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chapter 6

Infection with soil-transmitted
helminths is associated with increased
insulin sensitivity. Experiments
of nature on immune modulation
and metabolism

Abstract

Systemic inflammation has been propagated an important phenomenon in the pathogenesis of type-2 diabetes. Remarkably helminth infections shift the immune system to an anti-inflammatory profile. We therefore hypothesized that helminth infections, as an experiment of nature, lead to decreased insulin resistance, which has not been studied before in humans. We performed a cross-sectional study in Flores, Indonesia, an area endemic for soil-transmitted helminths to explore whether helminth infections are associated with increased insulin sensitivity. Stool samples from 646 participants aged 18-80 years were collected and screened for Trichuris trichiura by microscopy and for Ascaris lumbricoides, Necator americanus, Ancylostoma duodenale, and Strongyloides stercoralis by qPCR. We documented data on body mass index (BMI), waist-to-hip ratio, fasting blood glucose, insulin, high sensitive C-reactive protein level and E. coli lipopolysaccharide stimulated cytokines (TNF and IL-10). The HOMA-IR was calculated and the association between helminth infection status and insulin resistance was tested by linear regression adjusted for age, sex and BMI. Participants with any helminth infection had lower BMI (kg/m2) (mean difference -0.63, 95%CI [-1.22, 0.02], p=0.044), WHR (-0.01, [-0.02, -0.00], p=0.020) as well as insulin (pmol/L) (0.85, [0.74, 0.98], p=0.023) and HOMA-IR (0.83, [0.73, 0.95], p=0.0075) than uninfected subjects. After adjustment for BMI the association between helminth infection and insulin (pmol/L) (mean difference 0.89, 95%CI [0.78, 1.01], p=0.081) as well as HOMA-IR (0.88, [0.77, 0.99], p=0.036) remained. We conclude that helminth infections are associated with improved insulin sensitivity, which may support a direct association between systemic inflammation and type-2 diabetes.

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Introduction

The prevalence of type-2 diabetes is rising in urban areas in low-to-middle income countries (LMIC) (1). In many Asian countries, rapid socioeconomic development has led to a shift in infrastructure, technology and food supply that promotes over nutrition and sedentary lifestyles (2, 3). Indeed, the increased prevalence of type-2 diabetes has traditionally been explained by decreased physical activity or excess consumption of high-energy foods, which lead to a disturbed energy balance. However, there is now compelling evidence that inflammation plays a role in chronic non-communicable diseases, including type-2 diabetes (4). Indeed, in type-2 diabetes, elevated levels of inflammation-related markers such as interleukin 6 (IL-6), IL-8, tumor necrosis factor (TNF), and C-reactive protein (CRP) (4) have been reported. In this respect, it is interesting that some chronic infections, such as helminth infections, which are highly prevalent in rural areas of LMIC, have been shown to induce anti-inflammatory and immune regulatory effects (5). One particular modifier are the production of T helper (Th) 2 cells and regulatory cytokines (IL-4, IL-5, IL-10, and IL-13), that are capable of keeping Th1 and TNF responses in balance. Therefore it has been hypothesized that chronic helminth infections by inducing anti-inflammatory responses decrease systemic inflammation and might be beneficial to the prevention of inflammatory diseases, such as allergy (6), inflammatory bowel disease (7), and metabolic diseases (8).

In addition, in animal models, the response associated with helminth infections can forestall obesity and enhance glucose tolerance (9). Currently, there are no published human studies on the relationship between helminth infections and glucose metabolism.

In the present study, we aim to investigate the relationship between helminth infections and insulin resistance, as assessed by HOMA-IR (10) in an area endemic for soil-transmitted helminths on Flores Island, Indonesia.

Material and Methods

Study objectives

The primary objective of the study was to investigate the association between helminth infections and HOMA-IR. Chronic inflammation is a central feature in the pathophysiology of IR and type-2 diabetes. Therefore our hypothesis was that, since helminths might induce an immune evasion strategy by inducing anti-inflammatory responses, HOMA-IR is lower in subjects with helminth infections than in subjects without helminth infections.

Study population

The study area is Nangapanda on Flores Island in Indonesia, highly endemic for soil-transmitted helminths (6, 11). In this area, a large investigational project is being conducted on the relationship between helminth infections and the immune system (ImmunoSPIN study (6, 11)). For the current study, a cross sectional representative sample was included from all inhabitants aged 18 years and above. Data were collected between May-August 2009.

