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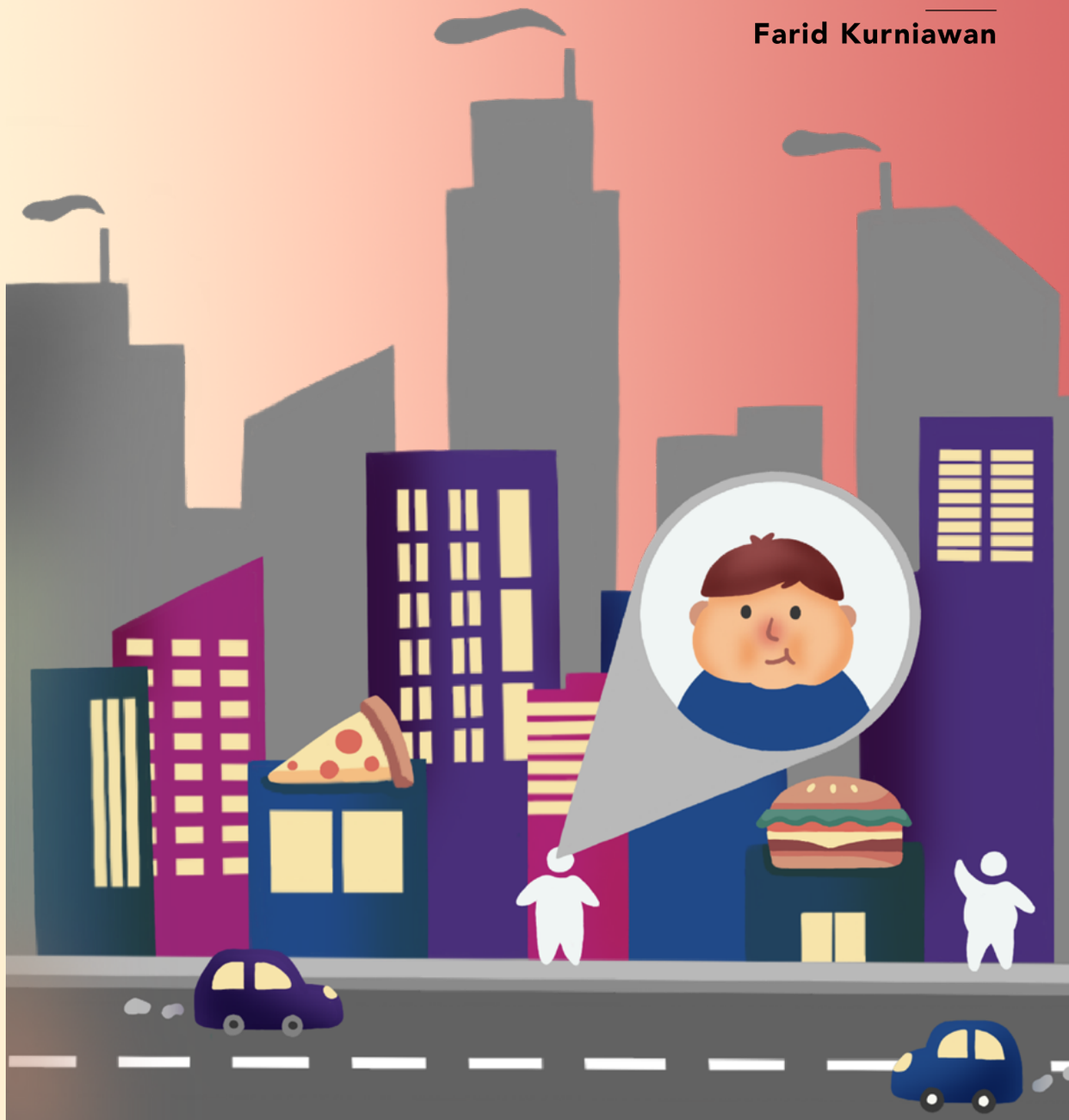

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Dedicated to the loving memory of my father,
M. Urifan Hasan
My inspiration and my role model in life

TABLE OF CONTENTS

Chapter 1

GENERAL INTRODUCTION

URBANIZATION IN INDONESIA AND ITS RELATED CHANGES

Indonesia, a low-middle income country (LMIC) with the largest economic power in the Southeast Asian region,[1] has experienced rapid economic growth in the last two decades.[2] This socio-economic growth consequently accelerates urbanization in Indonesia, a phenomenon which is also observed in other developing countries.[3-5] In 2021, more than 57% of Indonesian population lived in urban areas, increasing from 30% in 1990, and projected to be around 67% in 2035.[6,7]

Urbanization is not only defined as migration of people from rural to urban areas, but also the changing status of previously rural areas that become more urban, along with the adoption of the urban lifestyles.[5] Urbanization is accompanied by significant changes in the social, environmental, and lifestyle aspects of human lives. Dietary pattern in urban population has shifted towards more fast and processed food with high-fat, high-calories, and less fiber containing diet.[8] Urban ecosystem also promotes sedentary behavior due to increased mechanization and digitalization, as well as reliance on transportation.[9] Meanwhile, increased exposure towards pollutions [10,11] with higher level of stress [12] are also often associated with urban environments. In addition, living in urban areas causes a relatively less exposure towards agricultural environments,[13] parasitic, including helminth infections,[14,15] and biodiversity.[16] All of these alterations related with urbanization could affect human microbiome,[17] the epigenome,[18] and the immune system,[19] and thus, potentially affect the disease prevalence and outcome (see conceptual framework in **Figure 1**).[20,21]

In relation to urbanization, the prevalence of infectious diseases in many LMICs has declined in the last few decades.[22] At the same time, the number of people with non-communicable diseases (NCD) such as obesity, diabetes, cardiovascular diseases, cancer, autoimmune diseases, and allergies has greatly increased.[23] This epidemiological transition is also observed in Indonesia. In 1990, the three leading causes for disability-adjusted life years were diarrheal diseases, lower respiratory tract infections, and tuberculosis. However, in 2016, these diseases were replaced by ischemic heart disease, cerebrovascular disease, and diabetes.[24] Moreover, Indonesia is currently experiencing a double burden of malnutrition, which is partly

related to urbanization. On the one hand, undernutrition and stunting are still quite prevalent with 21.6% of the children under five years of age diagnosed with stunting in 2022,[25] even though this prevalence has significantly decreased from 37.2% in 2013.[26] On the other hand, the prevalence of obesity, as manifestation of overnutrition, has almost doubled from 19.1% in 2007 to 35.4% in 2018.[27,28]

Similarly, this changing pattern of disease prevalence has also been seen for diabetes, which increased from 5.7% in 2007 to 10.9% in 2018.[27,28] Additionally, in term of allergic diseases, Indonesia previously had one of the lowest global prevalence of allergic rhinitis (AR), 5.2%, based on the report by The International Study of Asthma and Allergies in Childhood (ISAAC) in 1998.[29] However, this number has increased significantly to 23% according to a study in 2019.[30]

The expanding prevalence of NCDs in many LMICs, including Indonesia, is associated with high burden at the individual, societal, and economic levels. At the individual level, the burden consists of morbidity due to such diseases and the related complications, disability, decreased quality of life, and lastly mortality.[31,32] The social burden includes the support needed from family caregivers and other community members, such as people working in the nursing home and elderly housing.[33,34] Finally, the economic burden constitutes the direct and indirect costs of diseases. Direct costs comprise of the capital allocated for medicines and visits to health care services, while the indirect costs are related with absenteeism and loss of work productivity due to illness.[35,36]

Given the increasing burden of non-communicable diseases in Indonesia, and their complex association with urbanization, extensive research is needed to unravel the relationships to be able to design interventions. Although many studies related to urbanization and health have been conducted in Indonesia, majority of these previous studies have only used secondary data and were cross-sectional in nature.

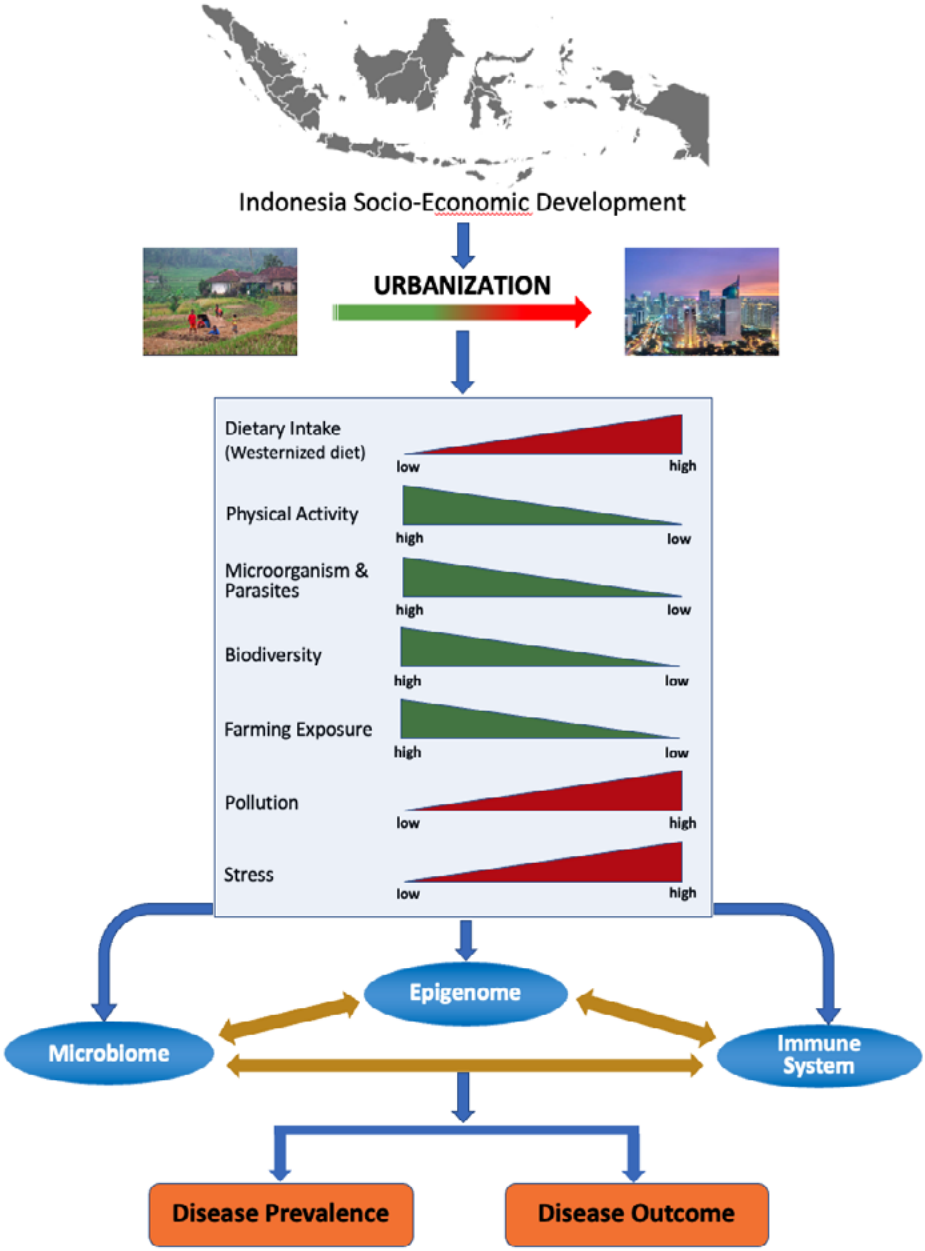

Figure 1. Conceptual Framework of Urbanization Related Changes and Its Effect on Health.

SCOPE AND AIM OF THE THESIS

The overall objective of this thesis is to evaluate the effects of urbanization on human health, focusing on metabolic health and allergy. In this thesis we combine clinical, epidemiological, and immunological approaches to unravel the relative contribution of urbanization and its associated sociodemographic, lifestyle, environmental, and clinical factors to human health and disease. For the clinical approach, a cluster-randomized clinical trial on anthelmintic treatment and an intervention study using short term high-fat high-calorie diet were conducted. This thesis also incorporates not only large scale secondary data for its epidemiological approach, but real world observation in the form of a prospective cohort study. In terms of immunology, mass cytometry has been utilized for in depth characterization of immune profiles associated with disease outcome.

Infection by soil-transmitted helminths (STH) is still highly prevalent in certain rural areas of Indonesia and is one of the prominent factors that distinguishes rural and urban areas. Thus, the first part of the thesis (**Chapter 2**) focuses on the effect of helminth infections and their treatment with albendazole on the metabolic-related hormones: free insulin-like growth factor (IGF)-1 and IGF binding protein (IGFBP)-3.

As the prevalence of NCDs are generally higher in urban compared to rural, we hypothesized that living in rural areas might be protective for developing metabolic diseases. Hence, in **Chapter 3**, we compare the differences in metabolic profiles between Indonesian rural and urban populations with same genetic background and a clinical intervention using short term high-fat high-calorie diet was undertaken to assess whether there are any differences in the metabolic and inflammatory responses between the two populations. In **Chapter 4**, the long-term effects of living in an urban area and adoption of associated lifestyles on the metabolic profiles (adiposity, insulin resistance, and adipokines) was evaluated in a prospective cohort study. Subsequently, **Chapter 5** describes the factors in urban and rural populations that associated with diabetes using a large scale secondary data from the 2018 Indonesian Basic Health Survey.

Aside from the relatively higher prevalence of allergic rhinitis (AR) in urban than rural population, previous studies also showed a relatively less severe clinical manifestation

of AR in rural compared to urban population.[37,38] Based on this, the immunological characteristics of Indonesian young adults, with and without allergic rhinitis, who originated from rural and urban areas, was studied in **Chapter 6**. High dimensional immunological data was generated using mass cytometry on peripheral blood and nasal mucosa to evaluate the association between clinical profiles and immune characteristics in these two populations. Lastly, **Chapter 7** summarizes and discusses our findings from previous chapters and provides directions for future research towards better understanding of the effect of urbanization on metabolic health and allergy.

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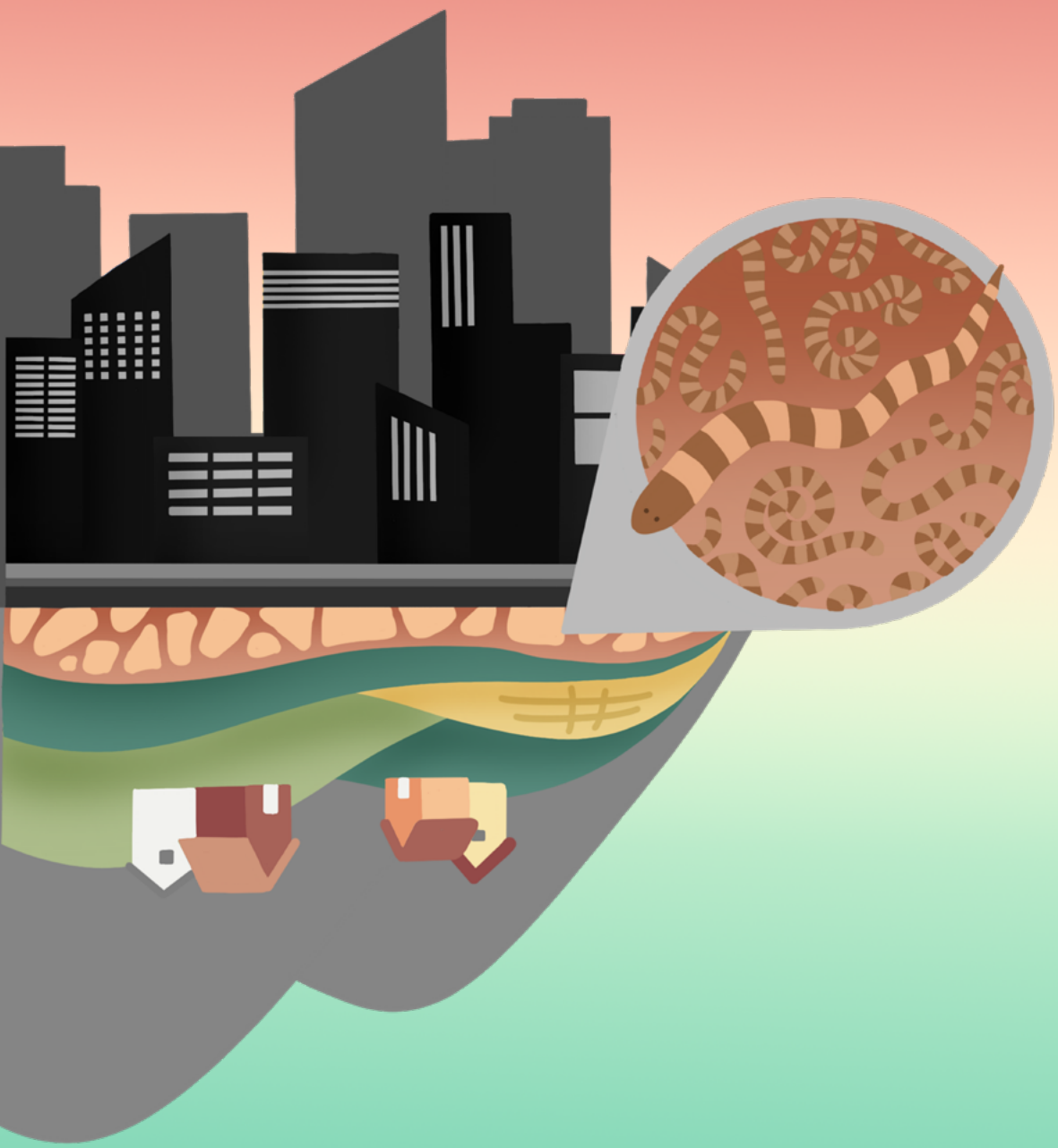

Chapter 2

EFFECT OF ANTHELMINTIC TREATMENT ON SERUM FREE IGF-1 AND IGFBP-3: A CLUSTER-RANDOMIZED-CONTROLLED TRIAL IN INDONESIA

Farid Kurniawan[1,2,3#], Dicky L. Tahapary[1,2#*], Karin de Ruiter[3], Em Yunir[1,2], Nienke R. Biermasz[4], Johannes WA. Smit[5,6], Taniawati Supali[7], Erliyani Sartono[3], Maria Yazdanbakhsh[3], Pradana Soewondo[1,2]

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ABSTRACT

In children, soil-transmitted helminth infections have been linked to poor nutritional status and growth retardation in association with lower levels of IGF-1. In adults, IGF-1 has an anabolic and metabolic function and is related to nutritional status. Here, we assessed the impact of helminth infection on free IGF-1 and its major binding protein, IGFBP-3, in adults. The levels of IGF-1 and IGFBP3 were measured in 1669 subjects aged ≥16 years, before and after receiving four rounds of albendazole 400 mg/day or matching placebo for three consecutive days. Helminth infection status was assessed by microscopy (Kato-Katz) and PCR. Serum free IGF-1 level was significantly lower in helminth-infected subjects [mean difference and 95%CI: -0.068 (-0.103;-0.033), P<0.001 after adjustment for age, sex, body mass index, and fasting insulin level]. There was no difference in IGFBP-3 level between helminth infected versus non-infected subjects. In the whole study population, albendazole treatment significantly increased serum free IGF-1 level [estimate and 95%CI: 0.031 (0.004;-0.057), P= 0.024] whereas no effect was found on the IGFBP-3 level. Our study showed that helminth infection in adults is associated with lower free IGF-1 levels but not with IGFBP-3 and albendazole treatment significantly increases free IGF-1 levels in the study population.

Clinical Trial Registration: https://www.isrctn.com/ISRCTN75636394.

INTRODUCTION

Soil-transmitted helminth (STH) infections are still highly prevalent, especially in low- to middle-income countries.[1] In 2010, more than 1,6 billion people were estimated to be infected with at least one of the three main STH species [*Ascaris lumbricoides*, hookworms (*Ancylostoma duodenale* and *Necator americanus*), and *Trichuris trichiura*]. [2] In Indonesia, overall, around 22% of the population were infected with *Ascaris*, 20% with hookworm, and 12% with *Trichuris*. The prevalence could be higher in certain region of Indonesia, especially in rural areas.[3] STH infections have been associated with adverse effects on health in children, such as malnutrition and growth disorders. [4] Conversely, in adults, STH infections have been associated with a lower insulin resistance (IR),[5] thus indicating a possible beneficial effect by counterbalancing modern health threats such as metabolic syndrome.[6]

Insulin-like growth factor 1 (IGF-1) is a hormone mainly produced by the liver under the influence of growth hormone (GH) from the pituitary gland. Most of circulating IGF-1 are bound to its six binding proteins with IGF-binding protein-3 (IGFBP-3) being the major one (>75%). Only IGF-1 in the free-form is considered biologically active.[7] IGF-I has important role on musculoskeletal growth and development,[8,9] cell proliferation,[10] and tissue repair,[11] through its binding to IGF-I receptors and activation of the Akt/protein kinase-B pathway.[12] Because of its structural homology with proinsulin,[13] IGF-1 also binds to the insulin receptor although with much lower affinity than insulin and exerts its metabolic properties.[14] These actions include promotes glucose uptake and transport in certain peripheral tissues,[15] modulates glycogen synthesis,[16] promotes fatty acid transport,[17] and regulates amino acid and glucose intestinal absorption.[18,19] Additionally, IGF-1 concentrations are dependent on nutritional state and decrease during calorie and protein restriction. [20] Inflammation could also significantly affect IGF-1 levels as IGF-1 values have been found to be reduced during systemic inflammation.[21]

As STH infections affect both nutritional and inflammatory status,[22] they could also affect IGF-1 concentration. A study in children has indeed shown that *Trichuris*-mediated dysentery syndrome was associated with lower IGF-1 levels and was related

to the observed growth disorder.[23] It is possible that STH infections also affect IGF-1 levels in adults. To address this, we conducted a study in the adult population living in an area endemic for helminth infection to evaluate the association between STH infections and free IGF-1 concentration as well as its main binding protein, IGFBP-3. Subsequently, to assess causality, we investigated the effect of anthelmintic treatment on serum free IGF-1 and IGFBP-3 levels to evaluate the effect of helminth infections on IGF system.

RESULTS

Study population

At baseline, there were 1669 subjects recruited in SUGARSPIN trial. After exclusion of 65 subjects (36 and 29 subjects from placebo and albendazole arms, respectively) with insufficient serum samples, a total of 1604 subjects were included in the analysis. At follow up time point, there were 1295 serum samples available for analysis because of incomplete follow up data and loss to follow up due to death, moving away from the study area, and refusal to continue participation in the study (see Consort Diagram in **Supplementary Fig. 1**).

Baseline characteristics for both treatment arms were similar. Majority of the study participants were female (61.8% vs 59.1%, for placebo and albendazole groups, respectively). Mean BMI in both genders were also similar between the two treatment arms (male: 21.8 vs. 21.9 kg/m2; female: 22.9 vs. 22.7 kg/m2, for placebo and albendazole groups, respectively). In addition, the levels of serum fasting insulin, free IGF-1, IGFBP-3, and hsCRP were also comparable between two groups. Based on microscopy results, at baseline 44.3% of the subjects in the placebo and 42.0% in the albendazole arms were infected with STH and when PCR was used for detection of parasites, again helminth infection prevalence was similar in the two arms (54.6% vs. 54.7% for placebo and albendazole arms, respectively). Proportion of subjects with single or multiple infections were also similar in both placebo and albendazole arms **(Table 1)**.

Table 1. Baseline characteristics.

	Placebo N=836	Albendazole N=768
Sex (n male, %)	319 (38.2)	308 (40.1)
Age (mean in years, SD)		
Male	44.45 (15.87)	42.91 (15.73)
Female	40.90 (15.21)	42.29 (15.63)
BMI (kg/m2) (mean, SD)		
Male	21.81 (3.81)	21.91 (3.58)
Female	22.90 (4.33)	22.73 (4.21)
Fasting insulin# (mU/L)	3.45 (3.22-3.73)	3.52 (3.27-3.79)
Free IGF-1# (ng/mL)	0.359 (0.337-0.382)	0.369 (0.345-0.395)
Proportion of free IGF-1 below detection limit (%, n/N)	35.9 (300/836)	35.8 (275/768)
IGFBP-3# (ng/mL)	424.75 (415.94-433.74)	423.73 (414.17-433.51)
hsCRP# (mg/L)	0.96 (0.89-1.04)	0.97 (0.90-1.06)
Helminth infection by microscopy (%infected, n/N)	44.3 (348/785)	42.0 (300/715)
Helminth infection by PCR (%infected, n/N)	54.6 (412/754)	54.7 (375/686)

#Non-normally distributed continuous variables, presented as geomean (95% CI)

BMI: body mass index; IGF-1: insulin-like growth factor-1; IGFBP-3: insulin-like growth factor binding protein-3

Baseline free IGF-1 and IGFBP-3 levels as a function of helminth infection

Male subjects had lower level of serum free IGF-1 compared with female subjects [mean difference (95% CI) −0.178 (−0.216; −0.138), P<0.001, **Fig. 1a**]. As expected, serum free IGF-1 levels decline with increasing age [−0.121 (−0.132; −0.109), P<0.001, **Fig. 1b**], but increased with increasing fasting insulin levels [0.087 (0.071; 0.104), P<0.001 in age, sex and BMI adjusted model, **Fig. 1c**]. In age and sex adjusted models, the levels of IGF-1 were also increased with increasing BMI [0.055 (0.038; 0.071), P<0.001, **Fig. 1d**].

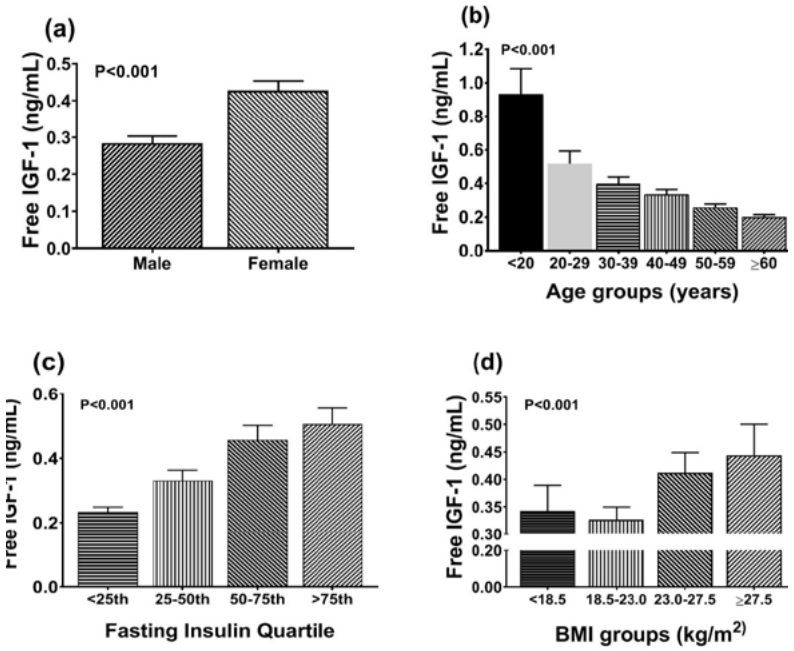

Figure 1. Serum free IGF-1 levels in males and females (A) and the association between free IGF-1 and -age groups (B), -fasting insulin quartiles (C), and -BMI groups (D). Serum free IGF-1 level was presented as geometric mean and its 95% confidence interval. Serum free IGF-1 and fasting insulin levels are log transformed for analysis. BMI grouping was based on WHO classification for Asian population.

When using PCR to detect helminth infection, we found a significantly lower serum free IGF-1 among STH-infected compared to uninfected subjects [−0.090 (−0.132; −0.048), P<0.001] and this did not change following adjustment with age and sex [−0.89 (−0.125; −0.053), P<0.001]. The mean differences, following adjustment with BMI [−0.078 (−0.115; −0.042), P<0.001] as well as a further adjustment with fasting insulin [−0.068 (−0.103; −0.033), P<0.001], were slightly attenuated (**Table 2a**). As inflammation is considered to be an important factor in assessing IGF-1 levels, we used hsCRP as a proxy for inflammation. Adjustment for hsCRP did not change the results, which still showed lower free IGF-1 levels in STH infected compared to uninfected groups [−0.071 (−0.107; −0.036), P<0.001)]. Regarding IGFBP-3, no differences were found between STH-infected and uninfected subjects [0.001 (−0.014; 0.015), P=0.937].

When helminth infection was detected based on microscopy, a less sensitive method that misses infection in 10.3% of the subjects, the difference in the level of free IGF-1 and IGFBP-3 between STH-infected and noninfected subjects fell short of statistical significance (**Table 2b**).

Interestingly, we also observed a decline in the levels of IGF-1 with increasing number of helminth infections, which was more pronounced when infection was detected by PCR [mean difference (95% CI) −0.044 (−0.064; −0.024), P<0.001 after adjustment for age, sex, BMI, and fasting insulin level) compared to by microscopy [−0.033 (−0.053; −0.013, P=0.001] (see also **Fig. 2a,b**).

Effect of anthelmintic treatment on serum free IGF-1 and IGFBP-3 levels

As reported in the main study,[24] 1 year of albendazole treatment significantly reduced the prevalence of helminth infections, either assessed by PCR (from 54.7% to 28.9%) or microscopy (from 43.2% to 20.2%) (**Supplementary Fig. 2)**.

In the whole study population, albendazole treatment resulted in an increase of serum free IGF-1 [estimate (95%CI): 0.031 (0.004; 0.057), P=0.024] but not IGFBP-3 levels [0.0001 (-0.0065; 0.0068), P=0.968] (Fig. 3). The treatment effect on serum free IGF-1 remained intact following adjustment with BMI [0.030 (0.003;0.057), P=0.028] or additional adjustment with fasting insulin [0.029 (0.002; 0.056), P=0.033].

When the population was stratified into helminth infected and uninfected at baseline to assess the effect of treatment in the two groups, no statistically significant treatment effect on free IGF-1 level was observed, either in the infected, [0.032 (-0.006; 0.070), P=0.084 when categorized by PCR, and 0.027 (-0.015; 0.069), P=0.07, when categorized by microscopy] or the uninfected groups [0.031 (0.092; 0.071), P=0.130 and 0.035 (-0.0004; 0.070), P=0.053; for PCR and microscopy, respectively].

