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

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

# Chapter 6

## SUMMARIZING DISCUSSION

Adapted from:

**Helminths, hygiene hypothesis and type 2 diabetes.**

Karin de Ruiter[1*], Dicky L. Tahapary[1,2*], Erliyani Sartono[2], Pradana Soewondo[2], Taniawati Supali[3], Johannes W.A. Smit[4,5], Maria Yazdanbakhsh[2]

Parasite Immunology 2017;39:e12404 DOI: 10.1111/pim.12404

## SUMMARY OF WHAT WAS ALREADY KNOWN

- The prevalence of obesity and type 2 diabetes is increasing, especially in urban areas of LMIC.
- Rural to urban migration is associated with increased risk of obesity
- In rural area, helminth infections are still highly prevalent, while the prevalence of T2D is low
- Studies in animal models have shown a protective effect of helminth infections on glucose metabolism
- Cross sectional studies have shown that past and current chronic helminth infections are associated with lower adiposity and insulin resistance.
- Recent animal studies reported an important role of adipose tissue in helminth-mediated protective metabolic effects.

## WHAT WAS ALREADY KNOWN ABOUT HELMINTH INFECTIONS, URBANIZATION, AND TYPE 2 DIABETES?

The prevalence of T2D is increasing worldwide, especially in urban areas of LMIC, whereby socioeconomic changes leads to increased adiposity and development of T2D.[1, 2] In animal models, a protective role for helminth infections on glucose metabolism was suggested.[3-8] In most rural areas of LMIC, where helmints are still highly prevalent, the prevalence of T2D is low, showing an inverse ecological association between STH and T2D.[9, 10] In line with this, several cross-sectional studies from different populations reported on the possible protective metabolic effects of past[11, 12] or current helminth infections[13, 14]. Despite the fact that a recent meta-analysis confirmed the protective metabolic effects of helminths[15], all available evidence from human studies were cross-sectional, thus, no causal relation could be drawn.

In general, the prevalence of T2D in rural areas is lower,[2] suggesting that living in rural area might give a relative protection against the development of T2D, in comparison to living in an urban area. It has been suggested that, in addition to the better known changes in diet and lifestyle, reduced biodiversity, commonly seen in urban areas, might also play some role in the increasing prevalence of inflammatory diseases,[16] including T2D. Furthermore, current deworming programs in many LMICs,[17, 18] might also contribute by removing helminth-associated beneficial effects,[9] which in turn alleviates helminth-associated induction of type 2 immune responses and regulatory network,[19] thus increasing the risk of inflammatory disorders, including T2D.[9, 10, 20-22]

Chronic low grade inflammation seems to be a key characteristic of obesity and T2D.[23] Whereas in obesity-associated development of IR, AT inflammation plays an important role,[23-26] experimental studies in animal model have shown that helminth infections cause changes in AT.[4-7] These changes are not only in terms of lowering fat mass, but also involve the immune cell composition in AT, shifting towards a more anti-inflammatory milieu, and better glucose homeostasis.[4-7]

## SUMMARY OF THE FINDINGS

- Repeated three-monthly treatment with triple dose of 400mg albendazole over 12 months reduced STH prevalence and intensity, thus to reach a substantial reduction in STH, a more intensive deworming than the current policy is needed.
- At community level, 12 months of anthelmintic treatment reduced total IgE level and eosinophil count, but did not affect insulin resistance.
- In STH-infected subjects, as assessed by microscopy, 12-month anthelmintic treatment increased insulin resistance, which was mediated by an increase in BMI and leptin to adiponectin ratio (L/A ratio), as well as reduction in eosinophil count.
- In comparison to those living in rural area, individuals living in urban area had higher whole body insulin resistance, which was mainly mediated by the higher adiposity and leptin level, which were progressively increased with increased duration of time spent in urban area.
- Different environmental factors (including past or current exposure to STH) did not seem to affect the metabolic response to HFD intervention, independent from adiposity.

