

Effect of experimental hookworm infection on insulin resistance in people at risk of Type 2 Diabetes: Supplementary Information

SUPPLEMENTARY TABLES

Supplementary Table 1. HOMA-IR change effect sizes at each evaluation visit

	Kruskal Wallis effect size	L3-20 vs Placebo	L3-40 vs Placebo
6 months	$\eta^2 = 0.04$	$r = 0.372$	$r = 0.235$
	$d_{Cohen} = 0.42^S$	$d_{Cohen} = 0.80^L$	$d_{Cohen} = 0.48^S$
12 months	$\eta^2 = 0.16$	$r = 0.556$	$r = 0.341$
	$d_{Cohen} = 0.86^L$	$d_{Cohen} = 1.34^L$	$d_{Cohen} = 0.73^M$
18 months	$\eta^2 = 0.23$	$r = 0.513$	$r = 0.088$
	$d_{Cohen} = 1.08^L$	$d_{Cohen} = 1.19^L$	$d_{Cohen} = 0.18$
24 months	$\eta^2 = 0.01$	$r = 0.409$	$r = 0.195$
	$d_{Cohen} = 0.20^S$	$d_{Cohen} = 0.90^L$	$d_{Cohen} = 0.40^S$

S, small effect size; M, medium effect size; L, large effect size.

**Supplementary Table 2. Absolute values and changes in glycated haemoglobin (HbA1c/IFCC) in each treatment group**

HbA1C IFCC (mmol/mol), Median and IQR (n)									
	baseline	6 months	Δ6 mo.	12 months	Δ12 mo.	18 months	Δ18 mo.	24 months	Δ24 mo.
Placebo	32	34	+1	34	+2	33	+3	35	+4
	30-33 (n = 12)	30-36 (n = 12)	0 to 3	30-37 (n = 9)	0 to 4	30-36 (n = 8)	0 to 4	33-37 (n = 6)	1 to 5
L3-20	33	32	0	32	0	33	+1	33	+2
	31-35 (n = 13)	30-34 (n = 11)	-1 to 1	31-35 (n = 11)	-1 to 1	32-36 (n = 8)	0 to 2	32-37 (n = 7)	1 to 4
L3-40	32	31	+1	33	+3	34 #	+4	32	+2
	29-34 (n = 11)	30-35 (n = 11)	0 to 3	32-35 (n = 10)	1 to 4	33-37 (n = 8)	2 to 7	31-37 (n = 9)	2 to 5

**Bolded data indicate presence of significantly different values, # Significantly different to baseline.**

**Supplementary Table 3. Absolute values and changes in serum cholesterol, high-density lipoprotein (HDL) and cholesterol/HDL ratio in each treatment group**

