Epidemiological transition in Indonesia : impact of helminths and urbanization on the development of Type 2 diabetes

Tahapary, D.L.; Tahapary D.L.

Chapter 3

EFFECT OF ANTHELMINTIC TREATMENT ON INSULIN RESISTANCE: A Cluster-randomized Placebo-controlled Trial in Indonesia

Dicky L. Tahapary,[1,2*] Karin de Ruiter,[2*] Ivonne Martin,[3,4] Eric A.T. Brienen,[2] Lisette van Lieshout,[2] Christa M Cobbaert,[5] Pradana Soewondo,[1] Yenny Djuardi,[6] Aprilianto E Wiria,[2,6] Jeanine J Houwing-Duistermaat,[3,7] Erliyani Sartono,[2] Johannes WA Smit,[8,9#] Maria Yazdanbakhsh,[2#$] Taniawati Supali[6#]

Clinical Infectious Disease. 2017. May 3. https://doi.org/10.1093/cid/cix416

ABSTRACT

Background. Emerging evidence suggests that helminth infections are associated with lower insulin resistance (IR). Current deworming programs might remove this helminth-associated protective effect. Therefore, we evaluated the anthelmintic treatment effect on changes in IR.

Methods. We conducted a double-blind, household-cluster-randomized, placebo-controlled clinical trial on Flores island, Indonesia, an area endemic for soil-transmitted helminths (STHs). All subjects received four rounds of albendazole or matching placebo with 3-month intervals, for 3 consecutive days. The primary outcome was the change in homeostatic model assessment of IR (HOMA-IR) in those aged ≥16 years. An intention-to-treat analysis was performed involving all subjects and ad hoc in the helminth-infected subjects.

Results. We examined 797 (in 329 households) and 872 (in 353 households) subjects, who were assigned randomly into the albendazole and placebo arm, respectively. Albendazole was associated with a significant reduction in STH prevalence, total Immunoglobulin E (IgE) and eosinophil count. Whereas albendazole had no effect on IR [estimated treatment effect, 0.006 (95% confidence interval, -0.010 – 0.021), p=0.48] at the community level, it was associated with a significant increase in IR [0.031 (0.004 – 0.059), p=0.04, p-value for interaction=0.01] among helminth-infected subjects as detected by microscopy. Pathway analysis suggested that this might in part be due to an increased body mass index or a reduced eosinophil count.

Conclusions. Anthelmintic treatment reduces STH prevalence, total IgE and eosinophil count but has no effect on IR at the community level. In helminth-infected subjects, treatment significantly increases IR, highlighting the need for metabolic health monitoring with ongoing deworming programs.

Clinical Trials Registration. ISRCTN 75636394.

Keywords. anthelmintic, insulin resistance, deworming, diabetes, helminths

INTRODUCTION

The increasing prevalence of type 2 diabetes mellitus (DM2) is a major health concern worldwide, in particular in low- and middle-income countries (LMIC).[1] The rapid socio-economic development in these countries has led to a shift in dietary habits and infrastructure which promotes overnutrition and decreased physical activity,[2] ultimately increasing the risk for DM2. DM2 is characterized by increased insulin resistance (IR). Although the pathophysiology of DM2 is complex and involves several defects,[3] there is evidence that in addition to an altered energy balance, chronic low-grade systemic inflammation plays a key role, linking the immune system and the impairment in metabolic homeostasis.[4]

Helminth infections, which are still endemic in many LMIC,[5] are associated with skewed immune responses towards type-2 and regulatory immune responses.[6] This may lead to a decreased systemic inflammation and consequently increased whole-body and tissue-specific insulin sensitivity.[7] In addition, helminths are associated with a lower body mass index (BMI),[8] which may be beneficial in terms of IR. Helminths may therefore improve insulin sensitivity via immunological and non-immunological pathways.[6, 9, 10]

Interleukin (IL)-4[11] and IL-10,[12] key cytokines in helminth infections, have been shown to regulate peripheral nutrient metabolism and insulin sensitivity.[11] Recent studies in animal models of diet-induced obesity [13-17] have also shown that helminth infections [13, 15-17] and helminth-derived molecules [14, 16-18] can increase insulin sensitivity through direct and indirect control of metabolic pathways.[18] Furthermore, several population based studies have reported a lower DM2 risk in subjects with previous [19, 20] or current [21, 22] chronic helminth infections. In a previous study on Flores island in Indonesia, we reported that chronic soil-transmitted helminth (STH) infections were associated with lower whole-body IR, independent of BMI.[8]

However, all human studies performed so far have been cross-sectional, preventing any insight on a causal relation between helminth infections and IR. Therefore, we performed a cluster-randomized controlled trial (RCT) of anthelmintic treatment in an area endemic for STHs, studying the hypothesis that a reduction of helminth infections will lead to a higher degree of IR.

METHODS

Study Overview

We conducted a household-based cluster-randomized double-blind trial in 3 villages in Nangapanda, Ende, Flores island, Indonesia. The trial was approved by the ethics committee of Faculty of Medicine, Universitas Indonesia (FKUI), filed by the ethics committee of Leiden University Medical Center (LUMC), and registered as a clinical trial (http://www.isrctn.com/ISRCTN75636394). The protocol was published previously.[23]

Participants

All subjects in the study area, except children <2 years of age and pregnant women, were included in the trial to avoid cross-contamination between household members. Subjects aged ≥16 years underwent clinical and laboratory examination, excluding subjects with active treatment for diabetes mellitus and serious concomitant diseases.

Study Design and Treatments

After obtaining written informed consent, the population was randomised by household blocks using Random Allocation Software for assignment to treatment. Both study investigators and participants were blinded for the treatment code. After randomisation, all study subjects received a tablet of albendazole (400 mg) or matching placebo (both manufactured by PT Indofarma Pharmaceutical, Bandung, Indonesia) for three consecutive days with direct supervision. This treatment regimen was given 4 times with 3-month intervals (week 9-10, 21-22, 33-34, and 45-46). Clinical measurements, as well as blood and stool sample collection, were performed during the first 8 weeks before the start of the drug administration(baseline or t=0) and 6 weeks after the last drug administration (follow-up or t=52 weeks) (**Supplementary Figure 1**). After completion of the study, the whole study population was treated with a tablet of albendazole (400 mg) for 3 consecutive days.

Study Procedures and Outcomes

All clinical measurements and blood sample collections were performed after an overnight fast. Detailed information on study procedures are available at the **Supplementary Appendix**. In brief, body weight, height, waist circumference, hip circumference were measured, from which BMI and Waist-to-hip-ratio were calculated. Fasting blood glucose was determined in capillary blood. All sera, plasma, whole blood, and stool samples were frozen at −20°C and subsequently

stored at −80°C. Insulin, haemoglobin A1c (HbA1c), total cholesterol, high-density lipoprotein cholesterol (HDL-C), triglycerides and high-sensitivity C-reactive protein (hs-CRP) were measured pairwise (baseline and follow-up) in the same analytical runs at the LUMC. A Giemsa-stained peripheral thin blood smear was read at FKUI to assess the differential white blood cell count, resulting in a relative percentage of basophils, eosinophils, neutrophils, lymphocytes and monocytes. Total IgE was measured at LUMC as described previously.[24]

Fresh stool samples were examined by microscopy using the Kato-Katz method to detect STHs (hookworm, *Ascaris lumbricoides,* and *Trichuris trichiura*). Multiplex real-time polymerase chain reaction (PCR) was performed to simultaneously detect the presence of hookworm (*Ancylostoma duodenale, Necator americanus*), *Ascaris lumbricoides, Trichuris trichiura,* and *Strongyloides stercoralis*. Stool samples were considered positive by PCR when cycle threshold (Ct) values were <50, and further grouped into 3 categories: Ct <30, 30 to <35, and ≥35 representing a high, moderate and low DNA load, respectively.[25]

Primary outcome was IR, assessed using the homeostatic model assessment of IR (HOMA-IR), a well-validated measure of whole body IR in humans (HOMA-IR= fasting serum insulin x fasting glucose / 22.5).[26] Secondary outcomes included BMI, waist circumference, fasting blood glucose, HbA1c, lipid levels, total IgE, eosinophil count, hs-CRP and prevalence of STHs as assessed by microscopy and stool PCR. Adverse events reported by subjects or observed by the investigators were monitored during the trial.

