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Supplementary Materials for

Mapping of host-parasite-microbiome interactions reveals metabolic determinants of tropism and tolerance in Chagas disease

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Published 22 July 2020, *Sci. Adv.* **6**, eaaz2015 (2020) DOI: 10.1126/sciadv.aaz2015

The PDF file includes:

Figs. S1 to S7 Tables S1 and S2 Legend for data file S1

Other Supplementary Material for this manuscript includes the following:

(available at advances.sciencemag.org/cgi/content/full/6/30/eaaz2015/DC1)

Data file S1

Supplementary Materials



Fig. S1. Overall higher luminescence, representing parasite burden, in infected samples than background luminescence from uninfected samples. (**A**) 12 days post-infection. (**B**) 89 days post-infection. RLU, relative luminescence units. Note that this data is not adjusted for section size (weight). For normalized data, refer to **Fig. 1A**.



Fig. S2. Limited overlap in chemical families differentially-modulated by infection, across organs. Metabolite features were combined into chemical families (as identified by feature-based molecular networking (*31*)). Chemical families with fold change in abundance >2 and Mann-Whitney p-value<0.05 were compared between sampling sites. (**A**) 12 days post-infection. (**B**) 89 days post-infection.



Fig. S3. Annotation support for infection-modulated molecules identified by random forest analysis and for microbially-derived and microbially-modified molecules. All annotations are level 2/3 according to the metabolomics standards initiative (33). Top (black), experimental MS2 spectrum. Bottom (green), library reference. (A) m/z 448.341 RT 2.90 min match to palmitovlcarnitine. (B) m/z 570.355 RT 2.96 min match to LPC(16:0). (C) m/z 329.268 RT 3.02 min match to monopalmitolein. (D) m/z 568.339 RT 2.90 min match to LPC(16:0). (E) m/z544.339 RT 2.90 min match to LPC(16:0). (F) m/z 388.305 RT 2.73 min match to lauryolcarnitine. (G) m/z 209.092 RT 0.63 min match to L-kynurenine. (H) m/z 442.352 RT 2.85 min match to laurvolcarnitine. (I) m/z 233.149 RT 0.82 min match to thr-leu. (J) m/z 510.391 RT 3.08 min match to LPC(16:0). (K) m/z 482.36 RT 2.97 min match to Lyso PAF C-18 (LPC(O-(18:0)). (L) m/z 508.376 RT 3.00 min match to 1-(1Z-Hexadecenyl)-sn-glycero-3-phosphocholine. (M) *m/z* 564.437 RT 3.26 min match to LPC(O-18:0). (N) *m/z* 550.386 RT 3.08 min match to LPC(16:0). (**O**) *m/z* 538,422 RT 3.24 min match to Lyso-PAF C-18. (**P**) *m/z* 232.154 RT 1.00 min match to acetyl-DL-carnitine. (O) m/z 204.123 RT 0.28 min match to acetyl-DL-carnitine. (R) m/z 373.273 RT 2.71 min match to cholic acid. (S) m/z 407.279 RT 2.68 min match to cholic acid. (T) *m/z* 572.37 RT 2.99 min match to LPC(16:0). (U) *m/z* 188.071 RT 1.19 min match to tryptophan. (V) m/z 205.097 RT 1.24 min match to tryptophan. (W) m/z 566.321 RT 2.91 min match to Lyso PAF C-18 (LPC(O-18:0)). (X) m/z 150.058 RT 0.38 min match to methionine. (Y) m/z 162.112 RT 0.26 min match to L-carnitine. (Z) m/z 357.278 RT 2.88 min match to chenodeoxycholic acid. (AA) m/z 817.518 RT 2.73 min match to cholic acid. (AB) m/z 538.519 RT 4.07 min match to Cer(d18:1/20:1). (AC) *m/z* 520.508 RT 4.06 min match to Cer(d18:1/16:1). (AD) *m/z* 279.231 RT 2.97 min match to 9(10)-EpOME. (AE) *m/z* 293.247 RT 3.15 min match to 9Z,11E,13E-octadecatrienoic acid ethyl ester. (AF) m/z 188.071 RT 2.18 min match to indole-llactate. (AG) m/z 527.158 RT 0.265 min match to maltotriose. (AH) m/z 785.589 RT 2.891 min

match to deoxycholic acid. (AI) m/z 307.083 RT 0.350 min match to oxidized glutathione (heart tissue, carnitine treatment). (AJ) m/z 165.055 RT 0.403 min match to tyrosine.



cycle number for 16S rRNA gene quantitative PCR. (**D-F**) Microbial community richness, and (**G-I**) relative abundance of microbial phyla. Sampling sites: Cecum (position 10), proximal large intestine (position 11), and central large intestine (position 12).