Table 2. Association between free IGF-1 or IGFBP-3 with STH infection status based on PCR (a) and microscopy (b).

		STH-infected	STH non-infected	Un-adjusted	Adjusted for age and sex	Adjusted for age, sex, and BMI	Adjusted for age, sex, BMI, and fasting insulin
(a)	Free IGF-1 (ng/mL)	0.33 (0.31-0.35)	0.41 (0.38-0.44)	-0.090 (-0.132;-0.048), P<0.001	0.089 (-0.125;-0.053), P<0.001	-0.078 (-0.115;-0.042), P<0.001	-0.068 (-0.103;-0.033), P<0.001
	IGFBP-3 (ng/mL)	424.50 (415.38-433.81)	423.94 (413.59-434.54)	0.001 (-0.014; 0.015), P=0.937			
(b)	Free IGF-1 (ng/mL)	0.35 (0.33-0.38)	0.37 0.35-0.400	-0.027 (-0.069;-0.015), P=0.202	-0.042 (-0.078;-0.006), P=0.022	-0.033 (-0.068;-0.003), P=0.074	-0.031 (-0.066;-0.003), P=0.075
	IGFBP-3 (ng/mL)	422.67 (412.79-432.79)	424.66 (415.51-434.01)	-0.002 (-0.016; 0.012), P=0.777			

All variables are presented as geometric mean and its 95% confidence interval and were log transformed for analysis. Analysis for the difference between STH-infected and non- infected subjects was performed using linear regression and presented as estimated mean difference and its 95% confidence interval.
BMI: body mass index; IGF-1: insulin-like growth factor-1; IGFBP-3: insulin-like growth factor binding protein-3; STH: soil-transmitted helminth.

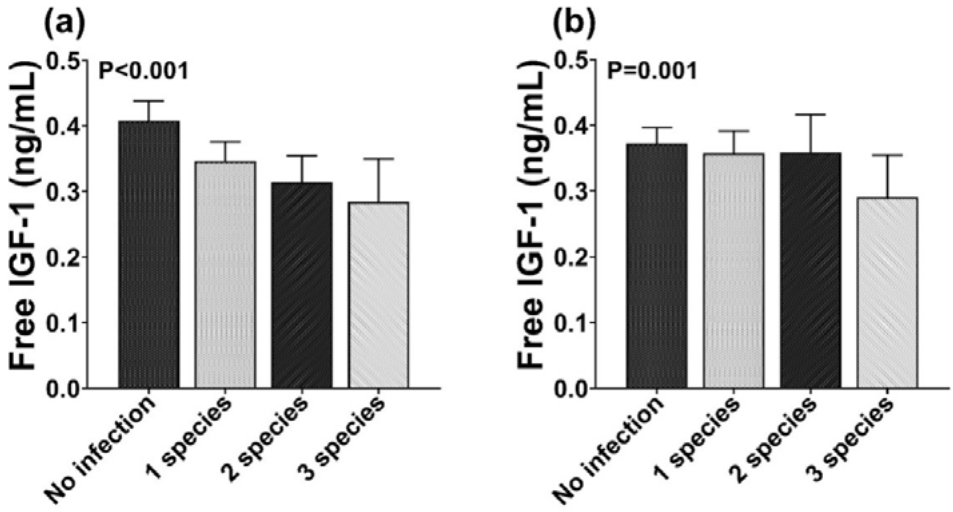

Figure 2. Serum free IGF-1 level based on the number of helminth species detected by PCR (a) and microscopy (b). Serum free IGF-1 level was presented as geometric mean and its 95% confidence interval and were log-transformed for analysis (N for PCR = 1140 subjects; N for microscopy = 1499 subjects).

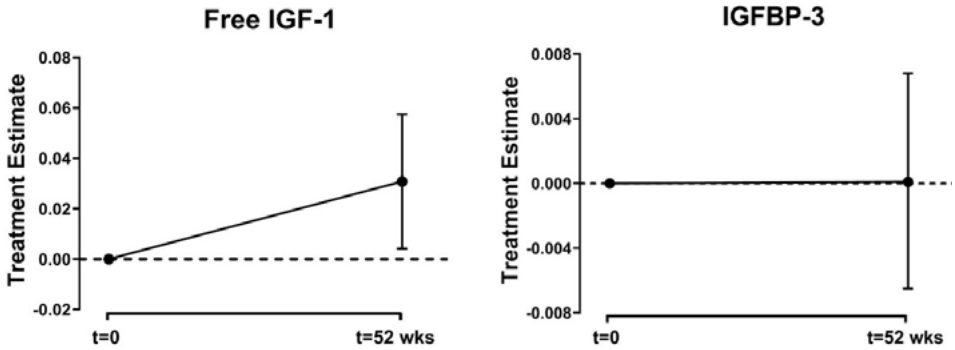

Figure 3. Effect of albendazole treatment on serum free IGF-1 and IGFBP-3 in the whole study population. The estimated treatment effect was obtained using linear mixed model and presented with its corresponding 95% confidence interval.

DISCUSSION

In this study, we observed a significantly lower serum free IGF-1, but not IGFBP-3 level, among STH-infected subjects. Intensive anthelmintic treatment significantly increased serum free IGF-1 level in the adult population studied.

The lower level of free IGF-1 in STH-infected compared to non-infected subjects observed in our study confirmed previous reports in children,[23,25] that showed a lower total IGF-1 level among helminth-infected subjects.

There are several possibilities as to how helminth infection could decrease serum free IGF-1 level. Firstly, calories and/or protein intake could be significantly affected by helminth infection.[26] IGF-1, as well as insulin, are anabolic hormones that promote energy storage and protein synthesis, especially in nutrient sufficient conditions.[27] Under a calorie or protein-restricted condition, a switch to catabolic state takes place by decreasing IGF-1 and insulin secretion with compensatory increased secretion of growth hormone (GH) and cortisol, to ensure sufficient substrates like glucose or fatty acids are available for basal metabolism.[28] It is known that under calorie or protein restricted condition, there is increasing GH but the IGF-1 levels remain low due to hepatic resistance against GH.[29] Thus, helminth infection could be seen as a model of long term calories and/or protein restriction in humans. Successful anthelmintic treatment might be considered as a way of nutrient uptake restitution.

The effect of helminth infection on lower free IGF-1 level seemed to be partly mediated through BMI, as adjustment for BMI, attenuated the differences between two groups although remaining significant. In line with previous studies,[5,21] our study also shows that helminth infection is associated with lower BMI and fat mass (data not shown), which in turn can be associated with lower adipose tissue mass. As human adipose tissue can secrete considerable amounts of IGF-1,[30,31] in helminth infected subjects, the lower adipose tissue mass can result in lower IGF-1 levels.

Another mechanism by which helminth infection could affect serum free IGF-1 levels might be mediated by insulin. A lower level of insulin in helminth infection could lead

to an increase in IGFBP-1 and -2 levels which eventually will result in a lower level of free IGF-1.[30,32] Our results, which show that, although remaining significant, there is attenuation of the mean differences on free IGF-1 level between STH-infected and non-infected groups after adjustment with fasting insulin level, supports this hypothesis.

Helminth infections have been reported to affect the immune system by shifting the phenotype of T cells towards the CD4+ T helper type 2 (TH2) cells and T regulatory (Treg) and reduction in TH1 phenotypes thereby, reduction in its associated pro-inflammatory responses.[33] As previous studies have shown that inflammation could significantly influence the IGF-1 level,[21] adjustment for hsCRP as an inflammatory marker was also performed in this study albeit no significant changes was observed in the differences between helminth infected and uninfected subjects. This is in line with the general concept that chronic helminth infections have no inflammatory but rather an anti-inflammatory impact[34] and shows that the effect of helminth infection on IGF-1 is not mediated by inflammation.

Previous animal and in-vitro studies have shown that IGF-1 could induce the secretion of interleukin(IL)-10 from immune cells.[35,36] However, the observed lower free IGF-1 level in our current study was not in line with the increase of IL-10 level usually observed during helminth infections due to activation of TH2 and Treg cells. Thus, it will be interesting to study the interaction between IGF-1 and IL-10 in helminth infected subjects.

Finally, several studies have shown that STH infections affect gut microbiota composition.[37] There is recent evidence that the gut microbiota can influence the production of IGF-1.[38] Therefore, it remains possible that STH infections might have their effect on serum free IGF-1 levels via modulation of the gut microbiota.

The observation regarding decreasing levels of IGF-1 with an increasing number of helminth infections in this study might suggest more disruption of nutrient intake, which in turn could directly or indirectly be linked to changes in the BMI and fasting insulin level.

In this study, we found a significant increase of serum free IGF-1 level in the whole population after albendazole treatment, even after adjustment for BMI or fasting insulin

levels. However, treatment effect on STH-infected and non-infected subjects seemed largely similar. These might suggest that albendazole has a broader effect than only on STH. It could affect the gut microbiome,[39] thus lead to the changes in IGF-1 levels after treatment. Additionally, intestinal protozoa, which have close interaction with gut microbiome,[40] could be significantly affected by albendazole treatment.[41] This might indirectly affect IGF-1 levels. Another reason for this finding might be because the SUGARSPIN trial was not originally designed for this study. From the original study result, we could observe that albendazole treatment significantly increased insulin resistance in helminth-infected but not in the helminth-uninfected subjects.[24] The complexity of the IGF system and the fact that insulin is also considered as a part of this system,[42] might explain the difference in our study findings compared to the original study.

This is the first human study that evaluated the effect of STH infection and the longitudinal assessment after anthelmintic therapy on serum levels of free IGF-1 and IGFBP-3 in adults. The relatively large number of study subjects and the randomized double-blind study design of anthelmintic treatment can be considered as the strength of this study. However, there are still some limitations. For example, measurement of IGFBP-1, GH and gut microbiome could have helped to get a clearer picture of the effect of STH infections and anthelmintic treatment on the whole IGF-system.

In conclusion, STH infections were associated with a significantly lower serum free IGF-1 level, which was partly explained by the lower BMI and a lower fasting insulin level but not inflammatory status. At population level, albendazole treatment increases serum free IGF-1 levels but not IGFBP-3. Further studies are needed to disentangle the complex mechanisms underlying the association between helminths and IGF-1 levels in adults.

MATERIAL AND METHODS

Study design

This present study was part of the SUGARSPIN trial, which has been described previously.[43] Briefly, we conducted a household-based cluster-randomized double blind anthelminthic trial in Flores Island, Indonesia, a region endemic for STH infections. The population was randomized using computer aided block randomization at household level, utilizing Random Allocation Software to assign treatment groups. After randomization, all subjects received three-monthly, of either a single dose of albendazole (400 mg) or matching placebo treatment for three consecutive days. This treatment regimen was given every three months for a total of four rounds.[43] Both study investigators and subjects were blinded to treatment codes. The treatment code was unblinded when all data needed for analysis were cleaned and entered into the database. The study was approved by the ethics committee of Faculty of Medicine, Universitas Indonesia (FKUI) (ref: 549/H2·F1/ETIK/2013), and filed by the ethics committee of Leiden University Medical Center (LUMC). The trial is registered as a clinical trial (***http://www.isrctn.com/ISRCTN75636394***). All participants in this study had signed the informed consent.

All measurements and sample collections were performed during the first 8 weeks before the start of the first treatment round (baseline) and 6 weeks after the end of the last treatment round (follow up). Clinical measurements and blood sample collections were performed after an overnight fast, as described previously. [19] Anthropometric measurements of body weight (SECA Model 876, Seca Gmbh Co, Hamburg, Germany) and height (SECA Model 213, Seca Gmbh Co, Hamburg, Germany,) were performed, of which body mass index (BMI) was then calculated.

Laboratory measurements

Quantification of serum free IGF-1 and IGFBP-3 was performed by enzyme-linked immunosorbent assays (ELISAs) using commercial reagents (DuoSet® ELISA R&D System Europe Ltd, Abingdon, UK). The standard range was 31.25-2000 pg/mL for free IGF-1 assay (CVa 5.5%) and 125-8000 pg/mL for IGFBP-3 assay (CVa 10.6%). For IGF-1, the level below detection limit of the assay is assigned a value of 0.15 ng/ml. Fasting

serum insulin was measured using a solid phase, enzyme-labeled chemiluminescent immunometric assay (Siemens IMMULITE 2000Xpi systems), with the measuring range of 2–300 mU/L (CVa <7% at all levels). A latex enhanced immunoturbidimetric method was used to measured high-sensitivity C-reactive protein (hsCRP) on Roche Modular P-instrumentation, the measuring range being 0.1-20.0 mg/L.

Identification of STH infection [hookworm (*Necator americanus, Ancylostoma duodenale), Ascaris lumbricoides, Trichuris trichiura, Strongyloides stercoralis*] was assessed using microscopy (Kato Katz) and PCR on stool samples as described elsewhere.[44]

Statistical analysis

Continuous variables with normal distribution were presented as mean and its standard deviation [mean (SD)]. Non-normally distributed data were presented as geometric mean and its 95% confidence interval [geomean (95%CI)] and were log-transformed for analysis.

We performed a linear regression on baseline data (IBM SPSS Statistics Version 23) to compare the differences in serum free IGF-1 and IGFBP-3 levels between STH-infected and uninfected subjects or between single vs. multiple helminth infections. These differences were presented as mean differences (95%CI) and P-value.

Meanwhile, to evaluate the effect of anthelminthic treatment on serum free IGF-1 and IGFBP-3 levels, linear mixed models [lme4 package (R software)] to account for the correlation within households were used, as described previously.[43] Two random effects were used: to model clustering within households, a random household specific intercept was used and to model correlation within subjects, a random subject-specific intercept was used. Treatment effect estimates and 95% confidence interval were reported, while P-value was generated from likelihood ratio test comparing the model with and without the treatment effect.

Data availability

The datasets generated and analyzed in the current study are available from the corresponding author on reasonable request.

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Author contributions

F.K. developed the study, performed ELISA measurement, analyzed the data, and wrote the paper. D.L.T. developed the study, supervised SUGARSPIN field study, and wrote the paper. K.R. supervised SUGARSPIN field study and data cleaning. E.Y. and N.R.B. contributed to writing the manuscript and advised on the metabolic aspect of the study. J.W.A.S. developed and also Dutch coordinator of the SUGARSPIN study. T.S. developed and also Indonesian coordinator of the SUGARSPIN study. E.S. developed and coordinated the study, advised on parasitological and immunological aspects of the study, supervised ELISA measurement and writing of the manuscript. M.Y. developed the study, supervised writing of the manuscript and the scientific coordinator of the SUGARSPIN study. P.S. supervised writing of the manuscript and advised on the metabolic aspect of the study.

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Competing interests

The authors declare no competing interests.

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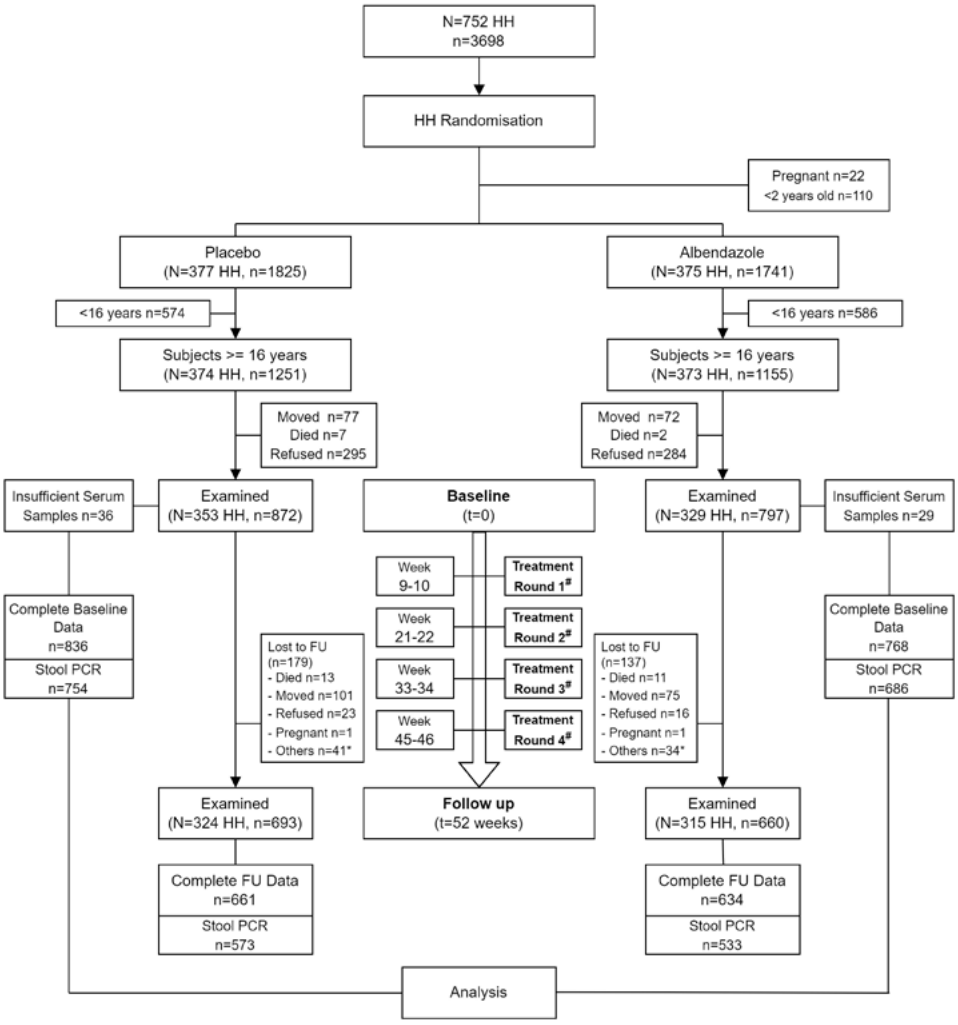
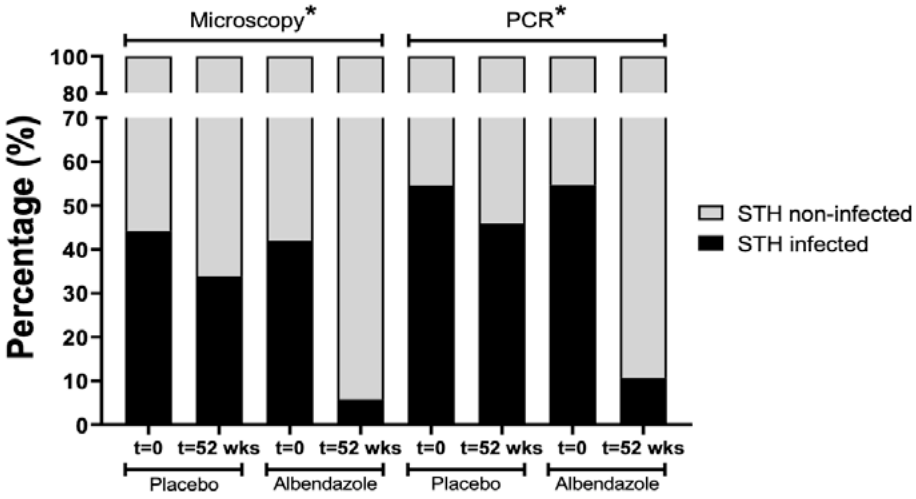
SUPPLEMENTARY MATERIALS



Supplementary Figure 1. Consort Diagram. 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 three consecutive days to all household members, except children below 2 years of age and pregnant women. *Other reasons of loss to follow up were harvesting cops, working on funeral ceremonies, severely ill, hospitalized, nursing mother. HH: Household; FU: Follow Up.



Supplementary Figure 2. The effect of albendazole treatment on the prevalence of helminth infection. Percentage of helminth infected subjects in the placebo and albendazole arms, as assessed by microscopy and polymerase chain reaction (PCR). p-values were calculated using a logistic model with random household effects and random subject effects.
*corresponds to P-value < 0.0001

Chapter 3

IMPACT OF RURAL-URBAN ENVIRONMENT ON METABOLIC PROFILE AND RESPONSE TO A 5-DAY HIGH-FAT DIET

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ABSTRACT

Epidemiological studies have indicated that rural living might be protective against type 2 diabetes development. We compared the metabolic profile and response to a short-term high-fat high-calorie diet (HFD) of men with the same genetic background living in an urban and rural area of Indonesia. First, we recruited 154 Floresian male subjects (18-65 years old), of whom 105 lived in a rural area (Flores) and 49 had migrated and lived in urban area (Jakarta) for more than 1 year. The urban group had significantly higher whole-body insulin resistance (IR), as assessed by homeostatic-model-assessment of IR (HOMA-IR), [mean difference (95%CI), p-value: 0.10 (0.02 – 0.17), p=0.010]. Next, we recruited 17 urban and 17 rural age-and-BMI-matched healthy-young-male volunteers for a 5-day HFD challenge. The HOMA-IR increased in both groups similarly [-0.77 (-2.03 - 0.49), p=0.223]. Neither rural living nor factors associated with rural living such as current helminth infection and total IgE were associated with protection against acute induction of IR by HFD.

INTRODUCTION

The prevalence of obesity and type 2 diabetes (T2D) is increasing worldwide, especially in low and middle-income countries (LMIC) that are currently facing rapid rate of urbanization.[1, 2] Rural-to-urban migration has indeed been shown to be associated with increased obesity and other cardiovascular (CV) risk factors, such as dyslipidemia and hypertension,[3-11] suggesting that living in rural environment might be protective against T2D development.

In addition to changes towards a sedentary lifestyle and increased dietary fat intake, migration to an urban environment is also associated with a reduction exposure to microorganism and parasites, such as helminth infections, which are still endemic in many rural areas of LMIC.[12] Recent data suggests that helminth infections might confer a protection against the development of obesity and T2D,[13-16] presumably by promoting type-2 and regulatory immune responses and subsequent reduction in systemic inflammation.[17-19] However, it is worth mentioning that the relative contribution of helminth infections in comparison to the more established factors such as a sedentary lifestyle and diet remains to be clarified.

Urban subjects have been reported to have longer sedentary periods and shorter active periods compared to those living in rural areas.[20] Furthermore, an increase in dietary fat intake, commonly observed upon rural-to-urban migration,[7,20] has been reported to be associated with impaired insulin resistance (IR) and glucose homeostasis.[21] Mice on high-fat diet (HFD) have provided models to study obesity and the development of IR.[22,23] Similarly, in humans, short-term HFD has been utilized to study the susceptibility to the development of IR.[24–28] Using this model, it has been possible to show how risk of IR is dependent on whether the participant is Caucasian or South Asian.[25,28] Short-term HFD has also been shown to induce organ-specific and systemic inflammation as evidenced by the increase in plasma cholesteryl ester transfer protein (CETP) levels,[24,29] predominantly produced by Kupffer cells (KC),[30] and plasma C-reactive protein (CRP) levels.[24]

Taken together, the chronic increase of energy rich diet, in addition to a more sedentary lifestyle, among people who migrate from a rural to urban areas,[20] might lead to the development of IR and T2D. However, there is still incomplete insight into the pathophysiology of the development of IR and T2D in rural-to-urban migration. In addition, there has been no study comparing the metabolic response towards a short-term HFD in terms of changes in glucose homeostasis and inflammation, between people living in urban and rural areas.

As some metabolic differences between individuals living in rural and urban area can be due to genetic differences, this study compared the metabolic profile between individuals with the same genetic background living in urban and rural areas. We also compared the metabolic and inflammatory response of individuals living in a rural and an urban area to a 5-day high-fat high-calorie (HFD) diet. Furthermore, since rural areas often go hand in hand with helminth infections and its associated IgE responses, we aimed to assess their contribution to metabolic profile. We hypothesized that individuals living in rural area would have a better metabolic profile and would be relatively more protected from the induction of IR and inflammation by the HFD compared to those living in an urban area.

RESULTS

The metabolic profile of rural and urban study participants.

The mean length of stay of urban subjects in Jakarta was 20.7 (range: 1-40) years. The differences in metabolic profile between subjects living in rural and urban are summarized in **Table 1**. Urban subjects had a significantly higher homeostatic model assessment (HOMA)-IR compared to rural subjects [1.45 (1.06 – 1.90) vs. 0.96 (0.80 – 1.13), respectively, p = 0.01]. Similarly, other metabolic parameters, such as 2-hour blood glucose, hemoglobin A1c (HbA1c), body mass index (BMI), waist circumference, and leptin level were significantly higher in urban subjects (**Table 1**). Interestingly, independent of age, increasing length of stay in urban area (in years) was positively associated with increasing BMI (in kg/m2) [estimate (95% CI), 0.15 (0.04 – 0.27), p = 0.01, **Figure 1A**], waist circumference (in cm) [0.45 (0.14 – 0.76) cm, p=0.006, **Figure 1B]** , but not HOMA-IR [0.005 (-0.003 – 0.013), p = 0.18]. Increasing length of stay in urban area was also associated with a trend of increase in leptin level (in ng/mL) [0.013 (-0.001 – 0.027), p = 0.07].

The prevalence of soil-transmitted helminth (STH) was significantly lower in the urban compared to rural subjects [5% (2/42) vs. 57% (52/92), respectively, $p < 0.0001$]. Similarly, the levels of total IgE, often driven by STH infections,[31] were lower in the urban compared to rural subjects (168 (105–271) IU/mL vs. 931 (702–1,235) IU/mL, respectively, $p < 0.0001$) (**Table 1**). As the number of subjects with current STH infections in urban area was very low ($n = 2$), it was not possible to assess the contribution of current STH infections to the HOMA-IR difference between urban and rural subjects. Therefore, we used the total IgE level as a proxy for past and current exposure to STH. The age-adjusted difference in HOMA-IR between urban and rural subjects was slightly attenuated [from estimated mean differences (95% CI), 0.09 (0.02–0.17), $p = 0.001$ to 0.08 (−0.00–0.17), $p = 0.06$] after further adjustment for total IgE level (**Table 2**). Moreover, adjustment for total IgE level also attenuated the age-adjusted difference in waist circumference [from 7.2 (2.0–11.3) cm, $p = 0.001$ to 4.2 (−0.5–8.8) cm, $p = 0.08$] and leptin level [from 0.36 (0.18–0.55) ng/mL, $p < 0.0001$ to 0.10 (−0.03–0.24) ng/mL, $p = 0.14$] (**Table 2**). When assessing the contribution of adiposity and leptin levels to the difference in HOMA-IR between urban and rural subjects, we observed that adjustment for waist circumference [to 0.02 (−0.04–0.08), $p = 0.55$] or both waist circumference and leptin level [to 0.01 (−0.06–0.07), $p = 0.77$] strongly attenuated the difference in HOMA-IR (**Table 2**).

Table 1. Comparison of metabolic profiles between subjects living in urban and rural area.

Variables	Urban (n=49)	Rural (n=105)
Duration in urban (in years)	20.7 (1.0-40.0)	-
Age (in years)	39.3 (13.5)	44.5 (12.2)*
HOMA-IR	1.45 (1.06 – 1.90)	0.96 (0.80 – 1.13)*
Fasting Insulin (mU/L)	4.9 (3.8 – 6.4)	3.1 (2.5 – 3.8)**
Fasting Blood Glucose (mmol/L)	5.7 (1.4)	5.4 (0.9)
2h-Blood Glucose (mmol/L)	7.7 (3.2)	5.9 (1.9)**
HbA1c# (mmol/L)	37.9 (14.3)	32.3 (6.6)*
HbA1c# (%)	5.6 (1.3)	5.1 (0.6)*
Body Mass Index (kg/m2)	24.3 (4.9)	22.7 (4.0)*
Waist Circumference (cm)	84.9 (13.8)	79.3 (11.9)*
Adiponectin (µg/mL)	4.38 (3.31 – 5.78)	3.54 (3.09 – 4.07)
Leptin (ng/mL)	5.62 (3.98 – 7.92)	2.64 (2.06 – 3.38)*
CRP (mg/L)	1.57 (1.17 – 2.05)	1.67 (1.29 – 2.11)
Total IgE (IU/mL)	168 (105 – 271)	931 (702 – 1,235)**
Prevalence of STH (%, n/N)	5 (2/42)	57 (52/92)**

All variables are presented as mean and its standard deviation, however, HOMA-IR, fasting insulin, adiponectin, leptin, CRP, and total IgE level are presented as geomean (95%CI) and were log transformed for analysis, while duration in urban is presented as mean (range). Analysis for the difference between urban and rural group was performed using independent t-test (*p<0.05, **p<0.0001) #Hba1c measurements were available in 42 and 95 of urban and rural subjects respectively. Abbreviation: HOMA-IR=the homeostatic model assessment of insulin resistance, CRP=C-reactive protein, STH=soil-transmitted helminth.

In addition, we stratified rural and urban subjects based on STH infection status into three groups, resulting in an urban group without STH infections, a rural group without STH infections, and a rural group with STH infections. The highest mean level of HOMA-IR, waist circumference, and leptin was observed in the urban group without STH infections, followed by the rural group without STH infections and the lowest among the rural group with STH infections (**Figure S1**). The opposite relationship was observed for total IgE level (**Figure S1)**.

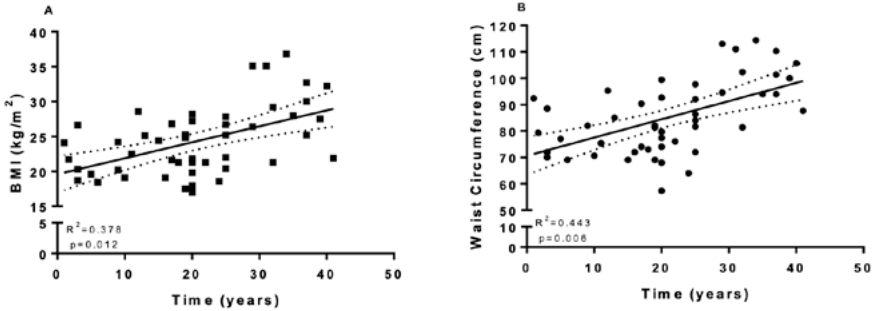

Figure 1. The association between length of stay in urban area with adiposity. The association between length of time in urban area with (A) body mass index (BMI) and (B) waist circumference are presented in scatter plot graphs (n=49), and analysed using age-adjusted linear regression. Each year increase of a time spent in urban area was associated with a significant increase in both (a) BMI [0.152 (0.036 – 0.269) kg/m^2, p=0.012] and (b) Waist Circumference [0.449 (0.135 – 0.762) cm, p=0.006].