Statistical Analysis

The sample size was calculated according to intention-to-treat analysis. Based on our previous study,[27] we assumed that the average household size is 4 and that around 20% of participants would be lost to follow-up after one year. We used a significance level of 5% and a power of 80%. Correlations within households were taken into account by using the correction factor 1 + (m-1) ICC, with *m* being the household size and ICC the intra-class correlation. The sample size was calculated to aim at a difference in mean HOMA-IR between the 2 treatment groups of 0.18 and an ICC of 0.1 indicating 1580 subjects in total.

For continuous variables, normally distributed data were summarized as mean and standard deviation [mean (SD)], while non-normally distributed data (HOMA-IR, insulin, hs-CRP, total IgE and eosinophil count) were summarized as geometric

mean and 95% confidence interval (CI), and log-transformed for analyses. HOMA-IR and hsCRP were log transformed as $\log_{10}(1+[value])$. Categorical data were expressed as proportions.

The effect of anthelmintic treatment on HOMA-IR was assessed at community level using an intention-to-treat approach, using mixed models to account for the correlation within households. As an ad hoc analysis, we stratified by infection status by including helminth infection status (no infection, any infection) at baseline and its interaction with treatment into the model. We also stratified by the number of helminth species a subject was infected with, by including the number of helminth species (no infection, single infection, multiple infection) at baseline and its interaction with treatment into the model. Two random effects were used: to model clustering within households a random household specific intercept was used and to model correlation within subjects random subject-specific intercept was used. Parameter estimates for treatment effect and 95% CIs were reported. The reported p-values were obtained using a likelihood ratio test comparing the model with and without the treatment effect. We used the same model for secondary outcomes. For the binary outcome (helminth infection status), a logistic model was used with random household effects and random subject effects. All models were fitted using the lme4 package (R software).

RESULTS

Between 1 April 2014 and 3 June 2014, we initially included 752 households with 3566 subjects in the trial. Randomization resulted in 1825 subjects assigned to placebo and 1741 subjects to albendazole (377 and 375 households, respectively). The overall trial profile is shown in **Figure 1**, with a total of 1669 subjects aged 16 and above who were examined at baseline [872 subjects (353 households) and 797 subjects (329 households) in the placebo and the albendazole group respectively]. Baseline (t=0) characteristics were similar between both treatment arms (**Table 1**).

The overall loss to follow-up, from baseline to 52 weeks, was 18.9%. The main reason for loss to follow-up was permanent or temporary movement out of the village for employment or study. Those who moved out and refused to come for follow-up, were younger in comparison to the whole population. There were no significant differences between both treatment arms in terms of loss to follow-up (**Supplementary Table S1**). With respect to compliance, 87.9% (1189/1353) of the subjects took the maximum of 12 tablets [87.0% (574/660) vs 88.7% (615/693), in

the albendazole and the placebo group, respectively]. We collected stool samples from 92.0% (1535/1669) of the subjects at baseline and 89.9% (1217/1353) of the subjects at follow-up. Data to calculate HOMA-IR were available for 1604 subjects at baseline, and for 1272 subjects at follow-up. Sixteen subjects who were receiving active treatment for DM2 were excluded from analysis.

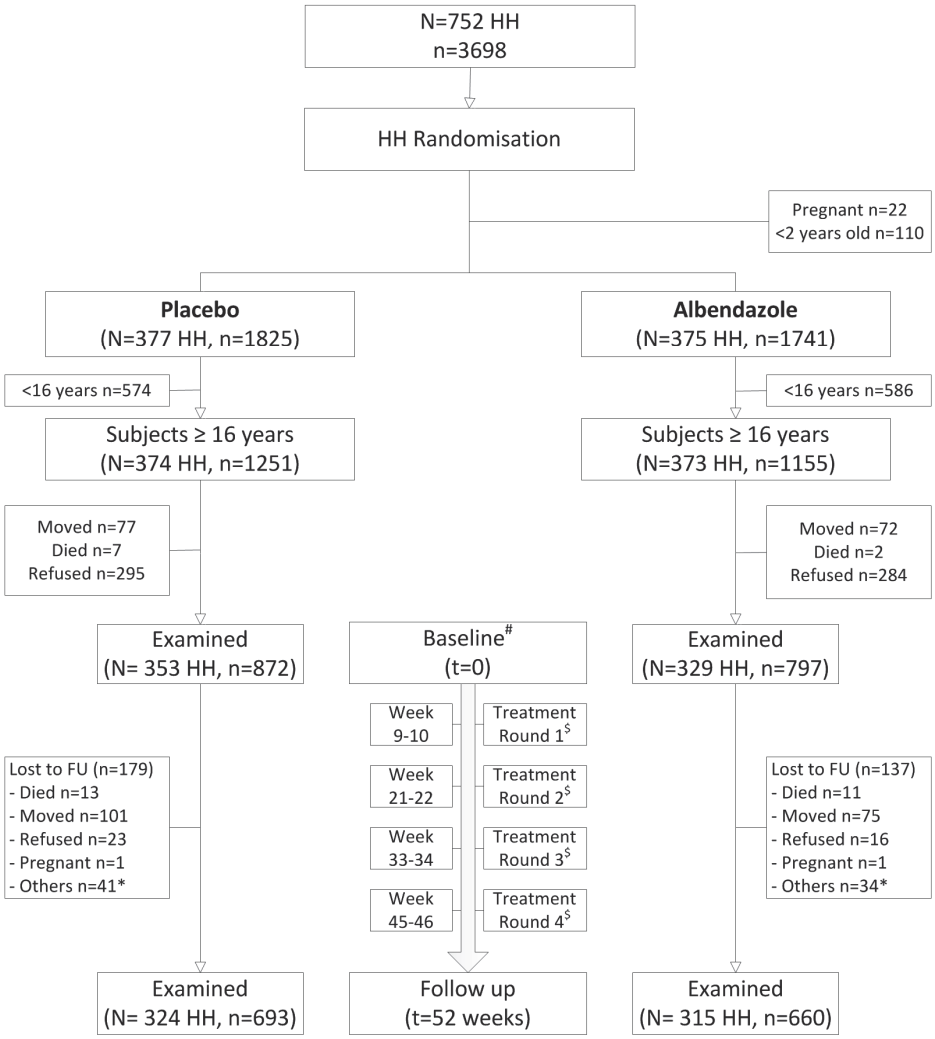

Figure 1. Trial profile. #Baseline data (t=0) were collected during the first 8 weeks before the start of the drug administration. $Single dose of albendazole or matching placebo was given for 3 consecutive days to all household members, except children <2 years of age and pregnant women. *Other reasons of lost to follow-up were harvesting crops, working on funeral ceremonies, severely ill, hospitalized, and nursing mother. Abbreviations: HH, households; FU, follow-up.