Weeks post-infection

Fig. S5. GI acylcarnitine abundance during infection and impact of carnitine

supplementation on low-dose *T. cruzi* infection. (A) GNPS mirror plot showing MS2 match of m/z 344.279 RT 2.752 min to laurovlcarnitine library reference (green, bottom). (B) Total acylcarnitine peak area across all GI sites, 12 days post-infection. (C) Total short-chain acylcarnitine peak area across all GI sites, 12 days post-infection. (**D**) Total acylcarnitine peak area at each sampling site, 12 days post-infection. (E) Short-chain acylcarnitine peak area at each sampling site, 12 days post-infection. (F) Mid-chain acylcarnitine peak area at each sampling site, 12 days post-infection. (G) Total acylcarnitine peak area at each sampling site, 89 days postinfection. (H) Short-chain acylcarnitine peak area at each sampling site, 89 days post-infection. (I) Long-chain acylcarnitine peak area at each sampling site, 89 days post-infection. (J) Acetylcarnitine peak area at each sampling site, 12 days post-infection. (K) Acetylcarnitine peak area at each sampling site, 89 days post-infection. (L) Carnitine peak area at each sampling site, 89 days post-infection. Black bars, FDR-corrected Mann-Whitney p<0.05. (M) and (N) Male C3H/HeJ mice were infected with 5,000 luciferase-expressing T. cruzi strain CL Brener trypomastigotes. Beginning 7 days post-infection, animals received drinking water supplemented with carnitine (100 mg/kg/day equivalent, based on water consumption) or remained on standard drinking water (vehicle group). (M) Acute-stage humane endpoints and mortality were only observed in the vehicle-treated group. (N) Comparable total body parasite burden between groups, weeks 1-7 post-infection. Mean + standard error of mean displayed.



Fig. S6. Carnitine treatment resets cardiovascular metabolism in the absence of similar effects on GI tract metabolism and on cardiac inflammation. (A) Reduced Brav-Curtis-Faith distance between plasma metabolite profiles of carnitine and benznidazole-treated animals and carnitine and uninfected animals, compared to vehicle-treated and benznidazole-treated animals and vehicle-treated and uninfected animals. (B) and (C) Reduced Bray-Curtis-Faith distance between heart metabolite profiles of carnitine and benznidazole-treated animals and carnitine and uninfected animals, compared to vehicle-treated and benznidazole-treated animals and vehicletreated and uninfected animals. (B) Replicate 1. (C) Replicate 2. (A), (B) and (C), Black lines, Mann-Whitney p<0.05. (**D**) PCoA analysis (Bray-Curtis-Faith distance metric) of heart samples. after 10 days of treatment (replicate 2). (E) and (F) Carnitine treatment does not mitigate infection-induced metabolic disturbances in the oesophagus (E) and the large intestine (F) after 10 days of treatment (both replicates combined). (G) Carnitine treatment alters metabolite profile but does not mitigate infection-induced metabolic disturbances in cell culture. PCoA analysis (Brav-Curtis-Faith distance metric) of *T. cruzi*-infected C2C12 cells, after 4 days of treatment (four replicates combined). (H) and (I) Heatmap showing cardiac metabolite features distinguishing vehicle-treated individuals from carnitine-treated and benznidazole-treated individuals (Kruskal-Wallis, FDR-corrected p < 0.05). (H) Replicate 1. (I) Replicate 2. Boxed: oxidized linoleic acid metabolites. (J) Comparable cellular infiltrate at the heart base between carnitine-treated and vehicle-treated animals. Representative H&E staining of histological sections at 7 days postinfection and 10 days post-treatment. Top, vehicle. Bottom, carnitine. (K) Comparable cardiac cytokine and chemokine profile between carnitine-treated and vehicle-treated animals (both replicates combined). (L) Carnitine treatment does not affect infection-induced increases in cardiac oxidized glutathione in infected animals (untargeted LC-MS/MS peak area, both replicates combined). Black line, Mann-Whitney p<0.05. (M) No significant induction of cardiac

fibrosis at 7 days post-infection and 10 days post-treatment (17 days post-infection). Collagen I

 Δ Ct to *Gapdh* gene expression (single replicate).



