were no significant changes in alcohol use and muscle-strengthening exercise, while a substantial decrease in smoking rate was observed. A favorable effect of muscle-strengthening exercise on both TG and HDL-C and an unfavorable impact of smoking on HDL-C were observed. Alcohol use was associated with higher HDL-C in both genders, but it showed opposite associations with TG between boys (unfavorable) and girls (favorable). Regarding dietary factors, there were increasing trends in total fat intakes and the percentage of energy supply from total dietary fat (〔total fat (%E)〕 in both genders. In boys, an increase in total fat (%E) was related to the higher HDL-C in normal-weight subjects (P<0.01 in both genders); however, it was associated with higher LDL-C in overweight girls (P=0.001). **Conclusions:** Increases in fat intakes and a decline in smoking rates appeared to have positively impacted HDL-C in Korean adolescents over the past 12 years. We confirmed a rise in fat intakes was linked with the increase in LDL-C among overweight adolescents. Therefore, close monitoring for the dyslipidemia prevalence is essential in Korean adolescents whose obesity prevalence is on the rise.

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ULK1 Regulates Hepatic Lipid Metabolism via Autophagy Independent Mechanisms

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Non-alcoholic steatohepatitis (NASH), a major complication of obesity, diabetes, and metabolic syndrome has emerged as a leading cause of chronic liver disease and a risk factor for hepatocellular carcinoma. Autophagy is a critical pathway for the degradation of intracellular components by lysosomes. Established functions for autophagy in hepatic lipid metabolism and insulin sensitivity suggest a mechanistic link between altered autophagy and NASH. However, the interactions between insulin sensitivity, NASH, and autophagy are incompletely understood. The Unc-51 Like Autophagy Activating Kinase 1 (ULK1) is the only serine/threonine kinase in the core autophagy pathway and thus represents an excellent drug target. In this study, we observed that ULK1 may directly regulate insulin signaling and lipid metabolism via mechanisms that might involve modulation of AKT dephosphorylation. Surprisingly, silencing ULK1 did not significantly alter autophagy in hepatocytes despite impairing insulin-stimulated activation of AKT. To further elucidate the autophagy-independent role of ULK1 in hepatic lipid metabolism and insulin action, ULK1 liver-specific knock-out mice were generated. L-ULK1 KO mice exhibited impaired glucose tolerance and insulin resistance on a normal chow diet or 60% high-fat diet (HFD). In young mice (4 weeks after birth), the expression of genes that regulate de novo lipogenesis, such as FAS, SCD1, and SREBP1-c were induced in livers of L-ULK1KO mice even prior to the development of insulin resistance and obesity. Hepatomegaly and lipid accumulation developed in L-ULK1KO on normal chow and was exacerbated relative to wild type mice on a HFD. Serum concentrations of insulin, triglyceride, cholesterol, AST and ALT were significantly increased. In contrast, L-ULK2 KO mice were phenotypically normal. To identify putative novel ULK1 targets, we conducted a phospho-proteomics screen in a ULK1 deficient hepatocyte cell line. We identified a relatively small number of novel proteins whose phosphorylation levels were reduced by ULK1 deficiency. The identification of these targets supports autophagy-independent mechanisms of action of ULK1. Recently, we confirmed that NCOA3, one of the targets regulates hepatic lipid metabolism by interacting directly with ULK1. These data suggest that ULK-1 may regulate cellular targets that regulate hepatic lipid metabolism and insulin sensitivity.

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Utility of Plasma Metanephrines in Adrenal Venous Sampling in Primary Aldosteronism

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Introduction: Adrenal Venous Sampling (AVS) is the most reliable means of identifying surgically curable subtypes of primary aldosteronism (PA). Cortisol levels are used to determine cannulation success and lateralization. However, cortisol has a variable secretion pattern and long-half life, and can be co-secreted by adrenal adenomas, leading to misinterpretation of results. Plasma metanephrines (MN) are a possible alternative analyte. MN levels are unaffected by stress, have a short half-life of 3–6 minutes and are released continuously by the adrenals, resulting in very high concentration gradients between the adrenal veins (AV) and peripheral veins (PV), thus providing a sensitive means to determine cannulation success. *Premise:*The objective of this study was to see if MN can be used in lieu of cortisol in AVS. A secondary end-point was to see if the data was particularly useful in patients who are known co-secretors of cortisol. **Methods:** Data from AVS carried out without cosyntropin stimulation, from October 2018 to March 2020, were analysed retrospectively. Of these, 51 had additional samples drawn for MN at the time of the procedure and were recruited. Six patients were identified as having autonomous cortisol secretion as they failed an overnight dexamethasone suppression test (ONDST). The data was analysed using cortisol and MN separately and then compared with regards to their selectivity and lateralization index. Data was also analysed to see if known co-secretors had an elevated cortisol/MN ratio of more than 2 on the affected side as described in previous papers. **Results:** When compared to cannulation and lateralization outcomes using cortisol, similar results were obtained using, a MN AV/PV ratio of more than 12 to indicate successful cannulation and an aldosterone/MN ratios of greater than 5 to confirm lateralization. Contralateral suppression