Table 1. Baseline Characteristics of Study Population

Characteristic	No.	Placebo	No.	Albendazole
Age, y, mean (SD)	872	42.5 (15.7)	797	42.5 (15.7)
Sex, female, No. (%)	872	534 (61.2)	797	478 (60.0)
Body mass index, kg/m2, mean (SD)	860	22.4 (4.2)	790	22.4 (4.0)
Waist circumference, cm, mean (SD)				
Female	532	76.5 (12.6)	476	77.0 (12.6)
Male	333	76.5 (11.6)	317	76.6 (11.3)
Waist-to-hip-ratio, mean (SD)				
Female	507	0.88 (0.07)	473	0.89 (0.08)
Male	317	0.94 (0.07)	315	0.94 (0.07)
Systolic BP, mmHg, mean (SD)	871	129.3 (23.4)	765	129.5 (23.8)
Diastolic BP, mmHg, mean (SD)	871	76.4 (12.1)	765	76.5 (12.1)
Total cholesterol, mmol/L, mean, (SD)	836	4.9 (1.1)	764	4. 9 (1.1)
HDL cholesterol, mmol/L, mean, (SD)				
Female	517	1.3 (0.4)	457	1.3 (0.4)
Male	319	1.1 (0.3)	307	1.1 (0.3)
LDL cholesterol, mmol/L, mean, (SD)	836	3.0 (0.9)	763	3.0 (0.9)
Triglycerides, mmol/L, mean, (SD)	836	1.4 (0.7)	764	1.5 (0.7)
HbA1c[a], mmol/mol, mean (SD)	715	32.5 (9.0)	683	32.3 (8.5)
Fasting blood glucose, mmol, mean, (SD)	836	5.5 (1.6)	768	5. 5 (1.6)
Fasting Insulin, mU/L, GM (95%CI)	836	3.5 (3.2 – 3.7)	768	3.5 (3.3 – 3.8)
HOMA-IR, GM (95%CI)	836	1.09 (1.02 – 1.15)	768	1.08 (1.01 – 1.14)
hs-CRP, mg/L, GM (95%CI)	836	1.26 (1.16 – 1.36)	764	1.26 (1.16 – 1.37)
Total IgE, IU/mL, GM (95%CI)	835	557.2 (498.1 – 623.3)	766	601.6 (534.8 – 676.7)
Eosinophil count, %, GM (95%CI)	829	5.9 (5.6 – 6.1)	763	6.1 (5.8 – 6.4)
Helminth infection by microscopy, No. (%)	655	283 (43.2)	602	251 (41.7)
Single		185 (28.2)		160 (26.6)
Multiple		98 (15.0)		91 (15.1)
Helminth infection by PCR, No. (%)	783	425 (54.3)	710	393 (55.4)
Single		256 (32.7)		252 (35.5)
Multiple		169 (21.6)		141 (19.9)

Abbreviations: BP, blood pressure; CI, confidence interval; GM, geometric mean; HDL, high-density lipoprotein; HbA1c, hemoglobin A1c; HOMA-IR, homeostasis model assessment for insulin resistance; hs-CRP, high-sensitivity C-reactive protein; IgE, immunoglobulin E; LDL, low-density lipoprotein; PCR, polymerase chain reaction; SD, standard deviation. [a]After excluding subjects with unidentified hemoglobinopathy on the Tosoh G8 high-performance liquid chromatography analyzer (13.9% [116/831] in the placebo group and 10.5% [80/763] in the albendazole group).

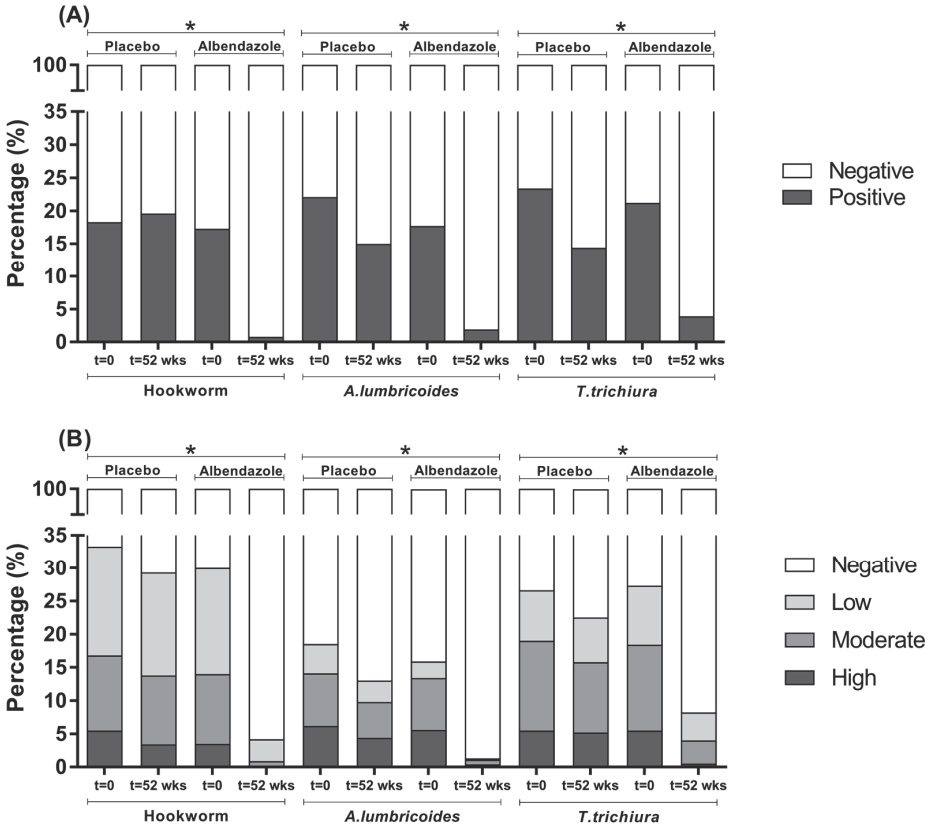

Figure 2. The effect of albendazole treatment on the prevalence and intensity of soil-transmitted helminths. Percentage of hookworm-, *Ascaris lumbricoides*-, and *Trichuris trichiura*-infected subjects at baseline (t=0) and following treatment (t=52 weeks), in placebo and albendazole treatment arms, as detected by microscopy (n=1011; A) and polymerase chain reaction (n=1144; B). Albendazole treatment was associated with a significant reduction of hookworm, *A. lumbricoides* and *T. trichiura*. p-values were calculated using a logistic model with random household effects and random subject effects. *corresponds to p-value <0.0001.

Effect of Treatment at the Community Level

Albendazole treatment reduced the percentage of subjects with any helminth infection as assessed by either microscopy [41.7% (251/602) to 5.6% (27/486) in the albendazole arm vs 43.2% (283/655) to 34.4% (181/526) in the placebo arm, p<0.0001] or PCR [55.4% (393/710) to 11.3% (62/550) in the albendazole arm vs 54.3% (425/783) to 46.8% (278/594) in the placebo arm, p<0.0001]. The highest reduction was seen for hookworm, followed by *A. lumbricoides* and *T. trichiura* infection **(Figure 2).** When assessing the infection intensity in categories based on PCR, albendazole treatment resulted in a reduction in intensity across these

three helminths species with the least effect on *T. trichiura* infection **(Figure 2B)**. S. stercoralis prevalence, which was already low, was eliminated in the albendazole group (**Supplementary Table S2**).

At the community level, neither HOMA-IR nor BMI, waist circumference, fasting blood glucose, HbA1c, total cholesterol, HDL-cholesterol, LDL-cholesterol, triglycerides and hsCRP were found to be affected by albendazole treatment **(Table 2)**. However, the significant reduction of infection prevalence and intensity by albendazole treatment, was accompanied by a significant decrease in total IgE level [Estimated treatment effect (95% CI), p value, -0.066 (-0.094 – -0.037), p<0.0001] and eosinophil count [-0.057 (-0.086 – -0.028), p=0.0001] **(Table 2)**.