Table 2. Associations between living in urban and rural area with HOMA-IR, leptin, and waist circumference.

Variables	Differences for each variable between urban and rural (rural group as the reference group)*					
	Crude	Model 1 (Age)	Model 2 (Age+Total IgE)	Model 3 (Age+Waist)	Model 4 (Age+Total IgE+Waist)	Model 5 (Age+Waist +Leptin)
HOMA-IR$	0.10 (0.02 – 0.17) p=0.010	0.09 (0.02 – 0.17) p=0.016	0.08 (-0.00 – 0.17) p=0.061	0.02 (-0.04 – 0.08) p=0.545	0.04 (-0.03 – 0.11) p=0.294	0.01 (-0.06 – 0.07) p=0.774
Leptin (ng/mL)$	0.33 (0.14 – 0.51) p=0.001	0.36 (0.18 – 0.55) p<0.0001	0.10 (-0.03 – 0.24) p=0.137	0.11 (-0.01 – 0.22) p=0.076	0.08 (-0.05 – 0.21) p=0.216	-
Waist Circumference (cm)	5.6 (1.3 – 9.9) p=0.010	7.2 (3.0 – 11.3) p=0.001	4.2 (-0.5 – 8.8) p=0.077	-	-	-

*Beta coefficient (95% CI) from linear regression. $HOMA-IR and leptin level were log transformed for analysis. Model 1: adjusted for age. Model 2: adjusted for model 1 plus total IgE level. Model 3: adjusted for model 1 plus waist circumference. Model 4: adjusted for model 2 plus waist circumference. Model 5: adjusted for model 3 plus leptin level. Abbreviation: HOMA-IR = the homeostatic model assessment of insulin resistance.

Comparison of metabolic responses after a short-term HFD intervention between subjects living in an urban and rural area

Among subjects who were included in the interventional part of the study (n = 34), we observed no significant differences between the age-and-BMI-matched urban (n = 17) and rural group (n = 17) in terms of HOMA-IR, adipose-IR index, CRP, and lipid levels at D-0 (Pre HFD). At this time point, serum CETP levels were significantly lower in the urban group [1.96 (0.58) μg/mL vs 2.59 (0.64) μg/mL, in urban and rural group respectively, p = 0.006]. Both groups showed a good compliance in terms of dietary intervention, all participants consumed all the cream provided and maintained their regular diet, resulting in a mean daily calorie intake that was ~60% higher compared to their regular diet, and ~56% of energy was derived from fat. The details of the dietary composition are shown in the **Supplementary Table S1**.

Intervention with a 5-day HFD resulted in a significant increase of HOMA-IR in both the urban [from 0.78 (0.51–1.09) to 1.13 (0.78–1.57), p = 0.03] and rural group [from 0.87 (0.59–1.21) to 1.69 (1.01–2.45), p = 0.001] (**Figure 2A, Table S1**), which was mainly driven by the increase in fasting insulin level in both urban [from 4.05 (2.98–5.52) mU/L to 5.59 (4.18–7.47) mU/L, p = 0.02] and rural group [from 4.63 (3.42–6.26) mU/L to 7.68 (5.70–10.34 mU/L), p = 0.001] (**Table S1**). Comparing the changes in IR before and after intervention between urban and rural group, we observed no significant differences for either HOMA-IR [estimated mean differences (95% CI), −0.77 (−1.95–0.41), p = 0.21] (**Figure 2A, Table S2**) or adipose-IR index [−41.2 (−115.1–32.7), p = 0.28] (**Figure 2B, Table S2**).

Interestingly, we observed a significant increase in CETP levels after HFD intervention in the urban group only [from 1.96 (0.58) μg/mL to 2.28 (0.63) μg/mL, p = 0.004 in urban group vs from 2.59 (0.64) μg/mL to 2.58 (0.72) μg/mL, p = 0.93 in rural group) (**Figure 2C**). Therefore, in comparison to the rural group, the increase in CETP level was significantly higher in urban group [0.33 (0.06–0.60) μg/mL, p = 0.02] (**Figure 2C, Table S2**). However, as indicated above, the CETP levels were already much higher in the rural group at D-0 (Pre HFD), even higher than the D-6 (post-HFD) CETP level in the urban group. Intervention with the HFD did not significantly increase CRP levels

in the two groups (**Table S2**). When assessing the effects of HFD on lipid levels, we observed no significant difference in changes in total cholesterol (TC), low-density lipoprotein cholesterol (LDL-C), and triglyceride (TG) levels between the urban and rural group, while the increase in high-density lipoprotein cholesterol (HDL-C) after intervention was significantly higher in the urban group compared to the rural group [0.09 (0.01–0.17) mmol/L, p = 0.04] (**Table S2**).

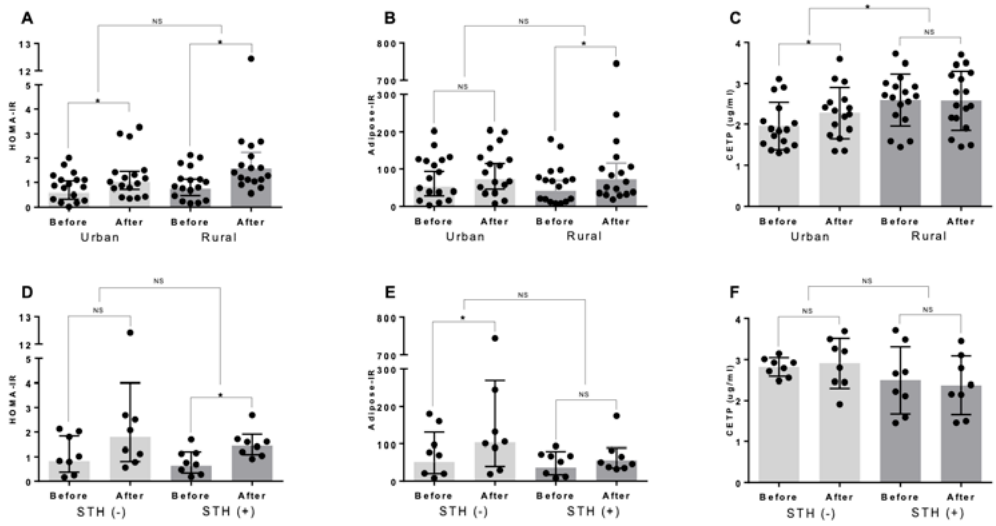

Figure 2. Comparison of metabolic responses to high-fat diet. HOMA-IR and adipose-IR index are presented as geometric mean and its corresponding 95% confidence interval, while CETP levels are presented as mean with its standard deviation. There were no significant differences in the increase of HOMA-IR (A), adipose-IR index (B) between urban and rural group, however, the increase in CETP level (C) was higher in the urban group. Furthermore, in rural group, there were no significant differences in the increase of HOMA-IR (D), adipose-IR index (E), and CETP level (F) between STH-infected and uninfected group. The difference between before and after intervention for each group was analysed using paired t-test, while the difference in the magnitude of changes for each parameter was analysed using linear mixed model (*p<0.05, NS: p>0.05).

The effect of current STH infections on the metabolic responses upon short-term HFD intervention.

Next, due to the very low prevalence of STH infections in the urban group [6% (1/17)], the effect of current STH infections on the metabolic response towards a short-term HFD intervention was only assessed in the rural group of which 50% was positive for STH infection (8/16). Thus, our study was underpowered (power of 56%) to detect any differences in metabolic responses between STH-infected and uninfected subjects.

Despite a significantly lower baseline body weight in STH-infected subjects in comparison to STH-uninfected subjects [51.1 (11.0) kg vs 63.3 (10.2) kg, p = 0.04], there was no significant difference in the magnitude of increase in HOMA-IR [−1.08 (−3.38–1.22), p = 0.36] (**Figure 2D**), adipose-IR [−87.8 (−222.1–46.4), p = 0.21] (**Figure 2E**), or CETP level [−0.21 (−0.62–0.20) μg/mL, p = 0.32] (**Figure 2F**) after HFD intervention. Interestingly, we observed a significantly higher increase in LDL-C level [0.27 (0.05–0.49) mmol/L, p = 0.03] after HFD intervention among STH-infected subjects in comparison to STH-uninfected subjects (**Table S3**). However, the level of LDL-C was much lower in the STH-infected group at D-0 in comparison to the STH-uninfected group [2.22 (0.31) mmol/L vs 2.97 (0.63) mmol/L, p = 0.01], and the level of LDL-C at D-6 in STH-infected group [2.44 (0.28) mmol/L] did not reach the level of LDL-C in the STH-uninfected group at D-0 (**Table S3**).

DISCUSSION

Our study showed that, in comparison to individuals living in a rural area, those living in an urban area had higher whole-body IR, as assessed by HOMA-IR, adiposity, and leptin levels. Whereas the higher whole-body IR was mainly mediated by the higher adiposity and leptin levels observed in urban individuals, differences in exposures to STH infection between urban and rural individuals, might contribute to a small extent to the differences observed in whole-body IR, adiposity or leptin levels. Intervention with a short-term HFD increased whole-body IR in both the urban and rural group. In comparison to the rural group, the CETP level was lower in the urban group, and the HFD intervention induced a stronger increase in CETP levels in this group. The presence of STH infections did not seem to have a

protective effect on the acute induction of IR from short-term HFD. However, it has to be noted that our study was underpowered to detect an effect of STH.

Our study found that the higher whole-body IR in individuals living in urban area was mediated by the higher adiposity, as well as a higher leptin level, a pro-inflammatory adipokine, which has been previously reported to be associated with glucose metabolism.[8,32] The increase of adiposity and, to a lesser extent, leptin level, was positively associated with the duration of time spent in the urban environment. This suggests that a higher degree of acculturation in terms of urban lifestyle, drifting away from their traditional lifestyle,[11] could lead to a positive energy balance,[20] hence increasing adiposity over time. In addition, reduced exposures to environmental factors, such as to STH infections, which have been shown to have beneficial metabolic effects[13] partly through the induction of type-2 and regulatory immune response,[18,19] might contribute to the difference in whole-body-IR, adiposity, and leptin level between urban and rural individuals. This was supported by our finding that the difference in whole-body IR, adiposity, and leptin level between urban and rural individuals was attenuated, but only slightly, after adjustment for total IgE level, a general marker for type-2 immune responses, and a proxy for past and current STH exposures.[31]

As expected, the overall metabolic profile of individuals living in a rural area, in term of adiposity and whole-body IR, was better in comparison to those living in an urban area. However, in contrast to our hypothesis, a short-term 5-day HFD intervention induced a similar increase of IR in both urban and rural individuals. As both groups were BMI-matched, these findings suggest that the direct protective metabolic effect of a combined past and current environmental exposures to helminths,[13] independent of their effect on adiposity, might be relatively weak in comparison to the strong induction of IR by the HFD intervention. Indeed, our group has recently reported that the increased IR in STH-infected subjects after deworming was mainly mediated by the increased adiposity.[16] Thus, adjusting for adiposity, in a way, remove the possible main pathway for STH-associated protection against the development for IR.

Although our study was underpowered to assess the effect of current STH

infection, it is possible that the presence of current STH infections might not be sufficient to protect against a strong induction of IR by short-term HFD, as in rural subjects, the increase in IR after HFD in STH-infected and STH-uninfected subjects was similar. However, it is also possible that the HFD intervention in STH-infected subjects with lower body weight would have a stronger impact than in STH-uninfected subjects, thereby masking any protective effects of STH infections.

Interestingly, we observed that the baseline serum CETP level was significantly lower in urban subjects. As CETP is mainly produced by KCs, higher CETP level may represent an increase in hepatic macrophage (KC) content, hence liver inflammation.[30] Also, environmental factors in the rural area, mainly exposure to various infectious agents, such as microorganisms and parasites, may explain the increased CETP level. For instance, it has been shown that subjects with chronic hepatitis C virus infection have elevated serum CETP levels.[33] Supporting this, the prevalence of hepatitis in our rural study area was higher than our urban study area (4.3% vs 0.8%).[34] However, currently, there are no available data connecting macrophage polarization status to CETP level and therefore further studies are needed.[35] In contrast to what is seen in urban subjects,[24,29] we found no increase in CETP levels in rural subjects after the HFD intervention. It is possible that the lack of an increase in CETP levels in rural subjects was caused by the already high baseline CETP levels, thus precluding its further increase after HFD intervention. Our results suggest an inflammation-independent mechanism of short-term HFD-associated induction of IR[23] as there was no significant increase in CRP following HFD. Studies on the role of inflammation in HFD-associated induction of IR have shown conflicting results. In one study, an increase in CRP and expression of M1 macrophage markers in skeletal muscle was reported,[24] while in another, no increase was seen in circulating pro-inflammatory cytokines.[36]

In terms of lipid levels, while no significant changes in lipid levels were observed in rural group, HFD intervention significantly increased HDL-C level in urban group. Our study and other observed that urban subjects had a relatively higher fat intake than rural subjects[20] at baseline. Thus, the relative difference in the changes of dietary composition before and after intervention[37–39] between

urban and rural individuals might potentially contribute to the difference in HDL-C level changes after intervention. In the rural group, we observed no significant changes in LDL-C in STH-uninfected subjects, whereas the HFD intervention resulted in a significant increase in LDL-C in STH-infected subjects. This might be related to the lower baseline LDL-C level and body weight in STH-infected subjects.

Our study is the first to compare the metabolic profile between people with the same genetic background, living in different environments (urban and rural) and to assess the metabolic responses to an intervention with a standardized short-term HFD. However, our study has several limitations. First, our study was only performed in male subjects, and potential differences in the outcomes might be observed in females. Next, due to the low prevalence of STH in urban area, our study could only assess the effect of current STH infections on HFD-induced IR in rural subjects. We also used a calculated HOMA-IR instead of the gold standard glycemic clamp to assess IR. In addition, there was no data available on physical activity, there were no biopsies of specific metabolic tissues (liver, muscle, adipose tissue), and we did not analyze the gut microbiota, all known to play an important role in metabolic profile and response.

In conclusion, in comparison to their rural ethnic counterparts, individuals living in an urban area had a higher whole-body IR, which was mainly mediated by their higher adiposity. The differences between urban and rural individuals in terms of past and current exposures to STH seem to have a relatively small contribution to the difference in whole-body IR. Contrary to our hypothesis, intervention with a short-term HFD induced similar increase in IR, in urban and rural individuals, and in helminth infected and uninfected subjects. However, well-powered larger studies are needed to determine which factors in terms of urbanization contribute to IR.

METHODS

Study Design and Population

The present study consisted of a cross-sectional and an interventional study. The cross-sectional study was performed in an urban (Jakarta) and a rural area (Nangapanda, Ende, Flores island) in Indonesia. We recruited 49 males (18-65

years old) with Floresian ethnic background who had migrated from Flores island and lived in Jakarta for more than 1 year (urban group). As their rural counterparts, we recruited 105 Floresian males with a similar age range, randomly selected from three villages in Nangapanda with age stratification, as described previously.[40]

For the HFD intervention study, 17 from urban and 17 from rural area, age-and-BMI-matched healthy young male volunteers (18-40 years old) were recruited via local healthcare workers who informed their community, in both Nangapanda and Jakarta, of the study. BMI-matching was performed to assess whether the difference between urban and rural in term of past or current exposure to STH infections affect the HFD-associated increase in IR, independent of adiposity. Exclusion criteria were T2D, recent body weight changes, intake of medication that could affect inflammation or IR.

The study was approved by the Medical Ethical Committee of the Faculty of Medicine, Universitas Indonesia (556/H2.F1/ETIK/2014) and performed in accordance with the principles of the revised Declaration of Helsinki. All volunteers gave written informed consent before participation.

Cross sectional Study

In the cross-sectional study, we invited all subjects to come to the Field Study Centre (FSC) in both rural and urban area to undergo clinical measurements and blood sample collections. Stool samples were also collected. All clinical measurements and blood sample collections were performed after an overnight fast. Anthropometric measurements of body weight, height, and waist circumference were performed. BMI was calculated as weight in kg divided by square of height in meter.

After collection of fasting blood samples, we performed an oral glucose tolerance test (OGTT), in which blood glucose levels were re-measured 2 hours after subjects were given 75g glucose dissolved in 200 mL of water (2h-BG). In this cross sectional study, we calculated HOMA-IR (homeostatic model assessment of insulin resistance), a well-validated measure of whole-body IR in humans (HOMA-IR = fasting serum insulin (mU/L) x fasting glucose (mmol/L) / 22.5),[41] as our primary outcome. We

also measured HbA1c, fasting blood glucose (FBG), fasting insulin, 2h-BG, BMI, waist circumference, adiponectin, leptin, high-sensitive C-reactive protein (hsCRP), total IgE, and prevalence of soil-transmitted helminths (STH) as our secondary outcomes.

Intervention Study

Subjects were examined before and after a 5-day HFD intervention, consisting of the subject's regular diet supplemented with 375 mL cream (Greenfields™ Whipping Cream, Greenfields Indonesia Ltd, Jakarta, Indonesia) per day [1,500 kcal/day, 83% fat (60% saturated fat)]. After baseline measurements, each subject received three bottles of 125 mL cream per day for five consecutive days. Subjects were instructed to continue their regular diet, and to consume one bottle of cream after each meal (3 meals per day) to make sure they could adhere to their regular dietary habits.

Subjects were asked to keep a food diary before and during the HFD intervention to estimate normal dietary intake and to check for compliance and compensatory behavior. Dietary assessment, using a 24 hours food recall, was performed by a trained dietician. Compliance was further assessed by interviewing the subject and collecting the bottles every day. During the study, subjects were asked not to change lifestyle habits. Measurements of clinical parameters and blood drawing were done on the day before starting the HFD intervention (D-0) and one day after the fifth day of the HFD intervention (D-6).

In this intervention study, we had HOMA-IR as our primary outcome. As our secondary outcomes, we measured adipose-IR index, a measure of adipose tissue IR, which was calculated as the product of the fasting serum free fatty acid (FFA) and insulin (Adipose-IR index = FFA[mM] x Insulin [pM]).[42,43] In addition, we also measured hsCRP, CETP, and lipid levels (TC, HDL-C, TG, LDL-C). Due to limited amount of sera after intervention, adiponectin and leptin level were measured only at baseline. All others measurements for the interventional study were performed pairwise (before and after intervention).

Laboratory measurements

Fasting blood glucose and 2h-post-load glucose were measured in capillary blood using Breeze®2 glucose meters (Bayer Health Care LLC, Basel, Switzerland) in the FSC. All sera, plasma and whole blood samples from rural area were frozen at −20°C in the FSC, and subsequently shipped and stored at −80°C in Faculty of Medicine Universitas Indonesia (FKUI), Jakarta, Indonesia and Leiden University Medical Centre (LUMC), Leiden, The Netherlands. All sera, plasma and whole blood samples from urban area were directly transported from FSC (Jakarta) to be stored at −80°C in FKUI, and subsequently shipped and stored at −80°C at LUMC.

Serum insulin concentrations were determined by a solid-phase, enzyme-labelled chemiluminescent immunometric assay, while HbA1c was measured using a cation-exchange chromatography (IC)-based high performance liquid chromatography (HPLC) assay. A latex-enhanced immunoturbidimetric method was used to measure hsCRP. Assays of TC, HDL-C, and TG were based on enzymatic colorimetric methods. These measurements have been described previously.[16]

Plasma CETP levels were measured with enzyme-linked immunosorbent assays (ELISA) kits according to the manufacturer's instructions (DAIICHI CETP ELISA, Daiichi, Tokyo, Japan). FFA were measured using ELISA kits according to the manufacturer's instructions (abcam ab 65341 FFA Quantification Assay Kit, Cambridge, UK). Adiponectin and leptin were also measured by using ELISA commercial reagents (DuoSet ELISA R&D System Europe Ltd, Abingdon, UK). The inter- and intra-assay coefficients of variance (CV) of adiponectin were 3.1% and 7.0% respectively. While for leptin, the inter- and intra-assay CV were 2.2% and 3.2%, respectively. The levels of total IgE, an important determinant of helminth infection,[31] were measured using ELISA as described previously.[44] The presence of STH [hookworm (*Necator americanus, Ancylostoma duodenale*), *Ascaris lumbricoides, Trichuris trichiura, Strongyloides stercoralis]* was assessed using PCR as described in detail elsewhere.[44,45]

Statistical Analysis

Normally distributed continuous variables were summarized as mean and standard deviation [mean (SD)], while non-normally distributed data were summarized

as geometric mean and its 95% confidence interval [geomean (95% CI)]. Based on previous studies,[14,25] we aimed to recruit 45 subjects from each urban and rural area for the cross-sectional study, while for the interventional study we aimed to recruit 15 subjects from each group (see **Supplementary Material**).

The original plan for the linear regressions was based on a conceptual framework (**Figure 3**) of the proposed causal pathways. In the cross-sectional study (A), we assessed whether the difference between urban and rural subjects, in term of past or current exposure to STH, by using total IgE level as a proxy, contributes to the difference in insulin resistance (IR) between subjects living in urban and rural area, and whether this difference in IR is independent from adiposity, by performing mediation analysis. Next, we further stratified the urban and rural group based on their STH infection status (see **Supplementary Material**). In addition, we also assessed the association between length of stay in urban area and metabolic profiles (IR, adiposity, and leptin) among subjects living in urban area using age-adjusted linear regression model.

In the HDF intervention study (B), first, we assessed whether the difference between urban and rural in term of past or current exposure to STH infections affect the HFD-associated increase in IR, independent of adiposity, by matching both groups for BMI. Next, among subjects living in rural area, similar model was used to further assess whether the presence of current STH infections protect against the HFD-associated increase in IR. The mixed model analysis was performed using R software (lme4).

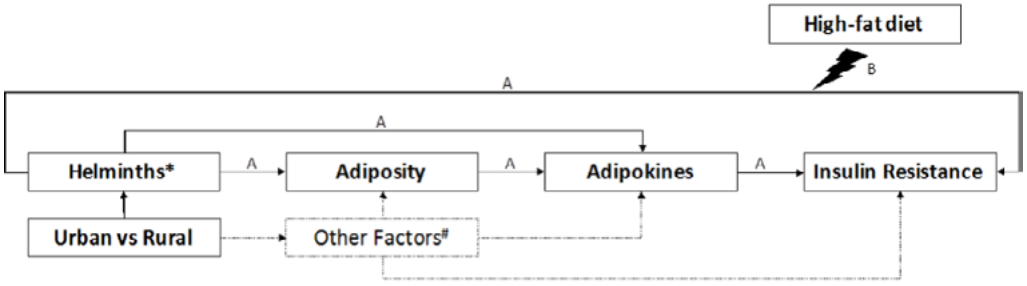

Figure 3. Conceptual framework. In the cross-sectional study (A), we assessed whether the differences in past or current exposure to helminths contribute to the difference in insulin resistance (IR) between subjects living in urban and rural area, and whether the observed difference in IR is independent from adiposity. In the high-fat diet (HFD) study (B), first, we assessed whether past or current exposure to helminths protect against the HFD-associated increase in IR, independent of adiposity. Next, we also assessed whether the presence of current helminth infection protect against the HFD-associated increase in IR. *Past and current exposure to helminths was assessed by measuring serum total IgE level, a general marker for Th2 responses, commonly induced by soil-transmitted helminth (STH). **Current exposure to helminths was assessed using stool PCR. #Other factors that were not specifically assessed in this study.

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Author Contributions Statement

Conception or study design: D.L.T., K.R., P.C.N.R., E.S., P.S., D.S.H., J.W.A.S., M.Y. Sample collection, data analysis: D.L.T., K.R., F.K., Y.D., Y.W., S.M.E.N., E.I., D.M., E.Y., P.C.N.R., E.S., J.W.A.S., M.Y. Interpretation of data: D.L.T., K.R., F.K., Y.D., Y.W., S.M.E.N., E.I., T.S., E.Y., B.G., P.C.N.R., E.S., P.S., D.S.H., J.WA.S., M.Y. Drafting the manuscript: D.L.T., E.S., M.Y., K.R. Revising the manuscript critically for important intellectual content; D.L.T., K.R., F.K., Y.D., Y.W., S.M.E.N., E.I., D.M., E.Y., B.G., T.S., P.C.N.R., E.S., P.S., D.S.H., J.W.A.S., M.Y. Principal investigator of this study: D.S.H. Scientific coordinator of this study: P.S., J.W.A.S., M.Y. All authors have given approval for publication.

Competing Interests

All authors declare no competing interests. The funder of the study had no role in study design, data collection, data analysis, data interpretation, or writing of the report.

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SUPPLEMENTARY MATERIALS

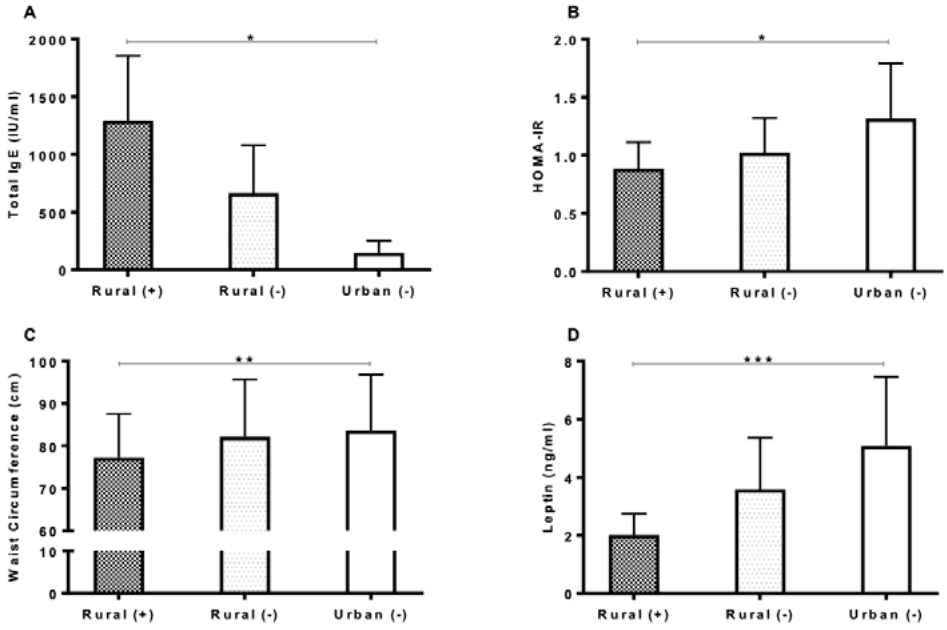

Figure S1. Comparison of metabolic profile between urban participants and rural participants (rural participants stratified by helminth infection status). The levels of total IgE, HOMA-IR, waist circumference, and leptin on different group of living area and soil-transmitted helminth (STH) infection status are presented as geometric mean and its 95% confidence interval, except for waist circumference which are presented as mean and its standard deviation. The number of urban subjects with helminth infections was very low (2/42) and was not included in this graph. Trend analysis was performed between three groups, namely: (1) rural subjects with STH infections [Rural (+)], (2) rural subjects without STH infections [Rural (-)], and (3) urban subjects without STH infections [Urban (-)]. Total IgE level was the lowest in Urban (-) group and progressively become higher in Rural (-) and Rural (+) groups (**A**). The contrary was observed for HOMA-IR (**B**), waist circumference (**C**), leptin level (**D**). *p<0.05 in unadjusted model, **p<0.05 in age-adjusted model, ***p<0.05 in age-waist circumference-adjusted model.

Table S1. Comparison of dietary compositions between urban and rural participants.

	Urban		Rural	
	Pre-HFD*	**HFD**	**Pre-HFD***	**HFD**
Energy (kcal)	1617.6 (78.2)	2533.0 (124.0)	1890.3 (433.7)	2822.6 (248.6)
Fat (g)	51.8 (23.4)	161.2 (7.5)	50.7 (25.1)	173.9 (13.9)
Carbohydrate (g)	222.1 (69.3)	200.1 (49.6)	268.9 (66.4)	220.2 (32.3)
Protein (g)	67.2 (9.1)	68.8 (5.6)	78.0 (24.3)	81.5 (16.8)
Fiber (g)	9.4 (3.9)	9.5 (1.5)	10.0 (3.1)	11.3 (2.3)

*At baseline, the proportion of energy derived from fat, carbohydrate, and protein for subjects living in urban area were 29%, 55%, and 16% respectively, while for subjects living in rural area the percentages were 24%, 58%, and 18% respectively.

Table S2. Comparison of metabolic responses towards a short-term HFD between subjects living in an urban and rural area.

