Adrenal

TRANSLATIONAL STUDIES ON ADRENOCORTICAL FUNCTION IN HEALTH AND DISEASE

Steroid:Corticosteroid-Binding Globulin Interactions; Effects of Neutrophil Elastase Cleavage, Pyrexia and Acidosis.

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Context Corticosteroid-binding globulin (CBG) transports cortisol and other steroid hormones[1,2]. High-affinity CBG (haCBG) undergoes proteolysis of the reactive centre loop (RCL) by neutrophil elastase (NE) at inflammatory sites, liberating immunomodulatory cortisol and altering conformation to low-affinity CBG (laCBG). Pyrexia reduces CBG:cortisol binding affinity, an interaction at the RCL is speculated[3]. **Objective** To measure the equilibrium binding constants of a panel of steroids to glycosylated haCBG and laCBG over temperature and pH ranges mimicking the pathophysiological conditions of septic shock. **Design** Surface plasmon resonance was used to determine the binding profiles of 19 steroid ligands to haCBG and laCBG at temperatures 25°C, 37°C and 39°C and pH 7.4 and 7.0. The RCL-recognizing 9G12 antibody was used to assess cleavage and epitope availability of the RCL across conditions. **Results** A 4–8 fold reduction in affinity for cortisol, cortisone, corticosterone, 11-deoxycortisol, progesterone, 17-hydroxyprogesterone and prednisolone occurred with NE-mediated haCBG-to-laCBG conversion, cortisol expectedly displayed the highest binding affinity. Binding affinity consistently decreased at higher temperatures and at acidic pH for both haCBG and laCBG. 9G12 antibody RCL binding was preserved for haCBG across temperatures. **Conclusions** These studies reveal that steroid binding to CBG is selective and in all cases reduced upon NE-mediated haCBG-to-laCBG transition. Moreover, reduced CBG:cortisol binding affinity at elevated temperature occurs with an intact and accessible RCL epitope, suggesting a non-RCL mechanism for the delivery of anti-inflammatory cortisol in pyrexia. Synergy of NE cleavage and pyrexia/acidosis may serve for local inflammatory site cortisol delivery and increase free cortisol. These findings demonstrate the modifiable hormone binding characteristics of CBG in (patho-)physiological conditions, supporting its significance in cortisol delivery in obviating systemic inflammation and multiorgan-organ failure in patients with septic shock and its association with mortality[4].

1. Pemberton PA, Stein PE, Pepys MB, et al. Hormone binding globulins undergo serpin conformational change in inflammation. Nature. 1988;336(6196):257–258.
2. Pugeat MM, Dunn JF, Nisula BC. Transport of steroid hormones: interaction of 70 drugs with testosterone-binding globulin and corticosteroid-binding globulin in human plasma. J Clin Endocrinol Metab. 1981;53(1):69–75.
3. Cameron A, Henley D, Carrell R, et al. Temperature-responsive release of cortisol from its binding globulin: a protein thermocouple. J Clin Endocrinol Metab. 2010;95(10):4689–4695.
4. Meyer EJ, Nenke MA, Rankin W, et al. Total and high-affinity corticosteroid-binding globulin depletion in septic shock is associated with mortality. Clin Endocrinol (Oxf). 2019;90(1):232–240.

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ADRENAL - HYPERTENSION

Validating and Optimizing the Guideline Criterion for Skipping Confirmatory Tests of Primary Aldosteronism

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MON-207

Abstract

Background: Confirmatory tests of primary aldosteronism (PA), including saline infusion test (SIT), captopril challenge test (CCT) and fludrocortisone suppression test (FST), are recommended by the Endocrine Society Clinical Practice Guideline. In order to simplifying diagnostic process, a criterion for skipping confirmatory tests was established by the guideline, while the evidence is lacking.

Objective: To validate and optimize the guideline criterion for skipping confirmatory tests.

Design: Prospective diagnostic study.

Setting: Chongqing, China.

Measurements: A total of 501 patients with high risk of PA were retrospectively enrolled. All of them completed at least two confirmatory tests (CCT, SIT, and FST). The guideline criterion is: history of spontaneous hypokalemia, plasma renin concentration (PRC) below detection levels and plasma aldosterone (PAC)>20ng/dl (550pmol/L). An optimized criterion (history of spontaneous hypokalemia, PRC <2.5 uIU/ml and PAC>20 ng/dl) was established based on the guideline criterion. Parameters such as sensitivity, specificity and area under the receiver-operator characteristic curves (AUC) were calculated to compare the diagnostic value of these two criteria.

Results: Using SIT, CCT and FST (cutoffs: PAC post-SIT 10 $ng{\cdot}dl^{-1}$; PAC post-CCT 11 $ng{\cdot}dl^{-1}$; PAC post-FST 6 $ng{\cdot}dl^{-1}$) for PA diagnosis, the specificity of the guideline criterion was 1.00 (0.98–1.00), 1.00(0.98–1.00) and 1.00(0.97–1.00) respectively, while the sensitivity was 0.12 (0.09–0.17), 0.12(0.08–0.16) and 0.09(0.06–0.12) respectively. Compared to the guideline criterion, the sensitivity of the optimized criterion was significantly improved [SIT: 0.42 (0.36–0.49); CCT: 0.41 (0.35–0.48); FST: 0.30 (0.25–0.34), all the *P values* < 0.001 when compared to the guideline criterion]. However, the specificity of the optimized criterion was similar to the guideline criterion (all the *P values*>0.05).