Effect of Treatment in STH-infected Subjects

Next, the effect of treatment was assessed only in those who were infected with helminths at baseline, as detected by microscopy. Albendazole treatment resulted in a significant increase in HOMA-IR [0.031 (0.004 – 0.059), p=0.04] **(Figure 3.A)**. This effect was greater in comparison to subjects without helminth infections at baseline (p=0.01 for the interaction between helminth infection status at baseline and treatment). Moreover, with an increasing number of helminth species infecting a subject at baseline, there was a gradual increase in HOMA-IR after treatment. Thus, whereas we saw no significant effect of treatment among those with no infection [-0.013 (-0.035 – 0.010), p=0.28] or those infected with single species [0.017 (-0.017 – 0.050), p=0.34], treatment in those infected with multiple helminth species resulted in a significantly higher HOMA-IR [0.061 (0.015 – 0.106), p=0.02, p value for interaction=0.005] **(Figure 3.A)**. These effects were also reflected for BMI **(Figure 3.B)**, eosinophil count **(Figure 3.C)**, and total IgE level (**Figure3.D**), but not for HbA1c, total cholesterol, HDL-cholesterol, LDL-cholesterol, triglycerides and hsCRP levels. Pathway analysis showed that adjustment for BMI, eosinophil count but not total IgE level, attenuated the treatment effect on HOMA-IR among helminth-infected subjects (**Supplementary Table S3**).

When using PCR to detect STH infection, a significant increase of HOMA-IR was only observed among subjects who were infected with multiple helminth species at baseline **(Supplementary Figure S2.A)**. A significant increase of BMI was also observed in these subjects (**Supplementary Figure S2.B**).The group of subjects infected with multiple helminth species had a significantly higher infection intensity in comparison to the group of subjects infected with single helminth species (**Supplementary Table S4**).

Table 2. Effect of Albendazole Treatment on Primary and Secondary Outcomes at the Community Level

		Placebo		Albendazole		Treatment Effect (95%CI)
Outcome	**Parameter**	**Baseline**	**Follow-up**	**Baseline**	**Follow-up**	**p-value**
Insulin resistance	HOMA-IR	1.09 (1.02 – 1.15)	1.16 (1.09 – 1.24)	1.08 (1.01 – 1.14)	1.17 (1.13 – 1.25)	0.006 (-0.010 – 0.021)
		n=836	n=659	n=768	n=635	p=0.48
Glucose-related	Fasting blood glucose	5.49 (1.61)	5.47 (1.09)	5.45 (1.58)	5.52 (1.49)	0.018 (-0.105 – 0.142)
	(mmol)	n=836	n=650	n=768	n=634	p=0.77
	Fasting insulin	3.5 (3.2 – 3.7)	3.8 (3.5 – 4.1)	3.5 (3.3 – 3.8)	3.9 (3.6 -4.2)	0.006 (-0.032 – 0.043)
	(mU/L)	n=836	n=646	n=768	n=628	p=0.77
	HbA1c	32.5 (9.0)	32.7 (7.4)	32.3 (8.5)	32.7 (8.4)	0.051 (-0.350 – 0.452)
	(mmol/mol)	n=715	n=564	n=683	n=556	p=0.80
Adiposity-related	Body mass index	22.4 (4.2)	22.8 (4.2)	22.4 (4.0)	22.9 (4.1)	0.104 (-0.011 – 0.220)
	(kg/m^2)	n=860	n=690	n=790	n=659	p=0.08
	Waist circumference	76.5 (12.2)	77.2 (11.5)	76.8 (12.1)	77.4 (11.1)	-0.229 (-0.855 – 0.397)
	(cm)	n=865	n=692	n=793	n=657	p=0.47
Lipid-related	Total cholesterol	4.9 (1.0)	5.0 (1.1)	4.9 (1.1)	5.0 (1.1)	-0.031 (-0.098 – 0.035)
	(mmol/L)	n=836	n=659	n=764	n=632	p=0.35
	HDL –C	1.2 (0.3)	1.3 (0.3)	1.2 (0.3)	1.3 (0.4)	-0.008 (-0.031 – 0.016)
	(mmol/L)	n=836	n=659	n=764	n=632	p=0.52
	LDL-C	3.0 (0.9)	3.1 (0.9)	3.0 (0.9)	3.1 (1.0)	-0.032 (-0.089 – 0.024)
	(mmol/L)	n=836	n=658	n=763	n=631	p=0.26
	Triglycerides	1.4 (0.7)	1.5 (0.7)	1.5 (0.7)	1.5 (0.7)	-0.003 (-0.023 – 0.090)
	(mmol/L)	n=836	n=659	n=764	n=632	p=0.25
Immune-related	Total IgE	557.2 (498.1 – 623.3)	441.8 (386.7 – 504.7)	601.6 (534.8 – 676.7)	399.0 (347.9 – 457.7)	-0.066 (-0.094 – -0.037)
	(IU/mL)	n=835	n=651	n=766	n=628	p<0.0001
	Eosinophil count	5.9 (5.6 – 6.1)	5.9 (5.6 – 6.1)	6.1 (5.8 – 6.4)	5.2 (5.0 -5.5)	-0.057 (-0.086 – -0.028)
	(%)	n=829	n=641	n=763	n=619	p=0.0001
	hs-CRP	1.26 (1.16 – 1.36)	1.30 (1.19 – 1.42)	1.26 (1.16 – 1.37)	1.34 (1.23 – 1.46)	0.010 (-0.017 – -0.038)
	(mg/L)	n=836	n=659	n=764	n=632	p=0.46

The estimated treatment effect after 12-months of follow-up for HOMA-IR and other glucose-related parameters, adiposity, lipid, and immunological parameters at community level is displayed with corresponding 95% CI. The estimated treatment effects were obtained by mixed models and p-values are indicated. HOMA-IR, fasting insulin, total IgE, eosinophil count and hs-CRP were log transformed.

Abbreviations: CI, confidence interval; HbA1c, hemoglobin A1c; HDL-C, high-density lipoprotein cholesterol; HOMA-IR, homeostatic model assessment for insulin resistance; hs-CRP, high sensitivity C-Reactive Protein. IgE, Immunoglobulin E; LDL-C, low-density lipoprotein cholesterol

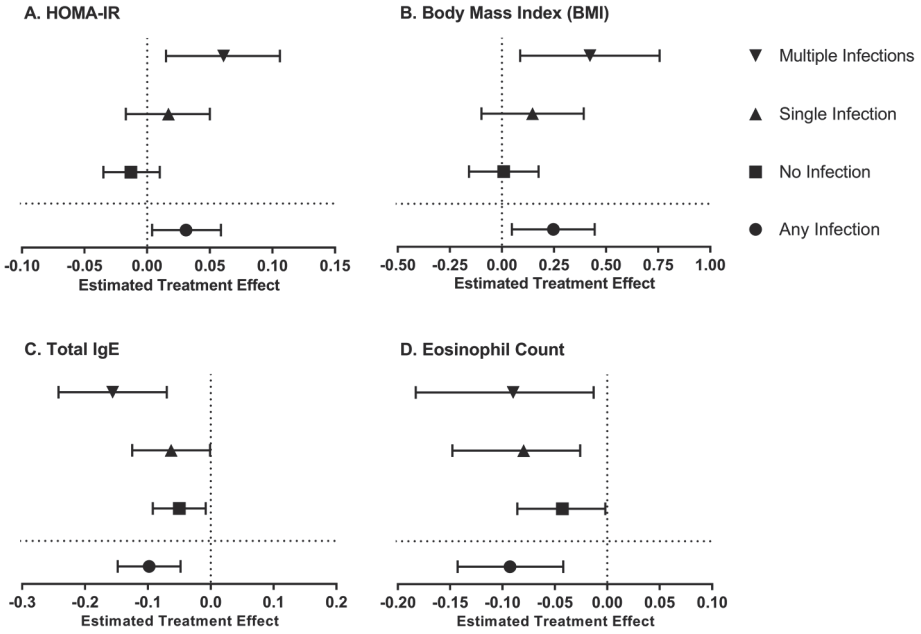

Figure 3. Effect of albendazole treatment on homeostatic model assessment for insulin resistance (HOMA-IR), body mass index (BMI), total immunoglobulin E (IgE), and eosinophil count, stratified by the number of helminth species carried by infected subjects at baseline as detected by microscopy. Effect of albendazole treatment on HOMA-IR (n = 1211; **A**), BMI (n = 1233; **B**), total IgE (n = 1209; **C**), and eosinophil count (n = 1200; **D**), stratified by the number of helminth species infecting subjects at baseline, as detected by microscopy. The estimated treatment effects are displayed with the corresponding 95% confidence interval. Circle, infected with at least 1 helminth species; square, no infection; triangle, infected with 1 helminth species; inverse triangle, infected with >1 helminth species.