	Urban n=17			Rural n=17			Comparison of the magnitude of changes between urban and rural subjects**	
Variables	**Pre HFD**	**Post HFD**	**p-value***	**Pre HFD**	**Post HFD**	**p-value***	**Estimated differences**	**p-value**
Age (years)	30.1 (6.4)	-	-	29.5 (8.0)	-	-	-	-
Body Mass Index (kg/m2)	23.1 (4.7)	-	-	21.6 (3.6)	-	-	-	-
HOMA-IR	0.78 (0.51 – 1.09)	1.13 (0.78 – 1.57)	**0.03**	0.87 (0.59 – 1.21)	1.69 (1.01 – 2.45)	**0.001**	-0.77 (-1.95 – 0.41)	0.21
Fasting Blood Glucose (mmol/L)	5.15 (0.44)	5.23 (0.50)	0.59	4.96 (0.19)	5.46 (0.73)	**0.005**	-0.42 (-0.82 - -0.03)	**0.04**
Fasting Insulin (mU/L)	4.05 (2.98 – 5.52)	5.59 (4.18 - 7.47)	**0.02**	4.63 (3.42 – 6.26)	7.68 (5.70 – 10.34)	**0.001**	-2.35 (-6.55 – 1.84)	0.28
Adipose-IR Index	51.6 (28.5 – 93.3)	71.9 (45.0 – 114.7)	0.23	40.5 (24.0 – 68.4)	72.0 (44.8 – 115.7)	**0.006**	-41.2 (-115.1 – 32.7)	0.28
Free Fatty Acid (mmol/L)	3.93 (3.37 – 4.59)	3.43 (2.91 – 4.04)	0.29	2.83 (2.42 – 3.30)	2.65 (2.27 – 3.09)	0.39	-0.32 (-1.24 – 0.59)	0.49
CETP (µg/mL)#	1.96 (0.58)	2.28 (0.63)	**0.004**	2.59 (0.64)	2.58 (0.72)	0.93	0.33 (0.06 – 0.60)	**0.02**
CRP (mg/L)	2.04 (0.94 – 4.74)	2.24 (1.21 – 3.75)	0.82	1.14 (0.59 – 1.89)	1.06 (0.58 – 1.71)	0.81	-0.31 (-3.61 – 2.98)	0.85
Total Cholesterol (mg/dL)	4.11 (0.61)	4.13 (0.59)	0.84	4.38 (0.61)	4.42 (0.71)	0.65	-0.02 (-0.29 – 0.24)	0.87
Triglyceride (mg/dL)	1.29 (0.53)	1.31 (0.54)	0.83	1.35 (0.36)	1.24 (0.43)	0.37	0.14 (-0.17 – 0.44)	0.38
HDL-C (mg/dL)	1.08 (0.25)	1.18 (0.23)	**0.01**	1.19 (0.25)	1.20 (0.24)	0.60	0.09 (0.01 – 0.17)	**0.04**
LDL-C (mg/dL)	2.44 (0.59)	2.35 (0.570	0.251	2.57 (0.61)	2.65 (0.59)	0.21	-0.17 (-0.36 – 0.01)	0.08

All variables are presented as mean and its standard deviation, however, HOMA-IR, Fasting Insulin, Adipose-IR Index, Free Fatty Acid, and CRP levels are presented as geomean (95%CI). *The differences between before and after HFD diet intervention were analysed using paired t-test. **The difference in changes (before and after HFD) of different parameters between urban and rural were analysed using linear mixed model and are presented as [Estimated Differences in Changes (95%CI), p-value]. #CETP measurements were only available for 33 subjects. Abbreviation: HOMA-IR= homeostatic model assessment of insulin resistance, CETP= cholesteryl ester transfer protein, CRP= C-reactive protein, HDL-C= high-density lipoprotein cholesterol, LDL-C= low-density lipoprotein cholesterol.

Table S3. Comparison of metabolic responses towards a short-term HFHC diet between STH-infected and uninfected subjects living in rural area.

	STH-infected n=8			STH-infected n=8			Comparison of the magnitude of changes between STH-infected and STH-uninfected subjects**	
Variables	**Pre HFD**	**Post HFD**	**p-value***	**Pre HFD**	**Post HFD**	**p-value***	**Estimated differences**	**p-value***
Age (years)	27.0 (9.6)	-	-	32.0 (6.3)	-	-	-	-
Body Mass Index (kg/m2)	20.1 (3.5)	-	-	23.1 (2.4)	-	-	-	-
HOMA-IR	0.73 (0.37 – 1.19)	1.47 (1.08 – 1.93)	**0.002**	1.00 (0.45 – 1.75)	2.03 (0.72 – 4.34)	**0.06**	-1.08 (-3.38 – 1.22)	0.36
Fasting Blood Glucose (mmol/L)	5.03 (0.22)	5.69 (0.88)	**0.04**	4.90 (0.17)	5.26 (0.57)	0.13	0.30 (-0.30 – 0.90)	0.34
Fasting Insulin (mU/L)	4.04 (2.60 – 6.29)	6.80 (5.17 – 8.95)	**0.008**	5.12 (2.89 – 9.08)	8.97 (4.69 – 17.17)	**0.04**	-3.74 (-11.67 – 4.18)	0.36
Adipose-IR Index	36.6 (16.9 – 79.6)	55.8 (34.9 – 89.0)	0.22	51.7 (20.4 – 73.4)	103.2 (39.6 – 268.8)	**0.02**	-87.8 (-222.1 – 46.4)	0.21
Free Fatty Acid (mmol/L)	2.88 (2.41 – 3.45)	2.44 (1.94 – 3.09)	0.21	3.03 (2.42 – 3.83)	2.99 (2.34 – 3.81)	0.87	-0.37 (-1.18 – 0.44)	0.37
CETP (µg/mL)	2.49 (0.82)	2.38 (0.72)	0.46	2.82 (0.23)	2.91 (0.61)	0.59	-0.21 (-0.62 – 0.20)	0.32
CRP (mg/L)	0.96 (0.28 – 2.00)	1.60 (1.48 – 3.57)	0.13	1.32 (0.29 – 3.17)	0.71 (0.39 – 1.11)	0.25	2.27 (-0.63 – 5.18)	0.13
Total Cholesterol (mg/dL)	4.07 (0.17)	4.23 (0.33)	0.20	4.77 (0.68)	4.70 (0.90)	0.69	0.23 (-0.12 – 0.58	0.21
Triglyceride (mg/dL)	1.32 (0.28)	1.14 (0.46)	0.40	1.40 (0.46)	1.38 (0.41)	0.92	-0.16 (-0.67 – 0.35)	0.54
HDL-C (mg/dL)	1.24 (0.25)	1.27 (0.28)	0.50	1.17 (0.26)	1.16 (0.20)	0.88	0.03 (-0.07 – 0.13)	0.54
LDL-C (mg/dL)	2.22 (0.31)	2.44 (0.28)	**0.01**	2.97 (0.63)	2.92 (0.74)	0.65	0.27 (0.05 – 0.49)	**0.03**

All variables are presented as mean and its standard deviation, however, HOMA-IR, Fasting Insulin, Adipose-IR Index, Free Fatty Acid, and CRP levels are presented as geomean (95%CI). *The differences between before and after HFD intervention were analysed using paired t-test. **The difference in changes (before and after HFD) of different parameters between STH-infected and STH-uninfected were analysed using linear mixed model and are presented as [Estimated Differences in Changes (95%CI), p-value]. Abbreviation: HOMA-IR= homeostatic model assessment of insulin resistance, CETP= cholesteryl ester transfer protein, CRP= C-reactive protein, HDL-C= high-density lipoprotein cholesterol, LDL-C= low-density lipoprotein cholesterol.

Supplementary Methods

Statistical Analysis

In the cross-sectional study, sample size was calculated to aim at a difference in HOMA-IR between urban and rural group of 0.5. The SD of HOMA-IR from previous study was 0.84. We used a significance level of 5% and a power of 80%, thus we needed at least 45 subjects for each group. For the interventional study, sample size was calculated to aim at a difference in changes of HOMA-IR between urban and rural group of 0.70. The SD of the HOMA-IR changes after HFD intervention from previous study was 0.68.2 We used a significance level of 5% and a power of 80%, thus we needed at least 15 subjects per group or 30 subjects in total. Next, to assess STH effect on the metabolic response upon HFD intervention we used similar calculation, aiming at having at least 15 subjects per group.

For the cross-sectional study, we further stratified the urban and rural group based on their STH infection status. However, as the number of urban subjects with STH infections was very low and therefore was excluded from analysis, eventually we had three groups: rural subjects with STH infections, rural subjects without STH infections, and urban subjects without STH infections. We calculated variance inflation factors (VIFs) to check multicollinearity in our regression models and VIF values below 4 were considered appropriate. Due to multicollinearity between BMI and WC, we used WC as clinical marker for adiposity. Analyses were performed using IBM Statistics 23.

For the HFD intervention study, to compare the parameter before and after the HFD intervention for each group, whenever appropriate, paired t-test or Wilcoxon-signed ranked test was performed. A mixed model was applied to assess mean differences before and after intervention between group. Groups were modelled as fixed effects, and to model correlation within subjects, random-specific intercept was used.

Chapter 4

URBANIZATION AND UNFAVORABLE CHANGES IN METABOLIC PROFILES: A PROSPECTIVE COHORT STUDY OF INDONESIAN YOUNG ADULTS

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ABSTRACT

The substantial increase in the prevalence of non-communicable diseases in Indonesia might be driven by rapid socio-economic development through urbanization. Here, we carried out a longitudinal study to evaluate the effect of urbanization, an important determinant of health, on metabolic profiles of young Indonesian adults. University freshmen/women in Jakarta, aged 16-25 years, who either had recently migrated from rural areas or originated from urban settings were studied. Anthropometry, dietary intake, and physical activity, as well as fasting blood glucose and insulin, leptin, and adiponectin were measured at baseline and repeated at one year follow-up. At baseline, 106 urban and 83 rural subjects were recruited, of which 81 urban and 66 rural were followed up. At baseline, rural subjects had better adiposity profiles, whole-body insulin resistance, and adipokine levels compared to their urban counterparts. After 1-year, rural subjects experienced an almost twice higher increase in BMI than urban subjects [estimate (95%CI): 1.23 (0.94;1.52) and 0.69 (0.43;0.95) for rural and urban subjects, respectively, P_{int} <0.01]. Fat intake served as the major dietary component, which partially mediates the differences in BMI between urban and rural group at baseline. It also contributes to the changes in BMI over time for both groups, although it does not explain the enhanced gain of BMI in rural subjects. A significantly higher increase of leptin/adiponectin ratio was also seen in rural subjects after 1-year of living in urban area. In conclusion, urbanization was associated with less favorable changes in adiposity and adipokine profiles in a population of young Indonesian adults.

INTRODUCTION

As a low-middle income country, Indonesia is facing two major health problems. On the one hand, an increasing prevalence of non-communicable diseases such as cardiovascular diseases (CVD), obesity, and type 2 diabetes (T2D) is becoming rampant. While on the other, infectious diseases such as helminth infections, malaria, and tuberculosis are still highly prevalent in some rural areas of Indonesia, resulting in stark differences of these disease patterns between urban and rural settings.[1,2]

People residing in urban areas are characterized by relatively high caloric and fat intake compared to their rural counterparts.[3] Moreover, urban people tend to be less physically active.[4] These factors can cause a disruption in energy homeostasis, with a surplus stored in the body as fat.[5] Increasing body fat increases the chance of obesity.[6] Previous studies have shown that higher adipose tissue mass is associated with higher inflammation and insulin resistance,[7] which eventually could lead to T2D[8] and CVD.[9]

Rapid socio-economic development in Indonesia has promoted the migration of people from rural to urban areas to seek a better life.[10] Previous studies have shown that urbanization is associated with new environmental and lifestyle changes that have the potential to put rural individuals at risk of deteriorating metabolic health.[11-13] The limitation of these previous studies evaluating the effect of urban-rural environment on metabolic health is their cross-sectional design, which lacks the power to show causality.

The worldwide increase of obesity is not only observed in older populations but also in young adults.[14] Based on the Indonesian National Basic Health Survey 2018, there is a high burden of obesity and prediabetes in the young adult population. [15] As this population constitutes a significant proportion of Indonesians,[16] the increase in the prevalence of these diseases would become a major health burden.

Early problem identification and intervention targeted towards these economically active young adult population in the context of metabolic health could have a great impact on decreasing the incidence rate, or even lowering the prevalence of non-communicable diseases. To this end, we conducted a prospective cohort study to assess the effect of urbanization over time and its contributing factors on the metabolic health profiles of the Indonesian young adult population.

METHODS

Study Design and Population

This prospective cohort study was conducted on the Depok campus of the University of Indonesia (UI). Freshmen/women UI bachelor students were recruited in this study. Baseline data were collected in the first three months of the start of the academic year, between August-November 2018, while the follow-up sample collection was performed one year later. Subjects' recruitment was started by providing information about the study during the medical examination of newly arrived students, via social media, and by spreading flyers/leaflets after classes, as well as in student dormitories. A short interview was performed to collect information regarding the areas where the students originated from. Afterwards, a detailed explanation of the study was given to the subjects who agreed to participate and fulfilled the criteria set in this study. After written informed consent, subjects were invited to visit the Makara UI Satellite Clinic to undergo clinical assessment, measurements, and blood sampling. The subjects were classified into urban group if they were born and lived in urban areas, such as in Jakarta metropolitan areas or in one of the provincial capital cities. The rural group comprised of subjects that were originally born and lived in rural areas, defined as the villages that are located at the district levels across Indonesia. Pregnancy and students with previously known diabetes, prediabetes, severe liver or kidney dysfunction, cardiovascular, and autoimmune diseases were excluded from the study. This study was approved by the Ethical Committee of Faculty of Medicine Universitas Indonesia (No. 1181/UN2.F1/ETIK/2017).

Anthropometric Measurements

Body height was measured using a portable stadiometer (SECA Model 213, Seca Gmbh Co., Hamburg, Germany), while body weight and body composition were measured using a Tanita body impedance analyzer (TBF-300A, Tanita Corp, Tokyo, Japan). Body mass index (BMI) was calculated in kg divided by squared height in meters. Three measurements of waist circumference were taken for each subject using an ergonomic circumference measuring tape (SECA Model 201, Seca Gmbh Co., Hamburg, Germany) and according to WHO standardized protocol. The average of all three measurements was then used for analysis.

Fasting Blood Glucose, HbA1c, Fasting Insulin, and HOMA-IR Measurement.

All clinical measurements and blood samples collection were performed after overnight fasting. Finger prick blood was used for measurement of fasting blood glucose (Accu-Check Performa, Roche Diagnostic GmBH, Germany) and HbA1c (A1c EZ 2.0 HbA1c Analyzer, BioHermes, Wuxi, China) levels. The results of fasting blood glucose (FBG) and HbA1c were used to detect subjects with undiagnosed diabetes and prediabetes that had to be excluded from the study. Serum fasting insulin levels were measured in a certified commercial laboratory (Prodia Lab) by a solid-phase, enzyme-labelled chemiluminescent immunometric assay (Siemens IMMULITE 2000XPi) with an assay range of 2-300 mU/L. For the levels below 2 mU/L, a standardized formula from the instrument manufacturer was used to interpolate the concentrations. Homeostatic model assessment for insulin resistance (HOMA-IR) as a validated measure for whole-body insulin resistance (IR) in human was calculated using the formula: HOMA-IR = fasting serum insulin x fasting glucose / 22.5).[17]

Leptin, Adiponectin, and Leptin/Adiponectin Ratio

Serum leptin and adiponectin levels were measured by ELISA using commercial reagents (DuoSet ELISA R&D System) according to the manufacturer's protocol. Leptin to adiponectin (L/A) ratio, a more sensitive marker for adipose tissue dysfunction, was calculated by L/A = leptin level (ng/mL) / adiponectin level (μg/mL).[18]

Dietary Intake Analysis

One week before the intended measurement date, each subject was informed and instructed on how to make a 3-days food record consisting of two working days and one day during the weekend. For each recording day, all participants were required to write down all of the foods and drinks they consumed throughout the day. The household servings portion for each meal, food preparation methods, brand name of the foods or beverages if applicable, as well as the addition of sugar, were recorded, as described previously.[19] On the study subjects' clinical measurement and blood sampling day, a certified dietician performed an interview with the subjects to review the completeness and validity of the food record data.

These dietary intake data were then analyzed using NutriSurvey 2007 (EBISpro, Germany) software. The amount of total calorie, carbohydrate, fat, and protein intake for each day were obtained and then averaged for further analysis, as published.[20]

Physical Activity Analysis

Physical activity was assessed using the adapted Global Physical Activity Questionnaire (GPAQ), which has been developed by the World Health Organization[21] and validated for the Indonesian population.[22] This self-reported questionnaire comprised 16 questions that were grouped to collect information regarding physical activity over a typical week in three domains: activity at work, transportation (travel to and from places), and recreational activity.[21] All subjects were asked to fill in the questionnaire based on their one-week activities before the measurement date. According to GPAQ analysis guidelines,[23] an estimation of the total weekly volume of moderate and vigorous physical activities (MVPA) was given as Metabolic Equivalent-minutes/week (MET.min/week), along with the total time spent on MVPA (minutes/week) and total time of sedentary activities in one week (minutes/week).[24] Furthermore, based on their total volume and time spent on MVPA, the subject's physical activity level was classified into three categories (low, moderate, and high).[23]

Statistical Analysis

Continuous variables with normal distribution were presented as mean and standard deviation [mean (SD)]. Meanwhile, non-normally distributed data were presented as geometric mean and 95% confidence interval (geomean (95%CI)) and were log-transformed (log2) for analysis. Linear regression (IBM SPSS Statistics ver. 25) was performed to compare the mean differences of independent variables between two groups at baseline when adjustment for covariates was needed. The Chi-square test was used to compare categorical data. Mediation analysis for evaluating the effect of dietary intake components on anthropometry parameter differences between rural and urban group at baseline was performed using PROCESS macro ver. 4.0 for SPSS, as described previously.[25] The changes in the parameters measured at baseline and 1-year follow-up for each group and the differences of these changes between urban and rural subjects were analyzed using linear-mixed model as implemented in the lme4 R package.[26] For

each parameter, the covariates used in the linear mixed model were origin (urban/rural), time, and their interaction. The within subject correlation was accounted for using a random-intercepts term. The statistical significance of the effects (i.e., changes from baseline within each group and between groups) were tested using the F-test with Satterthwaite's degree-of-freedom as implemented in lmerTest.[27] Mediation analysis for the BMI and adipokines changes was performed using 5000 bootstrap samples to obtain the 95% confidence interval for the indirect effect of the covariates. In particular, we evaluated the statistical significance in the decrease/increase of the estimate of the outcome variables after correcting for the changes in certain covariates. Linear mixed model analyses and bootstrapping were performed using R version 4.1.2 in RStudio version 1.4. For all tests, statistical significance was considered at the two-sided 5% level.

RESULTS

Study Population

A total of 189 (106 urban; 83 rural) subjects were recruited at baseline. For urban subjects, 87.7% originated from Jakarta metropolitan areas, while the rest were from other provincial capital cities. The overall loss to follow-up was 22.1%, leaving 81 urban and 66 rural subjects at the one-year assessment time point. The main reasons for loss to follow-up were: refusal to continue (18 subjects/9.4%), could not be contacted (22 subjects/11.6%), and moved to study at another university (2 subjects/1.1%). The proportion of loss to follow-up was similar between rural and urban groups (see flow-chart of the study in **Figure S1**).

Metabolic Profiles of Urban vs. Rural Subjects at Baseline

Age and proportion of males and females were similar between the rural and urban groups. Adiposity indices (BMI, waist circumference, and fat percentage) were significantly higher in urban compared to rural subjects [mean differences (95%CI) after adjustment for age and sex: 2.81 (1.55;4.07) kg/m^2, P<0.001; 6.37 (3.25;9.50) cm, P<0.001; and 5.07 (2.70;7.44) %, P<0.001, for BMI, waist circumference and fat percentage, respectively]. Moreover, if BMI was grouped based on WHO cut-off for Asian population,[28] we observed a higher proportion of overweight/obese in urban

compared to rural subjects. Conversely, the proportion of underweight subjects was almost three times higher in the rural than in the urban group (**Table 1**).

There was no difference in the fasting blood glucose and HbA1c levels between two groups. Urban subjects had double the HOMA-IR, leptin levels, and L/A ratio than their rural counterparts. The opposite was observed for adiponectin levels. Further

Table 1. Baseline characteristics of the study population.

Variables	Urban N=106	Rural N=83	P-values# (adjusted for age and sex)	P-values# (adjusted for age, sex, and BMI)
Age, yrs old (mean, SD)	18.4 (0.7)	18.6 (0.7)	0.09	
Sex, n male (%)	39 (36.8)	31 (37.3)	0.94	
BMI, kg/m2 (mean, SD)	22.9 (5.0)	20.0 (3.2)	**<0.001**	
BMI grouping, n (%)				
- Underweight (<18.5)	14 (13.2)	28 (33.7)		
- Normoweight (18.5-22.9)	50 (47.2)	41 (49.4)		
- Overweight (23-24.9)	17 (16.0)	7 (8.4)		
- Obese (≥25.0)	25 (23.6)	7 (8.4)	**0.001**	
Waist circumference, cm (mean, SD)	78.5 (12.8)	72.1 (8.2)	**<0.001**	
Fat percentage, % (mean, SD)	28.2 (9.1)	22.8 (8.3)	**<0.001**	
FBG, mg/dL (mean, SD)	87.1 (8.2)	86.7 (7.8)	0.54	
HbA1c, % (mean, SD)	5.1 (0.4)	5.1 (0.3)	**0.24**	
Fasting insulin†, IU/mL	5.3 (4.3-6.6)	2.9 (2.2-3.8)	**0.001**	0.06
HOMA-IR†	1.1 (0.9-1.4)	0.6 (0.5-0.8)	**0.001**	0.06
Leptin†, ng/mL	11.6 (9.7-13.8)	6.9 (5.3-9.1)	**<0.001**	0.07
Adiponectin†, µg/mL	4.1 (3.7-4.5)	4.9 (4.4-5.3)	**0.02**	0.19
Leptin-Adiponectin (L/A) Ratio†	2.9 (2.3-3.5)	1.4 (1.1-1.9)	**<0.001**	**0.03**
Dietary intake, mean (SD)				
- Total calories, kcal	1444 (335)	1289 (422)	**0.002**	**0.009**
- Fat, gram	52 (15)	44 (16)	**<0.001**	**0.01**
- Protein, gram	50 (14)	41 (13)	**<0.001**	**0.001**
- Carbohydrate, gram	193 (55)	179 (73)	0.08	0.06

†Not normally distributed continuous variables, presented as geomean (95% CI) and log transformed for analysis.
#Analyzed with linear regression for continuous variables and Chi-square test for categorical variables. The p-values shown in bold represent the statistically significant differences with p<0.05.
BMI: body mass index; FBG: fasting blood glucose; HOMA-IR: homeostatic model assessment for insulin resistance

adjustment for BMI revealed that the differences remained intact for L/A ratio, while HOMA-IR, leptin, and adiponectin became not statistically significant (**Table 1**).

Dietary Intake and Physical Activity at Baseline

Regarding dietary intake, we observed that urban subjects had significantly higher total calorie, fat, and protein intake compared to their rural counterparts [mean differences (95%CI) after adjustment for age and sex: 162.0 (59.4; 264.7) kcal, P=0.002; 8.2 (3.7; 12.6) gram, P<0.001; and 8.4 (4.7; 12.2) gram, P<0.001), for total calorie, fat, and protein intake, respectively] (**Table 1**). Additionally, the differences in BMI, waist circumference, and fat percentage between the two groups were slightly attenuated after further adjustment for fat and protein intake, despite remaining statistically significant [(2.22 (0.92; 3.52) kg/m^2, P=0.001 for BMI; 4.95 (1.74; 8.16) cm, P=0.003 for waist circumference; and 4.38 (1.91; 6.85)%, P=0.001 for fat percentage)]. Moreover, mediation analysis showed that fat intake, compared to the other dietary intake components, might be the major driver of the differences in the adiposity profiles between urban and rural subjects at baseline (**Table S1**).

Next, we compared the physical activity profiles between two groups at baseline based on the GPAQ analysis. The results showed that urban subjects had higher total volume and total time spent on MVPA compared to their rural counterparts. However, if these parameters were categorized as low, moderate, or high physical activity levels, no statistical significant differences were observed between two groups. Meanwhile, for the total time of sedentary activities, we observed lower values for urban compared to rural subjects (**Table S2**).

Effect of Urbanization over Time on Adiposity Profiles, Insulin Resistance, and Adipokines

At follow-up, after one year, both groups experienced an increase in their BMI. When we compared the degree of changes over time, we found that the increase of BMI in rural subjects was almost double what was seen in their urban counterparts [estimate (95%CI) after adjustment for age and sex: 1.23 (0.94;1.52), P<0.001 and 0.69 (0.43;0.95), P<0.001, for rural and urban subjects, respectively, P_{int} <0.01]. Although

a similar pattern was observed for fat percentage, the difference between the groups did not reach statistical significance [2.18 (1.39;2.97), P<0.001 in rural subjects vs. 1.33 (0.62;2.04), P<0.001 in urban subjects, P_{int}=0.12]. Meanwhile, HOMA-IR at one-year follow-up did not change significantly compared to baseline in either rural or urban groups (**Figure 1**).

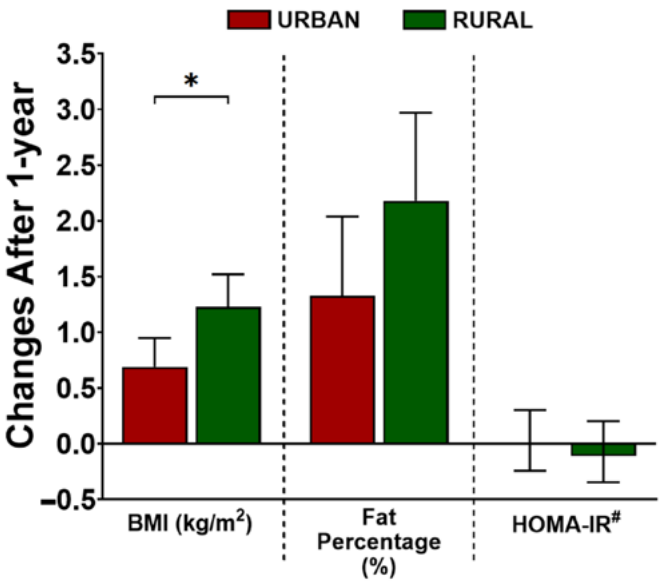

Figure 1. Changes of BMI, fat percentage, and whole-body insulin resistance (HOMA-IR) in urban and rural subjects after 1-year of living in an urban environment. The changes are presented as estimate and 95% confidence interval (95%CI). The changes in each group and the differences of changes between the urban and rural group for each parameter were analyzed using a linear-mixed model, adjusted for age and sex. The p-value depicted in the figure represents the p-value for interaction (P_{int}), the level of significance in the differences of changes between the two groups. *p < 0.05. #HOMA-IR was log-transformed (base 2) for analysis. The estimates (95%CI) were back-transformed (2β) and presented as a multiplicative scale compared to baseline. BMI: body mass index; HOMA-IR: homeostatic model assessment for insulin resistance.

Similar analysis was performed for adipokines data, which revealed that both groups had increased leptin levels at 1-year follow up, with a trend towards a higher increase in rural than urban subjects. (**Figure 2A, Table 2**). Additionally, no changes in the adiponectin level were observed in urban subjects at the follow-up time point, but a significant

decrease was found in the rural subjects (**Figure 2B, Table 2**). These changes caused no differences in the adiponectin levels between the two groups at 1-year follow-up time point (**Table S3**). When L/A ratio was considered, a significant, three times higher increase was seen in the rural compared to the urban group (**Figure 2C, Table 2**). After further adjustment with the changes in BMI over time, these changes of leptin, adiponectin, and L/A ratio were attenuated, and became non-significant for urban subjects (**Table 2**).

Effect of Urbanization over Time on Dietary Intake and Physical Activity

The changes over time in two important factors associated with urbanization-related lifestyle, namely, dietary intake and physical activity, were considered next. At follow-up time point, a significant increase in total calorie, fat, and protein intake was seen in both groups. However, only the increase in protein consumption was significantly higher in rural than in urban subjects [7.99 (4.42; 11.56), $P<0.001$ vs. 14.03 (9.95; 18.10), $P<0.001$, for urban and rural, respectively, $P_{int}=0.03$] (**see Figure 3**). These changes also resulted in the loss of differences in the protein intake between the two groups at the 1-year follow-up time point (**Table S3**). Similar to the findings at baseline, adjustment for the increase in fat intake after one year contributed to the largest attenuation of the BMI increase in both groups (in urban: 29.0% vs. 5.8% vs. 1.4%, and in rural: 19.5% vs. 8.9% vs. 7.3%, for fat, protein, and carbohydrate intake, respectively) (**Table 3**). Although the increase in protein intake was almost twice as high in the rural group compared to the urban group after 1-year, adjustment for protein intake changes did not attenuate the differences in the increase in BMI between the two groups (**Table S4**).

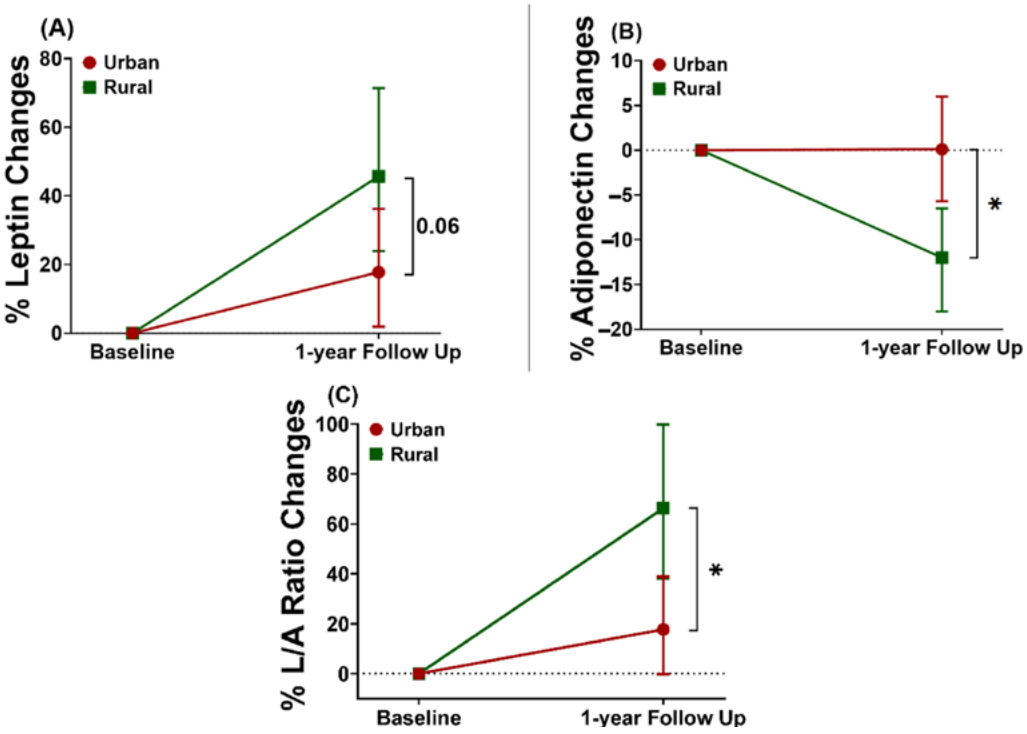

Figure 2. Changes of leptin levels (A), adiponectin levels (B), and leptin-adiponectin (L/A) ratio (C) in urban and rural subjects after 1-year of living in an urban environment. The changes are presented as estimate and 95% confidence interval (95%CI). The changes in each group and the differences of changes between urban and rural group for each parameter were analyzed using a linear-mixed model, adjusted for age and sex. All parameters were log-transformed (base 2) for analysis. The estimates (95%CIs) were back-transformed (2β) and presented as percent changes compared to baseline. The p-value depicted in the figure represents the p-value for interaction (P_{int}), the level of significance in the differences of changes between the two groups. *$p < 0.05$.