Limitation: This study was carried out in a single center.

Conclusions: The guideline criterion shows high specificity but low sensitivity for direct diagnosis of PA. The

optimized criterion has greatly improved the sensitivity and may be more suitable for skipping confirmatory tests of PA.

Diabetes Mellitus and Glucose Metabolism

METABOLIC INTERACTIONS IN DIABETES

Body Composition Assessment in Clinical Practice: Use in Rheumatoid Arthritis and Hypogonadism

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SUN-650

BACKGROUND: DXA is an accessible, non-invasive method, also used for body composition assessment, standing out for regional composition analysis. In clinical practice, the analysis of body composition is relevant by differentiating lean (fat-free) mass from fat mass. The higher the fat to lean mass ratio, the greater the obesity-related comorbidities.

CLINICAL CASE:

Case 1: A 22-year-old male, BMI 21kg/m2, with rheumatoid arthritis (RA) and on chronic glucocorticoid (GC) performed a DXA to evaluated body composition. The first analysis, during GC use, showed 26.1% fat (14.6kg) despite the low BMI. The patient, evolved stable from RA, and was able to stay out of GC for 2 years, with no other interventions. A new DXA showed a decrease in fat percentage to 12.6% (6.2kg), a reduction in total body weight (-7kg) and an increase in lean mass (+1.2kg). Within 16 months of GC reintroduction, the fat percentage increased up to 36.8% (23.8kg), the total weight increased by 15.6kg and the lean mass decreased by 2.1kg.

Case 2: A 40-year-old male with hypogonadism showed 37% fat (33.8kg) on first DXA evaluation. Testosterone replacement was started, and a new DXA was performed after 10 weeks, and although the total weight increased by 3.1kg, there was a decrease in fat mass to 33.5% (31.6kg) and an increase of 5.3kg in lean mass. After 3 years, there was a reduction to 27.1% of fat (24.5kg) and, after 4 years of therapy initiation, the percentage of fat was 26.9% (24.5kg). There was no change in diet or exercise.

CONCLUSION:

The exposed cases highlight the importance of body composition assessment in patients with conditions that interferes with energy metabolism. The patient on chronic GC use, after medication withdrawal, presented a significant decrease in fat mass, more pronounced in the android percentage. The reintroduction of the CG showed an increase in fat percentage, with android predominance. The patient with hypogonadism, in the second evaluation performed with only 10 weeks of treatment with testosterone, evolved with a reduction in fat mass associated with an increase in lean mass, besides a reduction in the android percentage.

The reported cases illustrate everyday clinical situations in which disease vs. treatment significantly changes body composition. Assessment of body composition is essential in patients exposed to conditions that interfere with energy metabolism since obesity is associated with chronic comorbidities and cardiovascular outcomes.

Adipose Tissue, Appetite, and Obesity

ADIPOSE TISSUE BIOLOGY AND OBESITY

Molecular Markers of Beige Adipose Following ESR1 Knockdown in the Mediobasal Hypothalamus of Adult Female Rhesus Monkeys

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Our studies in female marmoset monkeys show that the ablation of ovarian estradiol (E_2) production fails to alter energy homeostasis or body fat accumulation. Peripheral E_2 may therefore not play a crucial role in metabolic regulation in female primates. shRNA-mediated knockdown of ESR1 expression in the hypothalamic ventromedial nucleus (VMN) in adult female rodents, however, induces obesity and suggests ESR1 is a hypothalamic target for E_2 regulation of energy homeostasis, and likely mediates thermogenesis in brown/beige adipose depots. In female primates, including humans, the hypothalamic estrogen receptor mediating metabolic regulation is unknown. To test the hypothesis that ESR1 mediates female primate regulation of energy homeostasis, 11 ovary intact, adult female rhesus macaques, pair housed with female peers, received five 12µl MRI-guided MBH infusions into the rostral-to-caudal extent of both right and left VMN. Each infusion comprised a gadolinium contrast agent and ~3–4 x 10^{10} adeno-associated virus 8 (AAV8) particles containing either an shRNA specific for ESR1 (n=6, ERaKD) or scrambled shRNA (n=5, control). Mid-surgery MRI scans identified targeting accuracy. ~ 1.5 yrs following AAV8 infusion, pronounced gain in BMI enabled conversion of 83% of ERaKD females to overweight/obese compared to 20% of controls (p=0.08). Percent increase in BMI remained intermittently greater ($p<0.05$) than controls thereafter. Adipose depots were harvested at necropsy ~2.5–3 yrs following treatment. Total RNA was isolated using the Qiagen AllPrep DNA/RNA/miRNA Universal kit. RNA was reverse transcribed with High-Capacity cDNA Reverse Transcription kit (Applied Biosystems). All quantitative real-time PCR (qRT-PCR) were performed on a StepOnePlus System using Power SYBR Green master mix (Applied Biosystems). Primer sequences were designed using NCBI Primer-Blast. Expression of TATA-box binding protein (TBP) was used as the internal control housekeeping gene. The relative expression of target genes was measured using the comparative cycle threshold (Ct) method with results expressed as target mRNA expression relative to TBP using the formula 2^-delta Ct. Upper body beige adipose represents an organ system in primates, including humans, involved in thermogenesis. Axillary beige adipose depots in ERaKD females, however, did not exhibit significantly diminished