rs28929474) and 16 age-, gender- and BMI-matched controls were recruited from the Generation Scotland Biobank. Participants underwent combined receptor antagonist stimulation of the HPA axis ('CRASH') testing using RU486 400mg and spironolactone 200mg, or placebo in a double blind randomised crossover design. Plasma free cortisol was measured by isotopic dilution and ultrafiltration, total cortisol by LC-MS/MS, total CBG by ELISA, CBG binding capacity by radioligand displacement assay, and ACTH by immunoassay. Serum A1AT was measured by ELISA. Tissue cortisol (LC-MS/MS) and expression of glucocorticoid dependent transcripts (qPCR) were measured in subcutaneous adipose samples collected by needle biopsy.
Results
Serum A1AT was confirmed lower in those with heterozygous mutations vs wild type controls (411.3 +/- 27.44 vs 565.1 +/- 23.38 mg/dL, p=0.0002). No measurable differences in total CBG or CBG binding capacity were observed. However, plasma free cortisol fraction was higher in those carrying A1AT mutations (16.13 +/- 0.2 vs 13.88 +/- 0.04 %, p<0.0001). Adipose cortisol concentrations were not significantly different but expression of glucocorticoid responsive genes e.g. *PER1* was 54% higher (p=0.014) in A1AT-deficient subjects. Plasma cortisol was elevated during CRASH testing in both groups, with the increment versus placebo tending to be lower in A1AT-deficient subjects (82.5 +/- 6.7 vs 126.7 +/- 6.8 nM).
Conclusion
Alpha-1 antitrypsin mutation heterozygosity, common in the general population, is associated with higher free cortisol fraction, consistent with enhanced cleavage of CBG. This is associated with evidence of enhanced delivery of glucocorticoid to adipose tissues but reduced HPA negative feedback, suggesting tissue-specific control of cortisol delivery by CBG.

Thyroid

THYROID DISORDERS CASE REPORTS III

Anti-Streptavidin Interference in Multiple Hormones Immunoassays: Case Report

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MON-477

Automated immunoassays are the most commonly current methods used in clinicial laboratory to hormone tests, due short turnaround time and high specificity and sensibility. However immunoassays are not free from interferences. Several immunoassays use biotin-streptavidin interaction to anchor antigen-antibody complexes in solid phase and so are susceptible to biotine intake or anti-streptavidin antibody. In sample with these interferents, the results in competitive assays are falsely high and in non-competitive assays are falsely low. OBJECTIVE: To report a case of multiple hormones immunoassay interference by an anti-streptavidin antibody. MATERIAL AND METHODS: A 11-years-old boy with child obesity, and no other symptoms, had discordant laboratory results with markedly elevated free T4, total T4 and total T3 with normal TSH. Repeated measurements in the same sample and in a new collected sample confirmed the initial results and revealed low PTH and high TRAb without hypocalcemia or hyperthiroidism clinical pictures. All the parameters were measured on the automated Cobas e602, Roche plataform.The patient and your family denied biotin ingestion, so anti-streptavidin interference could explain the multiple interference.The serum was pre-incubated with streptavidin microparticles during an hour and centrifuged for 10min (3000 rpm) to removed the analytical interference. RESULTS: The results of post-incubation with microparticles were all in the normal range. Three control samples were not affected by the incubation. Since luminescence is inversely proportional to analyte concentration in competitive assays, low signals lead to falsely high levels (like in free T4, total T4, Total T3 and TRAb in our patient). The reverse occurs with immunometric assays, in which low signals result in falsely reduced values (like in PTH in our patient). CONCLUSION: In literature, streptavidin antibodies seem to be a rare occurrence. In samples with biotin or anti-streptavidin suspection, pre-incubation with streptavidin microparticles is a simple and effective procedure to remove this interference.

Genetics and Development (including Gene Regulation)

GENETICS AND DEVELOPMENT AND NON-STEROID HORMONE SIGNALING II

ACVR1 Activation in Primary and iPS-Derived Human Skeletal Muscle Stem Cells Impairs Myogenic Transcriptional Signature and Function

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MON-710

Developing optimal strategies for skeletal muscle regeneration and repair requires a detailed understanding of how these processes are regulated. The number of primary human satellite cells that can be obtained is usually extremely low, and may be impaired in disease of impaired skeletal muscle repair. One such condition is fibrodysplasia ossificans progressiva (FOP), a progressive disease characterized by massive heterotopic ossification in skeletal muscles and aberrant skeletal muscle repair after injury. FOP patients have activating mutations in the Activin A Type I receptor (ACVR1), a bone morphogenetic protein (BMP) receptor. Our overall hypothesis is that activated ACVR1 signaling caused by the ACVR1 R206H mutation incites inappropriate activation of human muscle stem cells (satellite cells, PAX7 expressing cells), causing loss of muscle cell fate and aberrant muscle repair. Since human satellite cells are difficult to obtain from live tissue donors, and injury can trigger heterotopic ossification, we created human induced pluripotent stem cell (iPSC)-derived muscle stem cells (iMuSCs) from FOP and control iPSC lines. We found that control and FOP iPSCs can