## HOW DID OUR STUDIES ADVANCE THE FIELD?

Through designing a randomized controlled trial (RCT) of anthelmintic treatment in a rural area of Indonesia[27], endemic for STH[28], as detailed in **Chapter 2**, it was possible, for the first time, to study the effect of anthelmintic treatment on the host metabolic homeostasis. Four rounds of three-monthly albendazole treatment given for three consecutive days lead to a significant reduction in infection intensity and prevalence but not elimination of STH.[29] Our triple doses of albendazole treatment regimen resulted in lower prevalence of helminth compared to single dose albendazole treatment previously given in the same study area.[28] Although not a major questions in our study, our data are important for the global deworming programs, as they indicate that the current annual single dose treatment[18] is unlikely to have an impact on the control, and in particular the elimination of STH. The issue of re infection needs to be considered,[30, 31] and in addition to intensive treatment, interruption of transmission by decontaminating the environment[32] will be essential for any global impact to be achieved.

Besides reduction in prevalence and intensity of STH infections, anthelmintic treatment also led to a significant reduction of Th2 responses at the community level, as measured by total IgE and eosinophil counts[29], which is in line with a previous report in Ecuador[33]. However, it did not affect insulin resistance nor any other metabolic parameter at the community level. However, in STH-infected subjects, anthelmintic treatment led to a significant increase in IR[29], providing the first evidence on the causal association between helminth infections and IR in humans (**Chapter 3**), further strengthening the evidence for the helminth-associated protective metabolic effects documented in cross-sectional studies[11-15] and animal models[3-8].

Interestingly, infection intensity and number of helminth species seems also to play a role. The increase in IR after anthelmintic treatment was higher in helminth-infected subjects, as assessed by microscopy, in comparison to helminth-infected subjects, as assessed by PCR, the later detecting less clinically relevant infections[34]. Moreover, subjects infected with multiple helminth species showed a more pronounced increase in IR after anthelminthic treatment, which suggests the importance of, not only infection intensity, as these subjects infected with multiple helminth species had a higher infection intensity in comparison to those infected with single species, but also the importance of polyparasitism for the down modulatory effect of helminth[35]. Previous cross- sectional studies in the

same population also support this notion, as increasing number of STH species was also associated with a progressively lower IR[14].

Following the evidence of a causal relation between helminth and IR, it would be important to be able to understand the pathways involved. Despite the significant reduction in Th2 responses, as assessed by serum total IgE level and eosinophil count, this pathway does not seem to play a role in the increased IR, in particular the total IgE. Although the presence of STH is an important determinant for total IgE level,[33] other environmental exposures or conditions can also affect total IgE. Our trial showed that the increased IR after anthelmintic treatment seems to be mainly mediated by the increased adiposity, as assessed by BMI, which is not surprising as adiposity has been closely associated with nutrition and the development of IR.[36] However, a role for eosinophils was shown when additional adjustment for eosinophil counts further attenuated the increased IR. This suggests that eosinophils, if anything, might be involved in the causal pathway. The possible role of eosinophils in affecting glucose homeostasis has been shown in animal models[3-6] and a human study[37].



Although our trial has shown that anthelmintic treatment increased adiposity, this only gives us limited information on the pathways involved in mediating the beneficial effects of helminths in humans. It is interesting to study whether the increase in adiposity is solely responsible for the increased IR, or it is also associated with changes in the physiology of AT. Studies in animal models of diet-induced obesity have shown that indeed chronic helminth infection is associated with less fat mass gain,[4] of which associated with increased white AT M2 macrophages and eosinophils, as well as less IR.[4] Interestingly, helminth-derived molecules also induce a similar type 2 responses and beneficial metabolic effects without any significant changes in body fat.[4, 5]