Study design

From 1841 inhabitants age 18 years and above in Nangapanda who participated in the ImmunoSPIN project, 683 were randomly selected to participate in the present cross-sectional

study and invited to provide data on BMI, WHR, and blood sampling for fasting glucose (FBG), insulin, High sensitive C-reactive protein (hs-CRP) and whole blood culture. 646 subjects of whom data on helminth infections, BMI and WHR ratio are available were included in the present analysis. In 584 of these subjects, laboratory measurements were performed.

The study was approved by the ethical committee of the Faculty of Medicine, University of Indonesia, ref: 194/PT02.FK/Etik/2006 with addendum ref: 96/PT02.FK/Etik/2010 and registered as clinical trial ref: ISRCTN83830814 and was filed by the Leiden University Medical Center Committee of Medical Ethics (CME). Because of the high rate of illiteracy amongst elderly participants, either written or verbal informed consent was obtained from each participant.

Clinical and laboratory assessment

Anthropometric measurements of body weight (SECA 761, SECA GMBH & Co. Kg., Hamburg, Germany), height (SECA 206, SECA GMBH & Co. Kg., Hamburg, Germany), waist and hip circumference (SECA 203, SECA GMBH & Co. Kg., Hamburg, Germany) were performed using the NHLBI practical guidelines (NHLBI web). Abnormal BMI is defined as ≥ 25 kg/m^2 and the Asian modified abnormal waist hip ratio (WHR) is >0.9 (men) and >0.8 (women) (12). Impaired fasting glucose (FBG) was defined as ≥5.6 mmol/L (13). A FBG ≥7.1 mmol/L of is indicative of diabetes mellitus (13). All participants were instructed to be fasting before venous sampling. FBG was analyzed using Breeze®2 glucose meter (Bayer Health Care LLC, Basel, Switzerland). Insulin was measured using MSD® 96-Well MULTI-ARRAY® Human insulin assay (Meso Scale Discovery, Gaithersburg, USA). HOMA-IR, a well-validated measure of IR was calculated to estimate insulin resistance (10). High sensitive C-reactive protein (Hs-CRP) level was measured using MSD® 96-Well MULTI-ARRAY® CRP Assay (Meso Scale Discovery, Gaithersburg, USA). Health questionnaires on smoking habits, medical history, and family history were collected.

Helminth status

Stool samples were collected and preserved in 4% formaldehyde for microscopy examination or frozen (-20°C) unpreserved for PCR detection. The formol-ether acetate concentration method was performed on the formalin preserved stool samples followed by microscopy examination for *Trichuris trichiura* infections (11). As described in detail before (11), DNA was isolated from approximately 100 mg unpreserved feces and a multiplex real-time PCR for the detection of *Ancylostoma duodenale*, *Necator americanus*, *Ascaris lumbricoides* and *Strongyloides stercoralis* was performed. The real-time PCR output from this system consisted of a cycle-threshold (CT) value, representing the amplification cycle in which the level of fluorescent signal exceeds the background fluorescence, and reflecting the parasite-specific DNA load in the sample tested. Negative and positive control samples were included in each run of the amplification. We defined a positive case for *T. trichiura* by the egg findings and for *A. duodenale*, *N. americanus*, *A. lumbricoides* and *S. stercoralis* by parasite-specific DNA amplification. Participants were also grouped by number of helminth species infections.

Whole blood stimulation and cytokine measurement, and haemoglobin count

The procedure of whole blood stimulation and cytokines measurement has been described previously (11). Briefly, heparinized blood was diluted 4x and stimulated within 6 hours after drawing.

Stimulations were performed with control medium or *E. coli* lipopolysaccharide (LPS, 1 ng/ml Sigma-Aldrich, Zwijndrecht, The Netherlands), incubated for 24 hours at 37^0C and 5% CO_2. The supernatants were frozen at -20^0C and transported to Jakarta where TNF and IL-10 supernatants were assessed by means of immunobead-based multiplex assays on a Liquichip 200® Workstation (Qiagen, Venlo, The Netherlands) using Liquichip analyzer software (Qiagen, Venlo, The Netherlands). Samples with TNF levels higher than 250 pg/ml in medium stimulation were excluded from further analyses (2 samples). Haemoglobin was determined using heparinized blood on a routine cell counter (Coulter® Ac-T™ diff Analyzer, Beckman Coulter Inc., Fullerton, CA, USA)

