Protein Modifications by Lactate Associate with Lower Risks of Neurocognitive Impairment and Death

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for the **H**IV Infection, **A**ging, and **I**mmune Function **L**ong-Term **O**bservational study (**HAILO) A5322 Study Team**

BACKGROUND: In response to HIV and other infections, macrophages shift their main metabolism from oxidative phosphorylation to aerobic glycolysis with lactate as the end-product[2]. Ensuing increases in glycolytic flux promotes the production of **advanced glycation end products (AGEs)**, reactive glucose metabolites that alter protein function via **non-enzymatic, post-translational modifications**.

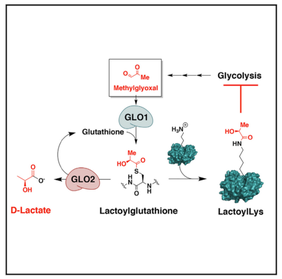
Methylglyoxal (MG) is a highly reactive glucose metabolite that is a major source of **AGEs**. Detoxification of **MG** occurs within the **glyoxalase cycle** [FIG 1] involving**:**

1) enzymatic transfer of the **lactate** moiety in **MG** to **glutathione** via glyoxalase (GLO)-1, generating ***Lactoyl-glutathione (LGSH)***.

2) non-enzymatic transfer of the **lactate** moiety from ***LGSH*** to free **lysine** residues on diverse proteins, generating ***Lactoyl-Lys***.

- Overrepresented by glycolytic enzymes, these modified proteins were previously shown to inhibit glycolysis[1].

Figure 1: **Glyoxalase Cycle**[1]



Lysine lactoylation (*Lactoyl-Lys*) describes a protein modification in which the lactate moiety from methylglyoxal (a glucose metabolite), is transferred to free lysine residues on diverse proteins via a glutathione intermediate, *Lactoyl-glutathione* (*LGSH;* Fig 1). Enriched for glycolytic enzymes, these modified proteins inhibit glycolysis[1].

In a prospective cohort study of PWH we found that higher plasma concentrations of *Lactoyl-Lys* proteins at entry were associated with lower hazards of NCI & death; higher *LGSH* was also associated with a lower hazard of NCI. These associations implicate the **glyoxalase cycle** in the pathogenesis of NCI & all-cause mortality in PWH.

HYPOTHESIS: Post-translational protein modifications arising from enhanced glucose metabolism in PWH contribute to adverse clinical outcomes including **neurocognitive impairment (NCI)** and increased **mortality**.

METHODS: The HIV infection, Aging, and Immune Function Long-Term Observational study (**HAILO**) is a multicenter, prospective cohort study of PWH, 40 years or older at entry, who previously initiated ART in randomized trials through the AIDS Clinical Trials Group (ACTG).

Plasma concentrations of **12 AGEs** and **3 glutathione** species were assayed by liquid chromatography-mass spectrometry in stored specimens that were collected **in the fasting state** from 375 participants at study entry, who were randomly selected among 970 participants with available specimens and at least one post-entry neurocognitive assessment. Associations by each of these metabolites with NCI and all-cause mortality were explored using multivariable Cox Proportional Hazard models in which the exploratory variables were boxcox transformed. Potential confounding variables included age, sex at birth, race/ethnicity, ART duration, education status, smoking history, prior efavirenz exposure, nadir CD4 & entry: CD4, HIV viral load, hemoglobin & BMI. These variables were included in the multivariable models if significant to $P<0.10$.

Neurocognition was assessed at entry and every 48 weeks by **Trailmaking A & B, Wechsler Adult Intelligence Scale-Revised Digit Symbol, & Hopkins Verbal Learning** tests. Demographically adjusted scores were converted to Z-scores, where NCI was defined by Z-scores in ≥2 domains ≥1S.D. below the norm or in ≥1 domain ≥2S.D.below the norm.

TABLE 1: Baseline Characteristics, n=375

Age yrs (median, IQR)		51 (46, 56)
Sex at Birth (n, %)	Female	57 (15%)
	Male	318 (85%)
Race/Ethnicity (n, %)	White (not Hispanic)	178 (47%)
	Black (not Hispanic)	107 (29%)
	Hispanic (regardless of race)	90 (24%)
Smoke (n, %)	Current	93 (25%)
	Past	132 (35%)
	Never	150 (40%)
CD4/µL (median, IQR)	Nadir	202 (68, 318)
	Entry	615 (449, 827)
Prior Antiretroviral Therapy yrs. (median, IQR)		7.7 (4.2, 11.8)
Entry HIV Viral Load < 50 copies/mL (n, %)		318 (85%)
Entry Hemoglobin mg/dL (median, IQR)		14.5 (13.6, 15.3)
Entry BMI (median, IQR)		27.2 (24.4, 30.1)