Total cholesterol (mmol/L), Median and IQR (n)									
	baseline	6 months	Δ6 mo.	12 months	Δ12 mo.	18 months	Δ18 mo.	24 months	Δ24 mo.
Placebo	5.4	4.9	-0.4	4.8	-0.4	4.9	-0.6	5.0	-0.2
	4.9-6.3 (n = 12)	4.4-6.0 (n = 12)	-0.7 to 0.0	4.4-5.4 (n = 9)	-0.6 to -0.1	4.3-5.8 (n = 8)	-1.1 to -0.1	4.5-5.3 (n = 6)	-0.6 to 0.1
L3-20	5.2	5.1	-0.3	5.0	-0.2	5.1	-0.2	4.9	-0.2
	4.6-6.3 (n = 13)	3.9-5.9 (n = 12)	-0.6 to -0.2	4.2-5.6 (n = 11)	-0.7 to 0.5	4.5-5.7 (n = 10)	-0.4 to 0.4	3.9-6.8 (n = 18)	-0.5 to 0.6
L3-40	5.4	6.0	+0.7	5.6	+0.2	5.7	+0.4 *	5.3	-0.1
	4.9-5.5 (n = 11)	4.9-6.3 (n = 11)	-0.2 to 0.9	4.8-5.7 (n = 10)	-0.2 to 0.3	4.6-6.1 (n = 8)	-0.2 to 0.6	4.3-5.8 (n = 9)	-0.8 to 0.6
HDL (mmol/L), Median and IQR (n)									
Placebo	1.2	1.1	-0.1	1.2	0.0	1.2	-0.1	1.2	0.0
	1.1-1.4 (n = 12)	1.0-1.3 (n = 12)	-0.1 to 0.0	1.1-1.3 (n = 9)	0.0 to 0.1	1.1-1.6 (n = 8)	-0.2 to 0.2	1.0-1.7 (n = 6)	-0.3 to 0.2
L3-20	1.2	1.3	-0.1	1.1	0.0	1.2	-0.2	1.1	+0.1
	1.0-1.5 (n = 13)	1.0-1.4 (n = 12)	-0.2 to -0.1	1.0-1.4 (n = 11)	-0.1 to 0.0	1.0-1.3 (n = 10)	-0.2 to 0.0	1.0-1.7 (n = 8)	-0.1 to 0.2
L3-40	1.3	1.3	0.0	1.3	0.0	1.2	-0.1	1.1	-0.1
	1.1-1.5 (n = 11)	1.1-1.5 (n = 11)	-0.2 to 0.2	1.1-1.3 (n = 10)	-0.1 to 0.1	1.1-1.3 (n = 8)	-0.2 to -0.1	1.0-1.4 (n = 9)	-0.3 to 0.0
Total cholesterol/HDL ratio, Median and IQR (n)									
Placebo	4.4	4.0	-0.1	4.0	-0.4	3.9	-0.2	3.9	+0.1
	3.8-5.2 (n = 12)	3.8-4.9 (n = 12)	-0.3 to 0.3	3.5 to 4.3 (n = 9)	-0.6 to -0.2	3.3-4.6 (n = 8)	-0.7 to 0.3	3.3-5.1 (n = 6)	-0.6 to 0.6
L3-20	4.1	4.2	+0.2	4.2	-0.1	4.3	+0.3	4.2	-0.2
	3.8-4.9 (n = 13)	3.7-4.9 (n = 12)	0.0 to 0.3	3.7-5.3 (n = 11)	-0.2 to 0.5	3.6-5.6 (n = 10)	-0.3 to 1.1	3.3-5.0 (n = 8)	-0.6 to 0.4
L3-40	4.0	4.4	+0.5	4.5	+0.1	4.8	+0.5	4.3	+0.2
	3.7-4.9 (n = 11)	4.0-4.7 (n = 11)	-0.2 to 0.8	3.9-5.1 (n = 10)	-0.4 to 0.8	4.1-5.0 (n = 8)	0.0 to 0.9	4.0-5.4 (n = 9)	-0.1 to 0.7

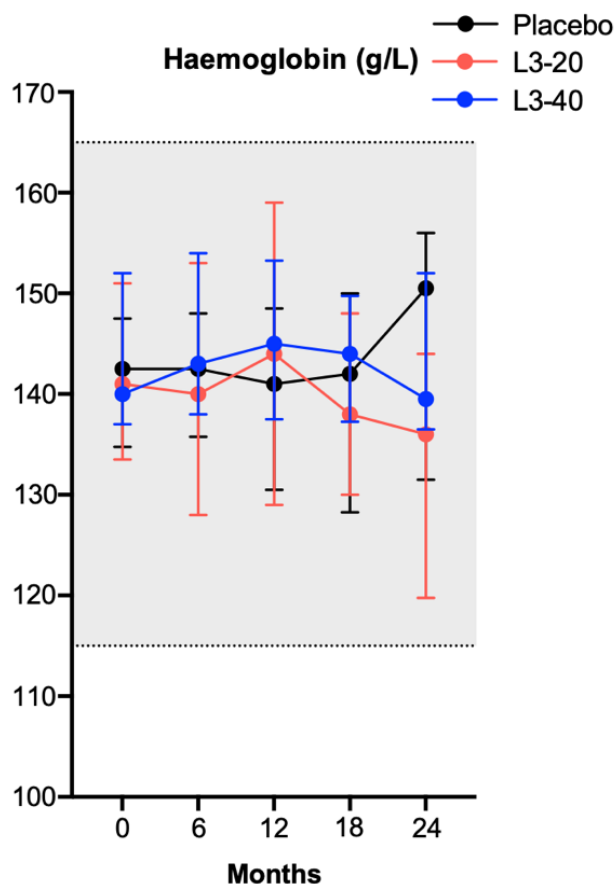
**Bolded data indicate presence of significantly different values, # Significantly different to baseline. \*Significantly different to Placebo.**

