

POPULATION: Washed platelets or platelet rich plasma from healthy human donors were treated with EPA and 12-HEPE to assess their ability to inhibit platelet activation. Platelets were stimulated with agonists targeting different steps of the hemostatic response to vascular injury. Platelet aggregation, dense granule secretion, surface expression of integrin $\alpha\text{IIb}\beta\text{3}$ and P-selectin, and clot retraction were analyzed. To assess signaling through G α s-GPCRs and protein kinase A activity, phosphorylation of vasodilator-stimulated phosphoprotein (VASP) was examined via western blot following treatment with EPA or 12-HEPE. **RESULTS/ANTICIPATED RESULTS:** EPA and 12-HEPE dose-dependently inhibit both collagen and thrombin-induced platelet aggregation. Furthermore, 12-HEPE more potently attenuates dense granule secretion and surface expression of platelet activation markers, integrin $\alpha\text{IIb}\beta\text{3}$ and P-selectin, in comparison to EPA. Plasma treated with EPA delayed thrombin-induced clot retraction, while 12-HEPE had no effect. Additionally, treatment with 12-HEPE increases phosphorylation of VASP, suggesting it could signal through the activation of the eicosanoid G α s-GPCRs. **DISCUSSION/SIGNIFICANCE:** Here, we show for the first time that EPA directly inhibits platelet activation through its 12-LOX metabolite, 12-HEPE. These findings provide further insight into the mechanisms underlying the cardioprotective effects of EPA. A better understanding of current PUFA supplementations can inform treatment and prevention of cardiovascular diseases.

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Intramuscular immunization with rVCG-MECA vaccine elicits stronger chlamydial specific immune response than intranasal immunization

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OBJECTIVES/GOALS: We prioritize Chlamydia's public health impact, aim to develop rVCG-MECA for practical use, study robust immunity for effective strategies, and assess animal models for human vaccination adaptation. Our work highlights rVCG-MECA's translational significance in public health. **METHODS/STUDY POPULATION:** Female Mice C57BL/6J mice (N=8) were immunized intramuscularly(IM) and intranasally(IN) and boosted twice, two weeks apart, with rVCG-MECA, once with live Chlamydia (*C. trachomatis* serovar D elementary bodies) and PBS. Specific mucosal and systemic immune responses were characterized. Vaccine efficacy was determined from chlamydia shedding following the transcervical challenge. Additionally, Chlamydia-specific cytokine (IFN- γ and IL-4) production by splenic and ILN T cells was assessed after 16 weeks **RESULTS/ANTICIPATED RESULTS:** Immunization with rVCG-MECA via intramuscular and intranasal routes triggered notable humoral responses in systemic and mucosal tissues. Intramuscular vaccination produced higher IgG2c levels in both tissues, while intranasal vaccination led to elevated IgA levels in mucosal tissues. rVCG-MECA-immunized mice exhibited significantly higher IFN- γ (Th1) secretion compared to IL-4 (Th2), with intramuscular immunization showing the highest IFN- γ levels. These findings anticipate robust immune responses, promising protection against Chlamydia, particularly through the intramuscular route. Overall, our results support rVCG-MECA as a promising Chlamydia vaccine, aligned with public health goals. **DISCUSSION/SIGNIFICANCE:** This study

suggests that IM and IN immunization with rVCG-MECA induces immune effectors such as IFN-gamma and IgG2c that mediate chlamydial clearance in the genital tract.

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Computable Phenotyping with "Big Data" as a Foundation for Artificial Intelligence Algorithm Construction: Puberty as a Transdisciplinary Case Example

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OBJECTIVES/GOALS: Artificial intelligence (AI) depends on quality machine learning (ML) algorithms constructed with high-quality training data. This TL1 trainee project develops a disease-agnostic computable phenotype framework for ML algorithm construction, modeling male puberty as a case example. **METHODS/STUDY POPULATION:** A computable phenotype of male puberty was constructed to answer the question: "Does early pubertal timing increase the risk of developing type II diabetes (T2D) in males?" A computable phenotype of males < 18 years old was created in the TriNetX[©] Diamond Network utilizing Boolean operator data queries. TriNetX[©] contains patient electronic health record information (ICD-10 diagnoses, anthropometric measures). An exploratory analysis of patient counts reflecting various computable phenotypes allowed for outcome (T2D) comparison of males diagnosed with precocious puberty (E30.1, ICD code for early pubertal timing) to those without, controlling for body mass index (BMI). **RESULTS/ANTICIPATED RESULTS:** Subjects (n=12,996,132) displayed the following computable phenotype: Male, < 18 years old, without ever having a BMI documented >85th percentile. Males diagnosed with precocious puberty (E30.1) were 6.89 times more likely to develop T2D when aged 14-18 years old than those without (OR 6.89, 95% CI: 5.17-9.19, p<0.0001). Next steps involve training a ML model on each computable phenotype groupings' health data, with anticipated results identifying underlying salient pathophysiologic variables. A generalized computable phenotype approach is further developed to: 1) explore clinical questions in large databases like TriNetX[©], and 2) model disease development with AI/ML algorithm construction. **DISCUSSION/SIGNIFICANCE:** Computed phenotypes reveal males with precocious puberty may have increased T2D risk. Next steps utilize subject data to train an AI/ML algorithm, model development to identify salient pathophysiologic variables, and synthesize a generalized AI/ML developmental research framework for dissemination.

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Innovation in MS Patient Care: Linking Cognitive Health and Myelin Integrity

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OBJECTIVES/GOALS: Our objective is to develop a patient-friendly application addressing the progression of cognitive impairments in multiple sclerosis (MS) patients. This initiative aims to augment individualized care and precision management of a major MS comorbidity by generating a cognitive health brain map for each patient. **METHODS/STUDY POPULATION:** Using the UAMS COMS