Adverse Events

Adverse events were reported in 3.9% (31/797) and 2.6% (23/872) of subjects in the albendazole and the placebo group respectively. Abdominal pain was the most commonly reported complaint [35% (11/31) vs 13% (3/23) in the albendazole and the placebo group respectively]. Other commonly reported complaints were diarrhea and nausea, which were similar in both treatment arms.

DISCUSSION

Here, we report the first cluster-randomized trial in humans investigating the causal relationship between helminth infections and whole-body IR in an area endemic for STHs. We found that after 12 months of follow-up, 4 rounds of anthelmintic treatment with 3-month intervals did not lead to an increase in IR or other parameters such as

BMI, waist circumference, fasting blood glucose, HbA1c, serum lipid levels and hs-CRP at the community level, when all participants irrespective of their helminth status were included in the analysis. This despite the fact that the prevalence and infection intensity of STHs, as well as its associated type 2 immune responses, measured by total IgE and eosinophil count, were significantly reduced in albendazole-treated subjects.

When considering helminth-infected subjects, we observed that albendazole treatment resulted in a significant increase of IR among helminth-infected subjects when infection was detected by microscopy. Moreover, the effect of treatment on IR was stronger in those infected with multiple STH species at baseline compared to those with a single STH infection. We observed a similar pattern of the treatment effect on BMI. Even though significant, it is important to note that the magnitude of the effect of 1 year deworming on IR was modest. The effect of deworming in increasing IR seemed to be partly mediated through an increase in BMI, as adjustment for BMI, a strong predictor of IR,[28] attenuated the treatment effect on IR. Similar to BMI, eosinophil count and total IgE were significantly decreased in helminth infected subjects and this was stronger in those with multiple helminth infections. The possible importance of eosinophils in IR, shown in animal models [13, 14, 16, 17] and in 1 epidemiological study,[29] is also seen in our study as correction for eosinophil count reduced the treatment effect on IR.

When infection was assessed by PCR, which in comparison to the Kato Katz method, has a better ability to detect low intensity infections that may be clinically less relevant,[25] we only observed a significant increase in IR in the group of subjects who were infected with multiple helminth species at baseline. The infection intensity (DNA load) in this group of subjects was significantly higher than in those infected with a single STH species. Albendazole treatment led to a strong reduction of infection intensity in those infected with multiple STH species, which might explain the significant increase in IR following albendazole treatment.

The observed modest increase of IR after treatment among helminth-infected subjects, as detected by microscopy, could also contribute to the lack of a significant effect of albendazole treatment on IR at the community level. Two recent meta-analysis on deworming in children support this notion as they show that whereas a mass deworming approach, thus irrespective of helminth infection status, resulted in no change in weight gain, targeted anthelmintic treatment of infected children resulted in a significant weight gain.[30, 31]

However, several other explanations for the absence of a treatment effect on IR at the community level need to be considered. Although our study design was successful in lowering STH infection prevalence and its associated Th2 responses, it is possible that longer treatment and follow up would show stronger effects. It is also possible that both immune and non-immune-related effects of helminths on IR are not only associated with current helminth infections [8] but also with exposure to helminth infections in the past and therefore sustained. [13, 19] The causal relationship between helminth infection and IR, as found in the subgroup of infected subjects, might have a relatively small contribution to the multifactorial pathogenesis of IR.[28] Therefore, longer follow-up studies involving assessment of other more established factors, such as diet and physical activity,[28] will be needed to investigate this.

The use of PCR in our study, in addition to microscopy for detection of helminths, has helped us realize that the burden of infections, in terms of the number of helminth species as well as the infection intensity (DNA load), might influence the effect of anthelmintic treatment on IR. Deworming in subjects with increasing burden of infections resulted in an increasing change in IR. In addition, treatment of uninfected subjects, as assessed by either microscopy or PCR, did not influence IR, which suggests that an undetectable or a low level of helminth infection might be irrelevant for IR. The question whether a high burden of helminth infection causes different modulating effects on the immune system or energy balance, remains to be answered.

In conclusion, intensive anthelmintic treatment in an STH endemic area significantly reduces both the STH infection prevalence and intensity, as well as its related type 2 responses. This treatment does not lead to an increase of whole-body IR at the community level, but it does increase IR among those with a microscopy-detected STH infection. Studies are needed to determine the long term metabolic consequences of anthelmintic treatment in communities where STH are highly prevalent. However, in terms of policy, countries implementing helminth control programmes need to be aware that this may exacerbate or accelerate the deterioration in metabolic health, and that education and prevention strategies for non-communicable diseases such as DM2 need to go hand in hand with infectious disease control measures.

Supplementary Data

Supplementary materials are available at Clinical Infectious Diseases online. Consisting of data provided by the authors to benefit the reader, the posted materials are not copyedited and are the sole responsibility of the authors, so questions or comments should be addressed to the corresponding author.

Notes

Author contributions. DLT is a medical doctor in charge of the field study, involved in setting up the study, supervising gathering of data, treatment, clinical care, follow up of the study population, analyze the data and wrote the manuscript. KR is a medical biologist in charge of the field study, involved in setting up the laboratory in the study area, performing the immunological analysis, supervising the data cleaning, follow up of the study population and involved in the writing of the manuscript. IM is a mathematician who is developing methods to analyze the complex data generated during the lifetime of the project and involved in the randomization and data analysis. LvL is a parasitologist who is involved in the performance and analysis of diagnostic assays for the detection of helminths in stool samples. EATB is a technician who develop, optimized and performed multiplex real time PCR for detection of helminth infection. CMC is a clinical chemist who advised on the type, quality and metrological traceability of medical tests and who was responsible for the measurements of the metabolic parameters at the Department of Clinical Chemistry and Laboratory Medicine at LUMC. PS is an endocrinologist who advised on the metabolic aspects of the study. YD is a medical doctor who is involved in coordinating the study and advises on the immunological and parasitological aspects of the study. AEW is a medical doctor who is involved in clinical care and setting up the database. JJH is a biostatistician who developed the study, and is involved in supervising sample size calculation, randomization and statistical analysis. ES is an immunoparasitologist who is involved in coordinating the study and advising on parasitological and immunological aspects of the study and supervised the writing of the manuscript. JWAS is an endocrinologist who developed the study, supervised the writing of the manuscript, and is the Dutch coordinator of the SUGARSPIN program. MY is an immunologist who developed the study, supervised the writing of the manuscript and is the scientific coordinator of the SUGARSPIN program. TS is a parasitologist who developed the study and is the Indonesian coordinator of the SUGARSPIN program. All authors read and approved the final manuscript. The senior authors TS, MY and JWAS had final responsibility for the decision to submit for publication.

Acknowledgements. The authors would like first to thank all study participants in Nangapanda, Ende, Flores, Indonesia. The authors would like to thank all local government and health officers in Nangapanda who supported this project, and also all field workers from Universitas Indonesia and Nangapanda. The authors would like to thank to Alison Elliott and Bruno Guigas for reading the manuscript critically. The authors would also like to thank Yvonne Kruize, Angga M. Fuady, and all colleagues at Department of Parasitology LUMC for their technical supports. The authors would also like to thank The Indonesian Directorate General of Higher Education (DIKTI) for providing scholarship to two PhD candidates involved in this project.

Disclaimer. The funder of the study had no role in study design, data collection, data analysis, data interpretation, or writing of the report.

Financial support. This work was supported by The Royal Netherlands Academy of Arts and Science (KNAW), Ref 57-SPIN3-JRP and Universitas Indonesia (Research Grant BOPTN 2742/H2.R12/HKP.05.00/2013.).