**Supplementary Table 4. Absolute values and changes in serum Triglyceride and Low-Density Lipoprotein (LDL) levels in each treatment group**

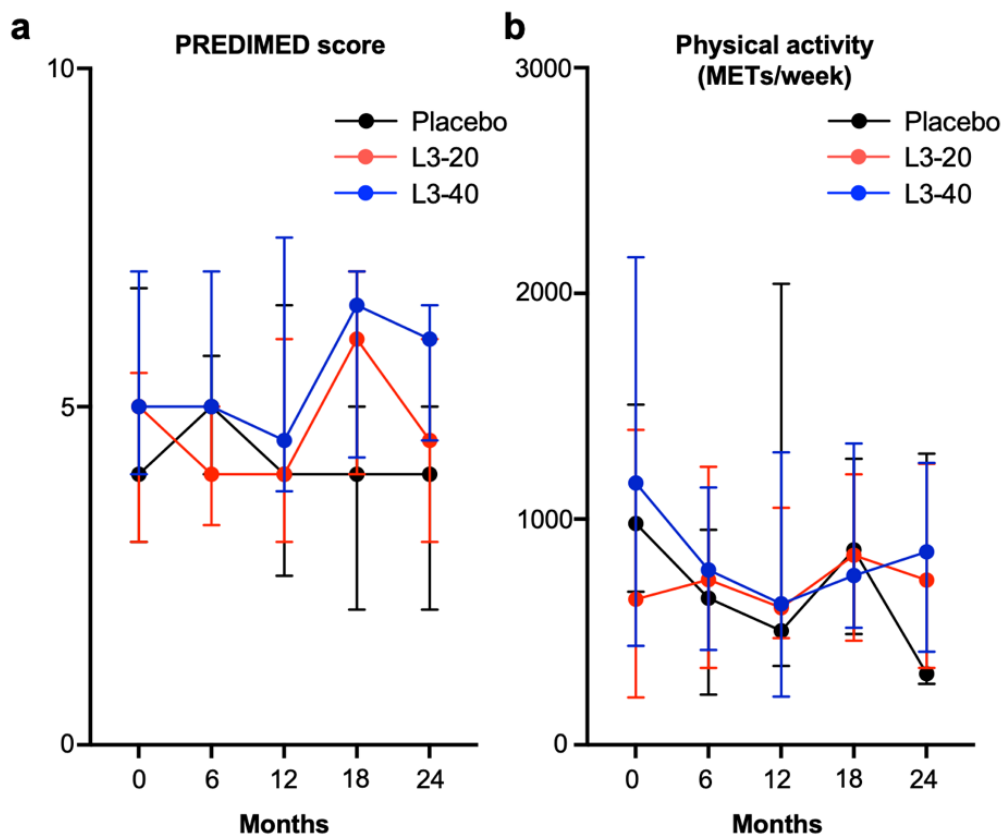
Triglycerides (mmol/L), Median and IQR (n)								
	baseline	6 months	Δ6 mo.	12 months	Δ12 mo.	18 months	Δ18 mo.	24 months
<b>Placebo</b>	1.4 0.9-2.0 (n = 12)	1.4 1.0-2.0 (n = 12)	+0.2 -0.4 to 0.6	1.3 0.9-1.6 (n = 9)	0.0 -0.2 to 0.3	1.4 1.0-1.9 (n = 8)	+0.3 -0.2 to 0.5	1.8 1.4-2.9 (n = 6)
<b>L3-20</b>	1.3 0.9-1.8 (n = 13)	1.3 0.8-1.7 (n = 12)	-0.2 -0.3 to 0.2	1.2 1.0-1.5 (n = 11)	-0.1 -0.4 to 0.2	1.4 0.8-2.5 (n = 10)	0.0 -0.2 to 0.8	+0.1 0.9-1.9 (n = 8)
<b>L3-40</b>	1.4 0.8-2.0 (n = 11)	1.3 1.0-2.7 (n = 11)	-0.2 -0.4 to 0.9	1.8 1.0-2.6 (n = 10)	+0.1 -0.4 to 0.6	1.7 1.1-1.9 (n = 8)	-0.1 -0.2 to 0.7	+0.1 0.9-2.1 (n = 9)
LDL (mmol/L), Median and IQR (n)								
<b>Placebo</b>	3.5 3.0-4.4 (n = 12)	3.1 2.6-3.9 (n = 12)	-0.2 -0.7 to 0.0	<b>2.9 #</b> 2.6-3.4 (n = 9)	-0.3 -0.8 to -0.1	<b>2.9#</b> 2.5-3.5 (n = 8)	-0.6 -0.8 to -0.3	2.7 2.6-3.2 (n = 6)
<b>L3-20</b>	3.3 2.9-4.3 (n = 13)	3.4 2.5-3.8 (n = 12)	-0.4 -0.6 to 0.0	3.2 2.7-3.7 (n = 11)	-0.1 -0.6 to 0.6	3.1 2.6-3.9 (n = 10)	-0.3 -0.5 to 0.5	-0.2 2.1-4.1 (n = 8)
<b>L3-40</b>	3.4 2.6-3.6 (n = 11)	3.4 3.0-4.0 (n = 11)	+0.3 -0.3 to 1.3	3.2 3.0-3.8 (n = 10)	+0.2 -0.3 to 0.4	3.8 2.9-4.0 (n = 8)	<b>+0.5*</b> 0.0 to 0.6	+0.1 2.7-3.7 (n = 9)