In human, however, it was not possible to perform AT biopsies. We therefore assessed the effect of anthelmintic treatment on adipokines, mediators secreted by human AT, especially leptin and adiponectin, two major adipokines which have been shown to have pro and anti-inflammatory properties, respectively, as well as resistin, which has been reported to be increased in helminth infected subjects[38] (**Chapter 4**). Indeed, anthelmintic treatment in helminth-infected subjects increased the leptin to adiponectin ratio, and through mediation analysis we showed that this increase may be involved in the anthelmintic-associated increase in IR. The shift toward a more proinflammatory, higher leptin to adiponectin ratio,

after anthelmintic treatment was mainly caused by the significant reduction in adiponectin level, an anti-inflammatory adipokine [39], and to a lesser extent, through an increase in leptin level, a pro-inflammatory adipokine [39]. No causal association between helminth infection and resistin, a pro-inflammatory adipokine, was observed in our trial, contrasting the previously reported finding.[38]

Furthermore, the increase in leptin level after anthelmintic treatment in STH-infected subjects might also suggest that STH suppresses leptin level, as levels recovered up to the levels in STH-uninfected subjects when STH were removed by anthelmintic treatment. Low leptin levels has been associated with higher susceptibility to infection[40-42] and lower autoimmunity.[43] Furthermore, as leptin can increase Th1 and suppress Th2 cytokine production,[40] and can act as a negative signal for the proliferation of human regulatory T cells [44], we may speculate that the supressed leptin level in helminth-infected subjects might contribute to helminth-associated induction of modified type 2 and regulatory immune responses,[21] and if anything, contribute to a reduced helminth clearance from the host.[45]

Next, as available evidence suggests that living in rural areas might provide relative protection against the development of T2D, in comparison to living in urban areas, we designed a study to compare the metabolic profile and responses to a short-term HFD intervention between individuals with similar genetic background living in rural and urban areas. **Chapter 5** describes that subjects living in the urban area have a significantly lower STH infections, as well as lower total IgE levels. These subjects had higher whole-body IR, which was mainly mediated by higher adiposity and leptin level. Past or current exposure to STH, as assessed by total IgE, albeit small, contributed to the differences in whole-body IR, adiposity, and leptin level. However, neither rural living nor current STH infections protected against acute induction of IR by high-fat diet intervention. Thus, living in rural area, as well as having helminth infections, might only give a relatively weak protection against the strong induction of IR by short-term HFD, independent of the effect on adiposity.

However, it is important to note that HFD did induce increased liver inflammation, as assessed by CETP level,[46-49] in individuals living in urban, but not in rural areas. The lack of increase in CETP level in rural subjects might be related to the fact that, in these subjects, the CETP levels were already high, thus precluding any further increase. Environmental factors in rural area may explain this high CETP levels, for instance, the higher prevalence of hepatitis in our rural study area may