Table 2. Mediation analysis of the effect of changes in BMI overtime on the leptin, adiponectin, and L/A ratio in urban and rural subjects at 1-year follow-up.

	Adjusted for age and sex			Adjusted for age, sex, and BMI				
			Pint					Pint
Variables[†]	Urban	Rural		Urban	Indirect effect[#]	Rural	Indirect effect[#]	
Leptin	0.24 (0.03;0.45), P=0.03	0.54 (0.31;0.78), P<0.001	0.06	0.09 (-0.11;0.29), P=0.38	-0.25;-0.07	0.33 (0.10;0.55), P=0.005	-0.29; -0.12	0.12
Adiponectin	0.002 (-0.08;0.09), P=0.97	-0.19 (-0.29;-0.10), P<0.001	0.003	0.04 (-0.04;0.12), P=0.34	0.01; 0.06	-0.12 (-0.22;-0.03), P=0.008	0.03; 0.10 -	0.008
L/A ratio	0.23 (-0.003;0.47), P=0.05	0.73 (0.47;1.00), P<0.001	0.006	0.06 (-0.16;0.28), P=0.60	-0.30, -0.08	0.45 (0.20;0.70), P<0.001	0.39; -0.17	0.02

[†]All variables were analyzed using linear mixed model on log transformed data, presented as estimate and its 95% confidence interval.
[#]Indirect effect of BMI on the variables analyzed, obtained by performing bootstrapping with 5000 iterations and presented as its 95% confidence interval.
BMI: body mass index; L/A ratio: leptin/adiponectin ratio; Pint: P-value for interaction.

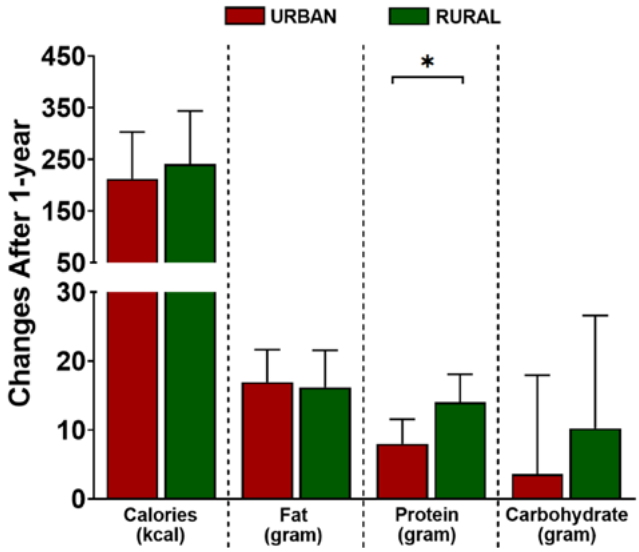

Figure 3. Changes of calorie-, fat-, protein-, and carbohydrate intake in urban and rural subjects after 1-year of living in an urban environment. The changes are presented as estimate and 95% confidence interval (95%CI). The changes in each group and the differences of changes between urban and rural group for each parameter were analyzed using a linear-mixed model, adjusted for age and sex. The p-value depicted in the figure represents the p-value for interaction (P_{int}), the level of significance in the differences of changes between the two groups. *$p < 0.05$.

With respect to physical activity, we found a significant decrease in the total volume of MVPA after one year in the urban group only. However, the difference of changes between the two groups was not statistically significant. A similar pattern was also observed for the total time spent on MVPA. Moreover, there was a significantly higher decrease in total sedentary time after one year in the rural group (**Figure S2**). Furthermore, addition of the physical activity parameters to the model with fat and protein intake did not significantly further attenuate the estimated changes of BMI in either group (**Table 3**).

Table 3. Mediation analysis of the effect of changes in dietary intake and physical activity over time on the changes of BMI at 1-year follow-up in both urban and rural subjects.

	Urban				Rural				
Model†	Estimate (95%CI)	P values	% changes††	Indirect effect# (95% CI)	Estimate (95%CI)	P values	% changes††	Indirect effect# (95% CI)	P_{int}
Adjusted for age and sex	0.69 (0.43, 0.95)	<0.001			1.23 (0.94; 1.52)	<0.001			0.007
Model with changes in dietary intake									
(+) Total calories intake	0.55 (0.28; 0.28)	<0.001	-20.3	-0.13 (-0.33;-0.02)	1.02 (0.71; 1.32)	<0.001	-17.1	-0.15 (-0.52;-0.04)	0.02
(+) Carbohydrate intake	0.68 (0.42; 0.93)	<0.001	-1.4	-0.01 (-0.12;0.03)	1.14 (0.85; 1.43)	<0.001	-7.3	-0.09 (-0.34;0.01)	0.02
(+) Fat intake	0.49 (0.20; 0.78)	<0.001	-29.0	-0.20 (-0.47;-0.04)	0.99 (0.67; 1.31)	<0.001	-19.5	-0.24 (-0.54;-0.06)	0.01
(+) Protein intake	0.65 (0.38; 0.93)	<0.001	-5.8	-0.04 (-0.22;0.08)	1.12 (0.78; 1.45)	<0.001	-8.9	-0.11 (-0.43; 0.14)	0.02
(+) Fat and protein intake	0.50 (0.21; 0.79)	<0.001	-27.5	-0.19 (-0.48; 0.02)	1.04 (0.70; 1.37)	<0.001	-15.4	-0.19 (-0.53;0.05)	0.007
Model with changes in physical activity				-					
(+) Total volume of MVPA	0.68 (0.42; 0.95)	<0.001	-1.4	0.01 (-0.14;0.05)	1.23 (0.94; 1.52)	<0.001	0.0	0.0 (-0.10;0.04)	0.007
(+) Total minutes of MVPA	0.68 (0.42; 0.95)	<0.001	-1.4	-0.01 (-0.15;0.06)	1.23 (0.94; 1.52)	<0.001	0.0	0.0 (-0.10;0.05)	0.007
(+) Total sedentary time	0.70 (0.44; 0.96)	<0.001	+1.4	0.01 (-0.03;0.10)	1.26 (0.94; 1.58)	<0.001	+2.4	0.03 (-0.11;0.22)	0.007
Model with changes in dietary intake and physical activity									
(+) Fat and protein intake and total volume of MVPA	0.48 (0.19; 0.78)	0.001	-30.4	-0.21 (-0.50;-0.01)	1.10 (0.75; 1.44)	<0.001	-10.6	-0.13 (-0.49;0.15)	0.003
(+) Fat and protein intake and total minutes of MVPA	0.49 (0.19; 0.78)	0.001	-29.0	-0.20 (-0.51; -0.01)	1.09 (0.75; 1.44)	<0.001	-11.4	-0.14 (-0.52;0.14)	0.003
(+) Fat and protein intake and total sedentary time	0.51 (0.22; 0.80)	<0.001	-26.1	-0.18 (-0.47;0.001)	1.15 (0.79; 1.50)	<0.001	-6.5	-0.08 (-0.47;0.21)	0.002

†All variables as an additional adjustment for age and sex, and all analyses were performed using linear-mixed model. The group of covariates used for model adjustment were shown in bold.

††Proportion of changes in the estimate of the model compared to the model adjusted for age and sex only.

#Indirect effect of covariate(s) on BMI, obtained by performing bootstrapping with 5000 iterations and presented as its 95% confidence interval.

BMI: body mass index; MVPA: moderate-vigorous physical activity; P_{int}: P-value for interaction.

DISCUSSION

Here, we report the first prospective cohort study in an Indonesian young adult population that evaluated the effect of urbanization on metabolic health profiles. Our study showed that rural subjects had overall better adiposity, insulin resistance, and adipokine profiles compared to their urban counterparts. Importantly, we observed a significantly higher increase in BMI and leptin/adiponectin ratios in the rural subjects migrating to an urban area compared to subjects originating from urban areas.

The higher adiposity indices, proportion of overweight/obese, and whole-body insulin resistance in urban compared to rural residents of Indonesia have been reported before.[29] Unhealthy dietary behavior, such as high intake of calories and fat-dense foods associated with urban living, is thought to contribute to the higher adiposity profiles [3]. Indeed, we confirmed this pattern of dietary intake in our study. Although further adjustment with total calorie, fat, or protein intake, all attenuated the anthropometric differences between rural and urban groups, our study showed that fat intake contributed the most. Additionally, the longitudinal follow up to see how urban lifestyle affects metabolic health in those migrating from rural areas compared to urban residents, first confirmed a significant increase of BMI after one-year follow-up in both groups, as seen in previous studies, showing that majority of freshmen gain weight during their first-year of university life.[30,31] The increase of total calorie, fat, and protein intake after one year in both groups, might partially explain these changes in BMI. Our study also demonstrated that fat intake changes as the dietary intake component that might be the major contributor for BMI increase after one year in both groups. Significantly, the rural group experienced almost a twice higher increase in BMI than the urban group. Although a significantly higher protein intake was observed in rural compared to urban group at one year follow-up, this could not explain the differences in the BMI increase between the two groups. Interestingly, previous studies have shown that higher meat or meat-products intake, which mostly consist of protein and fat, is associated with more weight gain independent of the total calorie intake.[32]

Another factor contributing to adiposity profiles is the level of physical activity,[33] as it promotes burning of calories, leading to negative energy balance and subsequently

less probability for fat deposition.[34] In our study, at baseline we found that rural group had lower total volume and time spent on MVPA with a higher sedentary time, compared to urban group. This suggests that physical activity does not explain the differences observed in BMI. However, it has to be noted that studies of physical activity in rural and urban areas can be influenced by factors such as level of education, ethnicity, or tools utilized.[35,36] In our study, the questionnaires used at baseline, which took place when the study subjects had already arrived in urban area, might not truly reflect the subjects' level of physical activity during their residence in rural areas. At the 1-year follow-up time point, we found no significant differences in the changes of total volume and time spent on MVPA between two groups. As for fat or protein intake, physical activity did not explain the higher gain in BMI seen in the rural group.

The addition of the physical activity parameters into the model with the adjustment of dietary intake also could not explain the higher increase of BMI in rural compared to urban group. This result implies that with similar changes of dietary intake and physical activity within one year, rural subjects experienced bigger changes in BMI than their urban counterparts. Hence, other factors, such as the gut microbiome[37] or epigenetic changes,[38] could potentially influence the adiposity changes in rural population upon migration to urban areas. Other factors that could potentially influence the changes of weight or BMI in our study subjects, as shown in previous studies, are psychological stress[39] and socioeconomic-cultural backgrounds.[40,41] These factors were not evaluated in our study.

The increase of BMI in both groups, if continued for the long term, could potentially cause obesity and induce other metabolic and cardiovascular diseases. In other cases, if the BMI increase does not lead to obesity, the distribution of body fat caused by the weight gain also needs to be considered. Previous studies have shown that Asian populations tend to have higher cardiometabolic risks compared to Caucasian populations with the same levels of BMI, in particular, due to central obesity or visceral adiposity.[42,43] These risks would potentially be higher in the rural group as a substantial number of subjects are underweight. Several studies showed that individuals with previous malnourished condition have an increased risk of obesity later in life, especially if they adopt unhealthy

lifestyles.[44,45] Moreover, individuals that have experienced a double burden of malnutrition or undernutrition in early life followed by later overweight/obesity, also pose a substantially enhanced risk of non-communicable diseases (NCDs).[46]

The observed differences in insulin resistance and adipokine levels at baseline between urban and rural groups aligned with the findings from previous studies, showing a higher HOMA-IR and lower adiponectin levels in urban compared to rural population.[47,48] Moreover, after adjustment for BMI, the differences in HOMA-IR, leptin, and adiponectin were no longer statistically significant, indicating a major contribution by BMI. Interestingly, after 1-year living in an urban area, a significant decrease of adiponectin levels, along with significant increase of L/A ratio was observed in rural group compared to the urban group. These changes were attenuated after adjustment for the changes in BMI. This finding showed that rural subjects also experienced worse changes in the adipokine profiles, which was partially mediated by the changes in BMI. It also indicates that there might be other factors than BMI, potentially contributing to the changes in adipokines in the rural subjects after 1-year living in an urban area. As shown from previous studies, gut microbiota has been associated with changes in leptin and adiponectin levels in response to a high-fat diet.[49,50]

Leptin and adiponectin have opposite effects on subclinical inflammation and insulin resistance. Leptin upregulates pro-inflammatory cytokines such as tumor necrosis factor-α and interleukin-6, while adiponectin has anti-inflammatory properties.[18] Adipose tissue dysfunction, marked by higher leptin and lower adiponectin levels, has been reported to be associated with insulin resistance and the incidence of T2D.[51] However, in our study, there were no significant changes in insulin resistance in the groups studied after one year of residence in an urban area. The relatively short follow-up period and preserved pancreatic beta-cells function in the young adult population might potentially explain this.[52]

The longitudinal study design, inclusion of several metabolic health parameters, and incorporation of dietary intake and physical activity measurements were several strong points of our study. There is only one previous prospective cohort study known to the

author that has evaluated the effect of urbanization on CVD risk factors and major NCDs.[12] However, this study did not incorporate dietary intake analysis and measurement of biological metabolic markers, such as insulin resistance index, leptin, and adiponectin. Our study also observed the importance of fat intake contribution in the increase of BMI in both the freshmen urban group and the rural individuals who recently migrated to an urban area. Previous study evaluating this freshmen weight gain only took into account eating behavior changes but did not perform detailed dietary intake analysis.[53]

However, the relatively small number of subjects and short duration of follow-up could be considered as limitations in our study. The addition of tools to evaluate the quality of dietary intake, such as the Healthy Eating Index and the utilization of health technology devices like an accelerometer to assess physical activity more objectively, could provide more accurate data in future studies. Additionally, the inclusion of psychological stress assessment and questionnaires or tools to accommodate the evaluation of the socio-economic and cultural aspects would also result in a more comprehensive data for future research. Moreover, investigation of the gut microbiome, epigenetic changes, as well as immunological factors, might shed more light on the mechanisms that underlie rapid changes in the metabolic profiles upon urbanization.

In conclusion, the findings in our study show that adoption of an urban lifestyle could potentially cause poorer metabolic health changes in rural individuals who migrate to an urban area. Our findings in part complements a previous study that showed the rising BMI in residents of increasingly urbanizing rural areas in low-middle income countries is due to an increase in low-quality diet.[54] However, it also indicates that there is a more rapid increase in BMI of subjects arriving from rural areas that could not be explained by either diet or physical activity. Therefore, further studies are needed, as it is important for policymakers to design innovative approaches to prevent this negative effect of urbanization in the young adult populations, with particular attention to those migrating from rural areas.

Author Contributions

Conceptualization, F.K. and M.Y.; Methodology, F.K., A.A., E.S., and D.L.T.; Investigation, F.K., D.L.T, D.V. and T.P.; Resources, E.S., M.D.M, and T.P.; Formal analysis, F.K. and M.D.M.; Funding acquisition, D.L.T., D.S.H., E.Y., M.Y., and P.S.; Supervision, M.Y., D.S.H., E.Y., and P.S.; Validation, R.T.; Writing - original draft, F.K. and E.S.; Writing - review and editing, D.L.T., M.Y., M.D.M, A.A., R.T., D.S.H., E.Y., and P.S. All authors have read and agreed to the published version of the manuscript.

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Institutional Review Board Statement

The study was conducted in accordance with the Declaration of Helsinki and approved by Ethical Committee for Health Research of Faculty of Medicine Universitas Indonesia (No. 1181/UN2.F1/ETIK/2017).

Informed Consent Statement

Informed consent was obtained from all subjects involved in the study.

Data Availability Statement

The data that support the findings of this study are available from the corresponding author (F.K.) upon reasonable request.

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Conflict of Interests

The authors declare that they have no conflict of interest.

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SUPPLEMENTARY MATERIALS

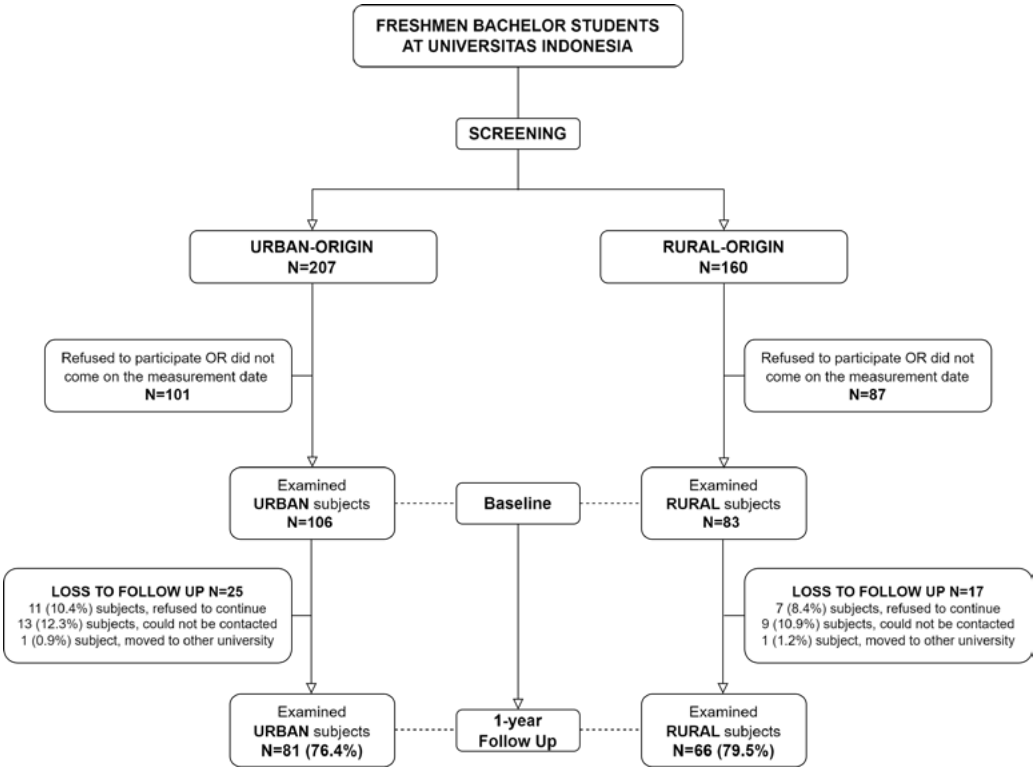

Figure S1. Flow Chart of The Study.

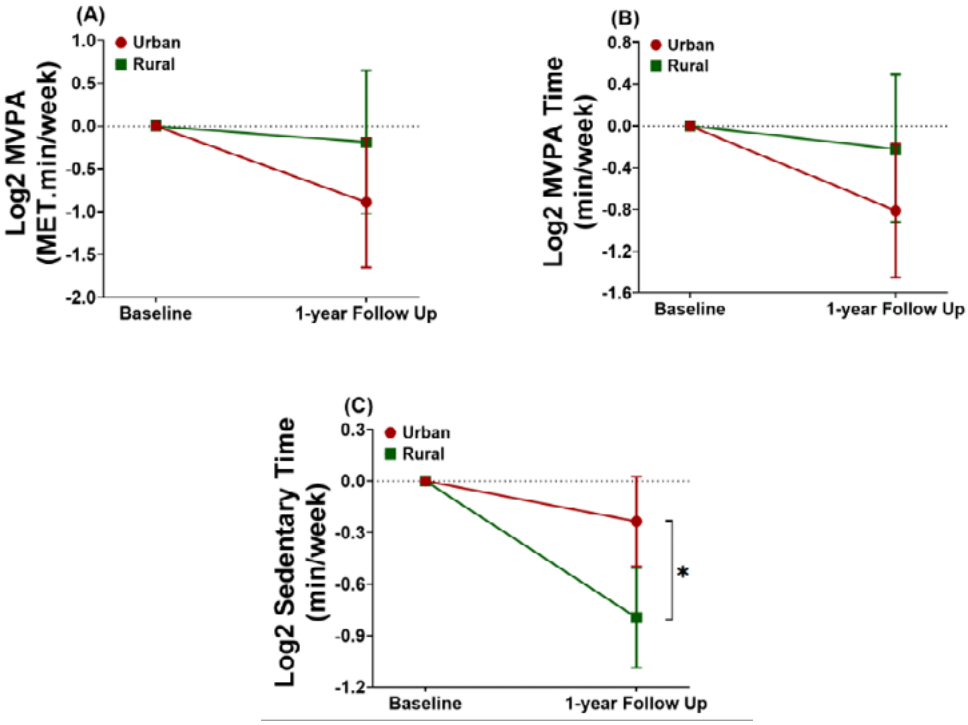

Figure S2. The changes of physical activity levels (A & B) and sedentary time (C) in urban and rural subjects after 1-year of living in an urban environment.(L/A) ratio (C) in urban and rural subjects after 1-year of living in an urban environment. The changes are presented as estimate and its 95% confidence interval and obtained from previously log-transformed variables. The changes in each group and the differences of changes between urban and rural group for each parameter was analyzed using linear-mixed model, adjusted for age and sex. The P-value depicted in the figure represents the P-value for interaction (P_{int}), the level of significance in the differences of changes between the two groups. *P<0.05
MVPA: moderate-vigorous physical activity

Table S1. Mediation analysis of the effect of dietary intake on the differences of adiposity profiles in urban and rural subjects at baseline.

Model†	Body mass index				Waist circumference				Fat percentage			
	Mean diff. (95%CI)	P-value	% changes ††	Indirect effect# (95% CI)	Mean diff. (95%CI)	P-value	% changes ††	Indirect effect# (95% CI)	Mean diff. (95%CI)	P-value	% changes ††	Indirect effect# (95% CI)
Unadjusted	2.85	<0.001			6.42	<0.001			5.29	<0.001		
	(1.60; 4.10)				(3.23; 9.62)				(2.75; 7.83)			
Adjusted for age and sex	2.81	<0.001			6.37	<0.001			5.07	<0.001		
	(1.55, 4.07)		-5.3		(3.25; 9.50)		-6.0		(2.70; 7.44)			
(+) Total calories intake	2.66	<0.001		-0.20	5.99	<0.001		-0.81	5.05	<0.001	-0.4	0.48
	(1.37; 3.96)		+2.5	(-0.62; 0.05)	(2.78; 9.21)		+3.1	(-2.14; -0.07)	(2.60; 7.50)			(-0.07; 1.19)
(+) Carbohydrate intake	2.88	<0.001		0.01	6.57	<0.001		-0.16	5.33	<0.001	+5.1	0.48
	(1.61; 4.16)		-20.3	(-0.14; 0.17)	(3.40; 9.75)		-22.3	(-0.71; 0.18)	(2.94; 7.73)			(-0.18; 1.24)
(+) Fat intake	2.24	0.001		-0.59	4.95	0.002		-1.70	4.23	0.001	-16.6	-0.52
	(0.97; 3.52)		-17.4	(-1.29; -0.15)	(1.81; 8.10)		-18.1	(-3.45; -0.47)	(1.81; 6.65)			(-1.41; 0.20)
(+) Protein intake	2.32	0.001		-0.54	5.22	0.002		-1.66	4.56	<0.001	-10.1	0.11
	(1.00; 3.63)		-21.0	(-1.24; -0.07)	(1.96; 8.48)		-22.3	(-3.46; -0.43)	(2.06; 7.06)			(-0.81; 0.93)
(+) Fat and protein intake	2.22	0.001		-0.64	4.95	0.003		-1.91	4.38	0.001	-13.6	-0.09
	(0.92; 3.52)			(-1.38; -0.09)	(1.74; 8.16)			(-3.81; -0.55)	(1.91; 6.85)			(-1.21; 0.89)

†All variables as an additional adjustment for age and sex.
††Proportion of changes in mean difference of the model compared to the model adjusted for age and sex only.
#Indirect effect of covariate(s) on anthropometry parameters, obtained by performing bootstrapping with 5000 iterations and presented as its 95% confidence interval.

Table S2. The levels of physical activity and sedentary time measured with GPAQ in urban and rural subjects at baseline.

	Urban N=106	Rural N=83	P-values
Total volume of MVPA, MET.min/week	1868	1046	**0.02#**
(geomean, 95%CI)	(1404-2486)	(618-1770)	
Total time spent for MVPA, min/week	423	255	**0.02#**
(geomean, 95%CI)	(328-546)	(164-397)	
Total sedentary time, min/week	441	490	**0.006#**
(geomean, 95%CI)	(411-472)	(444-540)	
Proportion of physical activity intensity categories, n (%)			
- Low	18 (17.0)	24 (29.3)	0.12
- Moderate	42 (39.6)	25 (30.5)	
- High	46 (43.4)	33 (40.2)	

#P-values derived from linear regression of log transformed data adjusted for age and sex.
The P-values shown in bold represent the statistically significant differences with P<0.05.
GPAQ: Global Physical Activity Questionnaires; MVPA: moderate-vigorous physical activity.

Table S3. Characteristics of study population at 1-year follow-up time.

Variables	Urban N=81	Rural N=66	P values[#] (adjusted for age and sex)	P values[#] (adjusted for age, sex, and BMI)
Age, yrs old (mean, SD)	19.4 (0.6)	19.5 (0.7)	0.23	
Sex, n male (%)	31 (38.3)	25 (37.9)	0.96	
BMI, kg/m2 (mean, SD)	23.7 (5.2)	21.2 (3.3)	**<0.001**	
BMI grouping, n (%)				
- Underweight (<18.5)	8 (9.9)	8 (12.1)	0.02	
- Normoweight (18.5-22.9)	34 (42.0)	42 (63.6)		
- Overweight (23-24.9)	13 (16.0)	8 (12.1)		
- Obese (≥25.0)	26 (32.1)	8 (12.1)		
Waist circumference, cm (mean, SD)	81.6 (12.4)	74.5 (8.2)	**<0.001**	
Fat percentage, % (mean, SD)	29.2 (9.0)	25.2 (8.8)	**<0.001**	
FBG, mg/dL (mean, SD)	91.1 (6.7)	90.4 (5.9)	0.56	
HbA1c, % (mean, SD)	NA	NA		
Fasting insulin[†], IU/mL	5.2 (4.0-7.0)	2.8 (2.0-3.9)	**0.006**	0.06
HOMA-IR[†]	1.2 (0.9-1.6)	0.6 (0.5-0.9)	**0.006**	0.07
Leptin[†], ng/mL	13.7 (11.3-16.8)	9.7 (7.4-12.7)	**0.01**	0.74
Adiponectin[†], μg/mL	4.0 (3.6-4.4)	4.4 (3.9-4.9)	0.28	0.95
Leptin-Adiponectin (L/A) Ratio[†]	3.5 (2.8-4.3)	2.2 (1.7-3.0)	**0.01**	0.75
Dietary intake, mean (SD)				
- Total calories, kcal	1659 (409)	1510 (422)	**0.04**	0.19
- Fat, gram	69 (25)	60 (21)	**0.03**	0.17
- Protein, gram	58 (16)	55 (17)	0.35	0.85
- Carbohydrate, gram	197 (49)	187 (60)	0.24	0.42

[†]Not normally distributed continuous variables, presented as geomean (95% CI) and log transformed for analysis.
[#]Analyzed with linear regression for continuous variables and Chi-square test for categorical variables.
The P-values shown in bold represent the statistically significant differences with P<0.05.
BMI: body mass index; FBG: fasting blood glucose; HOMA-IR: homeostatic model assessment for insulin resistance

Table S4. The effect of the differences in protein intake changes on the differences of BMI increase after 1-year between urban and rural subjects.

	BMI††Δ		
	Estimated differences# (95%CI)	**P values**	**Indirect effect (95%CI)**
Adjusted for age and sex	0.53 (0.22; 0.84)	<0.001	
Adjusted for age, sex, and Δprotein intake†	0.55 (0.24; 0.86)	<0.001	-0.02 (-0.10; 0.02)

†Δprotein intake = the differences of protein intake at 1-year follow-up time point with protein intake at baseline.

††ΔBMI = the differences of body mass index at 1-year follow-up time point with body mass index at baseline.

#Estimated differences of ΔBMI between urban and rural subjects, analyzed using linear-mixed model.

Chapter 5

LIFESTYLE AND CLINICAL RISK FACTORS IN RELATION WITH THE PREVALENCE OF DIABETES IN THE INDONESIAN URBAN AND RURAL POPULATIONS: THE 2018 INDONESIAN NATIONAL HEALTH SURVEY

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ABSTRACT

Aims: To investigate the differences between the Indonesian urban and rural populations in the presence of lifestyle and clinical risk factors and their relation with the prevalence of diabetes.

Methods: Using the 2018 Indonesian Basic Health Survey data, the diagnosis of diabetes was based on the combination of known diabetes, i.e., a previous history of diabetes or use of anti-diabetes medication, and unknown diabetes based on blood glucose criteria according to American Diabetes Association 2022 guidelines. We performed logistic regression analyses separately for the urban and rural populations to examine the association of lifestyle and clinical factors with prevalent diabetes.

Results: Our study comprised 17,129 urban and 16,585 rural participants. Indonesian urban population was less physically active [proportion differences (95% confidence interval/CI): -11.8% (-13.5; -0.1)] and had a lower proportion of adequate fruit and vegetable intake [-0.8% (-1.5; -0.1)], than the rural population. Higher participants with obesity [12.8% (11.4; 14.1)] were also observed in urban compared to rural population. Although there were no differences in the total prevalence of diabetes between the two populations [10.9% (10.4; 11.5) vs. 11.0% (10.4; 11.7) for urban and rural, respectively], the prevalence of known diabetes was twice higher in the urban [proportion (95%CI): 3.8% (3.5; 4.2)] than in the rural population [1.9% (1.6; 2.1). Physical inactivity was associated with the prevalence of diabetes, especially in urban population [prevalence odds ratio (95%CI): 1.15 (1.01; 1.31) and 1.05 (0.89; 1.24) for urban and rural, respectively). Overweight/obesity, abdominal obesity, hypertension, and dyslipidemia were risk factors for prevalent diabetes in both populations.

