, A., Langenbach, J., Willenberg, H. S., Barthel, A., Hauner, H., McCann, S. M., Scherbaum, W. A., & Bornstein, S. R. (2003). Human adipocytes secrete mineralocorticoid-releasing factors. *Proceedings of the National Academy of Sciences, 100*(24), 14211–14216. https://doi.org/10.1073/pnas.2336140100
7. Kargi, A. Y., & Iacobellis, G. (2014). Adipose tissue and adrenal glands: Novel pathophysiological mechanisms and clinical applications. *International Journal of Endocrinology, 2014*, Article 614074. https://doi.org/10.1155/2014/614074
8. Kilkenny, C., Browne, W. J., Cuthill, I. C., Emerson, M., & Altman, D. G. (2010). Improving bioscience research reporting: The ARRIVE guidelines for reporting animal research. *PLoS Biology, 8*(6), Article e1000412. https://doi.org/10.1371/journal.pbio.1000412
9. Panchal, S. K., Poudyal, H., Iyer, A., Nazer, R., Alam, M. A., Diwan, V., Kauter, K., Sernia, C., Campbell, F., Ward, L., Gobe, G., Fenning, A., & Brown, L. (2011). High-carbohydrate, high-fat diet-induced metabolic syndrome and cardiovascular remodeling in rats. *Journal of Cardiovascular Pharmacology, 57*(5), 611–624. https://doi.org/10.1097/FJC.0b013e3181feb90a
10. Rosmond, R. (2005). Role of stress in the pathogenesis of the metabolic syndrome. *Psychoneuroendocrinology, 30*(1), 1–10. https://doi.org/10.1016/j.psyneuen.2004.05.007
11. Saklayen, M. G. (2018). The global epidemic of the metabolic syndrome. *Current Hypertension Reports, 20*(2), Article 12. https://doi.org/10.1007/s11906-018-0812-z
12. Schneider, C. A., Rasband, W. S., & Eliceiri, K. W. (2012). NIH Image to ImageJ: 25 years of image analysis. *Nature Methods, 9*(7), 671–675. https://doi.org/10.1038/nmeth.2089
13. Suvarna, S. K., Layton, C., & Bancroft, J. D. (Eds.). (2019). *Bancroft's theory and practice of histological techniques* (8th ed.). Elsevier.
14. Swierczynska, M. M., Mateska, I., Peitzsch, M., Bornstein, S. R., Chavakis, T., Eisenhofer, G., Lamounier-Zepter, V., & Eaton, S. (2015). Changes in morphology and function of adrenal cortex in mice fed a high-fat diet. *International Journal of Obesity, 39*(2), 321–330. https://doi.org/10.1038/ijo.2014.102
15. Tappy, L., & Lê, K.-A. (2010). Metabolic effects of fructose and the worldwide increase in obesity. *Physiological Reviews, 90*(1), 23–46. https://doi.org/10.1152/physrev.00019.2009
16. Topal, F., Goren, H., Yucel, F., Şahintürk, V., & Aydar, Y. (2019). Effect of consuming high-fat diet on the morphological parameters of adrenal gland. *Bratislava Medical Journal, 120*(8), 593–600. https://doi.org/10.4149/BLL_2019_097
17. Vinson, G. P. (2016). Functional zonation of the adult mammalian adrenal cortex. *Frontiers in Neuroscience, 10*, Article 238. https://doi.org/10.3389/fnins.2016.00238
18. Walker, B. R. (2006). Cortisol – cause and cure for metabolic syndrome? *Diabetic Medicine, 23*(12), 1281–1288. https://doi.org/10.1111/j.1464-5491.2006.01998.x
19. Wong, S. K., Chin, K.-Y., Suhaimi, F. H., Fairus, A., & Ima-Nirwana, S. (2016). Animal models of metabolic syndrome: A review. *Nutrition & Metabolism, 13*, Article 65. https://doi.org/10.1186/s12986-016-0123-