Introduction Inflammation is implicated in endothelial dysfunction in CVD. A key mechanism mediating endothelial dysfunction is a reduction in bioavailable (eNOS-derived) nitric oxide (NO). Evidence has shown that increasing NO delivery via activation of the non-canonical pathway might increase anti-inflammatory and vascular protective NO. We hypothesise that inorganic nitrate (NO3-) will increase bioavailable NO and attenuate the inflammatory pathways leading to endothelial dysfunction in healthy volunteers. To identify mechanisms in the immune response we have undertaken 2 prospective double-blind RCTs: Blister-NITRATE (NCT0318383) and Typhoid-NITRATE (NCT02715635).

Methods Blister-NITRATE; using a cantharidin-induced skin blister model, 24hr and 72hr blisters were harvested pre- and post- 8 days of 8-12mmol of nitrate or placebo. Blister exudate was analysed for leukocyte activation state (CD11b, CD62L, CD162) using flow cytometry and for cytokines/chemokines (n=36). Typhoid-NITRATE; using a model of typhoid vaccine-induced systemic inflammation, flow-mediated dilatation (FMD) and GTN-induced brachial artery dilatation were measured pre- and post- 6 days of 8-12mmol of nitrate or placebo. Blood was collected 8hr after vaccination for flow cytometry, using the same markers (n=78). Blood, urine, and saliva were collected for nitrite (NO2-) and NO3- quantification.

Results Dietary nitrate increases plasma, urine, and saliva [NO2-] and [NO3-], indicating intact activation of the non-canonical pathway. FMD was preserved in the group treated with dietary nitrate, compared with placebo (absolute reduction 1.4%±1.6%, P<0.0001), with no difference in GTN-induced brachial artery dilatation (P=0.931) (figure 1). A decreased systemic proportion of intermediate monocytes (P=0.0425), with reduced neutrophils (P=0.017), intermediate monocytes (P=0.001) and expression of inflammatory (CD11b P=0.03, CD162 P=0.01, CD62L P=0.053) and intermediate monocytes (CD11b P=0.01, CD62L P=0.03) surface markers at 72hrs.

Conclusions Inorganic nitrate suppresses endothelium-mediated vascular dysfunction. Dietary nitrate influences the localised and systemic inflammatory response through suppressing of pro-inflammatory cell types with reduced inflammatory and intermediate monocytes, and attenuated inflammatory cytokines and chemokines. The intervention induces a pro-resolution phenotype. Inorganic dietary nitrate influences endothelial function through the modulation of inflammatory responses and might be of potential therapeutic benefit in patients with established CAD.

Abstract B538 Figure 2 (A) Attenuation of pro-inflammatory MCP-1/CCL2. (B) Enhancement of anti-inflammatory TGFβ. Statistical significance determined by 1-way ANOVA, with Dunnett’s post hoc analysis for multiple group analysis and unpaired t-test for comparison of change in response at 8h compared to baseline. Data are expressed as mean ± SEM. N=62.

Skin blisters resolved more rapidly in volunteers treated with dietary nitrate (P=0.0425), with reduced neutrophils (P=0.017), intermediate monocytes (P=0.001) and expression of inflammatory (CD11b P=0.03, CD162 P=0.05, CD62L P=0.053) and intermediate monocytes (CD11b P=0.01, CD62L P=0.03) surface markers at 72hrs.

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