Conclusions: Indonesian rural population showed relatively better lifestyle and clinical profiles than their urban counterparts. However, no differences were observed between the two populations in the relation between risk factors and diabetes. Special attention needs to be addressed to the high prevalence of undiagnosed and untreated diabetes in Indonesia.

INTRODUCTION

The prevalence of diabetes is increasing worldwide, from 8.3% in 2011 to 10.5% in 2021, and is projected to become 12.2% in 2045.[1] Currently, more than 80% of people with diabetes live in low and middle-income countries (LMICs) and the greatest relative increase in the prevalence of diabetes is expected to occur in middle-income countries.[1,2] Indonesia is the fourth most populated LMIC with a rising prevalence of diabetes. With more than 19 million people suffering from diabetes in 2021, it ranked as the 5th highest country of people with diabetes in the world, compared to the 7th in 2019.[1]

Diabetes causes significant morbidity and mortality [3] and is an established risk factor for other diseases such as cardiovascular diseases,[4] end-stage renal diseases,[5] and cancers.[6] In 2016, diabetes became the third leading cause of disability-adjusted life year (DALY) in Indonesia.[7] Diabetes not only has deleterious effects on an individual and society level, but also has become a national economic burden due to its high health care costs.[8]

The worldwide prevalence of diabetes was estimated to be higher in urban (12.1%) than in rural (8.3%) areas.[2] The rapid socio-economic development in many LMICs that promote rapid urbanization and influence the environmental and social changes, may lead to an increase in diabetes prevalence.[9] Previous studies have shown that urbanization is associated with relatively unhealthy dietary patterns [10] and less physical activity,[11] resulting in surplus of energy that will be stored as body fat.[12] This excess storage of body fat may result in obesity and consequent low-grade inflammatory state and insulin resistance, which eventually could lead to type 2 diabetes (T2D).[13] Our previous study in the Indonesian young adult population showed a higher prevalence of obesity, in the urban compared to the rural population. [10]

Besides obesity, previous studies also showed that hypertension and dyslipidemia differed greatly in prevalence between rural and urban populations.[14,15] Apart from the lifestyle and biological determinants mentioned above, the level of education,

type of employment, and socio-economic status usually differs between urban and rural populations [16] and could potentially influence the incidence of diabetes.[17] We hypothesized that these urban-rural discrepancies in lifestyle, clinical, and socio-demographic factors contribute to the differences in the prevalence of diabetes between these two populations. Therefore, the aim of our study was to investigate the differences in these risk factors between Indonesian urban and rural populations and their relationship with the prevalence of diabetes (**Supplementary Figure 1**).

METHODS

Study design and population

To investigate the objectives mentioned above, the data from The 2018 Indonesian Basic Health Survey (Riset Kesehatan Dasar, RISKESDAS) was used in this cross-sectional study. RISKESDAS is a five-yearly national health survey conducted by the Ministry of Health, Indonesia, the latest in 2018. This survey incorporated questionnaires and biomedical data collection to evaluate the prevalence of communicable and non-communicable diseases, as well as the health-related risk factors in the Indonesian population.

The 2018 RISKESDAS population comprises 1,017,290 individuals of all ages, of whom 713,783 were ≥15 years old during the time the survey was commenced. This present study included non-pregnant individuals aged ≥15 years who were randomly sampled for blood glucose measurement (n=37,135). Individuals with missing data on clinical factors (body mass index, waist circumference, systolic/diastolic blood pressure, and lipid profile) and lifestyle factors (physical activity level, fruit and vegetable intake, smoking status, and alcohol consumption) were excluded. This study was approved by and registered in the National Institute of Health Research and Development (NIHRD), Ministry of Health, Republic of Indonesia.[18]

Data collection

The design for the data collection and selection of respondents in the 2018 RISKESDAS was integrated with the data from The National Economic Survey held by The Indonesian Central Bureau Statistics (Biro Pusat Statistik/BPS). A detailed

explanation of the methodological sampling has been described previously.[19,20] Briefly, the participants were selected using a multistage systematic random sampling design. By considering urban-rural distribution using the 2010 BPS criteria,[21] 30.000 survey blocks were randomly selected from 34 provinces, each consisting of 10 census buildings. From each census building, one household was randomly selected. All household members of each selected household were asked to participate in the survey. A set of multiple blocks interviewer-assisted questionnaires were used to record data on socio-demographics, history of diseases, and behavioral/lifestyle determinants.[22] The participants who underwent biomedical data collection, including blood glucose measurements, were randomly selected from 2500 census blocks across 26 provinces, with 1446 urban and 1054 rural sites representing the overall Indonesian population.[19,20]

Assessment of socio-demographic determinants

Age, sex, marital status, level of education, employment status, and type of employment were obtained using standardized questionnaires. The level of education was categorized as low (no formal education after primary school); intermediate (high school); and high (college/university). The type of employment was categorized as currently in education, unemployed/retired, working in the formal sector (civil servant, army, police, private employee, entrepreneur), and working in the informal sector (farmer, fisherman, labor, driver, domestic helper). A socio-economic status score was based on the ownership of household assets, as well as average income and expenditure, from the data previously obtained by BPS and was divided into quintiles. A higher number represents a higher socio-economic status.[23] The urban and rural areas were defined based on the criteria established by BPS in 2010,[21] including population density/km^2, farming household percentage, and availability/ accessibility for urban-related facilities (school, market, shop, hospital, movie theatre, hotel, and percentage of household with telephone or electricity). Each criteria has a certain score and a total score ≥10 was considered an urban area, and people living in those areas were considered member of the urban population (**Supplementary Table 1**).

Assessment of lifestyle factors

Physical activity was measured by the adapted Short Questionnaire to Assess Health-Enhancing (SQUASH) physical activity integrated into the RISKESDAS questionnaire, as the frequency and duration of moderate and vigorous activity within four domains, which were restructured to hours per week of metabolic equivalents.[24] Being physically active was defined as moderate to vigorous physical activity (MVPA) of ≥30 minutes/day for 5 days or ≥150 minutes/week.[25]

In the RISKESDAS questionnaire, fruit and vegetable intake was measured as the number of portions eaten per day, with display cards of common dishes provided by the interviewers as visual aids.[22] The recommended intake for fruits and vegetables is ≥400 grams/day or ≥5 portions/day.[25]

Smoking status was assessed as never, former, and current smoker. Additionally, the pack-years of smoking was calculated by multiplying the number of packs of cigarettes smoked per day by the number of years the person smoked. Alcohol consumption was estimated by the number of portion glasses per day, with display cards as visual aids, summed across all types of alcohol and restructured to the unit of alcohol per day.[22]

Assessment of clinical factors

Body weight was measured by a calibrated digital FESCO™ weight scale to the nearest 0.1 kg. Body height was measured without shoes using a calibrated, vertically fixed tape measure to the nearest 0.1 cm. BMI was calculated by dividing body weight (kg) by the square of height (m2) and categorized based on the WHO criteria for the Asia-Pacific population [26] Waist circumference was measured halfway between the iliac crest and the lowest rib using a flexible steel tape measure to the nearest 0.1 cm (SECA Model 201, Seca Gmbh Co, Hamburg, Germany).[20]

Blood pressure was obtained by a digital sphygmomanometer at the left arm and upright sitting position after 5 minutes of rest (HEM-7200, Omron Healthcare Co, Ltd, Kyoto, Japan). The average of three measurements was used for analysis. Hypertension

was defined as systolic blood pressure (SBP) ≥140 mmHg and/or diastolic blood pressure (DBP) ≥90 mmHg or previous diagnosis of hypertension with current use of anti-hypertensive medications.[27] Serum total, HDL-, and LDL-cholesterol, as well as triglyceride levels were measured using standard clinical chemistry methods (Roche® enzymatic assay).[19] Based on the criteria from The Indonesian Society of Endocrinology 2021, dyslipidemia was defined as one or more of the following criteria: total cholesterol ≥200 mg/dL, LDL-cholesterol ≥130 mmHg, HDL-cholesterol <40 mmHg in men or <50 mmHg in women, and triglyceride ≥150 mg/dL.[28]

Assessment of diabetes status

The definition of diabetes was based on the combination of known diabetes, i.e., a previous diagnosis of diabetes or use of anti-diabetes medication, and unknown diabetes based on blood glucose criteria according to the American Diabetes Association (ADA) 2022 guidelines for the diagnostic criteria of diabetes, which include one or more of the following [29]: fasting plasma glucose (FPG) ≥126 mg/dL, OR 2-hour plasma glucose (2h-PG) ≥200 mg/dL during oral glucose tolerance test (OGTT), OR random blood glucose ≥200 mg/dL with classic symptoms of hyperglycemia or hyperglycemia crisis. In the survey, random, fasting, and 2-hour post OGTT blood glucose were measured using capillary blood samples (Accu-Chek Performa, Roche Diagnostics GmbH, Mannheim, Germany). HbA1c was not measured during the survey.

Statistical analysis

All analyses in our study were weighted towards municipality/provincial density to correct for the differences in geographical density and urban/rural distribution across the 34 provinces in Indonesia.[20] As a result of the weighted analyses, percentages and proportions were given instead of the number of participants.

Study population characteristics and diabetes prevalence were presented for the Indonesian urban and rural populations. Continuous variables were summarized as mean with standard deviation (SD) for normally distributed data and median (25th, 75th percentile) for non-normally distributed data. Categorical variables were

presented as proportions with 95% confidence intervals (95% CI). Additionally, the differences between urban and rural population were presented as mean or proportion differences with 95% CI.

We performed multivariable logistic regression analyses to calculate odds ratios (OR) with 95% confidence intervals, stratified by the urban and rural population to examine the associations between lifestyle and clinical determinants with the total prevalence of diabetes. The associations between lifestyle factors and diabetes were adjusted for socio-demographic determinants (age, sex, education, occupation, marital status, and socio-economic status) and BMI. The associations between clinical factors and diabetes were adjusted for socio-demographic, lifestyle factors, and BMI. The lifestyle and clinical factors were both modeled as continuous and as categorical variables based on known cut-offs from previous literatures. All continuous variables were modeled based on their actual unit, except for MVPA duration and smoking pack-years, which used per standardized (SD) unit for better interpretation. For lifestyle factors, the behaviors that are considered a part of a healthy lifestyle based on national guidelines recommendation [25] will serve as reference. These include as follows: physically active, defined as ≥150 minutes/week (≥30 minutes/day for 5 days) of moderate-vigorous physical activity; adequate intake of fruits and vegetables, defined as intake of ≥5 portions/day; never smoker; and no alcohol consumption.

To examine the differences between the populations within one analysis, we generated new categorical variables for the combinations of each risk factor and the population, using the non-exposed ('healthy') urban population as the reference. All analyses were performed using STATA (version 16.0, StataCorp, College Station, TX, USA).

RESULTS

Socio-demographic, lifestyle, and clinical factors in Indonesian urban and rural populations

In this study, we included 33,714 participants (17,129 urban and 16,585 rural) who were non-pregnant and ≥15 years old from the 2018 RISKESDAS database after excluding participants without blood glucose measurements and with missing data

on lifestyle and clinical factors (**Figure 1**). The rural Indonesian population was slightly older than its urban counterpart. More participants in the urban population had a higher education [proportion difference (95% confidence interval/CI): 5.5% (4.8; 6.3)] and were in the highest quintile of socio-economic status [23.4% (21.4; 25.3)] compared to rural population. In terms of lifestyle factors, the rural population was more physically active [-11.8% (-13.5; -0.1)] and more often had an adequate fruit and vegetable intake [-0.8% (-1.5; -0.1)] than the urban population. In comparison with the urban population, the rural population more often were current smokers (**Table 1**).

BMI and waist circumference were higher in urban than rural population. This also applied to the proportion of participants with obesity, either by BMI categories (40.7% vs. 28.9%, for urban and rural, respectively) or abdominal obesity criteria (41.2% for urban and 28.4% for rural population). Systolic blood pressure was higher in rural compared to urban population, and the opposite was observed for DBP, resulting in no differences of hypertension status between the two groups. In addition, no differences were observed for total cholesterol, LDL-cholesterol, HDL-cholesterol, and triglyceride levels between the two populations. Nevertheless, the proportions of hypercholesterolemia, high LDL-cholesterol, hypertriglyceridemia, and dyslipidemia were higher in the urban than in the rural population. (**Table 1**).

Diabetes prevalence in the Indonesian urban and rural populations

There were no differences in the total prevalence of diabetes between Indonesian urban and rural population [proportion (95%CI): 10.9% (10.4; 11.5) and 11.0% (10.4; 11.7) for urban and rural, respectively]. Nevertheless, the proportion of individuals with a previous diabetes diagnosis and using anti-diabetes medication was twice as high in the urban population [3.8% (3.5; 4.2)] than in the rural population [1.9% (1.6; 2.1]. This resulted in a relatively high prevalence of undiagnosed and untreated diabetes, especially in the rural population [7.1% (6.7; 7.6) in urban and 9.1% (8.6; 9.8) in rural population] (**Figure 2**).

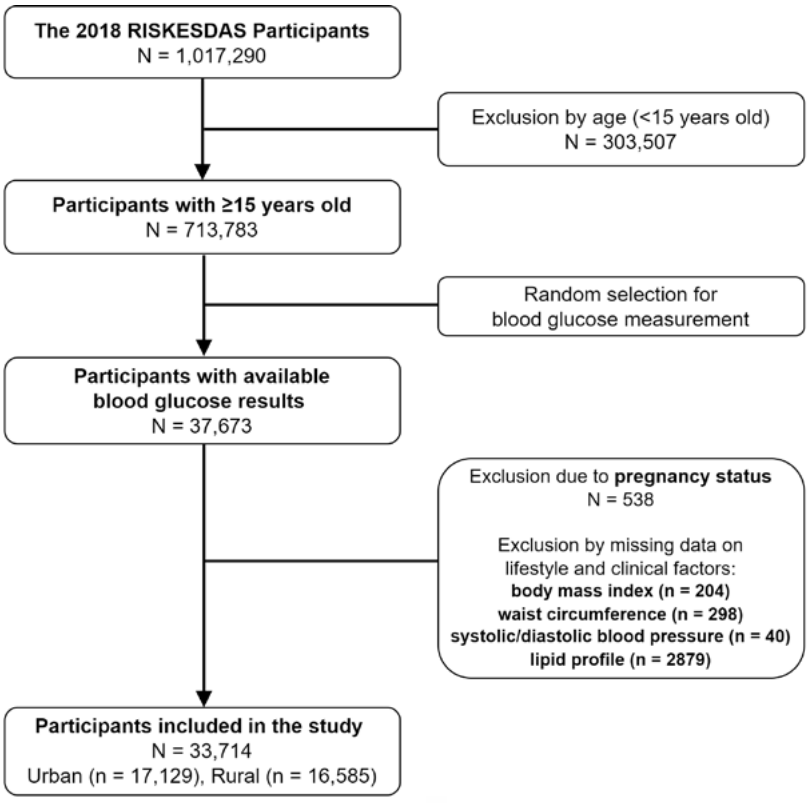

Figure 1. Flow chart for the inclusion of study participants using the data from The 2018 Indonesian Basic Health Survey.

Table 1. Differences in socio-demographic characteristics, lifestyle factors, and clinical factors between Indonesian urban (n = 17,129) and rural (n = 16,585) population.

	Urban (55%)	Rural (45%)	Differences[13] (95 CI)
Socio-demographic			
Age, years	42.6 (14.9)	44.5 (16.7)	-1.8 (-2.2; -1.4)
Sex (% men)	50.3 (49.6; 51.0)	50.5 (48.8; 50.1)	-0.2 (-1.1; 0.7)
Level of education (% high[1])	8.2 (7.5; 8.9)	2.6 (2.3; 3.0)	5.5 (4.8; 6.3)
Type of employment (% informal sector[2])	26.4 (25.2; 27.5)	53.0 (51.7; 54.2)	-26.7 (-28.3; -24.9)
Marital status (% married)	73.8 (73.0; 74.6)	79.1 (78.3; 79.8)	-5.3 (-6.4; -4.2)
Socio-economic status (% highest/5th quintile)	32.9 (31.2; 34.7)	9.5 (8.7; 10.4)	23.4 (21.4; 25.3)
Lifestyle Factors			
Physically active (%)	73.5 (72.1; 74.9)	85.3 (84.2; 86.3)	-11.8 (-13.5; -0.10)
Moderate-vigorous physical activity duration* (hours/week)	11.5 (2; 28)	21 (7; 42)	-8.1 (-9.1; -7.1)
Adequate fruit and vegetable intake (%)	3.4 (3.0; 3.8)	4.2 (3.6; 4.9)	-0.8 (-1.5; -0.1)
Fruit and vegetable intake* (portion/day)	1.4 (0.9; 2.1)	1.4 (1.0; 2.6)	-0.2 (-0.2; -0.1)
Smoking behaviour (% current smoker)	31.8 (30.9; 32.7)	37.1 (36.2; 37.9)	-5.3 (-6.5; -4.0)
Pack years*[3]	10.1 (4.2; 19.2)	12 (5.8; 21.5)	-1.7 (-2.5; -0.9)
Alcohol consumption (% current drinker)	2.2 (2.0; 2.5)	1.7 (1.5; 2.0)	0.5 (0.1; 0.9)
Quantity*[4] (unit alcohol/day)	0.2 (0.1; 1.0)	0.3 (0.1; 1.5)	0.2 (-0.5; 1.0)
Clinical Factors			
BMI (kg/m2)	24.4 (4.7)	23.1 (4.6)	1.3 (1.2; 1.4)
BMI categories[5] (%)			
Underweight (<18.5 kg/m2)	9.3 (8.8; 9.9)	11.9 (11.3; 12.6)	-2.6 (-3.4; -1.7)
Normo-weight (18.5-22.9 kg/m2)	33.1 (32.2; 34.0)	44.1 (43.2; 45.1)	-11.1 (-12.3; -9.8)
Overweight (23.0-24.9 kg/m2)	16.8 (16.2; 17.4)	15.0 (14.4; 15.6)	1.8 (1.0; 2.7)
Obesity (≥25.0 kg/m2)	40.7 (39.8; 41.7)	28.9 (28.0; 29.8)	12.8 (11.4; 14.1)
Waist circumference, cm	81.8 (11.8)	77.7 (12.2)	4.1 (3.7; 4.5)
Men	81.3 (10.8)	76.3 (10.3)	5.0 (4.5; 5.6)
Women	82.3 (12.7)	79.2 (13.9)	3.1 (2.6; 3.6)
Abdominal obesity[6] (%)	41.2 (40.1; 42.2)	28.4 (27.5; 29.3)	12.8 (11.4; 14.1)
Men	24.5 (23.2; 25.7)	10.4 (9.6; 11.3)	14.0 (12.5; 15.5)
Women	58.1 (56.8; 59.3)	46.7 (45.4; 48.0)	11.4 (9.5; 13.2)
Systolic blood pressure, mmHg	131.3 (23.0)	132.7 (25.0)	-1.4 (-2.1; -0.8)
Diastolic blood pressure, mmHg	84.6 (12.4)	83.9 (13.2)	0.7 (0.3; 1.1)
Hypertension[7] (%)	40.2 (39.2; 41.1)	39.3 (38.3; 40.3)	0.9 (-0.5; 2.2)
Total cholesterol, mmol/L	4.7 (1.0)	4.6 (1.1)	0.1 (0.1; 0.1)
Hypercholesterolemia[8] (%)	29.9 (29.0; 30.8)	26.4 (25.5; 27.3)	3.5 (2.2; 4.7)
LDL-cholesterol, mmol/L	3.2 (0.8)	3.1 (0.9)	0.1 (0.1; 0.1)
High LDL-cholesterol[9] (%)	38.7 (37.7; 39.7)	35.0 (34.0; 36.0)	3.7 (2.2; 5.1)
HDL-cholesterol, mmol/L	1.2 (0.3)	1.2 (0.3)	0.0 (-0.0; 0.0)
Low HDL-cholesterol[10] (%)	40.6 (39.6; 41-6)	41.2 (40.2; 42.2)	-0.6 (-2.0; 0.9)
Triglyceride, mmol/L	1.5 (1.1)	1.4 (1.0)	0.1 (0.1; 0.1)
Hypertriglyceridemia[11] (%)	28.4 (27.5; 29.2)	25.7 (24.8; 26.5)	2.7 (1.5; 3.9)
Dyslipidemia[12] (%)	69.5 (68.7; 70.4)	68.0 (67.1; 68.9)	1.5 (0.3; 2.8)
Random blood glucose, mmol/L	6.2 (2.4)	6.1 (2.4)	0.1 (-0.0; 0.2)
Fasting blood glucose, mmol/L	5.7 (1.8)	5.6 (1.6)	0.1 (0.1; 0.2)
2-hour glucose post OGTT, mmol/L	8.1 (2.9)	8.1 (2.9)	-0.0 (-0.2; 0.1)

Data were presented as mean (SD) for normally distributed continuous variables and median (25th-75th percentiles) for not- normally distributed continuous variables. Categorical variables were presented as percentage (95% confidence interval). Results were based on analyses weighted towards geographical density across 34 provinces in Indonesia.

*not-normally distributed continuous variables

[1]High education level includes participants who currently studying or having degree in college or university.

[2]Informal sector employment includes farmer, fisherman, labor, driver, and domestic helper

[3]calculated from individuals who smoke.

[4]calculated from individuals who drink alcohol.

[5]BMI categories were based on the WHO cut-offs for Asian population.

[6]Ethnic-Specific (Asian) waist-circumference cut-offs for abdominal obesity were >90 cm for men and >80 cm for women.

[7]Hypertension was defined as systolic blood pressure >140 mmHg AND/OR diastolic blood pressure >90 mmHg OR previous hypertension diagnosis with current use of anti-hypertensive medications.

[8]Hypercholesterolemia was defined as total cholesterol levels ≥5.2 mmol/L ((≥200 mg/dL).

[9]High LDL-cholesterol was defined as LDL-cholesterol levels ≥3.4 mmol/L (≥130 mg/dL).

[10]Low HDL-cholesterol was defined as HDL-cholesterol levels <1.0 mmol/L (<40 mg/dL) in men or <1.3 mmol/L (<50 mg/dL) in women.

[11]Hypertriglyceridemia was defined as triglyceride levels ≥1.7 mmol/L (≥150 mg/dL).

[12]Dyslipidemia was defined based of one or more of the following criteria: total cholesterol ≥200 mg/dL, OR LDL-cholesterol ≥130 mg/dL, OR low triglyceride (≥150 mg/dL), OR low HDL-cholesterol (<40 mg/dL in men or <50 mg/dL in women).

[13]Differences were calculated as values in urban minus values in rural. For not normally distributed continuous variables, the differences were calculated using mean and standard error to obtain the mean differences and its 95% confidence intervals.

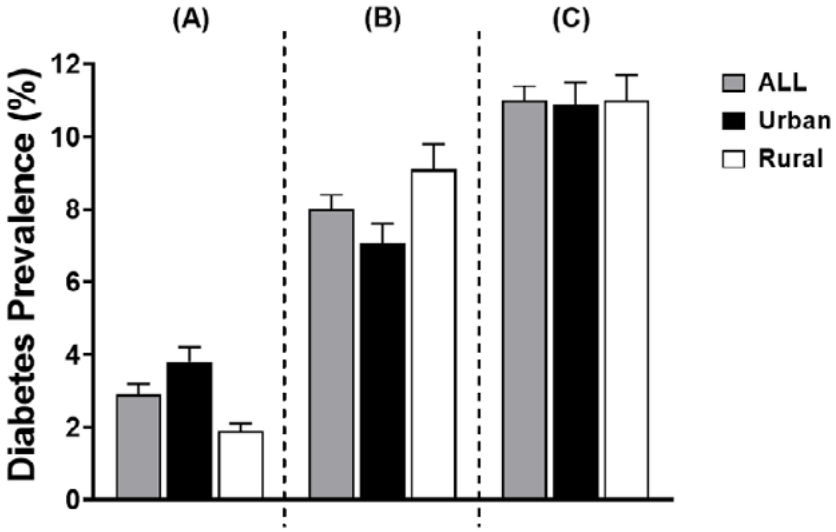

Figure 2. The prevalence of diabetes between Indonesian urban and rural population, A. Known (previously diagnosed and treated) diabetes; B. Unknown (undiagnosed and untreated) diabetes; C. Total prevalence* *The combination of prevalence of known diabetes and unknown diabetes using blood glucose criteria as follows: fasting plasma glucose (FPG) ≥126 mg/dL or 7 mmol/L, OR 2-hour plasma glucose (2h-PG) ≥200 mg/dL or 11.1 mmol/L after an oral glucose tolerance test (OGTT), OR random blood glucose ≥200 mg/dL or 11.1 mmol/L with classic symptoms of hyperglycemia or hyperglycemia crisis.

Lifestyle factors and prevalent diabetes in Indonesian urban and rural populations

Longer duration of moderate/vigorous physical activity was associated with lower risk of prevalent diabetes [prevalence odds ratio (95% confidence interval): 0.91 (0.85; 0.98) for urban and [0.94 (0.89; 1.00) for rural population, per 1 SD unit=21.4 hours/week] (**Table 2A**). The results were similar when using categorical variables in the models, showing a higher risk of prevalent diabetes with physical inactivity, especially in the urban population (**Table 2B**). In contrast with majority of previous findings, we found a positive correlation between fruit and vegetable intake with prevalent diabetes in the urban population (**Table 2A**). Although, sensitivity analysis showed a possible confounding of sex, age, and BMI in this association since additional adjustment for these factors resulted in the attenuation of the ORs (**Supplementary Tables 2 and 3**). Additionally, in this urban population, inverse associations between smoking pack-

years and alcohol consumption with the prevalence of diabetes were also observed (**Table 2A**). Moreover, when compared to the non-smoker group, current smoker was inversely associated with prevalent diabetes in urban and rural populations (**Table 2B**). Further sensitivity analysis showed that this current smoker group has a lower BMI and higher proportion of men than the non-smoker group in both populations (**Supplementary Tables 4 and 5**).

Clinical factors and prevalent diabetes in Indonesian urban and rural populations

All clinical factors, either modelled as continuous or as categorical variables, were associated with prevalent diabetes both in urban and rural populations (**Table 3A and 3B**).

The differences in the association of lifestyle and clinical factors with diabetes prevalence between Indonesian urban and rural populations

In comparison with the urban-physically active group, a higher prevalence odds ratio of diabetes was observed for urban-inactive [prevalence OR (95%CI): 1.17 (1.03; 1.33) but not for the rural-inactive group (0.97, 0.81; 1.16). No differences were observed between urban and rural populations who did not consume adequate fruit and vegetable compared with the urban-adequate group as the reference category. There were also no differences between urban and rural current smokers in comparison with the urban reference group (**Figure 3A**).

With regard to clinical factors, the urban and rural populations with overweight or obesity had higher prevalence ORs than the urban-normo-weight reference group, although there were no differences between the two groups [1.79 (1.56; 2.06) vs. 1.84 (1.57; 2.15) for urban-overweight/obese and rural-overweight/obese, respectively]. Similar patterns were observed for all other clinical factors, showing no differences in the prevalence ORs of diabetes between the urban and rural populations with clinical risk factors compared to the urban population without risk factor (**Figure 3B**). Additionally, it can be observed that when compared with the urban population without lifestyle or clinical risk factors, the rural population without risk factors had a higher risk of prevalent diabetes. Further analyses showed that this rural population without risk factors was somewhat older than the urban reference group (**Supplementary Table 6**).

Table 2A. Association of lifestyle factors as continuous variables with prevalent diabetes in Indonesian urban and rural population.

Variables	Model[3]	Prevalence Odds Ratio (95%CI)	
		Urban	Rural
Moderate/vigorous physical activity,	Crude OR	0.86 (0.81; 0.92)	0.90 (0.85; 0.95)
per 1 SD=21.4 hours/week	Model 1	0.92 (0.87; 0.99)	0.97 (0.91; 1.02)
	Model 2	0.92 (0.86; 0.98)	0.96 (0.90; 1.02)
	Model 3	0.91 (0.85; 0.98)	0.94 (0.89; 1.00)
Fruit and vegetable intake (portion/day)	Crude OR	1.11 (1.07; 1.14)	1.01 (0.97; 1.05)
	Model 1	1.08 (1.04; 1.12)	1.01 (0.97; 1.05)
	Model 2	1.07 (1.03; 1.11)	1.02 (0.97; 1.06)
	Model 3	1.06 (1.02; 1.11)	1.01 (0.96; 1.05)
Smoking[1],	Crude OR	1.35 (1.23; 1.49)	1.26 (1.15; 1.37)
per 1 SD=15.2 pack-years	Model 1	0.97 (0.85; 1.11)	1.05 (0.93; 1.19)
	Model 2	0.94 (0.81; 1.10)	1.01 (0.88; 1.17)
	Model 3	0.94 (0.81; 1.10)	1.00 (0.86; 1.16)
Alcohol consumption[2] (unit alcohol/day)	Crude OR	0.87 (0.78; 0.96)	1.01 (0.91; 1.11)
	Model 1	0.85 (0.78; 0.93)	1.02 (0.95; 1.10)
	Model 2	0.86 (0.77; 0.96)	1.02 (0.95; 1.10)
	Model 3	0.86 (0.76; 0.96)	1.02 (0.95; 1.10)

Data were presented as prevalence odds ratio (OR) and its 95% confidence interval (CI).

[1]calculated from individuals who smoke.

[2]calculated from individuals who drink alcohol.

[3]Model for adjustment:

Model 1: adjusted for age and sex.

Model 2: adjusted for model 1 + other socio-demographic determinants (education, employment, marital, and socio-economic status).

