0402

PLASMA CITRATE AND SUCCINATE PREDICT NEUROCOGNITIVE IMPAIRMENT IN OLDER PWH

The A5322 (HAILO) Study

Corrilynn O. Hileman[1,2], Sausan Azzam[2], Kunling Wu[3], Katherine Tassiopoulos[3], Roger Bedimo[4], Ronald J. Ellis[5], Kristine M. Erlandson[6], Asha Kallianpur[2,7], Susan L. Koletar[8], Alan L. Landay[9], Frank J. Palella[10], Muralidhar Pallaki[2,11], Babafemi Taiwo[10], Charles L. Hoppel[2], and Robert Kalayjian[1,2] for the HIV Infection, Aging, and Immune Function Long-Term Observational Study (HAILO) (ACTG A5322) Research Team

[1]MetroHealth Medical Center, Cleveland, OH, [2]Case Western Reserve University School of Medicine, Cleveland, OH, [3]Harvard T.H. Chan School of Public Health, Boston, MA, [4]Trinity Health and Wellness Center, Dallas, TX, [5]University of California, San Diego, CA, [6]University of Colorado Anschutz Medical Campus, Aurora, CO, [7]Cleveland Clinic Lerner Research Institute, Cleveland, OH, [8]The Ohio State University, Columbus, OH, [9]Rush University Medical Center, Chicago, IL, [10]Northwestern University Feinberg School of Medicine, Chicago, IL, [11]Cleveland Veterans Administration Medical Center, Cleveland, OH

BACKGROUND

- Upon activation by lipopolysaccharide (LPS) and other stimuli, macrophages and microglia substantially increase glycolysis and reduce oxidative phosphorylation (akin to the Warburg effect) which results in accumulation of tricarboxylic acid (TCA) cycle metabolites including citrate and succinate.[1]
- Actively transported from mitochondria to cytosol, these metabolites may directly promote disease by inducing reactive oxygen species (ROS) formation, while also engaging pro-inflammatory pathways.[1]
- In metabolomic analyses of cerebrospinal fluid (CSF), higher concentrations of citrate and succinate were associated with neurocognitive impairment (NCI) in people with HIV (PWH).[2,3]
- Based on this, we hypothesized that a metabolic shift like the Warburg effect may contribute to prevalent and incident NCI and slowing gait speed in PWH.

METHODS

- HAILO is a multicenter, prospective, observational study of 1,035 PWH ≥40 years who received initial ART by randomized allocation in ACTG trials. A random sample with plasma available at entry were included here.
- Fasting citrate and succinate were assayed from frozen plasma at entry by liquid chromatography mass spectrometry (MassHunter qualitative/quantitative analysis software; version 8.0; Agilent Technologies, CA).
- Neurocognitive performance was assessed at entry and every 48 weeks using normalized, demographic-adjusted scores from:

TEST:	DOMAIN
Trail Making A:	Speed of information processing
Trail Making B:	Executive functioning
Hopkins Verbal Learning:	Verbal learning and memory
Wechsler Adult Intelligence Scale-Revised Digit Symbol:	Speed of information processing

- NCI defined by at least 1 z-scores ≥2 SD below the mean or at least 2 scores ≥1 SD below the mean.
- Gait speed was assessed at entry and every 48 weeks by time to walk 4 meters at usual pace from standing start.
- Statistical methods employed included: logistic regression, proportional hazards regression, and mixed-effects linear regression.

Higher fasting plasma TCA cycle metabolites **citrate and succinate** are associated with cross-sectional and longitudinal **neurocognitive impairment and slower gait speed** in people with HIV; however, the associations are age-dependent. These findings support the importance of monocyte activation and altered mitochondrial function in the pathogenesis of NCI, and imply differential mechanisms of NCI by age.

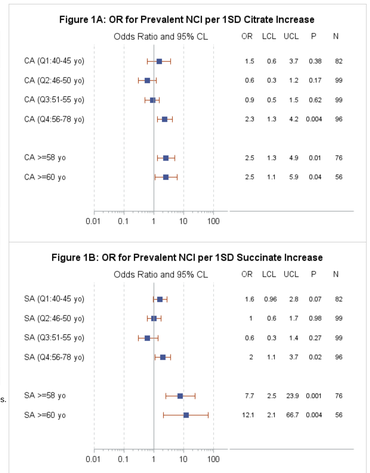
RESULTS

TABLE 1: Baseline demographics, HIV-related factors, TCA cycle metabolites and gait speed

	NCI absent n=274	NCI present n=100	P
Age, years	51	52	0.04
Male	227 (83%)	77(77%)	0.2
Race/Ethnicity			0.01
White-not Hispanic	135 (49%)	42 (42%)	
Black-not Hispanic	83 (30%)	23 (23%)	
Hispanic regardless of race	56 (21%)	35 (35%)	
Education, years	14	13	0.22
Viral load < 50 copies/mL	252 (93%)	92 (93%)	0.99
CD4+ count, cells/mm^3	613	613	0.72
Nadir CD4+ count, cells/mm^3	189	224	0.62
ART duration, years	7.6	6.8	0.14
Smoking History			0.36
Current	114 (42%)	33 (34%)	
Past	93 (34%)	37 (38%)	
Never	66 (24%)	28 (28%)	
Citrate, μg/ml	9.63	9.10	0.65
Succinate, μg/ml	0.66	0.82	0.08
Gait speed, seconds	3.81	4.08	0.0002

Categorical variables are presented as frequency (column percent), continuous variables as median values.