**Bolded data indicate presence of significantly different values, # Significantly different to baseline. \*Significantly different to Placebo**

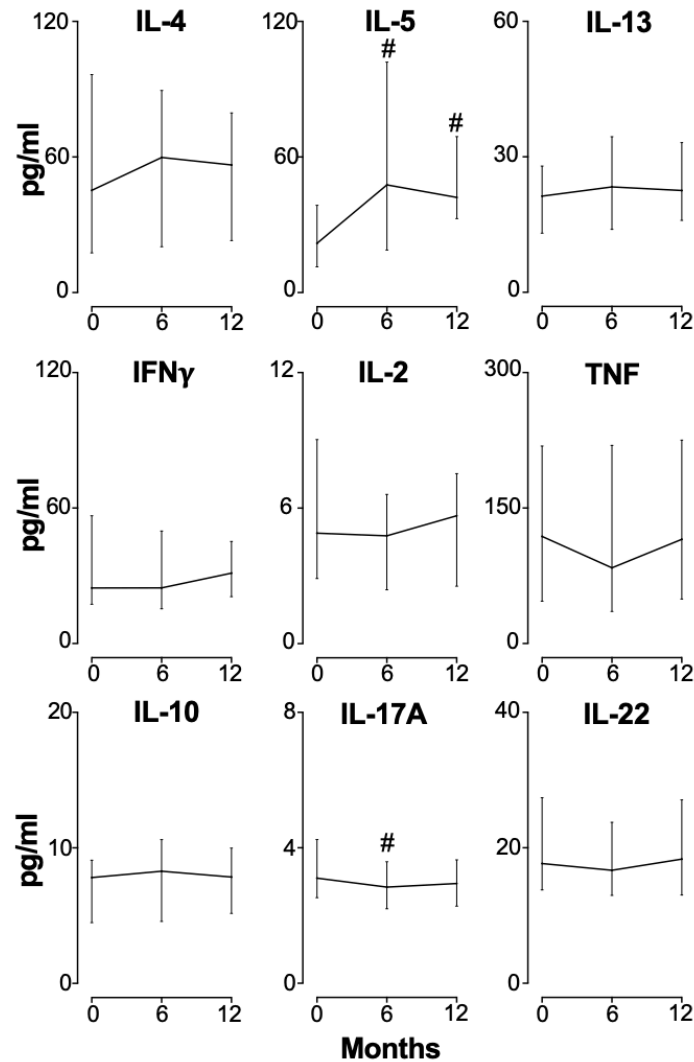
## SUPPLEMENTARY FIGURES AND LEGENDS



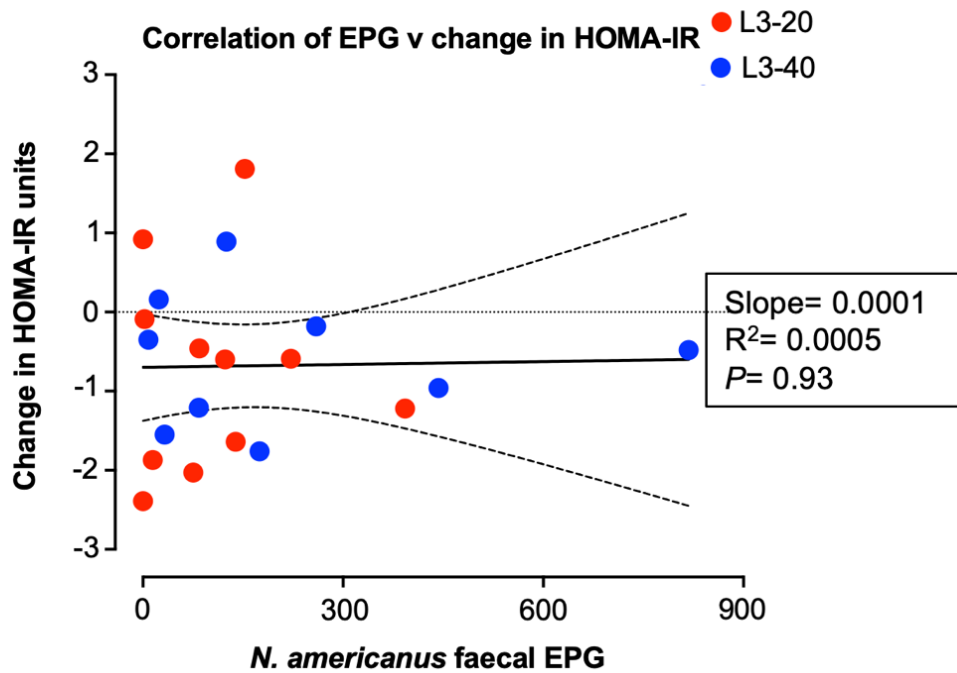
**Supplementary Figure 1. Hookworm infection was not associated with anaemia.** Blood hemoglobin concentration at each evaluation visit (median and IQR), shaded area indicates combined normal range for males and females. (Sample size for month 0, 6, 12, 18 and 24: Placebo n=12, 12, 9, 8 and 6; L3-20 n=13, 12, 11, 11 and 8; L3-40 n=11, 11, 10, 8 and 8, respectively). Source data are provided as a Source Data File.



**Supplementary Figure 2. Diet (PREDIMED score) and Physical Activity remained relatively stable in all groups throughout the study. (A)** PREDIMED total scores in each treatment group at each evaluation visit (median and IQR). (Sample size for month 0, 6, 12, 18 and 24: Placebo n=12, 12, 9, 8 and 7; L3-20 n=13, 12, 12, 10 and 6; L3-40 n=11, 11, 10, 8 and 9, respectively). **(B)** Physical activity (Metabolic Equivalents of task, METs) per week assessed at each evaluation visit (median and IQR). (Sample size for month 0, 6, 12, 18 and 24: Placebo n=12, 12, 9, 8 and 7; L3-20 n=13, 12, 11, 10 and 8; L3-40 n=11, 11, 10, 8 and 9, respectively). Source data are provided as a Source Data File.