Potential conflicts of interests. All authors: No reported conflicts of interest. All authors have submitted the ICMJE Form for Disclosure of Potential Conflicts of Interest. Conflicts that the editors consider relevant to the content of the manuscript have been disclosed.

Author details

[1]Department of Internal Medicine, Division of Endocrinology, Dr. Cipto Mangunkusumo National General Hospital, Faculty of Medicine Universitas Indonesia, Jakarta, Indonesia. [2]Department of Parasitology, Leiden University Medical Center, Leiden, The Netherlands. [3]Department of Medical Statistics and Bioinformatics, Leiden University Medical Center, Leiden, The Netherlands. [4]Department of Mathematics, Parahyangan Catholic University, Bandung, Indonesia. [5]Department of Clinical Chemistry and Laboratory Medicine, Leiden University Medical Center, Leiden, The Netherlands. [6]Department of Parasitology, Faculty of Medicine Universitas Indonesia, Jakarta, Indonesia. [7]Department of Statistics, University of Leeds, Leeds, United Kingdom. [8]Department of Internal Medicine, Radboud University Medical Centre, Nijmegen, The Netherlands. [9]Department of Internal Medicine, Leiden University Medical Center, Leiden, The Netherlands.

*# These authors have contributed equally. $ Corresponding author

REFERENCES

1. Collaboration NCDRF. Worldwide trends in diabetes since 1980: a pooled analysis of 751 population-based studies with 4.4 million participants. Lancet **2016**; 387(10027): 1513-30.
2. Hu FB. Globalization of diabetes: the role of diet, lifestyle, and genes. Diabetes Care **2011**; 34(6): 1249-57.
3. Defronzo RA. Banting Lecture. From the triumvirate to the ominous octet: a new paradigm for the treatment of type 2 diabetes mellitus. Diabetes **2009**; 58(4): 773-95.
4. Hotamisligil GS. Inflammation and metabolic disorders. Nature **2006**; 444(7121): 860-7.
5. Hotez PJ, Brindley PJ, Bethony JM, King CH, Pearce EJ, Jacobson J. Helminth infections: the great neglected tropical diseases. J Clin Invest **2008**; 118(4): 1311-21.
6. Wammes LJ, Mpairwe H, Elliott AM, Yazdanbakhsh M. Helminth therapy or elimination: epidemiological, immunological, and clinical considerations. The Lancet Infectious diseases **2014**; 14(11): 1150-62.
7. de Ruiter K, Tahapary DL, Sartono E, et al. Helminths, hygiene hypothesis and type 2 diabetes. Parasite Immunol **2016**.
8. Wiria AE, Hamid F, Wammes LJ, et al. Infection with Soil-Transmitted Helminths Is Associated with Increased Insulin Sensitivity. PLoS One **2015**; 10(6): e0127746.
9. Wiria AE, Sartono E, Supali T, Yazdanbakhsh M. Helminth infections, type-2 immune response, and metabolic syndrome. PLoS Pathog **2014**; 10(7): e1004140.
10. Berbudi A, Ajendra J, Wardani AP, Hoerauf A, Hubner MP. Parasitic helminths and their beneficial impact on type 1 and type 2 diabetes. Diabetes Metab Res Rev **2015**.
11. Ricardo-Gonzalez RR, Red Eagle A, Odegaard JI, et al. IL-4/STAT6 immune axis regulates peripheral nutrient metabolism and insulin sensitivity. Proc Natl Acad Sci U S A **2010**; 107(52): 22617-22.
12. Hong EG, Ko HJ, Cho YR, et al. Interleukin-10 prevents diet-induced insulin resistance by attenuating macrophage and cytokine response in skeletal muscle. Diabetes **2009**; 58(11): 2525-35.
13. Wu D, Molofsky AB, Liang HE, et al. Eosinophils sustain adipose alternatively activated macrophages associated with glucose homeostasis. Science **2011**; 332(6026): 243-7.
14. Hams E, Bermingham R, Wurlod FA, et al. The helminth T2 RNase omega1 promotes metabolic homeostasis in an IL-33- and group 2 innate lymphoid cell-dependent mechanism. FASEB J **2016**; 30(2): 824-35.

15. Yang Z, Grinchuk V, Smith A, et al. Parasitic nematode-induced modulation of body weight and associated metabolic dysfunction in mouse models of obesity. Infect Immun **2013**; 81(6): 1905-14.
16. Hussaarts L, Garcia-Tardon N, van Beek L, et al. Chronic helminth infection and helminth-derived egg antigens promote adipose tissue M2 macrophages and improve insulin sensitivity in obese mice. FASEB J **2015**; 29(7): 3027-39.
17. Berbudi A, Surendar J, Ajendra J, et al. Filarial Infection or Antigen Administration Improves Glucose Tolerance in Diet-Induced Obese Mice. J Innate Immun **2016**; 8(6).
18. Bhargava P, Li C, Stanya KJ, et al. Immunomodulatory glycan LNFPIII alleviates hepatosteatosis and insulin resistance through direct and indirect control of metabolic pathways. Nat Med **2012**; 18(11): 1665-72.
19. Chen Y, Lu J, Huang Y, et al. Association of previous schistosome infection with diabetes and metabolic syndrome: a cross-sectional study in rural China. J Clin Endocrinol Metab **2013**; 98(2): E283-7.
20. Shen SW, Lu Y, Li F, et al. The potential long-term effect of previous schistosome infection reduces the risk of metabolic syndrome among Chinese men. Parasite Immunol **2015**; 37(7): 333-9.
21. Aravindhan V, Mohan V, Surendar J, et al. Decreased prevalence of lymphatic filariasis among diabetic subjects associated with a diminished pro-inflammatory cytokine response (CURES 83). PLoS Negl Trop Dis **2010**; 4(6): e707.
22. Hays R, Esterman A, Giacomin P, Loukas A, McDermott R. Does Strongyloides stercoralis infection protect against type 2 diabetes in humans? Evidence from Australian Aboriginal adults. Diabetes Res Clin Pract **2015**; 107(3): 355-61.
23. Tahapary DL, de Ruiter K, Martin I, et al. Helminth infections and type 2 diabetes: a cluster-randomized placebo controlled SUGARSPIN trial in Nangapanda, Flores, Indonesia. BMC Infect Dis **2015**; 15: 133.
24. Wiria AE, Prasetyani MA, Hamid F, et al. Does treatment of intestinal helminth infections influence malaria? Background and methodology of a longitudinal study of clinical, parasitological and immunological parameters in Nangapanda, Flores, Indonesia (ImmunoSPIN Study). BMC Infect Dis **2010**; 10: 77.
25. Arndt MB, John-Stewart G, Richardson BA, et al. Impact of helminth diagnostic test performance on estimation of risk factors and outcomes in HIV-positive adults. PLoS One **2013**; 8(12): e81915.
26. Wallace TM, Levy JC, Matthews DR. Use and abuse of HOMA modeling. Diabetes Care **2004**; 27(6): 1487-95.
27. Wiria AE, Hamid F, Wammes LJ, et al. The effect of three-monthly albendazole treatment on malarial parasitemia and allergy: a household-based cluster-randomized, double-blind, placebo-controlled trial. PLoS One **2013**; 8(3): e57899.
28. Riserus U, Arnlov J, Berglund L. Long-term predictors of insulin resistance: role of lifestyle and metabolic factors in middle-aged men. Diabetes Care **2007**; 30(11): 2928-33.
29. Zhu L, Su T, Xu M, et al. Eosinophil inversely associates with type 2 diabetes and insulin resistance in Chinese adults. PLoS One **2013**; 8(7): e67613.
30. Welch VA, Ghogomu E, Hossain A, et al. Mass deworming to improve developmental health and wellbeing of children in low-income and middle-income countries: a systematic review and network meta-analysis. Lancet Glob Health **2017**; 5(1): e40-e50.
31. Taylor-Robinson DC, Maayan N, Soares-Weiser K, Donegan S, Garner P. Deworming drugs for soil-transmitted intestinal worms in children: effects on nutritional indicators, haemoglobin, and school performance. The Cochrane database of systematic reviews **2015**; 7: CD000371.