Statistical analysis

Participant characteristics were stratified for helminth uninfected and infected. Insulin, HOMA-IR, cytokines and CRP concentrations were normalized by log-transformation. Analyses were performed with these log transformed values but results were presented as geometric means after exponentiation of the values on a logarithmic scale. Linear regression was used to study the associations between infection with any helminth species as well as the number of helminth species and HOMA-IR and adjusted for age and sex. To assess whether a potential association between helminth infection and HOMA-IR is mediated through an effect on BMI or WHR, we adjusted for BMI or WHR. Furthermore, in the helminth infected group we tested the association of helminth intensity per species with HOMA-IR. Differences between infected and uninfected participants were reported as mean differences with 95% confidence intervals (95% CI). P values <0.05 were considered to be statistically significant. Statistical analysis was performed with SPSS statistics 17.0.2 (SPSS Inc., Chicago, Illinois, The USA).

Results

Characteristics of study participants

A total of 424 participants infected with at least one species of soil-transmitted helminths were compared to 222 uninfected participants (Table 1). There were slightly more males in the infected group (38%) than in the uninfected group (34%), whereas the mean age was similar (45.1 vs 44.7 years). The most prevalent soil-transmitted helminth species were *N. americanus* 334/646 (51.7%), *A. lumbricoides* 141/646 (21.8%) and *T. trichiura* 127/646 (19.7%). The proportion of participants infected with *A. duodenale* 24/646 (3.7%) and with *S. stercoralis* 4/646 (0.6%) was clearly lower. 261 participants were infected with one helminth species only, 124 with two helminth species and 39 with 3 or more helminth species. 322 of 584 (55.1%) participants had elevated FBG (≥5.6 mmol/L) of whom 27 (4.6%) with FBG ≥7-11 mmol/L and 10 (1.7%) ≥11 mmol/L. We found no significant differences in hemoglobin levels between participants infected with helminths and uninfected participants.

Association between helminth infection and glucose metabolism

Participants with any helminth infection had lower BMI (kg/m2) (mean difference -0.63, 95%CI [-1.22, -0.02]), WHR (-0.01, [-0.02, -0.00]), insulin (pmol/L) (0.85, [0.74, 0.98]) and HOMA-IR (0.83, [0.73, 0.95]) than uninfected participants (Table 1). After adjustment for BMI, the association between helminth infection and insulin (0.89, [0.78, 1.01]) as well as HOMA-IR (0.88,

Insulin sensitivity in helminths infected subjects

Table 1. Characteristics of the study population

	Helminth uninfected (n=222)	Helminth infected (n=424)	Mean difference adjusted for age and sex (95% confidence interval)	Mean difference adjusted for age, sex and BMI (95% confidence interval)
Age (year) (mean, Range)	44.4 (18.2-79.4)	45.2 (18.0-79.4)	-	-
Male (%)	33.5	37.8	-	-
Trichuris trichiura[1] (%)	0	30.7	-	-
Ascaris lumbricoides[2] (%)	0	33.1	-	-
Necator americanus[2] (%)	0	78.2	-	-
Ancylostoma duodenale[2] (%)	0	5.3	-	-
Strongyloides stercoralis[2] (%)	0	0.9	-	-
BMI (Kg/m2) (mean, SD)	23.2 (3.7)	22.5 (3.8)	-0.63 (-1.22, -0.02), p=0.044	-
WHR (mean, SD)	0.89 (0.07)	0.88 (0.06)	-0.01 (-0.02, -0.00), p=0.020	-
FBG (mmol/L) (mean, SD)	5.9 (1.5)	5.9 (1.6)	-0.05 (-0.31, 0.22), p=0.73	0.01 (-0.25, 0.27), p=0.93
Haemoglobin (g/dl) (mean, SD)	14.5 (2.5)	14.5 (2.7)	0.05 (-0.46, 0.56), p=0.84	0.07 (-0.44, 0.58), p=0.80
Insulin (pmol/L) (geometric mean [95%CI])	38.1 (34.1-42.5)	32.0 (29.5-34.8)	*0.85 (0.74, 0.98), p=0.023	*0.89 (0.78, 1.01), p=0.081
HOMA-IR (geometric mean [95%CI])	0.7 (0.7-0.8)	0.6 (0.6-0.7)	*0.83 (0.73, 0.95), p=0.0075	*0.88 (0.77, 0.99), p=0.036
Hs-CRP (mg/l) (geometric mean [95%CI])	0.5 (0.4-0.6)	0.5 (0.4-0.6)	*0.95 (0.75, 1.30), p=0.96	*1.02 (0.77, 1.36), p=0.88
TNF (pg/ml)* (geometric mean [95%CI])	222.5 (180.0-275.1)	281.2 (245.4-322.2)	*1.27 (0.99, 1.63), p=0.056	*1.26 (0.98, 1.61), p=0.073
IL-10 (pg/ml)* (geometric mean [95%CI])	132.3 (109.7-159.7)	127.9 (114.2-143.2)	*0.98 (0.79, 1.21), p=0.82	*0.96 (0.78, 1.19), p=0.74