Figure 2A: Kaplan-Meier Plot of All-Cause Mortality by Lactoyl-Lys
1=low,2=medium 3=high

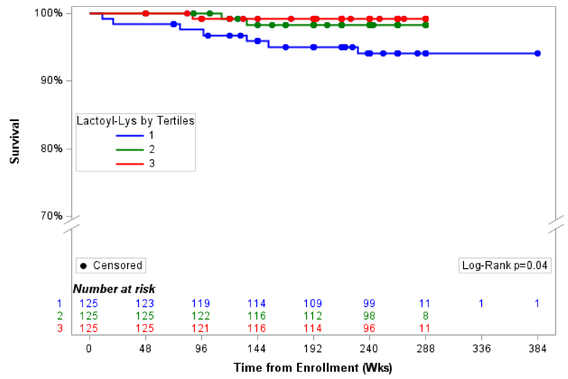


Figure 2B: Kaplan-Meier Plot of NCI by LGSH
1=low,2=medium 3=high

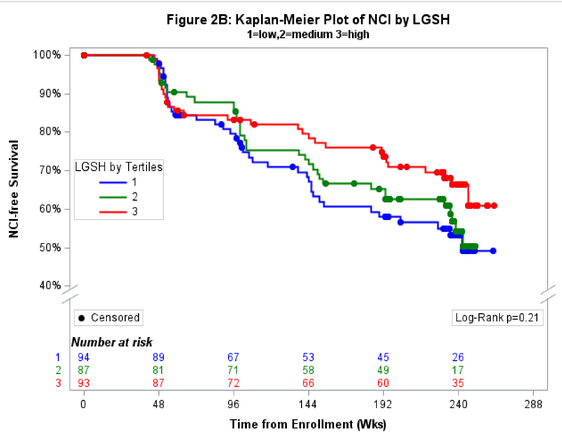


Figure 2C: Kaplan-Meier Plot of NCI by Lactoy-Lys
1=low,2=medium 3=high

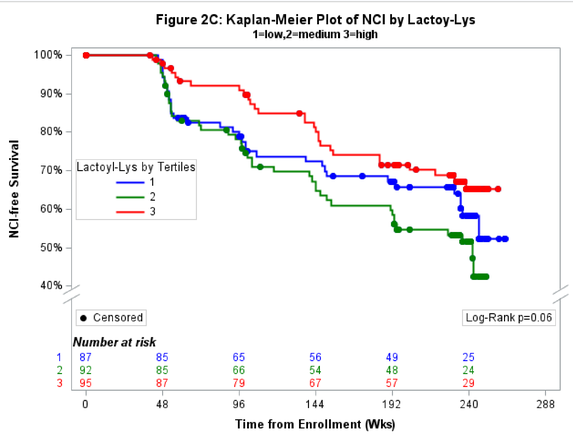


Table 2: Adjusted Hazard Ratios by Cox PH models

	Metabolite	aHR 95% C.I.	*P*
NCI*	*Lactoyl-Lys*	0.66 [0.45, 0.97]	0.03
	LGSH	0.72 [0.54, 0.94]	0.02
Mortality§	*Lactoyl-Lys*	0.41 [0.19, 0.89]	0.02

Adjusted for: *race/ethnicity & entry BMI; §ART duration & hemoglobin

RESULTS:

NCI: There were 103 incident NCI diagnoses among 274 participants at risk, over a median follow-up duration of 199 weeks. Each 1 S.D. increase in the entry plasma concentrations of ***Lactoyl-Lys*** & ***LGSH*** was associated with a 34% & a 28% lower hazard of NCI, respectively [Table 2, FIG 2B & 2C].

Mortality: There were 10 deaths among 375 participants over a median follow-up duration of 240 weeks. A 1 S.D. increase in the entry ***Lactoyl-Lys*** plasma protein concentration was associated with a 59% lower hazard of death [Table 2; FIG 2A].

CONCLUSION: Present in all mammalian cells, the **glyoxalase cycle** is an essential system that detoxifies **methylglyoxal** and other harmful metabolites[3]. The apparent beneficial effects on cognition and mortality by variations in this cycle may represent a negative feedback response to enhanced glycolytic flux in PWH. These observations support an important role of oxidative stress involving the **glyoxalase cycle** in the pathogenesis of NCI and all-cause mortality in PWH.

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