Fig. S7. Impact of carnitine treatment on gastrointestinal and cardiac acylcarnitines. (A-C)

Carnitine. (D-F) Acetylcarnitine. (G-J) Short-chain acylcarnitines. (K-N) Mid-chain acylcarnitines. (O-R) Long-chain acylcarnitines. (S-V) Total acylcarnitines. (A), (D), (G), (K), (O), (S) Heart. (B), (E), (H), (L), (P), (T) Oesophagus. (C), (F), (I), (M), (Q), (U) Small intestine. (J), (N), (R), (V) Large intestine. Carnitine and acetylcarnitine were not detected under our filtering criteria in the large intestine. Black lines, Mann-Whitney p<0.05.

Table S1. Detected acylcarnitines in GI tract samples.

Short-chain acylcarnitines					
m/z	RT (min)	Putative annotation	ppm error		
204.123	0.282	C2:0 acylcarnitine (acetylcarnitine)	2.94		
218.138	0.466	C3:0 acylcarnitine (propionylcarnitine)	5.50		
232.154	0.349	C4:0 acylcarnitine (butyrylcarnitine)	0		
232.154	1.002	C4:0 acylcarnitine (butyrylcarnitine)	0		
248.149	0.404	C4:0-OH acylcarnitine	3.22		
248.149	0.267	C4:0-OH acylcarnitine	3.22		
		Mid-chain acylcarnitines			
m/z	RT (min)	Putative annotation	ppm error		
246.17	2.205	C5:0 acylcarnitine (valerylcarnitine)	2.03		
260.185	2.304	C6:0 acylcarnitine	4.61		
260.186	2.383	C6:0 acylcarnitine	0.77		
288.216	2.514	C8:0 acylcarnitine	4.20		
314.233	2.599	C10:1 acylcarnitine	0.32		
316.247	2.645	C10:0 acylcarnitine	5.69		
332.243	2.546	C10:0-OH acylcarnitine	2.11		
	Long-chain acylcarnitines				
m/z	RT (min)	Putative annotation	ppm error		
342.263	2.865	C12:1 acylcarnitine	4.09		
344.279	2.752	C12:0 acylcarnitine (lauroylcarnitine) ^a	3.20		
360.273	2.655	C12:0-OH acylcarnitine	2.78		
368.279	2.808	C14:2 acylcarnitine	2.99		
370.294	2.789	C14:1 acylcarnitine	4.59		
370.295	2.982	C14:1 acylcarnitine	1.89		
372.237	2.399	C12:2-DC acylcarnitine	4.30		
372.310	2.998	C14:0 acylcarnitine	3.76		
372.310	2.835	C14:0 acylcarnitine	3.76		
388.305	2.732	C14:0-OH acylcarnitine	1.80		

396.310	2.829	C16:2 acylcarnitine	3.53
400.269	2.605	C14:2-DC acylcarnitine	2.25
400.341	2.929	C16:0 acylcarnitine (palmitoylcarnitine)	4.25
402.284	2.636	C14:1-DC acylcarnitine	3.98
412.305	2.72	C16:2-OH acylcarnitine	3.15
414.32	2.773	C16:1-OH acylcarnitine	4.59
414.321	2.841	C16:1-OH acylcarnitine	2.17
422.326	2.848	C18:3 acylcarnitine	2.37
424.341	2.896	C18:2 acylcarnitine	4.01
426.357	2.953	C18:1 acylcarnitine (oleoylcarnitine)	3.05
428.3	2.67	C16:2-DC acylcarnitine	2.80
428.373	3.018	C18:0 acylcarnitine	2.33
430.316	2.698	C16:1-DC acylcarnitine	2.09
440.336	2.823	C18:2-OH acylcarnitine	3.63
442.352	2.853	C18:1-OH acylcarnitine	2.71
444.331	2.773	C17:1-DC acylcarnitine	3.38
444.367	2.911	C18:0-OH acylcarnitine	2.25
448.341	2.901	C20:4 acylcarnitine	3.79
452.373	2.977	C20:2 acylcarnitine	2.21
454.316	2.706	C18:3-DC acylcarnitine	1.98
454.388	3.037	C20:1 acylcarnitine	3.52
456.331	2.725	C18:2-DC acylcarnitine	3.29
456.404	3.099	C20:0 acylcarnitine	2.84
458.348	2.726	C18:1-DC acylcarnitine	0.44
458.348	2.76	C18:1-DC acylcarnitine	0.44
512.466	3.52	C24:0 acylcarnitine	3.71

^a GNPS library match (see Figure S5a).

Table S2. Instrumental and LC-MS data processing methods.