SUPPLEMENTARY APPENDIX

Supplementary Methods

Study Procedures

All clinical measurements and blood sample collections were performed after an overnight fast. Anthropometric measurements of body weight (SECA Model 876, Seca Gmbh Co, Hamburg, Germany), height (SECA Model 213, Seca Gmbh Co, Hamburg, Germany), waist and hip circumference (SECA Model 201, Seca Gmbh Co, Hamburg, Germany) were obtained using the National Heart, Lung, and Blood Institute (NHLBI) practical guidelines by a team of trained researchers. BMI was calculated as weight in kg divided by square of height in meter, while Waist Hip Ratio (WHR) was calculated as waist circumference divided by hip circumference. Three blood pressure measurements (left arm, sitting upright position, after resting 5 minutes) were taken from each subject, using a digital sphygmomanometer (HEM-7200, Omron Healthcare Co, Ltd, Kyoto, Japan). The average of all three measurements was used for analysis.

Fasting blood glucose was measured in capillary blood using Breeze®2 glucose meters (Bayer Health Care LLC, Basel, Switzerland). All sera, plasma and whole blood samples were frozen at −20°C in the field study centre and subsequently stored at −80°C at the Department of Parasitology of FKUI and LUMC. Insulin, HbA1c, total cholesterol, high-density lipoprotein (HDL)-cholesterol, triglycerides and high-sensitive C-reactive protein (hsCRP) were measured pairwise (baseline and follow-up) in the same analytical runs at the Department of Clinical Chemistry and Laboratory Medicine at LUMC, which is ISO 15189:2012 accredited. Accuracy of test results is periodically and independently verified in EQA-schemes organized by the Dutch EQA-organization, the SKML.

Serum insulin concentrations were determined by a solid-phase, enzyme-labeled chemiluminescent immunometric assay (Siemens IMMULITE 2000XPi). The measuring range of the insulin assay was 2–300 mU/L (CV_a <7% at all levels). IR was assessed by HOMA-IR, a well-validated measure of whole body IR in humans (HOMA-IR = fasting serum insulin x fasting glucose / 22·5).[26] HbA1c was measured using a cation-exchange chromatography (IC)-based high performance liquid chromatogtaphy (HPLC) assay (Tosoh G8 HPLC Analyzer, from Tosoh Corporation, Tokyo, Japan and distributed through Sysmex in the Netherlands), with a measuring range of 20–125 mmol/mol Hb (CV_a<5% at all levels). Test results are in accordance to the IFCC Reference Measurement System for HbA1c. The

non-porous ion exchange HPLC-column and four-steps buffer gradient enables a clear separation of HbA1c from other fractions and haemoglobin variants such as HbC, HbD and HbS. As these variants can be detected, the software automatically corrects the HbA1c results for these variants. However, other variants which may typically be present in non-Caucasian populations, are not detected and lead to inaccurate HbA1c results. Therefore, all chromatograms were visually inspected by experienced technicians in order to detect the unidentified Hb-variants. HbA1c results from unidentified Hb-variants were excluded as these test results were inaccurate.

Total cholesterol, HDL-cholesterol and triglycerides assays were based on enzymatic colorimetric methods (Modular P analyzers, Roche Diagnostics, Mannheim, Germany). The measuring range of total cholesterol, HDL-cholesterol and triglycerides were 0·08–20·7 mmol/L (CV_a<2%), 0·08–3·10 mmol/L (CV_a<2%) and 0·05–11·4 mmol/L (CV_a<5%), respectively. Lipid test results are standardized to internationally recognized CDC Reference Measurement Systems. Low-density lipoprotein (LDL)-cholesterol (in mmol/L) was calculated using The Friedewald formula, [LDL-chol, mmol/L] = [Total chol] - [HDL-chol] - (0·456 X [TG]). A latex-enhanced immunoturbidimetric method was used to measure hsCRP on Roche Modular P-instrumentation, the measuring range being 0·1–20·0 mg/L. HsCRP test results are ERM-DA470k/IFCC standardized.

A Giemsa-stained peripheral thin blood smear was read at the Department of Clinical Pathology, FKUI to assess differential white blood cell count, resulting in a relative percentage of basophils, eosinophils, neutrophils, lymphocytes and monocytes. Total IgE was measured at the Department of Parasitology, LUMC using ELISA with rabbit anti-human IgE antibody (Ab) (Dako, Glostrup, Denmark) as capture Ab and goat anti-human IgE biotinylated Ab (Vector Laboratories, Burlingname, CA, USA) as detection Ab.[24]

Fresh stool samples were examined by the Kato Katz method to identify and quantify STH (hookworm, *Ascaris lumbricoides* and *Trichuris trichiura*) eggs using 2 slides for each sample. Aliquots of fresh stool samples were frozen at –20°C in the field study centre and subsequently at –80°C at the Department of Parasitology of FKUI and LUMC for DNA extraction. Stool DNA isolation and real-time PCR were performed pairwise (baseline and follow-up). DNA isolation from stool was performed as described elsewhere,[24] with an additional step of bead beating (1800 rpm for 3 minutes) inside the Fastprep-96™ system to optimize DNA

extraction. Multiplex real-time PCR was performed to simultaneously detect the presence of hookworm (*Ancylostoma duodenale, Necator americanus*), *Ascaris lumbricoides, Trichuris trichiura* and *Strongyloides stercoralis* using a method described previously[24] with some modifications, adding the *T. trichiura* primers and probe, and combining the fluorescence of *A. duodenale* and *N. americanus* as one reading of hookworm. Stool samples were considered positive by PCR when cycle threshold (Ct) values were below 50. Positive Ct values were further grouped into 3 categories: $Ct<30$, $30\leq Ct<35$ and $Ct\geq 35$ representing a high, moderate and low DNA load, respectively.[25] An additional grouping was made based on the number of different STH species that subjects were infected with: no infection, single infection (one of the four STHs: hookworm, *A.lumbricoides*, *T.trichiura, S.stercoralis*) and multiple infections (more than one of these STHs).

Supplementary Figures

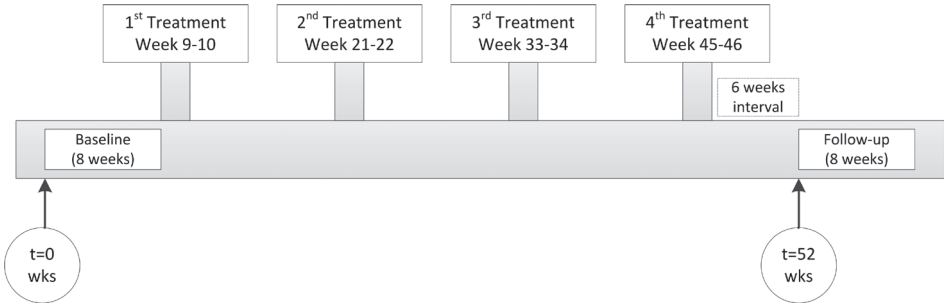

Figure S1. Trial time frame. Baseline data (t=0) were collected during the first 8 weeks before the start of the drug administration. Treatment regimen was given 4 times with three months time intervals (week 9-10, 21-22, 33-34, and 45-46). Follow-up data (t=52 weeks) were collected 6 weeks after the last treatment round.

