Changes in Gut Microbiome Composition with Healthy Aging in Humans: Links to Vascular Endothelial Function

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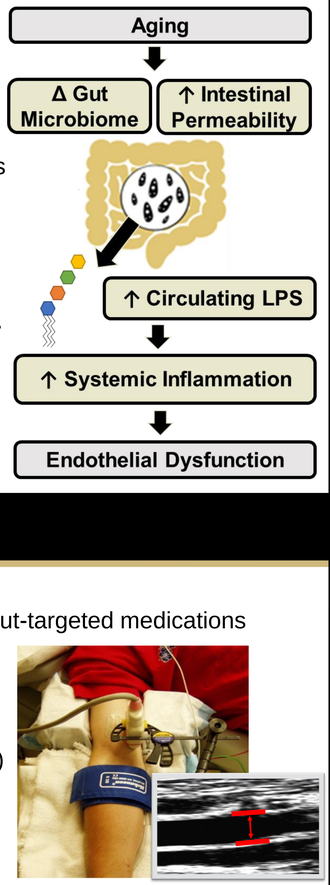
Abstract

Aging is the primary risk factor for cardiovascular diseases, primarily due to development of vascular endothelial dysfunction. The gut microbiome is a strong influencer of host physiology, but few studies have investigated how gut microbiome composition changes with primary (healthy) aging in humans, or how such changes may influence endothelial function. PURPOSE: To: 1) determine changes in gut microbiome composition and their relation to endothelial function in healthy late middle-aged to older (MA/O) vs. young (Y) adults; and 2) investigate potential mechanisms of this link. METHODS & RESULTS: N=14/group (MA/O: 60-79 yrs; Y: 18-29 yrs). Data are mean ± SE. Gut microbiome composition was assessed via fecal 16S rRNA sequencing. α-diversity, phylogenetic diversity within each sample, was higher in MA/O vs Y adults (Faith's PD: 22.2 ± 1.8 vs 15.5 ± 1.3, P = 0.02). β-diversity, difference in overall composition between samples, was also altered with aging (PERMANOVA: P < 0.05; unweighted UniFrac). Both α-diversity (R = -0.60, P = 0.04) and β-diversity (R = -0.58, P = 0.04) were inversely related to age-related impairments in endothelial function, measured by brachial artery flow-mediated dilation (MA/O: 4.5 ± 0.4 vs Y: 7.9 ± 1.7%, P < 0.05). Potential Mechanisms: In preliminary analyses (unpaired t-tests), changes in gut microbiome composition were accompanied by altered relative abundance of gram-negative bacteria (e.g., Bacteroides [MA/O: 33 ± 4% vs Y: 20 ± 4%, P = 0.02]) and Enterobacteriaceae [MA/O: 1.5 ± 0.1% vs Y: 0.4 ± 0.1%, P = 0.02]), which contain pro-inflammatory lipopolysaccharide (LPS) in their cell walls. Translocation of LPS into systemic circulation is facilitated by increased intestinal permeability, which we found was higher with aging, as measured by a lactulose-mannitol (L:M) test (1-hour serum L:M ratio, MA/O: 0.012 ± 0.002 vs Y: 0.007 ± 0.001, P = 0.04). As such, plasma LPS-binding protein, a readily detectable marker of LPS in peripheral blood, was higher in MA/O vs Y adults (31.3 ± 4.1 vs 17.7 ± 2.0 ng/mL, P = 0.01). Once in circulation, LPS is recognized as a pathogen-associated molecular pattern and can trigger an inflammatory response. Consistent with this, circulating levels of the pro-inflammatory markers IL-6 [MA/O: 1.56 ± 0.29 vs Y: 0.70 ± 0.13 pg/ml, P = 0.01] and CRP [MA/O: 1.65 ± 0.27 vs Y: 0.78 ± 0.11 pg/ml, P = 0.02]) were increased with aging. In biopsied venous endothelial cells from a subset of subjects (n = 4-8/group), abundance of phosphorylated (i.e., activated) NFκB was higher in MA/O vs Y adults (0.40 ± 0.05 vs 0.31 ± 0.04 ng/ml, P = 0.24), with no difference in total NFkB, indicating potentially increased vascular inflammation.
CONCLUSIONS: Our findings represent initial evidence in humans that the gut microbiome changes with healthy aging and may be an important mediator of age-related endothelial dysfunction, possibly via increasing circulating LPS and vascular inflammation. **Program # : 850.4.**

Introduction

- Aging is the primary risk factor for cardiovascular diseases (CVD) due in part to the development of vascular endothelial dysfunction.
- Changes in the gut / gut microbiome with aging may contribute to endothelial dysfunction, including changes in:
  - *Alpha diversity* = species diversity within a sample
  - *Beta diversity* = differences in overall composition between samples
  - *Differential abundance* = taxonomic differences between samples
  - *Intestinal Permeability* = tight junctions in the gut epithelial wall lose their integrity, allowing for translocation of lipopolysaccharide (LPS).
- **Purpose:**

1. **To determine changes in gut microbiome composition and their relation to endothelial function in healthy late middle-aged to older (MA/O) vs. young (Y) adults; and**
2. **Investigate potential mechanisms, including the role of translocation of bacterial-derived lipopolysaccharide (LPS) and resultant inflammation.**



Methods

HUMAN SUBJECTS

- Young (N=14) and MA/O (N=14) healthy adult subjects not taking gut-targeted medications