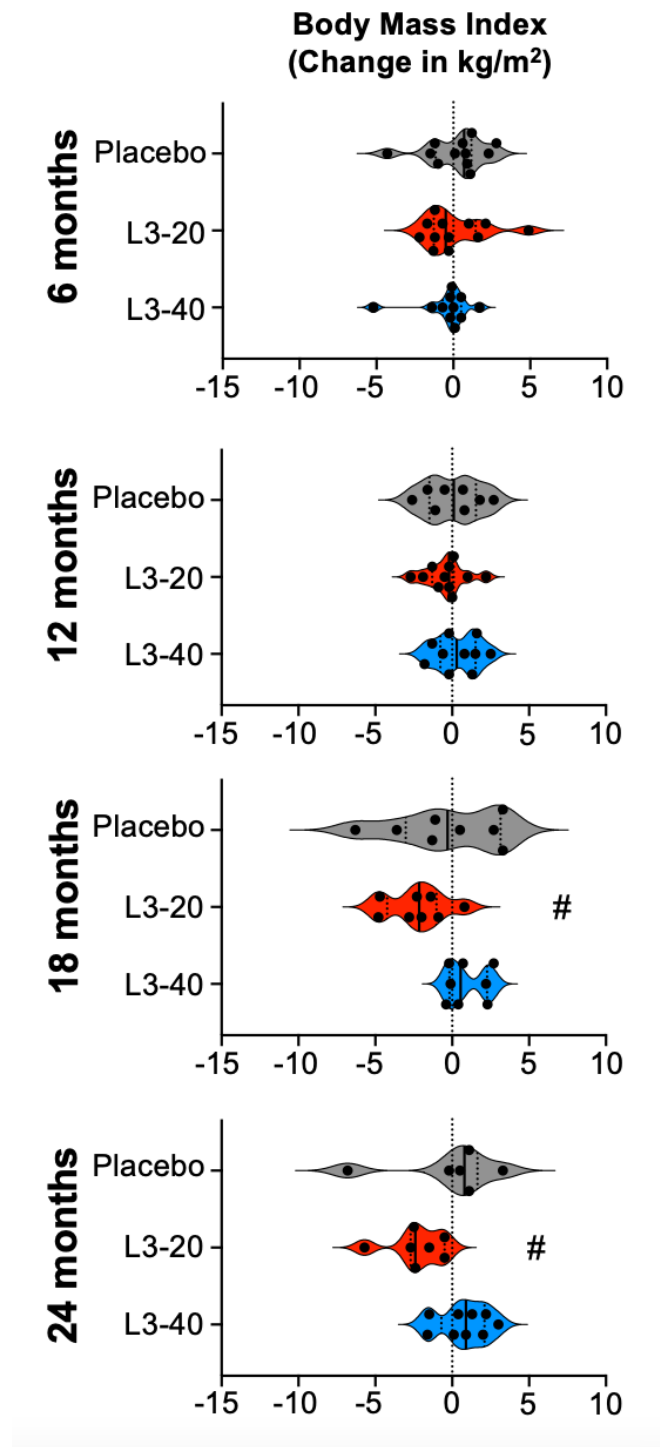


**Supplementary Figure 3. Serum cytokine levels in combined hookworm-treated cohorts.** Serum samples taken from L3-20 and L3-40 participants (n=17) at baseline, 6 months and 12 months were thawed and batch analysed using a multiplex human cytokine panel. Levels of nine different cytokines are depicted as median and IQR. # Significant difference to month 0 baseline value, Friedman's test with Dunn's multiple comparisons. IL-5 p= 0.0001 (6 months) and p= 0.02 (12 months); IL-17A p=0.026 (6 months). Source data are provided as a Source Data File.



**Supplementary Figure 4. Correlation analysis of hookworm infection intensity and changes in HOMA-IR.** Hookworm eggs per gram (EPG) in faecal samples at 6 months were plotted against change in HOMA-IR from baseline to 6 months for L3-20 and L3-40 hookworm-treated participants. Simple linear regression analysis (solid line, dotted lines represent 95% CI) revealed no significant associations. Source data are provided as a Source Data File.





**Supplementary Figure 5. Impact of experimental hookworm infection on body mass index (BMI).** Changes in participant's body mass index (kg/m<sup>2</sup>) from baseline values at each 6-monthly evaluation visit. Violin plots display each individual data point, median and IQR. Dotted vertical line indicates baseline levels (0 change). # Median value significant difference to baseline. Durbin, Skillings-Mack test and Wilcoxon signed-rank test: L3-20 at

18 months  $p=0.016$ , L3-20 at 24 months  $p=0.016$ . Source data are provided as a Source Data File.