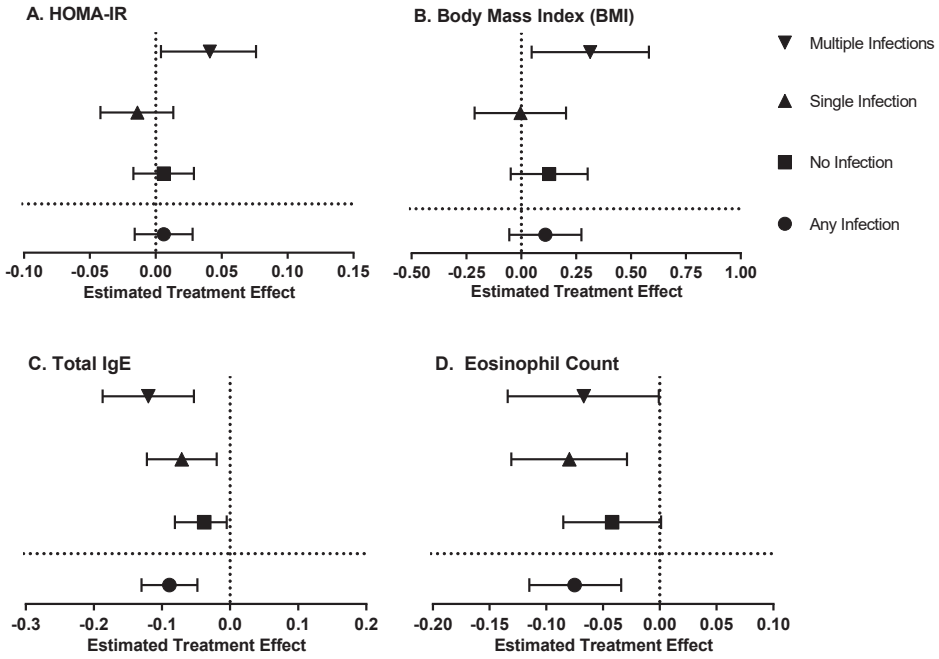

Figure S2. Effect of albendazole treatment on HOMA-IR, BMI, Total IgE and Eosinophil count stratified by the number of helminth species carried by infected subjects at baseline as detected by PCR. Effect of albendazole treatment on (A) HOMA-IR (n= 1433), (B) BMI (n= 1464), (C) Total IgE (n=1430), and Eosinophil count (n=1422) stratified by the number of helminth species infecting subjects at baseline (t=0), as detected by PCR. The estimated treatment effects are displayed with corresponding 95% confidence interval. Circle= infected with at least one helminth species, square= no infection, triangle= infected with one helminth species, inverse triangle= infected with more than one helminth species. Abbreviations: HOMA-IR=Homeostatic Model Assessment for Insulin Resistance, BMI=Body Mass Index, IgE= Immunoglobulin E.

Supplementary Tables

Table S1. Summary of subjects lost to follow-up

	Died		Moved		Refused		Other Reasons		Total Lost to Follow Up	
	Pla	Alb	Pla	Alb	Pla	Alb	Pla	Alb	Pla	Alb
Subjects (n, %)*	13 (1.5)	11 (1.4)	101 (11.5)	75 (9.4)	23 (2.6)	16 (2.0)	42 (4.8)	35 (4.4)	179 (20.5)	137 (17.2)
Age (mean, SD)	56.8 (10.1)	63.1 (10.7)	29.7 (14.1)	29.5 (14.0)	36.6 (13.9)	39.9 (13.8)	42.4 (17.2)	39.7 (41.1)	35.5 (16.5)	36.0 (17.1)
Sex (male) (n/N, %)	10/13 (77)	6/11 (55)	40/101 (40)	34/75 (45)	14/23 (61)	11/16 (69)	22/42 (52)	19/35 (54)	86/179 (48.0)	70/137 (51.1)

*% was calculated from all subjects in each treatment arm (placebo n=872, albendazole n=797
Abbreviations: Pla, Placebo; Alb, Albendazole

Table S2. Effect of albendazole treatment on infection intensity detected by PCR

		Baseline (t=0)		Follow-Up (t=52 weeks)	
Type of STH	Infection Intensity*	Placebo n=594	Albendazole n=550	Placebo n=594	Albendazole n=550
Hookworm n (%)	Negative	396 (66.7)	385 (70.0)	420 (70.7)	527 (95.8)
	Low	98 (16.5)	88 (16)	92 (15.5)	18 (3.3)
	Moderate	67 (11.3)	58 (10.5)	62 (10.4)	5 (0.9)
	High	33 (5.5)	19 (3.5)	20 (3.4)	0 (0.0)
A. lumbricoides n (%)	Negative	484 (81.5)	462 (84.0)	517 (87.0)	543 (98.7)
	Low	26 (4.4)	14 (2.5)	19 (3.2)	1 (0.2)
	Moderate	47 (7.9)	43 (7.8)	32 (5.4)	4 (0.7)
	High	37 (6.2)	31 (5.6)	26 (4.4)	2 (0.4)
T. trichiura n (%)	Negative	436 (73.4)	400 (72.7)	460 (77.4)	505 (91.8)
	Low	45 (7.6)	49 (8.9)	40 (6.7)	23 (4.2)
	Moderate	80 (13.5)	71 (12.9)	63 (10.6)	19 (3.5)
	High	33 (5.5)	30 (5.5)	31 (5.2)	3 (0.5)
S. stercoralis n (%)	Negative	588 (99.0)	547 (99.5)	589 (99.1)	550 (100.0)
	Low	3 (0.5)	1 (0.2)	3 (0.5)	0 (0.0)
	Moderate	2 (0.3)	1 (0.2)	1 (0.2)	0 (0.0)
	High	1 (0.2)	1 (0.2)	1 (0.2)	0 (0.0)

*Ct value of 50 indicates no infection (negative). Ct values <50 were further grouped into 3 categories: Ct<30, 30≤Ct<35 and Ct≥35 representing a high, moderate and low DNA load, respectively. Abbreviations: Ct=Cycle threshold

Table S3. Pathway analysis of the treatment effect on insulin resistance in helminth-infected subjects as detected by microscopy

Model	Estimated Treatment Effect (95%CI), p value
Unadjusted	0.031 (0.004 – 0.059), p=0.04
Adjusted for BMI changes	0.025 (-0.001 – 0.051), p=0.10
Adjusted for total IgE changes	0.030 (0.002 – 0.057), p=0.06
Adjusted for eosinophil count changes	0.026 (-0.002 – 0.053), p=0.13
Adjusted for BMI and eosinophil changes	0.020 (-0.007 – 0.046), p=0.23

Abbreviations: BMI=Body Mass Index, IgE= Immunoglobulin E.

Table S4.A. Comparison of baseline infection intensity in subjects infected with single and multiple helminth species as detected by PCR

Helminth species	Single (n=491) [Ct value, mean (SD)]	Multiple (n=296) [Ct value, mean (SD)]	Mean differences (95%CI), p value*
Hookworm	35.9 (4.0), n=259	34.3 (4.4), n=226	-1.6 (-2.3 – -0.8), p<0.0001
A.lumbricoides	32.9 (3.2), n=75	31.0 (3.7), n=179	-1.9 (-2.9 – -0.9), p<0.0001
T.trichiura	33.7 (2.9), n=156	32.3 (3.3) n=247	-1.4 (-2.0 – -0.8), p<0.0001

This analysis was conducted in subjects analyzed in figure S1. Infection intensity was assessed using Ct values of PCR, which represent the amount of DNA in the stool sample. The lower the Ct value, the higher the amount of DNA. Number of subjects with *S.stercoralis* was very low (one within the single infection group and nine within the multiple infection group). *Independent t-test.

Table S4.B. Comparison of baseline infection intensity in subjects infected with single and multiple helminth species as detected by microscopy

Helminth species	Single (n=334) [epg, median (IQR), n]	Multiple (n=180) [epg, median (IQR), n]	p value*
Hookworm	96 (48 – 267), n=108	138 (72 – 474), n=108	0.087
A·lumbricoides	360 (72 – 1596), n=103	1980 (432 – 6264), n=143	<0.001
T·trichiura	36 (12 – 120), n=123	84 (36 – 261), n=152	<0.001

This analysis was conducted in subjects analyzed in figure 3. Infection intensity was assessed using the number of egg per gram of stool sample (epg) from Kato Katz method. *Independent-samples Mann Whitney U Test.