differentiate into PAX7+ cells with high efficiency. Control and FOP iMuSCs can regenerate injured mouse muscle and form new human fibers, but both showed few PAX7 cells after transplant. Single cell RNA sequencing showed cell heterogeneity, and specific subsets of PAX7+ cells. FOP iMuSCs showed a chondrogenic/osteogenic signature (e.g *COL1A1*, *DCN*, *OGN*) with higher p38 pathway signaling activity. Skeletal muscle samples from autopsies of patients with FOP also showed increased expression of *COL1A1*. Additionally, we found that primary human FOP satellite cells can engraft and regenerate injured muscle, but with lower efficiency than control satellite cells. These studies used a novel iMuSC strategy to elucidate how increased ACVR1 activity affects human satellite cells function, and compare these iMuSCs to primary human satellite cells. These approaches will be useful to identify new therapeutic targets for conditions affecting skeletal muscle, and will improve our understanding of how muscle and bone interact in development and disease pathophysiology.

Adrenal

ADRENAL - CORTISOL EXCESS AND DEFICIENCIES

Circadian Misalignment of the 24-H Profiles of Melatonin and Cortisol in Adrenal Insufficiency

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MON-174

Patients with adrenal insufficiency (AI), in whom cortisol release is absent, need to be on lifelong replacement therapy. Depending on the modality of glucocorticoid replacement, the resulting 24-h profile of circulating cortisol levels maybe dampened, enhanced, abnormally timed or inconsistent. The 24-h cortisol profile is a major internal synchronizing signal between central and peripheral circadian clocks. Misalignment between central and peripheral clocks has a host of adverse effects, particularly on metabolism and cardiovascular function. Melatonin, normally secreted by the pineal gland exclusively at night and considered a robust marker of central circadian timing, also plays a role as an internal synchronizing signal. Conditions like AI, where the 24-h profile of glucocorticoid levels deviates from normality, could produce misalignment between central and peripheral oscillators.

We examined whether AI patients are at higher risk of disturbances of the circadian system. Participants were 13 AI patients (11 females, 2 men; mean age 45.8 yrs old; mean BMI 25.5) and 13 controls (11 females, 2 males; mean age 48.5 yrs old, mean BMI 26.4) matched for age, sex, and BMI. 10 of the AI patients were on hydrocortisone treatment (total dose range: 20 to 40mg/day, number of doses: 1-5 doses/day) and 3 of the AI patients were on prednisone treatment. Cortisol and melatonin were measured over a 24-h period every 30-60 minutes in all patients and controls. The 24-h profile of cortisol was quite variable across patients, dependent on replacement therapy. Those on prednisone had very little detectable cortisol with small peaks that occurred around dosing. Those on hydrocortisone, had multiple peaks across the 24-h cycle dependent on medication regime and dosing. As expected, control subjects had a quiescent period of cortisol release from early evening to mid-sleep (18:00 to 02:00) and a mid-sleep rise in serum cortisol that peaks in the morning and subsequently dissipates across the day. In contrast, AI patients have low levels of cortisol across the entire sleep period, with a sharp morning rise, delayed compared to controls. Unexpectedly, we observed a marked difference in the melatonin profile in AI subjects compared to controls. Indeed, a significant daytime phase of elevated melatonin levels was detected in 7 of the 13 AI patients. The nocturnal elevation in the patients was similar to that observed in controls albeit advanced.

In conclusion, the abnormalities of the circadian profile of glucocorticoid levels in AI are associated with an abnormal 24-hr profile of melatonin release, a marker of central circadian function, suggesting that the disruption of the glucocorticoid rhythm may affect central circadian function. It is possible that this circadian dysfunction contributes to the well-known adverse cardio-metabolic outcomes in AI.

Adrenal

ADRENAL - HYPERTENSION

New Methods for Primary Aldosteronism Screening by Exploring the Values of Different Indicators and in Combination with Predictive Model

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MON-217

Background: Primary Aldosteronism (PA) is the most common cause of secondary hypertension. The endocrine society guidelines recommend case detection of PA by determining the aldosterone-renin ratio (ARR) under standard conditions, but the cut-off value of upright ARR varies greatly. In addition, as with all other biochemical tests, there are false positives and false negatives for upright ARR. In order to avoid false negative, some researchers have proposed plasma renin activity (PRA) < 1.0 ng/ml/h as a screening indicator. In order to avoid false positive caused by low PRA, Professor Young of the Mayo Clinic have proposed that the ARR combined with the plasma aldosterone concentration (PAC) > 15 ng / dL for PA screening. In 2019, Professor Young proposed PA screening positive when PAC≥10ng/dL and PRA<1.0 ng/ml/h. In addition, angiotensin II (AT-II) directly stimulates the synthesis of aldosterone. Compared with renin, the decrease of AT-II level and the increase of aldosterone to AT-II ratio (AA2R) may reflect the aldosterone autonomy secretion. However, whether these screening indicators are superior to upright ARR remains to be further verified. Therefore, either exploring the diagnostic efficacy of PAC, PRA, or AA2R as a screening indicator or even better developing a clinical prediction model combined with multiple indicators may provide a more accurate method for PA screening. **Objective:** To explore the value of different indicators and the logistic regression model (nomogram) for primary aldosteronism screening. **Methods:** The clinical data of 499 patients with PA and 479 patients