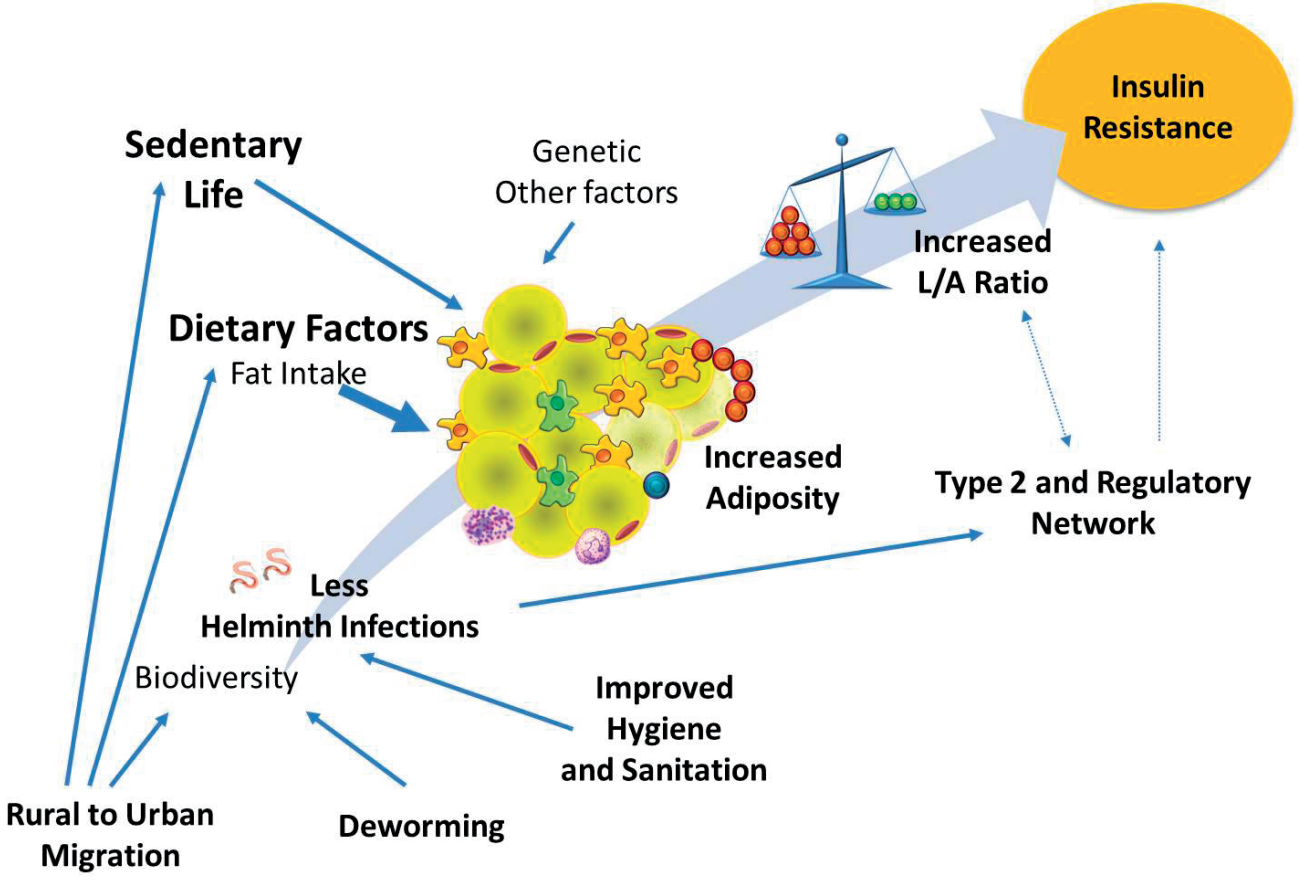


**Figure 1. Simplified Schematic Overview on The Contribution of Helminths and Urbanization on The Development of Insulin Resistance.** L/A: leptin to adiponectin

also contribute.[50] However, the currently available data shows inconsistent results of the effect of different infection or inflammatory exposures on CETP level,[50, 51] thus further studies are needed to assess this. Next, as CETP also play a role in lipoprotein metabolism[47, 52-54], the questions whether this high CETP level in rural will also affect lipoprotein metabolism and subsequent development of atherosclerosis remains.

Our data, from the RCT and from the HFD study, have shown that adiposity seems to play a central role in the development of IR, as well as in mediating the helminth-associated beneficial effects. Helminth parasites, through affecting food intake or absorption, could indeed influence adiposity and thereby IR. Taken together, a simplified schematic overview on the contribution of helminth infections on the development of IR in the light of urbanisation is summarized in **Figure 1**.

## DIRECTIONS FOR FUTURE RESEARCH

### Controlled Human Infections

Experimental infections with helminths or helminth-derived molecules in diet-induced obese mice have shown to improve glucose tolerance and increase insulin sensitivity compared to controls [4-7]. Importantly, these experimental studies enable us to investigate the potential mechanisms by which helminths can influence metabolic outcomes. Therefore, controlled human infection (CHI) models will be an excellent approach to unravel the mechanism by which helminth infections can improve metabolic profiles in human.