PRIMARY MEASUREMENTS

- Gut Microbiome Composition (16s rRNA; α/β diversity & taxa-level relative abundance of *Bacteroides* & *Enterobacteriaceae)*
- Endothelial Function (brachial artery flow-mediated dilation, FMD_{BA})

MECHANISTIC MEASUREMENTS

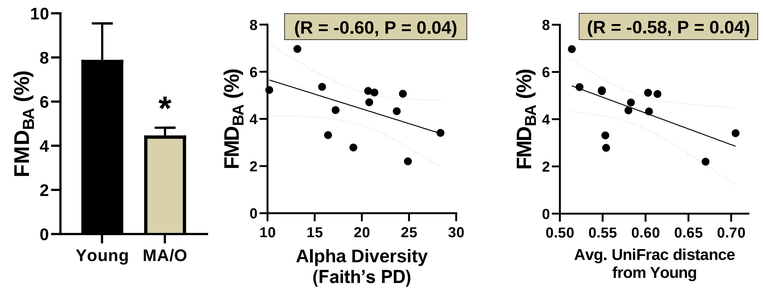
- Intestinal Permeability (lactulose-mannitol test)
- Translocation of LPS (ELISA; LPS-binding protein)
- Circulating Concentrations of Pro-Inflammatory Markers (ELISA; IL-6 & CRP)
- Endothelial Cell Abundance of Total and Phosphorylated NFκB (Immunofluorescence)

Results

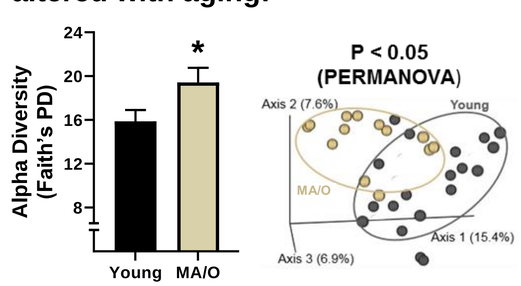
Key Subject Characteristics:

	Young	Older
N	14	14
Male/Female	7/7	4/10
Age (yrs)	23.6 ± 0.6	68.1 ± 1.5
VO_2 Max (ml/min/kg)	50.4 ± 3.5	* 30.3 ± 2.0
Systolic BP (mmHg)	108.4 ± 2.3	* 116.0 ± 2.7
Diastolic BP (mmHg)	62.1 ± 1.7	* 70.9 ± 1.7
Body Mass Index	23.2 ± 0.9	24.7 ± 0.6

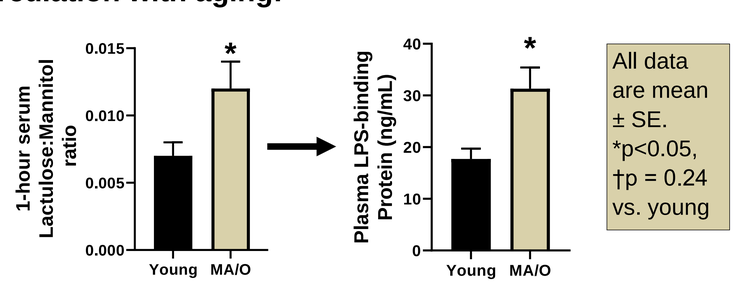
Age-related reductions in endothelial function are related to changes in microbiome composition:



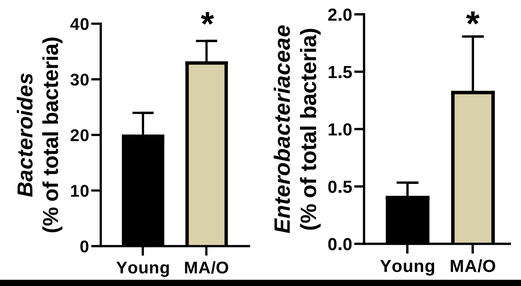
Gut microbiome composition is altered with aging:



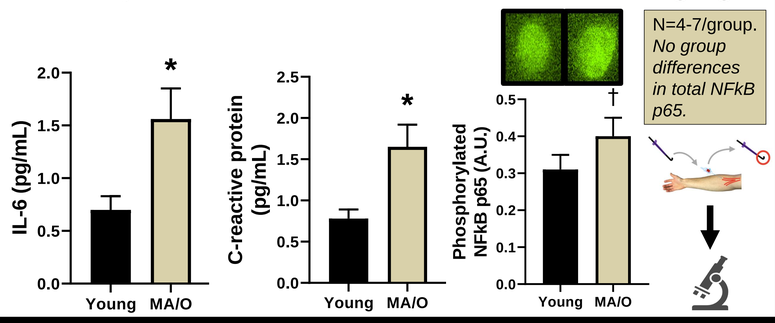
Increased intestinal permeability and LPS in circulation with aging:



Relative abundance of key gram-negative bacteria increase with aging:



Circulating levels of pro-inflammatory markers and potentially vascular inflammation increase with aging:



Conclusions

- Gut microbiome composition is altered with aging and is related to reductions (impairments) in vascular endothelial function.
- Aging increases intestinal permeability and abundance of gram-negative bacteria, leading to increased circulation of LPS which may induce vascular inflammation.
- Age-related changes in the gut microbiome may be a mechanism by which aging impairs endothelial function.
- The gut microbiome may be a promising target for the treatment of age-related endothelial dysfunction and subsequent CVD risk.

Acknowledgements

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