1. positive by microscopy
2. positive by PCR

Abbreviations: BMI = body mass index, WHR = waist to hips ratio, FBG = fasting blood glucose, HOMA-IR = Homeostasis model assessment for insulin resistance, Hs-CRP = High sensitive C reactive protein, TNF = tumor necrosis factor, IL-10 = interleukin 10. *The cytokines were measured from stimulated whole blood for 24h with *E. coli* lipopolysaccharide (LPS). *Adjusted mean difference for insulin, HOMA-IR, hs-CRP and cytokines were anti-log transformed and represent ratio of the measurement between uninfected and infected group.

[0.77, 0.99]) remained (Figure 1). No clear associations were found between helminth infection and FBG, hs-CRP or TNF-LPS. Using WHR as a marker of central obesity gave similar results that there is negative association between helminth and insulin as well HOMA-IR (data not shown). Additional correction for smoking, medical history, and familial history did not change the association between helminth infection and HOMA-IR (data not shown).

When considering the relationship between the number of helminth species, we found that BMI, WHR, insulin and HOMA-IR were negatively associated with the number of helminth species (p=0.036, p=0.010, p<0.001, p<0.001, respectively). Adjustment for BMI attenuated the association between number of infections and insulin as well as HOMA-IR but both associations remained significant (p=0.0015 and p<0.001, respectively). The results were also similar after adjustment for WHR (p=0.0024 and p=0.0013, respectively). TNF-LPS was positively associated with increasing number of helminth species with the highest LPS-levels in participants with 3 or more infections. This association was not attenuated after adjustment for BMI or WHR (p=0.035 and p=0.032, respectively).
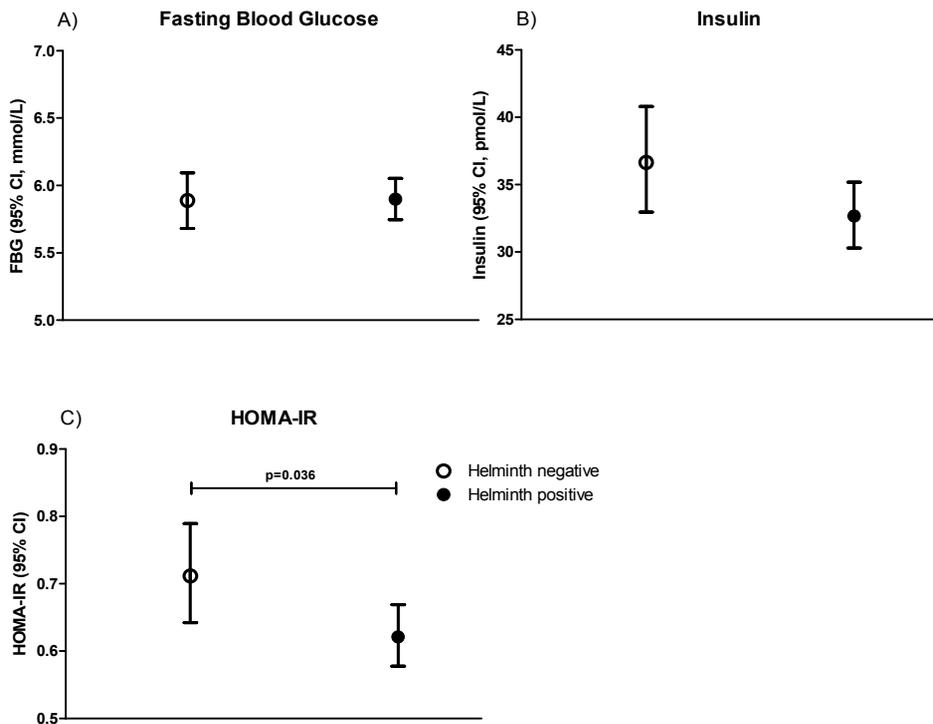
Figure 1. The association of soil-transmitted helminths infection and metabolic parameters. Relation of helminth infection with (A) fasting blood glucose (FBG), (B) insulin* and (C) HOMA-IR* with correction for age, sex, and BMI. 208 participants had no helminth infection and 376 were infected with at least one helminth species. Mean FBG was 5.9 mmol/L 95%CI (5.7, 6.1) and 5.90 (5.8, 6.1), insulin was 36.6 pmol/L (33.0, 40.8) and 32.7 (30.3, 35.2), and HOMA-IR was 0.7 (0.6, 0.8) and 0.6 (0.6, 0.7) for helminth-uninfected and infected group, respectively. *Adjusted mean for insulin and HOMA-IR were anti-log transformed and represents geometric means.