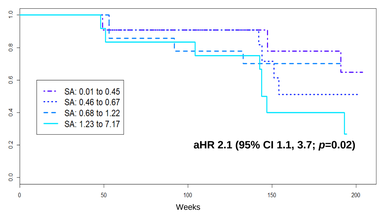
FIGURE 1A and 1B: Odds of NCI at baseline per 1 SD increase in plasma TCA cycle metabolite concentration by age quartile



Multivariable logistic regression models adjusted for age, sex, race/ethnicity, smoking, education status, ART duration, plasma viral load, and nadir CD4+ cell counts.

Odds ratio, OR; citrate, CA; succinate, SA; lower confidence limit, LCL; upper confidence limit, UCL

FIGURE 2: Kaplan-Meier curves for incident NCI with baseline succinate divided into quartiles in PWH aged ≥56 years



Newly diagnosed NCI was established in 89 of 274 without NCI at baseline over a median of 2.8 years. Neither citrate nor succinate were associated with incident NCI among all participants.

Multivariable proportional hazards models adjusted for age, sex, race/ethnicity.

TABLE 2: Association between TCA cycle metabolites and time-updated normalized neuropsychologic test scores in PWH aged ≥56 years

	Citrate		Succinate	
	Score	P	Score	P
NPZ-4*	**-0.2**	**<0.01**	**-0.2**	**0.04**
Trail Making A	**-0.2**	**<0.01**	-0.3	0.11
Trail Making B	**-0.1**	**0.03**	**-0.3**	**0.03**
Hopkins Verbal Learning	-0.2	0.06	-0.2	0.08
Wechsler Adult Intelligence Scale-Revised Digit Symbol	**-0.2**	**0.02**	-0.3	0.1

Multivariable mixed effects linear models adjusted for age, sex, race/ethnicity, smoking, education status, ART duration, plasma viral load, and nadir CD4+ cell counts.

*Individual test scores were averaged as a single score (NPZ-4).

FIGURE 3A and 3B: Receiver operating characteristic (ROC) curves for TCA cycle metabolites overall and in PWH aged ≥60 years

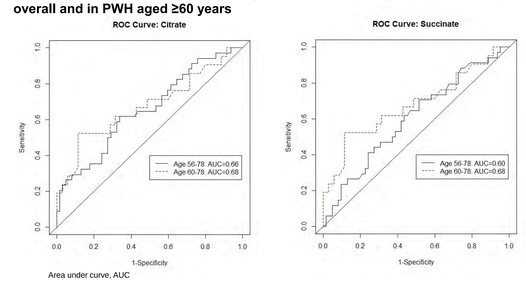
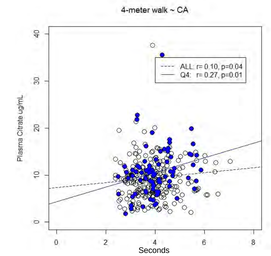
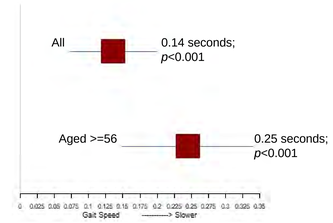


FIGURE 4: Correlation between 4-meter gait speed with plasma citrate at baseline overall and in PWH aged ≥56 years



Succinate concentrations were not associated with gait speed.

FIGURE 5: 4-meter gait speed per 1 SD increase in plasma citrate at baseline overall and in PWH aged ≥56 years



Citrate-by-age interaction p=0.03 in full model including all participants.

Above estimates adjusted for age, sex, race, ART duration, smoking, hemoglobin, ART duration, plasma viral load, nadir CD1+ count, and study week.

CONCLUSIONS

In people with HIV on ART:

- Higher plasma citrate is associated with prevalent NCI, lower individual neuropsychological test scores and slower gait speeds.
- Higher plasma succinate is associated with prevalent and incident NCI and lower individual neuropsychological test scores.
- These associations are age-dependent and mainly evident in persons aged 56 years and older.
- Despite robust associations, these metabolites are expected to perform poorly as diagnostic markers for NCI in older PWH, as determined by ROC curves.

Through different cellular processes both citrate and succinate lead to stabilization of hypoxia inducible factor (HIF)-1α, which promotes glycolysis and sustains transcription of the pro-inflammatory cytokine IL-1β. The local and systemic effects of these TCA cycle metabolites, which accumulate in the presence of inflammatory mediators and mitochondrial dysfunction, serve to mechanistically link monocyte activation with NCI among PWH.

A5322 is supported by the following: the National Institute of Allergy and Infectious Diseases (NIAID) of the National Institutes of Health under Award Numbers UM1 AI068634, UM1 AI068636 and UM1 AI106701. Additional funding for this work by Veterans Administration: VISN10 Geriatric Research Education and Clinical Centers, Louis Stokes Cleveland Veterans Administration Medical Center, Case Western Reserve University Center for AIDS Research AI036219 and R01 AG054366 to KME.

ACTG AIDS CLINICAL TRIALS GROUP

REFERENCES

1. Kelly B, O'Neill LAJ. *Cell Research*. 2015;25:771-84.
2. Cassol E, et al. *AIDS*. 2014;28(11):1579-91.
3. Dickens AM, et al. *AIDS*. 2015;29(5):559-69.