Model 3: adjusted for model 2 + body mass index

SD: standardized unit

Table 2B. Association of lifestyle factors as categorical variables with prevalent diabetes in Indonesian urban and rural population.

Variables	Urban				Rural			
	Crude OR	Model 1[1]	Model 2[2]	Model 3[3]	Crude OR	Model 1[1]	Model 2[2]	Model 3[3]
Moderate/vigorous physical activity								
- Active	1	1	1	1	1	1	1	1
- Inactive	1.24	1.13	1.12	1.15	1.10	0.98	1.00	1.05
	(1.11; 1.40)	(1.00; 1.28)	(0.99; 1.28)	(1.01; 1.31)	(0.95; 1.28)	(0.84; 1.14)	(0.85; 1.18)	0.89; 1.24)
Fruit and vegetable intake								
- Adequate	1	1	1	1	1	1	1	1
- Not adequate	0.82	0.94	1.00	1.00	0.85	0.81	0.79	0.82
	(0.62; 1.07)	(0.71; 1.26)	(0.73; 1.36)	(0.73; 1.36)	(0.66; 1.08)	(0.63; 1.05)	(0.60; 1.04)	(0.62; 1.07)
Smoking								
- Never smoke	1	1	1	1	1	1	1	1
- Former smoker	1.26	0.98	0.96	0.96	0.93	0.85	0.89	0.89
	(1.06; 1.49)	(0.80; 1.20)	(0.77; 1.21)	(0.77; 1.20)	(0.72; 1.20)	(0.64; 1.13)	(0.66; 1.20)	(0.66; 1.20)
- Current smoker	0.52	0.56	0.57	0.61	0.55	0.62	0.63	0.68
	(0.45; 0.60)	(0.46; 0.67)	(0.47; 0.70)	(0.50; 0.74)	(0.48; 0.63)	(0.51; 0.76)	(0.51; 0.78)	(0.55; 0.84)
Alcohol consumption								
- Non-drinker	1	1	1	1	1	1	1	1
- Drinker	0.47	1.00	0.95	0.91	0.42	0.86	0.82	0.85
	(0.29; 0.76)	(0.61; 1.64)	(0.55; 1.64)	(0.53; 1.58)	(0.24; 0.75)	(0.48; 1.55)	(0.44; 1.54)	(0.46; 1.59)

Data were presented as prevalence odds ratio (OR) and its 95% confidence interval (CI).

[1]Model 1: adjusted for age and sex.

[2]Model 2: adjusted for model 1 + other socio-demographic determinants (education, employment, marital, and socio-economic status)

[3]Model 3: adjusted for model 2 + body mass index

Table 3A. Association of clinical factors as continuous variables with prevalent diabetes in Indonesian urban and rural population.

Variables	Model[1]	Prevalence Odds Ratio (95%CI)	
		Urban	Rural
BMI (kg/m^2)	Crude OR	1.06 (1.05; 1.07)	1.06 (1.05; 1.07)
	Model 1	1.06 (1.05; 1.07)	1.06 (1.05; 1.08)
	Model 2	1.06 (1.05; 1.07)	1.06 (1.05; 1.08)
	Model 3	1.06 (1.05; 1.07)	1.06 (1.05; 1.08)
Waist circumference (cm), per 5 unit increase	Crude OR	1.19 (1.16; 1.21)	1.15 (1.12; 1.18)
	Model 1	1.15 (1.13; 1.18)	1.14 (1.11; 1.17)
	Model 2	1.15 (1.12; 1.17)	1.14 (1.11; 1.17)
	Model 3	1.14 (1.12; 1.17)	1.14 (1.11; 1.16)
	Model 4	1.11 (1.07; 1.15)	1.11 (1.07; 1.15)
Systolic blood pressure (mmHg), per 10 unit increase	Crude OR	1.25 (1.23; 1.27)	1.20 (1.17; 1.22)
	Model 1	1.12 (1.10; 1.15)	1.10 (1.08; 1.13)
	Model 2	1.12 (1.10; 1.15)	1.10 (1.07; 1.13)
	Model 3	1.12 (1.10; 1.15)	1.09 (1.07; 1.12)
	Model 4	1.10 (1.08; 1.13)	1.07 (1.05; 1.10)
Diastolic blood pressure (mmHg), per 5 unit increase	Crude OR	1.15 (1.12; 1.17)	1.15 (1.13; 1.18)
	Model 1	1.09 (1.07; 1.12)	1.11 (1.09; 1.13)
	Model 2	1.10 (1.08; 1.12)	1.10 (1.08; 1.13)
	Model 3	1.10 (1.07; 1.12)	1.10 (1.08; 1.12)
	Model 4	1.07 (1.05; 1.09)	1.07 (1.05; 1.10)
Total cholesterol (mmol/L)	Crude OR	1.67 (1.58; 1.75)	1.49 (1.42; 1.58)
	Model 1	1.40 (1.33; 1.48)	1.30 (1.23; 1.38)
	Model 2	1.40 (1.32; 1.49)	1.30 (1.22; 1.37)
	Model 3	1.40 (1.33; 1.49)	1.29 (1.22; 1.37)
	Model 4	1.37 (1.29; 1.45)	1.25 (1.18; 1.32)
LDL cholesterol (mmol/L)	Crude OR	1.66 (1.56; 1.76)	1.49 (1.40; 1.58)
	Model 1	1.40 (1.32; 1.49)	1.30 (1.22; 1.39)
	Model 2	1.39 (1.30; 1.48)	1.30 (1.22; 1.39)
	Model 3	1.39 (1.29; 1.48)	1.30 (1.21; 1.38)
	Model 4	1.34 (1.25; 1.43)	1.23 (1.15; 1.32)
HDL cholesterol (mmol/L)	Crude OR	0.65 (0.54; 0.78)	0.82 (0.67; 0.99)
	Model 1	0.35 (0.29; 0.43)	0.47 (0.38; 0.58)
	Model 2	0.36 (0.29; 0.44)	0.46 (0.37; 0.57)
	Model 3	0.34 (0.28; 0.42)	0.45 (0.36; 0.55)
	Model 4	0.40 (0.32; 0.50)	0.52 (0.42; 0.65)
Triglyceride (mmol/L)	Crude OR	1.32 (1.25; 1.40)	1.32 (1.24; 1.41)
	Model 1	1.30 (1.22; 1.39)	1.32 (1.24; 1.40)
	Model 2	1.31 (1.23; 1.41)	1.32 (1.24; 1.41)
	Model 3	1.33 (1.24; 1.42)	1.33 (1.25; 1.42)
	Model 4	1.28 (1.20; 1.37)	1.28 (1.20; 1.36)

Data were presented as prevalence odds ratio (OR) and its 95% confidence interval (CI).
[1]Model for adjustment:
Model 1: adjusted for age and sex.
Model 2: adjusted for model 1 + other socio-demographic determinants (education, employment, marital, and socio-economic status).
Model 3: adjusted for model 2 + lifestyle determinants (physical activity, fruit and vegetable intake, smoking behaviour, and alcohol consumption).
Model 4: adjusted for model 3 + body mass index.

Table 3B. Association of clinical factors as categorical variables with prevalent diabetes in Indonesian urban and rural populations.

Variables	Urban					Rural				
	Crude OR	Model 1	Model 2	Model 3	Model 4	Crude OR	Model 1	Model 2	Model 3	Model 4
BMI categories[1]										
- Underweight (<18.5 kg/m2)	0.72 (0.56; 0.95)	0.74 (0.57; 0.97)	0.78 (0.58; 1.03)	0.77 (0.59; 1.03)		1.10 (0.92; 1.32)	0.94 (0.79; 1.13)	0.95 (0.79; 1.15)	0.96 (0.79; 1.16)	
- Normo-weight (18.5-22.9 kg/m2)	1	1	1	1		1	1	1	1	
- Overweight (23.0-24.9 kg/m2)	1.60 (1.35; 1.89)	1.58 (1.33; 1.87)	1.53 (1.27; 1.84)	1.51 (1.25; 1.81)		1.31 (1.10; 1.55)	1.31 (1.10; 1.57)	1.29 (1.07; 1.56)	1.27 (1.06; 1.54)	
- Obese (≥25.0 kg/m2)	2.05 (1.80; 2.34)	1.99 (1.73; 2.29)	1.98 (1.71; 2.30)	1.94 (1.67; 2.25)		1.82 (1.59; 2.07)	1.80 (1.56; 2.07)	1.80 (1.55; 2.10)	1.77 (1.52; 2.05)	
Abdominal obesity[2]										
- No	1	1	1	1	1	1	1	1	1	1
- Yes	2.46 (2.22; 2.73)	2.09 (1.86; 2.34)	2.08 (1.84; 2.35)	2.05 (1.81; 2.31)	1.70 (1.46; 1.97)	2.00 (1.78; 2.24)	1.77 (1.56; 2.01)	1.75 (1.54; 2.00)	1.72 (1.51; 1.95)	1.30 (1.11; 1.52)
Hypertension[3]										
- No	1	1	1	1	1	1	1	1	1	1
- Yes	3.12 (2.82; 3.46)	1.90 (1.69; 2.13)	1.88 (1.66; 2.12)	1.85 (1.63; 2.09)	1.66 (1.46; 1.88)	2.47 (2.22; 2.76)	1.73 (1.54; 1.95)	1.68 (1.49; 1.90)	1.66 (1.47; 1.88)	1.49 (1.31; 1.68)
High total cholesterol[4]										
- No	1	1	1	1	1	1	1	1	1	1
- Yes	2.51 (2.26; 2.79)	1.76 (1.57; 1.98)	1.74 (1.54; 1.96)	1.75 (1.55; 1.97)	1.65 (1.46; 1.87)	2.12 (1.89; 2.38)	1.59 (1.41; 1.80)	1.55 (1.36; 1.76)	1.54 (1.36; 1.75)	1.44 (1.27; 1.63)
High LDL-cholesterol[5]										
- No	1	1	1	1	1	1	1	1	1	1
- Yes	2.10 (1.89; 2.33)	1.56 (1.40; 1.74)	1.54 (1.37; 1.73)	1.54 (1.37; 1.73)	1.44 (1.28; 1.62)	1.76 (1.58; 1.96)	1.42 (1.27; 1.59)	1.39 (1.24; 1.57)	1.39 (1.24; 1.56)	1.29 (1.14; 1.44)
Low HDL-cholesterol[6]										
- No	1	1	1	1	1	1	1	1	1	1
- Yes	1.41 (1.27; 1.56)	1.56 (1.41; 1.74)	1.56 (1.40; 1.75)	1.59 (1.43; 1.78)	1.46 (1.30; 1.64)	1.41 (1.27; 1.56)	1.46 (1.31; 1.63)	1.48 (1.32; 1.66)	1.50 (1.33; 1.68)	1.39 (1.24; 1.56)
High triglyceride[7]										
- No	1	1	1	1	1	1	1	1	1	1
- Yes	2.16 (1.94; 2.41)	2.01 (1.79; 2.26)	2.06 (1.82; 2.32)	2.09 (1.85; 2.36)	1.90 (1.68; 2.15)	1.96 (1.75; 2.20)	1.88 (1.67; 2.12)	1.87 (1.65; 2.12)	1.88 (1.66; 2.13)	1.71 (1.51; 1.94)
Dyslipidemia[8]										
- No	1	1	1	1	1	1	1	1	1	1
- Yes	2.38 (2.08; 2.72)	1.95 (1.70; 2.23)	1.92 (1.66; 2.22)	1.95 (1.69; 2.26)	1.75 (1.51; 2.04)	1.76 (1.54; 2.01)	1.55 (1.35; 1.77)	1.54 (1.34; 1.77)	1.54 (1.34; 1.78)	1.39 (1.21; 1.60)

[1]BMI categories were based on the WHO cut-offs for Asian Population
[2]Ethnic-Specific (Asian) waist-circumference cut-offs for abdominal obesity were >90 cm for men and >80 cm for women.
[3]Hypertension was defined as systolic blood pressure >140 mmHg AND/OR diastolic blood pressure >90 mmHg OR previous hypertension diagnosis with current use of anti-hypertensive medications.
[4]High total cholesterol (hypercholesterolemia) was defined as total cholesterol levels ≥5.2 mmol/L ((≥200 mg/dL).
[5]High LDL-cholesterol was defined as LDL-cholesterol levels ≥3.4 mmol/L (≥130 mg/dL).
[6]Low HDL-cholesetrol was defined as HDL-cholesterol levels <1.0 mmol/L (<40 mg/dL) in men or <1.3 mmol/L (<50 mg/dL) in women.
[7]Hypertriglyceridemia was defined as triglyceride levels ≥1.7 mmol/L (≥150 mg/dL).
[8]Dyslipidemia was defined based of one or more of the following criteria: total cholesterol ≥200 mg/dL, OR LDL-cholesterol ≥130 mg/dL, OR low triglyceride (≥150 mg/dL), OR low HDL-cholesterol (<40 mg/dL in men or <50 mg/dL in women).
Model 1: adjusted for age and sex.
Model 2: adjusted for model 1 + other socio-demographic determinants (education, employment, marital, and socio-economic status).
Model 3: adjusted for model 2 + lifestyle determinants (physical activity, fruit and vegetable intake, smoking behavior, and alcohol consumption).
Model 4: adjusted for model 3 + body mass index

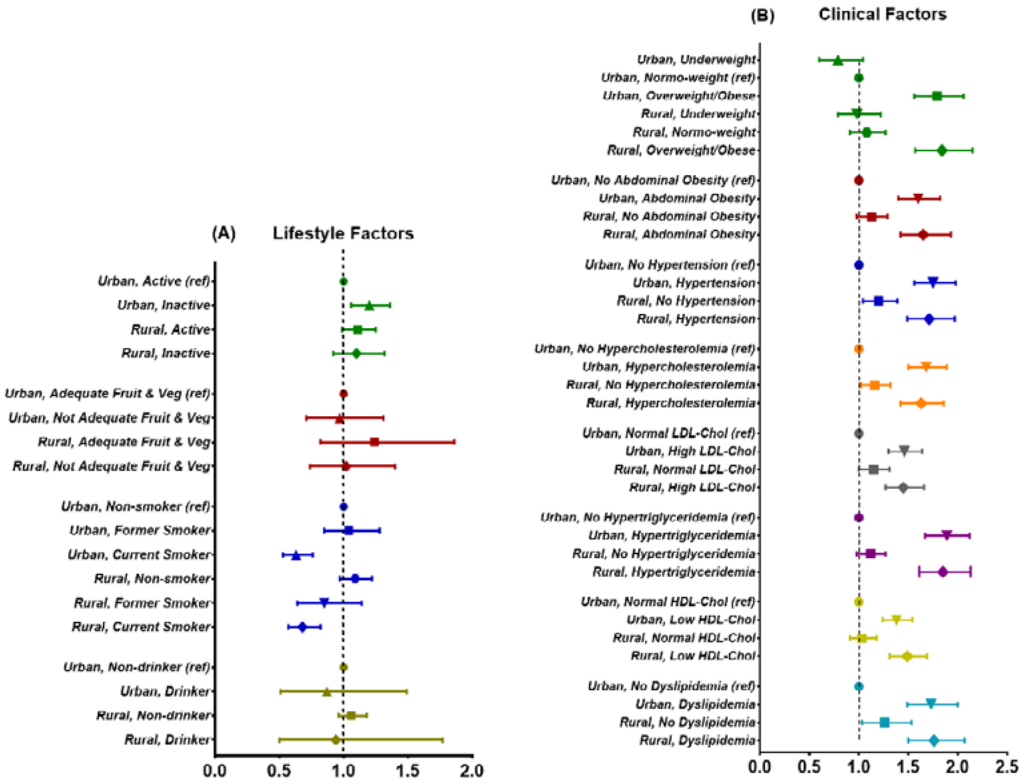

Figure 3. The differences in the association of lifestyle (A) and clinical (B) factors with prevalent diabetes between Indonesian urban and rural population. Data were presented as prevalence odds ratio (OR) with its 95% confidence interval (95%CI) compared with the reference category (ref), i.e., urban population without risk factors.
For models in A, Associations were adjusted for age, sex, socio-demographic determinants (level of education, type of employment, marital status, and socio-economic status), and body mass index (BMI).
For models in B, associations were adjusted for age, sex, socio-demographic determinants (level of education, type of employment, marital status, and socio-economic status), lifestyle factors (moderate/vigorous physical activity, fruit and vegetable intake, smoking, and alcohol consumption), and BMI.
Inactive was defined as moderate/vigorous physical activity <150 minutes/week.
Not adequate fruit and vegetable intake was defined as fruit and vegetable consumption <5 portions/day.
BMI categories were based on the WHO cut-offs for Asian population: underweight (BMI <18.5 kg/m2), normo-weight (BMI 18.5-22.9 kg/m2), and overweight/obese (BMI ≥23.0 kg/m2).
Ethnic-Specific (Asian) waist-circumference cut-offs for abdominal obesity were >90 cm for men and >80 cm for women.
Hypertension was defined as systolic blood pressure >140 mmHg AND/OR diastolic blood pressure >90 mmHg OR previous hypertension diagnosis with current use of anti-hypertensive medications.
Hypercholesterolemia was defined as total cholesterol levels ≥5.2 mmol/L ((≥200 mg/dL).
High LDL-cholesterol was defined as LDL-cholesterol levels ≥3.4 mmol/L (≥130 mg/dL).
Low HDL-cholesetrol was defined as HDL-cholesterol levels <1.0 mmol/L (<40 mg/dL) in men or <1.3 mmol/L (<50 mg/dL) in women.
Hypertriglyceridemia was defined as triglyceride levels ≥1.7 mmol/L (≥150 mg/dL).
Dyslipidemia was defined based of one or more of the following criteria: total cholesterol ≥200 mg/dL, OR LDL-cholesterol ≥130 mg/dL, OR low triglyceride (≥150 mg/dL), OR low HDL-cholesterol (<40 mg/dL in men or <50 mg/dL in women).

DISCUSSION

In this study utilizing the data from RISKESDAS 2018, we observed several differences in lifestyle and clinical determinants between Indonesian urban and rural population aged ≥15 years old. Compared with the urban population, the rural population had a better profile of lifestyle and clinical factors. Whereas there was no difference in the total prevalence of diabetes between the populations, a higher prevalence of previously diagnosed diabetes individuals using anti-diabetes medication was observed in the urban than in the rural population. In terms of lifestyle, physical inactivity was a risk factor for diabetes, and most strongly in the urban population. Whereas overweight/obesity, abdominal obesity, hypertension, and dyslipidemia were all risk factors for diabetes in both populations, there were no differences in the relation between these risk factors and the prevalence of diabetes between the urban and rural population.

The observed higher physical activity levels in rural compared to urban population had been shown in previous study.[30] This finding could be explained by the greater proportion of individuals in rural areas who work in the informal sector, which need more physical work, as shown in our previous study.[31] Our study also confirmed that longer duration of MVPA in a week is inversely associated with diabetes and physical inactivity is associated with higher risk of diabetes, which were more pronounced in the urban population. This could be explained by previous findings that leisure time, but not occupational physical activity, is associated with a lower risk of diabetes.[32]

Contrary to the finding from previous study,[33] we observed a positive association between fruit and vegetable consumption and diabetes in the urban population. One potential explanation for this finding may be reverse causation due to the cross-sectional nature of our study: patients with diabetes may have adjusted their diet after the diagnosis of diabetes. In addition, in our urban population, sex, age, and BMI seemed strong confounding factors in this association. More women, with higher age and BMI were observed for the highest tertile of fruit and vegetable intake compared to the lowest tertile. Furthermore, the types of fruit or vegetable and serving methods were not evaluated in this study. Previous study showed that certain types fruits or vegetables and juices were positively associated with diabetes.[33,34]

The finding of current smokers that is inversely associated with diabetes compared to the non-smoker group was also reported to be confounded by sex and BMI by several previous studies.[35,36] Indeed, in our study, the current smoker group had a lower BMI and male predominance compared with the non-smokers. Although, adjustment for sex and BMI did not fully attenuate the associations. The observed inverse association between alcohol consumption and diabetes in the urban population supports the findings from previous studies showing that light/moderate drinking might lower the risk of diabetes.[37,38] However, this finding must be interpreted carefully since current drinkers may represent a small selective group of the Indonesian urban population who drink alcohol, and may have a lower risk of diabetes because of other reasons than alcohol consumption.

The higher BMI and waist circumference, as well as the higher proportion of obesity in urban compared to rural population observed in the current study, confirmed the finding from our previous study.[10] Our present study also found these adiposity indices and obesity are positively correlated with diabetes in both populations, similar to what had been observed previously.[39,40] In addition, higher blood pressure and hypertension status as well-established clinical risk factors for diabetes,[39,41] were also confirmed in this study for both urban and rural populations. Subsequently, as reported before,[15] our study also showed a higher prevalence of hypercholesterolemia, high LDL-cholesterol, hypertriglyceridemia, and dyslipidemia in the urban than rural population. In concordance with the finding from previous study,[42] our study also observed positive associations between these lipid abnormalities and prevalent diabetes.

Interestingly, although rural population had a better lifestyle and clinical profile than the urban population, there were no differences in the associations with diabetes between the two populations, except for physical activity. Nevertheless, it must be noted there is an alarming increase of BMI in the rural areas of low-middle income countries, possibly due to transition from undernutrition to complex malnutrition with over consumption of low-quality calories,[43] which may lead to increased future rates of diabetes in rural populations. Our previous studies also support this postulate,

showing more unfavorable metabolic changes in rural compared to urban subjects, when exposed towards short-term high-fat high-calories diet intervention,[44] as well as a relatively long-term urban lifestyle.[10]

We observed no differences in the prevalence of total diabetes between urban and rural population. This supports the finding from The 2014 Indonesia Family Life Survey (IFLS) which showed a similar pattern (7.5% in urban vs. 6.8% in rural population) using HbA1c measurement.[45] Interestingly, another study using the same IFLS database reported a twice higher prevalence of known diabetes in the Indonesian urban compared to the rural population (2.9% vs. 1.4%, for urban and rural, respectively), similar to what was found in our current study.[46]

Based on the findings from our current study and The 2014 IFLS database, we could observe that majority of individuals with diabetes in Indonesia were undiagnosed and untreated, especially in the rural population. The higher proportion of undiagnosed and untreated diabetes observed in the rural population might be due to several factors: limited availability and difficulties to access of healthcare facilities,[47,48] relatively poor socio-economic factors requiring prioritization of household resources for needs other than health,[45] and the lower level of education may lead to a lack of knowledge in the importance of diabetes screening.[49] In addition, this number of undiagnosed diabetes in Indonesia is higher than the global prevalence of 44%, as reported by IDF in 2021.[1] Strikingly, compared with the 2007 Indonesian Basic Health Survey report showing approximately 74% out of the 5.7% Indonesian population with diabetes being undiagnosed,[50] there has been no improvement in the last decade regarding undiagnosed diabetes in Indonesia. Thus, concrete actions need to be taken by all related stakeholders to improve this condition since diabetes is associated with many health complications,[51] even worse if left untreated or sub-optimally managed.[52,53] In the long term, this could lead to deleterious outcomes and an even higher burden on the Indonesian health and economic system.

The relatively large number of participants and nationally representative data are some of the strengths of our current study. Thus, the findings in this study could be

generalized to the whole Indonesian population. Another added point offered by this study is the attempt to evaluate the magnitude of differences between urban and rural population on the association of lifestyle and clinical factors with diabetes. Our study also has some limitations that need to be considered. First, the unavailability of HbA1c data for diabetes diagnosis might lead to an underestimation of the total prevalence of diabetes in our study. Second, the observational and cross-sectional design of this study does not allow to evaluate the temporal relationship between exposures and outcome and may lead to reverse causation and residual confounding that may explain the unexpected associations between certain lifestyle factors with diabetes. Third, the possibility of information bias, including social desirability bias, and possible measurement error, could not be fully excluded in this study. Fourth, the unavailability of lipid lowering agent usage data might cause an underestimation of the prevalence of dyslipidemia/lipid-associated disorders. Lastly, there are other factors that might differ characteristically and in the association with diabetes between rural and urban population but not included in this study, such as consumption of high-risk foods,[54] macronutrients intake,[55] pollution,[56] parasitic infection,[57] and psychological stress.[58]

In conclusion, our study showed a better profile of lifestyle and clinical factors in the Indonesian rural compared to the urban population. Although there were no differences in the total prevalence of diabetes between the two populations, a high proportion of undiagnosed and untreated diabetes was observed, especially in the rural population. Moderate/vigorous physical activity needs to be encouraged more in the Indonesian population. Although there were no differences in the associations between clinical risk factor and diabetes between the two populations, all risk factors were associated with higher prevalence of diabetes. All these findings warrant extensive action, along with supportive government health policies, to overcome the diabetes pandemic in the Indonesian population. In particular, attention needs to be addressed to the high prevalence of undiagnosed and untreated diabetes in Indonesia.

Author contributions

Conceptualization: F.K., F.S.S., R.dM.; Methodology: F.K., F.S.S., S.T., D.L.T, E.S., R.dM.; Data acquisition: F.K., D.L.T, E.Y., T.J.E.T, D.S.H., P.S.; Formal analysis: F.K., F.S.S.; Supervision: E.S., P.S., R.dM.; Writing – original draft: F.K.; Writing – review and editing: F.S.S., S.T., D.L.T, E.Y., T.J.E.T, D.S.H., P.S., E.S., R.dM.

Conflict of Interest

The authors declare that they have no conflict of interest.

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SUPPLEMENTARY MATERIALS

Supplementary Table 1. The 2010 Indonesian Central Bureau Statistics criteria for defining urban and rural areas in Indonesia.

Criteria				Availability/accessibility for urban-related facilities		
Population density/km^2	Score	Farming household percentage	Score	Urban-related facilities	Criteria	Score
<500	1	>70.00	1	a. Kindergarten	• Yes OR <2.5 km	1
500 – 1249	2	50.00 – 69.99	2	b. Junior high school	• >2.5 km	0
1250 – 2499	3	30.00 – 49.99	3	c. Senior high school		
2500 – 3999	4	20.00 – 29.99	4	d. Market	• Yes OR <2.0 km	1
4000 – 5999	5	15.00 – 19.99	5	e. Shops	• >2.0 km	0
6000 – 7499	6	10.00 – 14.99	6	f. Movie theatre	• Yes OR <5.0 km	1
7500 – 8499	7	5.00 – 9.99	7	g. Hospital	• 5.0 km	0
> 8500	8	<5.00	8	h. Hotel/Pool/Nightclub/	• Yes	1
				Massage parlors/Salon	• No	0
				i. Percentage of house-hold	• ≥8.00	1
				with telephone	• <8.00	0
				j. Percentage of house-hold	• ≥90.00	1
				with electricity	• <9000	0

Total score ≥10 was categorized as urban area.

Supplementary Table 2. Age, sex, and BMI between tertiles of fruit and vegetable intake in Indonesian urban and rural population.

	Lowest tertile	Mid-tertile	Highest tertile
		URBAN	
Age*, years old	41.4 (15.2)	42.6 (14.5)	43.9 (14.7)
Sex, %male	54.6 (53.2; 56.0)	50.2 (48.9; 51.6)	46.1 (44.8; 47.4)
BMI*, kg/m^2	23.8 (4.6)	24.5 (4.7)	24.9 (4.8)
Fruit and vegetable intake#, portion/day	0.6 (0.4; 0.9)	1.4 (1.1; 1.6)	2.7 (2.1; 3.6)
		RURAL	
Age*, years old	45.2 (17.6)	43.7 (16.5)	44.5 (16.0)
Sex, %male	52.0 (50.7; 53.4)	49.5 (48.1; 50.9)	50.1 (49.0; 51.2)
BMI*, kg/m^2	22.7 (4.5)	23.2 (4.7)	23.4 (4.7)
Fruit and vegetable intake#, portion/day	0.7 (0.4; 1.0)	1.3 (1.1; 1.5)	3 (2.3; 3.7)

*normally distributed continuous variable, presented as mean and its standard deviation.
#non-normally distributed continuous variable, presented as median (25th, 75th percentile)
BMI: body mass index

Supplementary Table 3. Association between fruit and vegetable intake tertiles and prevalent diabetes in Indonesian urban and rural population adjusted for sex, age, and BMI.

Fruit and vegetable intake tertiles	Crude OR	Adjusted for sex	Adjusted for age	Adjusted for BMI	Adjusted for sex, age & BMI
			URBAN		
Lowest tertile	1	1	1	1	1
Mid-tertile	1.14 (0.99; 1.31)	1.12 (0.97; 1.29)	1.12 (0.97; 1.30)	1.09 (0.95; 1.26)	1.07 (0.92; 1.24)
Highest tertile	1.44 (1.26; 1.65)	1.40 (1.23; 1.60)	1.37 (1.19; 1.57)	1.36 (1.19; 1.55)	1.26 (1.09; 1.45)
			RURAL		
Lowest tertile	1	1	1	1	1
Mid-tertile	0.93 (0.81; 1.07)	0.91 (0.79; 1.05)	0.98 (0.85; 1.14)	0.95 (0.82; 1.10)	0.95 (0.82; 1.09)
Highest tertile	0.97 (0.85; 1.11)	0.96 (0.84; 1.10)	1.01 (0.88; 1.16)	0.96 (0.84; 1.10)	0.96 (0.84; 1.10)

Data were presented as prevalence odds ratio (OR) and its 95% confidence interval (CI).

Supplementary Table 4. Age, sex, BMI, and pack-years between the three categories of smoking habit in Indonesian urban and rural population.

	Non-smoker	Former smoker	Current smoker
		URBAN	
Age*, years old	41.8 (15.9)	49.0 (14.5)	42.6 (12.7)
Sex, %male	22.1 (21.1; 23.1)	83.5 (81.1; 85.7)	95.1 (94.4; 95.8)
BMI*, kg/m2	25.1 (5.1)	24.6 (4.6)	22.9 (3.7)
Pack-years#	0	14.4 (6.5; 30)	9.6 (4.0; 18.6)
		RURAL	
Age*, years old	43.1 (17.5)	51.0 (16.5)	45.8 (15.0)
Sex, %male	18.6 (17.7; 19.6)	87.1 (84.3; 89.5)	96.4 (95.8; 96.9)
BMI*, kg/m2	24.0 (5.2)	22.6 (4.4)	21.7 (3.4)
Pack-years#	0	16.2 (8.4; 28.2)	12 (5.7; 21)

*normally distributed continuous variable, presented as mean and its standard deviation.
#non-normally distributed continuous variable, presented as median (25th, 75th percentile)
BMI: body mass index

Supplementary Table 5. Association between smoking habit and prevalent diabetes in Indonesian urban and rural population adjusted for sex, age, and BMI.