### Human Fat Biopsies

It is important to note that, in helminth-infected subjects, anthelmintic-associated increased IR seems to be mainly mediated by the increase in adiposity, as well as an increase in leptin to adiponectin ratio. Moreover, the difference in IR between subjects living in urban and rural areas is also mainly mediated by the difference in adiposity. Thus, assessments of human adipose tissue biopsies[55-57] could give a powerful grip on the underlying mechanism that mediate the effect of helminths and urbanization on the development of IR.

### Gut Microbiome

Intestinal helminths and bacteria reside in the same niche, the human intestine, and might interact with each other and affect the host immune system, nutrition, and metabolism.[58] Interestingly, emerging evidence suggests that the composition of gut microbiota plays a role in the pathogenesis of obesity-associated insulin resistance by affecting energy homeostasis and inflammation [59]. Therefore, alteration of the gut microbiome in the presence of helminth parasites,[60] might contribute to the observed difference in IR. Interestingly, whereas less bacterial diversity was commonly observed among obese people or diabetics [59, 61], greater bacterial diversity was recently reported among subjects infected with *Schistosoma haematobium* [62] and STHs [63]. However, the findings on the effects of STH infections on gut microbiota has not been consistent, as some studies showed them to be associated with a reduced bacterial diversity[64].

The effect of deworming on gut microbiota has also been inconsistent, whereas two studies reported no changes in gut microbiota composition[62, 64], one study reported alteration in gut microbiota composition, most notably, reduced levels of protective bacteria, *Clostridiales*[65]. Furthermore, human experimental

hookworm infection failed to induce changes in bacterial diversity, however it did induce a minor increase in the richness of the microbial species [66]. Interestingly, in mice, helminth infections are associated with an increase of intestinal short chain fatty acids (SCFA) [67], the end products of dietary carbohydrate fermentation, which has been shown to play an important role in the control of body weight and insulin sensitivity [68].

Taken together, helminth-associated diversity of the gut microbiome and amount of SCFAs might mediate the effects of helminths on whole-body IR. However, further studies are needed to confirm these findings, and unravel the complex interaction between helminth infections, gut microbiome, the host immune system and metabolism.



### Rural to urban study

With the increasing burden of NCDs, which are mostly reflect the increasing rate of urbanization, additional efforts should be invested in assessing urbanization-associated changes in metabolic homeostasis, immune responses, and inflammation. Specific focus should be put into urbanization-associated decreased biodiversity, which might be potentially related to the emergence of inflammatory diseases,[16] including T2D. This is supported by two studies showing different immune activation profiles between subjects living in rural and urban areas,[69, 70] which suggests that environmental differences may contribute to immunological footprints, thus affecting the development of inflammatory diseases. However, the contribution of these differences in the immune system to the development of IR, hence T2D, remain to be clarified.

## DIRECTIONS FOR FUTURE HEALTH POLICY AND CARE

In terms of health policy, control measures for communicable diseases, such as elimination of STH, need to go hand in hand with providing education, prevention, and monitoring the development of major NCDs, especially obesity and T2D. More attention to urbanization-related changes in metabolic health, which is currently lacking, is needed. In terms of health care, it is important to develop locally or nationally practical and sensitive diagnostic tools to detect the presence of NCDs, such as T2D, applicable to many resource-limited rural areas of Indonesia. This, hopefully, will enable us to monitor the development of obesity and T2D, as well as enabling us to reduce the high number of undiagnosed T2D in Indonesia.[71]

**Author Details**

Helminths, hygiene hypothesis and type 2 diabetes.

*These authors have contributed equally. [1]Department of Parasitology, Leiden University Medical Center, Leiden, The Netherlands; [2]Department of Internal Medicine, Dr. Cipto Mangunkusumo National General Hospital, Faculty of Medicine, Universitas Indonesia, Jakarta, Indonesia; [3]Department of Parasitology, Faculty of Medicine, Universitas Indonesia, Jakarta, Indonesia; [4]Department of Internal Medicine, Radboud University Medical Centre, Nijmegen, The Netherlands; [5]Department of Internal Medicine, Leiden University Medical Center, Leiden, The Netherlands.

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