	Instrumental methods				
Use Lock Masses	Off				
Chromatogram Peak Width		6 second	S		
Method Duration	7.50 min (GI tract), 12.50 min (Plasma and cell culture)				
Exclusion list	371.101				
	235.206				
	311.084				
	314.131				
	285.0135				
	144.9822				
		LC parameters			
		Tissue samples			
Time (min)	Flow	%B (Acetonitrile + 0.1%	Curve		
	(mL/min)	Formic Acid)			
0.00	0.500	2	5		
1.00	0.500	2	5		
2.50	0.500	98	5		
4.50	0.500	98	5		
5.50	0.500	2	5		
7.50	0.500	2	5		
7.50		Stop Rur	1		
	Plasma sam	ples and cell culture samples			
Time (min)	Flow	%B	Curve		
	(mL/min				
)				
0.00	0.500	5	5		
1.00	0.500	5	5		
9.00	0.500	100	5		
11.00	0.500	100	5		
11.50	0.500	5	5		
12.50	0.500	5	5		
12.50		Stop Rur	1		
	Dive	rt valve parameters			
Switch at	Switch at 0.2 min				
MS parameters					
		Source			
Sheath Gas Flow Rate	35 L/min				
Aux Gas Flow Rate	10 L/min				
Sweep Gas Flow Rate	0 L/min				
Spray Voltage	3.80 (+) / 3.0 (-) kV				
Capillary Temperature	320°C				
S-lens RF Level	50.0				
Aux Gas Heater Temperature	Temperature 350.0°C				
Full MS					
Resolution	Resolution 17,500 (cell culture samples), 70,000 (all other samples)				

Maximum IT 100 milliseconds (cell culture samples), 246 milliseconds (all other samples) Scan Range 70 to 1050 m² (GI tract, plasma), 100 to 1050 m² (GI tract, plasma), 100 to 1050 m² (GI tract, plasma), 101 to 1050 m² (GI tract, plasma), 115 (carnitine treatment tissue samples) Maximum IT 100 milliseconds (cell culture samples), 25 (GI tract, plasma), 105 (carnitine treatment tissue samples) Maximum IT 100 milliseconds (cell culture samples), 54 milliseconds (all other samples) Fixed First Mass (N)CE Stepped NCE: 20, 40, 60 Maximum IT 100 milliseconds (cell culture samples), 1.5E5 (all other samples) Fixed First Mass (N)CE Stepped NCE: 20, 40, 60 Maximum Tireshold 8.0E4 (cell culture samples), 1.5E5 (all other samples) Apact Trigger 2 to 15 seconds (cell culture samples), (all other samples) Paptide Match (cell culture samples), (all other samples) Dynamic Exclusion 5 seconds (cell culture samples), not (all other samples) Dynamic Exclusion 5 seconds (cell culture samples), (all other samples) Definite Maximum Time Span (min) 0.01 0.01 MS ¹ Noise Level 2.0E6 2.0E5 MS ²	AGC Target		1E6 (GI tract, plasma, cell culture), 3E6 (carnitine treatment tissue samples)			
Scan Range 70 to 1050 m/z (GI tract, plasma), 100 to 1500 m/z (carnitine treatment tissue samples, cell culture samples) dd-MS ² Resolution 17,500 AGC Target 5E5 (cell culture samples), 2E5 (GI tract, plasma), 1E5 (carnitine treatment tissue samples), 5 Isolation Window 3 m/z (cell culture samples), 5 Isolation Window 3 m/z (cell culture samples), 5 Isolation Window 3 m/z (cell culture samples), 10 m/z (all other samples) Fired First Mass	Maximum IT		100 milliseconds (cell culture samples), 246 milliseconds (all other samples)			
samples, cell culture samples) dd/MS ² Resolution 17,500 AGC Target SE5 (cell culture samples), 2E5 (GI tract, plasma), IE5 (carnitine treatment tissue samples), Data malification window Maximum IT 100 milliseconds (cell culture samples), 54 milliseconds (all other samples) Fixed First Mass	Scan Rang	ge	70 to 1050 m/z (GI tract, plasma), 100 to 1500 m/z (carnitine treatment tissue			
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Monotonic ShapeCheckedCheckedMaximum Charge33Representative IsotopeLowest m/zLowest m/zMinimum Charge110.010.0Meight for m/z11Weight for m/z11Weight for Retention Time11Retention Time Tolerance (min)0.5 (tissue samples), 0.2 (C2C12 samples)0.5Row FilteringMinimum Peaks in a Row6 (tissue samples), 36	Deisotoning			samples)		
Maximum Charge33Representative IsotopeLowest m/z Lowest m/z m/z Tolerance (ppm)10.010.0Weight for m/z 11Weight for Retention Time11Retention Time Tolerance (min)0.5 (tissue samples), 0.2 (C2C12 samples)0.5Row FilteringMinimum Peaks in a Row6 (tissue samples), 36	Densotoping	Monotonic Shape		Checked	Checked	
Representative IsotopeLowest m/z Lowest m/z m/z Tolerance (ppm)10.010.0Weight for m/z 11Weight for Retention Time11Retention Time Tolerance (min)0.5 (tissue samples), 0.2 (C2C12 samples)0.5Row FilteringMinimum Peaks in a Row6 (tissue samples), 36		Maximum Charge		3	3	
m/z Tolerance (ppm)10.010.0Weight for m/z 1Weight for m/z 1Use of the second se		Representative Isotone		Lowest m/z	Lowest m/z	
AlignmentWeight for m/z 11Weight for m/z 11Weight for Retention Time10.5 (tissue samples), 0.2 (C2C12 samples)Retention Time Tolerance (min)0.5 (tissue samples), 0.2 (C2C12 samples)Row FilteringMinimum Peaks in a Row6 (tissue samples), 36		m/z Tolerance (ppm)		10.0	10.0	
Alignment Weight for Retention Time 1 Retention Time Tolerance (min) 0.5 (tissue samples), 0.2 (C2C12 samples) 0.5 Row Filtering Minimum Peaks in a Row 6 (tissue samples), 3 (C2C12 samples) 6		Weight for m/z		1	1	
Augminent0.5 (tissue samples), 0.2 (C2C12 samples)0.5Row FilteringMinimum Peaks in a Row6 (tissue samples), 3 (C2C12 samples)6	Alignmont	We	ight for Retention Time	1	1	
Retention Time Tolerance (min) 0.2 (C2C12 samples) Row Filtering Minimum Peaks in a Row 6 (tissue samples), 3 (C2C12 samples)	Angninent			0.5 (tissue samples),	0.5	
Row Filtering Minimum Peaks in a Row 6 (tissue samples), 3 6		Retention Time Tolerance (min)		0.2 (C2C12		
Row FilteringMinimum Peaks in a Row6 (tissue samples), 36				samples)		
	Row Filtering M		nimum Peaks in a Row	6 (tissue samples), 3	6	