We also investigated whether there were differences in the association between different species of helminths with HOMA-IR. We found no association between *N. americanus*, *A. lumbricoides*, or *T. trichiura*, individually with HOMA-IR (prevalence of *S. stercoralis* and *A. duodenale* were too low to be considered).

Discussion

The objective of this study was to examine the association between helminth infections and insulin resistance in a population residing in an area highly endemic for soil-transmitted helminths. The hypothesis being tested is that helminth infections may have a direct beneficial effect on glucose metabolism, by influencing systemic inflammation.

The influence of helminth infections has been shown in animal models of type-1 diabetes (T1D) (14, 15) or type-2 diabetes (9) but has not been studied in humans. In our study, we found a negative association between HOMA-IR with helminth infections, which was independent of BMI or WHR.

While both helminth infected and uninfected participants in our study had relatively low HOMA-IR, we found that helminth infection was associated with even lower HOMA-IR. No clear association was found for each single helminth species in the absence of infection with other helminths.

Studies in experimental models have shown that injecting helminth antigens to young non-obese-diabetic mice prevented the onset of T1D. This inhibition of T1D development appeared to be due to the ability of helminth and its products to induce IL-10 production by dendritic cells, B cells, alternatively activated macrophages, as well as regulatory T cells (16). In the model of type-2 diabetes, mice with high fat diet that were infected with helminths, had eosinophilia, became less obese and less insulin resistant which seemed to be in conjunction with maintenance of alternative activated macrophages in adipose tissues (9). Ricardo-Gonzales *et al*, have also shown that the IL-4/STAT6 immune axis, which is a key pathway affected by helminths, promotes control on peripheral nutrient metabolism and insulin sensitivity (17).

Inflammation is known to be an important factor in the pathogenesis of type-2 diabetes (4) and increased CRP has been shown to be either a causal or a prediction marker of metabolic syndrome and IR (18-22). Individuals with Asian descent may exhibit the characteristics of inflammation while relatively lean (2, 23, 24), however, our participants who were lean, had also very low hs-CRP level (0.4-0.6 mg/L) and even lower than what has been reported in China (0.6-0.8 mg/L) (21, 22), South Asia (0.9-2.8 mg/L in rural and in urban 2.2-2.6 mg/L) (19, 20) or US population of various ethnicities (1.1-4.5 mg/L) (18, 24, 25).

We acknowledge the limitation of this cross-sectional study in nature. As the development of type-2 diabetes is a chronic process, ideally, lifetime exposure to helminth infections should be related to the disease development, which is however not feasible. The causal relationship between helminth infection and glucose metabolism could be studied in a placebo-controlled trial with anti-helminth treatment.

Conclusion

In a large cross-sectional study in an area endemic for helminth infections, we found an association between soil-transmitted helminths infection and decreased insulin resistance. We

believe that this experiment of nature supports the notion of a direct relationship between systemic inflammation and the pathogenesis of type-2 diabetes, although further studies are needed to assess the causal relationships.

Acknowledgments

Transparency declaration

Authorship/Contribution

Conceived and designed the experiments: AEW, FP, ES, TS, MY and JWAS. Patient enrolment and performed the experiment: AEW, FH, LJW, and MAP. PCR detection on helminths: MMMK and JJV. Analyzed and interpreted the data: AEW LM OMD MY JWAS. Wrote the paper: AEW. Review of paper: ES, OMD, BG, MY and JWAS. All authors revised the report for important intellectual content and have seen and approved the final version. MY and TS had full access to all the data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis.

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Insulin sensitivity in helminths infected subjects