to less than 0.5 for aldosterone/MN below the PV was seen in unilateral disease. With regards to the six co-secretors, all had elevated cortisol/MN ratios of more than 2 on the affected side. Three had concordant results but the other three had discrepant results, with MN analysis suggesting unilateral disease and cortisol measurements suggesting bilateral disease. Two had undergone surgery with biopsy confirming unilateral disease that correlated with MN analysis. The third is under medical management. **Conclusion:** This is the first study evaluating the use of MN to determine lateralisation of aldosterone production in PA. Further studies are needed, but using MN may be a more reliable alternative to cortisol in the analysis of AVS before definitive surgery in particular in patients with cortisol co-secretion.

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Visualization of Ca Channel Blocker on Human Adrenal Tissue by Mass Spectrometry Imaging ~Its Predominant Distribution at Aldosterone-Producing Cells ~

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Primary aldosteronism (PA) is the main cause of secondary hypertension, accounting for approximately 5–10% of all hypertension. Amlodipine, a third-generation calcium channel blocker, is one of the most frequently administered pharmaceuticals medications of hypertension, binds specifically to Cav1.2, a calcium channel primarily localized in the cardiovascular system, and exerts antihypertensive effects through inhibiting calcium influx into the vascular smooth muscle cells. In addition, calcium influx also plays important roles in aldosterone production and amlodipine was also reported to influence *in vitro* functions of Cav1.3, a calcium channel involved in aldosterone secretion. Ca channel blockers were also reported to reduce plasma aldosterone concentration by some clinical studies although with mild degrees. However, *in vivo* effects of amlodipine to aldosterone secretion has remained virtually unknown. A novel technique "Matrix-Assisted Laser Desorption/Ionization Mass Spectrometry Imaging (MALDI-MSI)" has been recently developed, which did make it possible to visualize non-labeled small molecules on tissue sections. Therefore, in this study, we firstly applied MALDI-MSI to visualize amlodipine on human adrenal glands including aldosterone producing adenoma (APA). We performed selective imaging of amlodipine using MALDI-MSI on the resected adrenal tissues from APA patients. Frozen sections containing whole representative tumor area were coated with a matrix called CHCA (α-Cyano-4-hydroxycinnamic acid) by deposition as a pretreatment. We subsequently analyzed and detected a precursor ion with MS at m/z 407.1 and then an amlodipine-specific ion with MS/MS at m/z 318.1. We also examined the concordance of amlodipine distribution obtained by this method with immunohistochemistry. Human resected adrenal tissues obtained from the patients APAs treated with and without amlodipine before adrenalectomy were examined. Periadrenal adipose tissues were also analyzed as a control tissue of non-aldosterone-producing tissues. Amlodipine was specifically detected and visualized only in the administered cases. Amlodipine was more abundantly detected in adrenal tissues than periadrenal adipose tissues. On the other hand, significant different was not detected between tumors and adjacent adrenal glands by semi-quantification using MALDI-MSI. In this study, we firstly visualized amlodipine directly in human tissue sections using MALDI-MSI. Increased accumulation of amlodipine in APAs treated with amlodipine did indicate direct effects of amlodipine on aldosterone production but further investigations are required for clarification between neoplastic and non-neoplastic aldosterone producing cells.

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Vitamin D Deficiency Induces Macrophage Pro-Inflammatory Phenotype via ER Stress-Mediated Activation of Renin-Angiotensin System

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Chronic inflammation and local activation of the renin-angiotensin-aldosterone system (RAAS) play a pivotal role in the pathogenesis and progression of diabetic complications. In patients with type 2 diabetes (T2DM), the prevalence of vitamin D deficiency is almost twice that of non-diabetics, and vitamin d deficiency nearly doubles the risk of developing hypertension and cardiovascular complications compared to diabetics with normal vitamin D levels. Interestingly, mice lacking the vitamin D receptor (VDR) in macrophages (KODMAC) develop renin-dependent hypertension, insulin resistance, and inflammation via up-regulation of macrophage ER stress. Macrophages also express all major components of the RAAS system. However, little is known about the regulation of macrophage-generated renin and its role in modulating the sequelae of VDR signaling in macrophage function and cytokine production. This study found that KODMAC macrophages and vitamin D-deficient macrophages have increased expression and secretion of renin, angiotensin II, ACE, and AT1 receptor and that adhesion, migration, and cytokine release were also increased. Inhibition of ER stress in KODMAC macrophages and vitamin D-deficient macrophages with 4-Phenylbutyric acid (PBA) reduced RAS gene expression and macrophage pro-inflammatory phenotype. Renin 1c gene deletion decreased macrophage