	Retention Time (min)	0.20 – 6.45 (tissue samples), 0.2-12.26 (C2C12 samples)	0.20 - 6.45	
	Keeps Only Peaks with MS ² Scans	Checked	Checked	
	Reset Peak No. ID	Checked	Checked	
Can Filling	Intensity Tolerance	75%	-	
Gap Filling	Retention Time Tolerance	0.2	-	
(C2C12 samples	RT Correction	Checked	-	
only)	<i>m/z</i> Tolerance (ppm)	10.0	-	
	Mouse carnitine treatment sa	mples	•	
			rity	
			Positive	
MS ¹	Noise Level	2.01	Ξ5	
MS ²	Noise Level	1.0E3		
	Mass List	mass	ses	
Chromatogram	Minimum Time Span (min)	0.0	1	
Builder	Minimum Height	3.01	3.0E5	
	<i>m/z</i> Tolerance (ppm)	10.	0	
	Algorithm	Local Minimum Search		
	m/z Range for MS ² Scan Pairing (Da)	0.01		
	RT Range for MS ² Scan Pairing (min)	0.2		
	Chromatographic Threshold	5%		
Chromatogram	Search Minimum in RT range	0.1		
Deconvolution	Minimum Relative Height	5%		
	Minimum Absolute Height	4E5		
	Minimum ratio of peak top/edge	1		
	Peak Duration range (min)	0.01 -3.5		
	<i>m/z</i> Tolerance (ppm)	10.0		
	Retention Time Tolerance (min)	0.5 (plasma), 0.05 (tissue)		
Deisotoping	Monotonic Shape	Checked		
	Maximum Charge	3		
	Representative Isotope	Lowest m/z		
	<i>m/z</i> Tolerance (ppm)	10.0		
Alionment	Weight for m/z	1		
¹ inglinient	Weight for Retention Time	1		
	Retention Time Tolerance (min)	0.1	0.1	
	Minimum Peaks in a Row	4		
Row Filtering	Retention Time (min)	0.20 – 12.00 (plasma), 0.2-6.45 (tissues)		
Now I mering	Keeps Only Peaks with MS ² Scans	Checked		
	Reset Peak No. ID	Checked		
	Intensity Tolerance	5%		
Gan Filling	Retention Time Tolerance	0.1		
Sap I ming	RT Correction	Checked		
	m/z Tolerance (ppm)	10.	0	

Data file S1. Impact of infection on GI metabolite profiles, including infection-modulated metabolites identified through random forest analysis.