Smoking categories	Crude OR	Adjusted for sex	Adjusted for age	Adjusted for BMI	Adjusted for sex, age & BMI
			URBAN		
- Non-smoker	1	1	1	1	1
- Former smoker	1.26	1.32	0.88	1.30	0.97
	(1.06; 1.49)	(1.10; 1.60)	(0.74; 1.05)	(1.09; 1.54)	(0.79; 1.19)
- Current smoker	0.52	0.55	0.49	0.59	0.60
	(0.45; 0.60)	(0.46; 0.66)	(0.43; 0.57)	(0.51; 0.68)	(0.50; 0.73)
			RURAL		
- Non-smoker	1	1	1	1	1
- Former smoker	0.93	1.12	0.70	0.99	0.85
	(0.72; 1.20)	(0.85; 1.50)	(0.54; 0.90)	(0.76; 1.28)	(0.64; 1.13)
- Current smoker	0.55	0.69	0.49	0.61	0.67
	(0.48; 0.63)	(0.56; 0.84)	(0.43; 0.56)	(0.54; 0.70)	(0.55; 0.81)

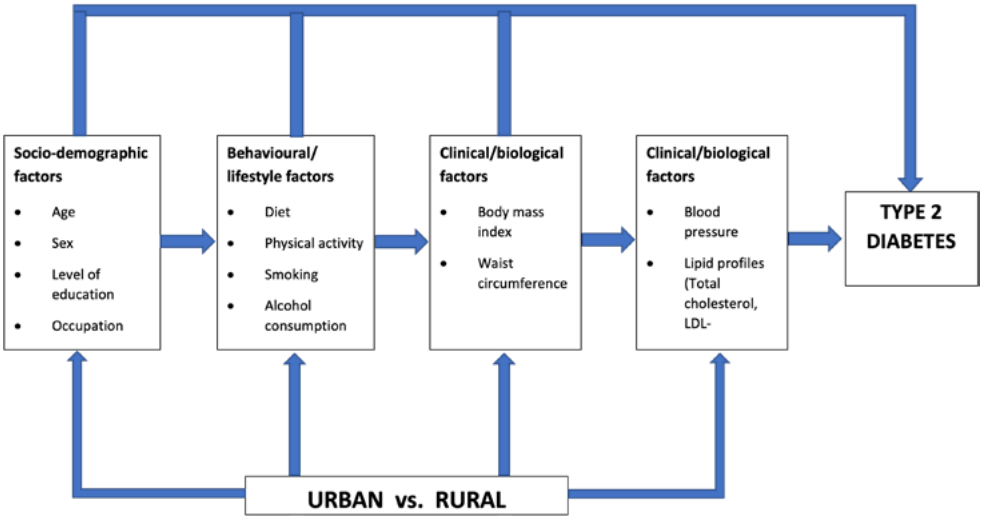
Data were presented as prevalence odds ratio (OR) and its 95% confidence interval (CI).

Supplementary Table 6. Age, sex, and BMI among the groups generated from the interaction between urban/rural and clinical factors.

Groups	Age	Sex (%male)	BMI
BMI Categories			
- Urban, Underweight	37.6 (18.6)	64.7 (62.1; 67.3)	17.1 (1.0)
- Urban, Normo-weight	41.5 (15.9)	60.1 (58.8; 61.4)	20.9 (1.2)
- Urban, Overweight/Obese	44.1 (13.1)	42.3 (41.3; 43.3)	27.6 (3.9)
- Rural, Underweight	46.7 (21.4)	62.6 (60.4; 64.8)	17.2 (1.2)
- Rural, Normo-weight	44.6 (17.4)	62.1 (60.9; 63.2)	20.8 (1.3)
- Rural, Overweight/Obese	43.7 (14.0)	35.6 (34.5; 36.7)	27.0 (3.7)
Abdominal Obesity			
- Urban, No abdominal obesity	40.6 (15.3)	64.6 (63.6; 65.5)	21.7 (3.0)
- Urban, Abdominal obesity	45.5 (13.4)	29.9 (28.7; 31.1)	28.3 (4.3)
- Rural, No abdominal obesity	44.2 (17.3)	63.2 (62.4; 64.0)	21.2 (3.1)
- Rural, Abdominal obesity	45.0 (14.5)	18.6 (17.4; 19.8)	27.8 (4.3)
Hypertension			
- Urban, No hypertension	37.9 (14.0)	52.8 (51.8; 53.8)	23.4 (4.3)
- Urban, Hypertension	49.7 (13.6)	46.6 (45.3; 47.8)	25.9 (4.9)
- Rural, No hypertension	40.2 (16.1)	54.9 (54.0; 55.8)	22.2 (4.2)
- Rural, Hypertension	51.0 (15.1)	43.8 (42.7; 44.9)	24.3 (4.9)
Hypercholesterolemia			
- Urban, No hypercholesterolemia	40.0 (14.9)	53.4 (52.5; 54.3)	23.8 (4.7)
- Urban, Hypercholesterolemia	48.8 (12.9)	43.0 (41.6; 44.5)	25.6 (4.7)
- Rural, No hypercholesterolemia	42.4 (16.8)	54.0 (53.2; 54.8)	22.7 (4.4)
- Rural, Hypercholesterolemia	50.3 (14.3)	40.8 (39.3; 42.3)	24.2 (4.9)
High LDL-Cholesterol			
- Urban, Low/normal LDL-cholesterol	39.8 (15.1)	53.0 (52.1; 53.9)	23.6 (4.6)
- Urban, High LDL-cholesterol	47.2 (13.4)	46.0 (44.8; 47.3)	25.6 (4.8)
- Rural, Low/normal LDL-cholesterol	42.3 (17.1)	53.9 (53.0; 54.8)	22.5 (4.4)
- Rural, High LDL-cholesterol	48.5 (14.8)	44.3 (43.0; 45.6)	24.2 (4.9)
Hypertriglyceridemia			
- Urban, No hypertriglyceridemia	41.3 (15.4)	46.4 (45.5; 47.3)	23.8 (4.7)
- Urban, Hypertriglyceridemia	46.0 (12.9)	60.1 (58.7; 61.5)	26.0 (4.4)
- Rural, No hypertriglyceridemia	43.5 (17.0)	48.1 (47.2; 48.9)	22.6 (4.5)
- Rural, Hypertriglyceridemia	47.2 (15.2)	57.7 (56.2; 59.2)	24.4 (4.8)
Low HDL-Cholesterol			
- Urban, High HDL-cholesterol	43.3 (15.3)	55.2 (54.2; 56.2)	23.5 (4.5)
- Urban, Low HDL-cholesterol	41.7 (14.2)	43.1 (41.9; 44.4)	25.6 (4.8)
- Rural, High HDL-cholesterol	45.8 (16.7)	58.4 (57.4; 59.3)	22.3 (4.2)
- Rural, Low HDL-cholesterol	42.5 (16.3)	39.3 (38.1; 40.5)	24.2 (5.0)
Dyslipidemia			
- Urban, No dyslipidemia	38.8 (15.5)	55.6 (54.1; 57.0)	22.5 (4.4)
- Urban, Dyslipidemia	44.3 (14.3)	48.0 (47.1; 48.9)	25.2 (4.7)
- Rural, No dyslipidemia	42.8 (17.5)	60.3 (58.9; 61.8)	21.6 (3.8)
- Rural, Dyslipidemia	45.2 (16.2)	45.9 (45.1; 46.8)	23.8 (4.8)

Data were presented as mean (standard deviation) for age and BMI variables; meanwhile proportion and its 95% confidence interval for sex variable.

BMI: body mass index



Supplementary Figure 1. Conceptual framework and hypothesis diagram of the association between socio-demographic, lifestyle, and clinical factors with diabetes in the urban and rural populations.

5

Chapter 6

TH2A AND CD38+ TH2A CELLS IN PERIPHERAL BLOOD AND NASAL MUCOSA OF INDIVIDUALS WITH ALLERGIC RHINITIS IN URBAN AND RURAL INDONESIA

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ABSTRACT

Background: The prevalence and severity of AR are generally higher in urban than in rural areas. It is hypothesized that urbanization alters the immune system and thereby, AR manifestation.

Aims: To evaluate the peripheral blood and nasal mucosal immune cells of Indonesian young adults with AR originating from rural and urban areas.

Methods: AR subjects were skin prick test (SPT) positive to allergens and had rhinitis symptoms based on ISAAC questionnaire. The healthy control (HC) subjects were negative for SPT and had no rhinitis symptoms. Total IgE levels were determined by ELISA and eosinophil counts through microscopy. Whole blood and nasal mucosal samples of 18 urban (10 AR and 8 HC) and 12 rural (6 AR and 6 HC) subjects were analyzed using mass cytometry.

Results: In comparison to HC, both urban and rural AR subjects had higher total IgE levels, while eosinophil counts were only elevated in urban AR group. Major differences were seen when examining the nasal mucosa, where basophils, mast cells, CD4 Th2, Th2A, and $CD38^+$ Th2A cells were upregulated, but only in the urban AR. In addition, the expression of the upper respiratory tract homing marker CCR3 on $CD38^+$ Th2A cells was restricted to the urban AR group. In contrast, the differences in peripheral blood were modest, with only a significantly higher $CD163^+$ mDCs in rural AR.

Conclusion: Urban AR showed strong inflammatory immune responses in the nasal mucosa compared to rural AR, which might explain the higher disease activity in urban areas globally.

INTRODUCTION

Allergic rhinitis (AR) is a chronic inflammatory allergic disease involving the nasal mucosa, characterized by rhinorrhea, nasal congestion, sneezing, and itchy eyes.[1] The worldwide prevalence of AR in the adult population is approximately 18.1% (ranging from 1.0% to 54.5%).[2] Although AR is not a life-threatening condition, it causes a high economic burden, including indirect costs due to high rates of absenteeism and decreased work productivity.[3] It also has major effects on sleep, daily activity, emotional well-being, and quality of life (QOL).[3,4]

Indonesia has one of the lowest AR prevalence (5.2%) globally based on the result of The International Study of Asthma and Allergies in Childhood (ISAAC) in 1998.[5] However, the prevalence of AR has increased significantly, as reported by several recent studies, between 13.5% to 38.4%.[6-9] This rising prevalence could be associated with the rapid urbanization associated with rapid socio-economic growth in Indonesia.[10]

Urbanization causes significant alterations in the social, environmental, and lifestyle aspects of human lives, such as: dietary intake,[11] farming exposure,[12] parasitic infections,[13] hygiene and sanitation,[14] biodiversity,[15] and pollution.[16] These changes affect the immune system and thereby, disease pathogenesis and outcome.[17] As a systemic immune disease with nasal mucosa as its effector site, AR manifestation is likely to be modified by these potential effects of urbanization on the immune system. Many studies have shown a higher prevalence of AR in urban compared to rural populations,[18-20] although no data is available for Indonesian. Additionally, a less severe manifestation of allergic rhinitis in rural compared to urban population has been reported previously.[21,22]

Numerous studies have been performed to evaluate the role of the immune system in AR pathophysiology, either in peripheral blood or nasal site. The majority of these studies reported an upregulation of basophils,[23,24] eosinophils,[25,26] mast cells,[27] group 2 innate lymphoid cells (ILC2s),[28,29] and CD4 Th2 cells in AR patients both in systemic and nasal compartments.[30,31] Recent studies have identified a specific subset of Th2 cells, the pathogenic Th2A cells, that play an important role in

AR.[32,33] However, despite the differences in the prevalence and disease severity, no study has compared the immune system in AR individuals of urban and rural areas. In this study, we analyzed the immune profiles of whole blood and nasal mucosal compartments using mass cytometry in Indonesian young adults originating from urban and rural areas, with and without AR.

METHODS

Study design and population

This cross-sectional study included part of the subjects from a larger cohort study evaluating the effect of urbanization on metabolic health and allergy in an Indonesian young adult population. This study was conducted in the Depok campus of the University of Indonesia (UI), involving freshmen UI bachelor students. The study was approved by the Health Research Ethical Committee of Faculty of Medicine Universitas Indonesia (No. 1181/UN2.F1/ETIK/2017). All participants provided informed consent prior to the study.

The detailed procedures for recruitment of study participants can be found in our previous study.[11] The subjects were classified into the urban group if they were born and lived in urban areas, such as in Jakarta metropolitan areas or in one of the provincial capital cities. While, the rural group comprised subjects that were originally born and lived in rural areas, defined as the villages that are located at the district levels across Indonesia, and just recently (less than three months) migrated to an urban area. Subjects with pregnancy, any current infections, history of autoimmune diseases, and the usage of anti-inflammatory drugs, anti-histamines, or steroids were excluded from the study. All subjects' measurements and biological samples collections were performed in the first three months of the start of the academic year, between August to November 2019.

From the larger cohort, 18 urban (8 allergic rhinitis/AR and 10 healthy control/HC) and 12 rural (6 AR and 6 HC) subjects with availability of whole blood and nasal samples were selected for mass cytometry measurements. These subjects were referred as mass cytometry cohort. To confirm the findings observed in the whole blood mass

cytometry measurements, flow cytometry was performed in the remaining subjects from the larger cohort, and referred as the flow-cytometry cohort. This latter dataset consisted of 13 urban (5 AR and 8 HC) and 18 rural (11 AR and 7 HC) subjects (see **Fig. S1** for the flow chart for the inclusion of study participants). Both cohorts compared AR and HC subjects from each urban and rural group.

Skin prick test, ISAAC questionnaire, and allergic rhinitis definition

All subjects underwent a skin prick test (SPT) using extracts of five common aeroallergens: two species of house dust mites (*Dermatophagoides farinae/Der F and Dermatophagoides pteronyssinus/Der P*), cockroach/*Blatella germanica*, dog epithelial, and cat epithelial (ALK-Abello BV, Almere, The Netherlands). Saline was used as a negative control and histamine chloride (10 mg/mL) as the positive control. The SPT was performed on the volar side of the subject's lower arm using skin prick lancets. After fifteen minutes of application, the wheal sizes were measured. Skin test reactivity was considered positive if the longest diameter plus the diameter perpendicular of wheal size divided by two was 3 mm or larger than the negative control.[34]

For evaluating rhinitis symptoms, all subjects were asked to filled in the core questionnaire for rhinitis from the ISAAC (International Study of Asthma and Allergies in Childhood) questionnaire, which has been validated previously for the Indonesian population.[35,36] Subjects were defined as having rhinitis symptoms if they answered 'Yes' to the questions *"Have you ever had a problem with sneezing, or a runny, or a blocked nose when you DID NOT have a cold or the flu?"* and *"In the past 12 months, have you had a problem with sneezing, or a runny, or a blocked nose when you DID NOT have a cold or the flu?"*.

Allergic rhinitis (AR) subjects were defined as having reactive SPT results for any of the aeroallergens and positive rhinitis symptoms based on the ISAAC questionnaire (SPT+RHI+). Additionally, the question *"In the past 12 months, how much did this nose problem interfere with your daily activities?"* in the ISAAC questionnaire was used to evaluate the severity of rhinitis symptoms in these AR subjects. This was categorized as no, mild, moderate, or severe activity disturbance. The subjects who had negative SPT results and without rhinitis symptoms (SPT-RHI-) were included as healthy control (HC) subjects.

Biological samples collection

Blood samples collection were performed after an overnight fasting. EDTA blood samples were used to prepare Giemsa-stained peripheral blood smear for evaluation of eosinophil counts. While, SST containing blood samples were centrifuged to obtain the serum samples and stored in a −80°C freezer until measurement. Approximately 200 µL sodium heparin whole blood sample was mixed together with 1 mL cryopreservation solution medium (CryoStor® CS10, STEMCELL Technologies, Cologne, Germany) in cryovials and was transferred to a −80°C freezer for a minimum of 4 hours. Subsequently, cryovials were stored in liquid nitrogen until analysis for immune cells profiling. Cellular frequencies of such whole blood cryopreserved samples were previously shown to correlate with immune frequencies measured on fresh blood samples.[37]

To obtain immune cells from the nasal mucosal layer, nasal curettages were collected using a small probe with a previously described protocol.[38] Briefly, the nasal inferior turbinate was visualised using a headlamp with the subject in a seated position and head slightly tilted posteriorly and then, curettage was performed using small probe/curette (ASL Rhino-Pro©, Arlington Scientific, Utah, USA) to collect the cells from the mucosal layer. A total of four scrapes, two from each nostril, were obtained from each subject. After each curettage, the nasal probe was flicked into 1 mL cryopreservation solution medium (CryoStor® CS10, STEMCELL Technologies, Cologne, Germany) inside a cryovial until all biological material was dislodged. Subsequently, these cryovials were transferred to a freezing unit in a −80°C freezer for a minimum of 4 hours and then stored in liquid nitrogen for further analysis.

Total IgE and eosinophil counts measurements

The levels of total IgE were measured in the serum samples by ELISA as described previously.[39] The results were expressed in International Units (IU/mL). As some parts of Indonesia are helminth endemic areas and previous study showed that helminth infections could increase the total IgE levels and upregulated the pathogenic Th2A immune cells population,[40] healthy subjects (SPT-RHI-) with total IgE >500 IU/mL were excluded for immune cells profiling. The cut-off of 500 IU/mL was based on the

median total IgE levels of all subjects without helminth infection in urban and rural population, calculated from our previous study.[41] The Giemsa-stained peripheral thin blood smears were assessed to obtain the differential white blood cell counts, resulting in a relative percentage of eosinophils (eosinophil counts).

Mass cytometry

Mass cytometry measurement was performed on the cryopreserved whole blood (WB) and nasal curettage samples of 18 urban and 12 rural mass cytometry cohort subjects. Two antibody panels were designed for this experiment, one for phenotyping the immune cells ex vivo (**Table S1**), while the other was for sample's barcoding (**Table S2**). Samples were measured with a HeliosTM mass cytometer (Fluidigm, USA) in several batches and the obtained .FCS files were exported and pre-processed using 'CyTOFclean' (v1.0.3.)[42] and 'CATALYST' (v1.22.0)[43] packages. For WB samples, granulocytes were defined as $EpCAM^{-}CD66b^{+}$ and other immune cells as $EpCAM^{-}CD45^{+}CD66b^{-}$. For nasal mucosal samples, epithelial cells were defined as additional population expressing $EpCAM^{+}CD45^{-}$, leading to two blood and three nasal populations exported (**Fig. S2**). The resulting .FCS files were then exported to the OMIQ software (Dotmatics, Boston, USA) separately for WB and nasal mucosal immune cells. Batch correction of the phenotypic markers was performed with CytoNorm (k=5 for WB, k=3 for nasal mucosal samples) using concatenated samples as reference control, and results were assessed visually.[44] We performed Uniform Manifold Approximation and Projection (UMAP) [45] algorithm to visualize the high dimensional data, and FlowSOM [46] consensus metaclustering (k=50 for WB, k=25 for nasal mucosal) algorithm was applied to generate immune cell clusters. A total of 49 immune cells clusters were identified from the WB samples (**Table S3**), while 23 clusters from nasal mucosa (**Table S4**), which were exported as .CSV files for further statistical analysis. For the identification of pathogenic Th2A in the WB and nasal mucosal mass cytometry dataset, manual gating was performed in the OMIQ software. The pathogenic Th2A was defined as $CD4^{+}CD27^{-}CCR7^{-}CRTH2^{+}CD161^{+}$, as described previously.[33] An additional gating for CD38 was applied in this pathogenic Th2A population to obtain the $CD38^{+}$ Th2A (see **Fig. S3A & S3B** for gating strategy).

Flow-cytometry

To confirm the findings regarding the pathogenic Th2A immune cells population from the mass cytometry analysis, we performed flow cytometry using cryopreserved WB samples from 13 urban and 18 rural flow cytometry cohort subjects. After preparation and staining with extracellular antibody cocktail (**Table S5**), the samples were acquired on a Cytek Aurora 5L spectral flow cytometer and unmixed using SpectroFlo software (Cytek Biosciences, USA). The .FCS files were then exported to the OMIQ software and a similar gating strategy for mass cytometry data was applied for the identification of pathogenic Th2A immune cells population. An additional gating for CCR3, an important marker for T-cell homing in the human upper airway mucosa,[47] was performed to obtain the CD4 Th2 $CCR3^{+,}$ Th2A $CCR3^{+,}$ and $CD38^{+}$ Th2A $CCR3^{+}$ immune cell populations (**Fig. S3C**).

Statistical analysis

For clinical variables, data were presented as means and its standard deviations (SD) if normally distributed and as median (25^{th}, 75^{th} percentile) if not normally distributed. To evaluate the differences of total IgE levels and eosinophil counts between AR and HC subjects for each urban and rural group, Mann-Whitney test was performed.

All the CSV files generated from OMIQ were imported and analysed in R software (x64 version 4.1.2) within RStudio version 1.4 and analyzed using a generalized linear mixed model (lme4 and lmerTest package v3.1-3) [48,49] to evaluate the differences between AR and HC subjects for each urban and rural group. We adjusted the P-values with Benjamini Hochberg procedure to correct for multiple testing hypothesis.[50] Lastly, for the differences of CD4 Th2, Th2A, and $CD38^{+}$ Th2A between AR vs. HC subjects for each urban and rural group, separate generalized linear mixed model test was applied. For all tests, statistical significance was considered at the two-sided 5% level.

More detailed information on mass cytometry and flow cytometry procedures, as well as statistical analysis, are available at the **Supplementary Methods.**

RESULTS

Characteristic of study population

As indicated in **Table 1**, the age and BMI were comparable between AR and HC subjects in both urban and rural groups, but the proportion of male participants was higher in the AR group. Additionally, higher total IgE levels were observed in both urban and rural AR compared to HC subjects. Eosinophil counts were higher only in the urban AR than HC subjects (**Fig. S4A**). Similar patterns for these clinical characteristics, total IgE levels, and eosinophil counts were also observed for the flow cytometry cohort (**Table 1**, **Fig. S4B**) as well as the larger cohort that the study subjects were selected from (**Table S6**), indicating a representative group were analyzed.

Systemic immune profiles of allergic rhinitis vs. healthy control subjects from urban and rural areas

A total of 2.19 x 10^6 WB immune cells from thirty subjects were included for unsupervised clustering using FlowSom algorithm in the OMIQ software to identify cell subsets, as indicated and visualized with UMAP. The expression of immune cell markers used for clustering can be seen in **Fig. 1A and S5**. Furthermore, 49 immune cell clusters were captured within B cells, CD4 and CD8 T cells, unconventional T cells, γδ T cells, NK and innate lymphoid cells, as well as monocytes and dendritic cells (**Fig. 1B and C, Table S3**).

A higher frequency of cluster 28 ($CD163^+$ myeloid dendritic cells/mDCs) was seen in rural but not in urban AR compared to HC counterparts (**Fig. 1D**). Similar trends were observed for clusters 29 ($CD4^+CD25^{hi}CD127^-$/Tregs) and 35 (non-classical monocytes), which were not statistically significant after fdr-correction. When considering the type-2 immune responses, which are known to be associated with allergic disorders, we found no differences in the frequency of cluster 19 (CD4 Th2), cluster 27 (basophils), and cluster 26 (ILC2s) between AR and HC subjects in both groups (**Fig. S6, Table S7**). Altogether, despite the clear differences in the systemic total IgE levels between AR and HC subjects in both urban and rural groups, there were less prominent differences in peripheral blood immune cells known to have an important role in the pathogenesis of AR. The only statistically significant difference was in the $CD163^+$ mDCs, which was upregulated in the rural AR individuals compared to HCs.

Table 1. Comparison between allergic rhinitis (AR) and healthy control (HC) subjects for urban and rural group in the subsets for mass cytometry and flow cytometry (confirmation study) measurement.

Variables	Mass cytometry subjects				Confirmation study (flow cytometry) subjects			
	Urban (N=18)		Rural (N=12)		Urban (N=13)		Rural (N=18)	
	SPT+RHI+ (AR) (n=8)	SPT+RHI+ (AR) (n=8)	SPT+RHI+ (AR) (n=6)	SPT-RHI- (HC) (n=6)	SPT+RHI+ (AR) (n=5)	SPT-RHI- (HC) (n=8)	SPT+RHI+ (AR) (n=11)	SPT-RHI- (HC) (n=7)
Age (years old)#, median (25^{th}; 75^{th} percentile)	18.1 (17.4; 18.6)	18.5 (18.1; 19.1)	18.7 (18.0; 19.1)	18.9 (18.6; 19.1)	18.5 (17.9; 19.6)	18.5 (18.1; 18.8)	18.9 (17.9; 19.3)	18.7 (17.9; 18.9)
Sex, n male (%)	4 (50.0)	5 (50.0)	5 (83.3)	3 (50.0)	2 (40.0)	3 (37.5)	9 (81.8)	2 (28.6)
BMI (kg/m2)#, median (25^{th}; 75^{th} percentile)	19.9 (18.3; 23.0)	22.6 (19.2; 24.1)	21.1 (19.4; 26.9)	19.6 (17.4; 22.2)	20.5 (18.1; 24.5)	18.9 (17.5; 23.0)	21.0 (18.8; 23.6)	19.0 (18.0; 23.6)
Activity disturbances, n positive (%)								
- No	2 (25.0)	NA	3 (50.0)	NA	2 (40.0)	NA	4 (36.4)	NA
- Mild	3 (37.5)		2 (33.3)		2 (40.0)		6 (54.5)	
- Moderate	3 (37.5)		1 (16.7)		1 (20.0)		1 (9.1)	

#non-normally distributed continuous variables, presented as median (25^{th}; 75^{th} percentile).

SD: standard deviation; BMI: body mass index; SPT+RHI+: reactive SPT result combined with the presence of rhinitis symptoms; SPT-RHI-: negative SPT results without any rhinitis symptoms; AR: allergic rhinitis; HC: healthy control; NA: not applicable.

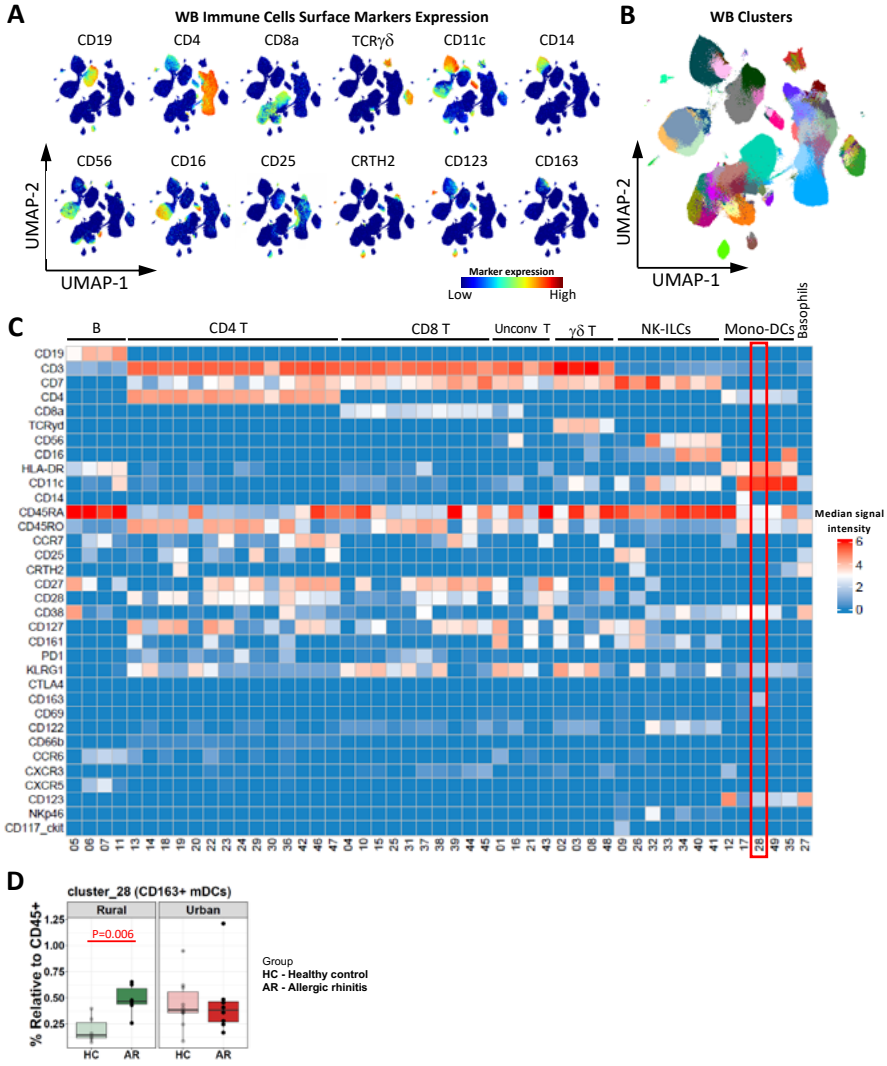

Figure 1. Higher frequency of systemic CD163+ mDCs was observed in rural allergic rhinitis compared to healthy control subjects but not in the urban group.

A. The expression of several immune cell surface markers obtained from mass-cytometry measurement, used for the clustering of whole-blood immune cell populations. **B.** Uniform Manifold Approximation and Projection (UMAP) of whole-blood immune cells clustering in mass-cytometry data based on the cell markers expression. **C**. A heatmap summary of median expression values of cell markers expressed by whole-blood immune cell clusters identified in hierarchical clustering. Red line box in the heatmap indicated the cluster with statistically significant differences (adjusted P-value <0.05) between AR vs. HC subjects. **D**. Box plots depicting the percentage of immune cell cluster (relative to CD45+ cells) significantly different between AR vs. HC subjects. To evaluate the differences between AR vs. HC subjects for each rural and urban group, generalized linear mixed model test was performed for all identified clusters with Benjamini-Hochberg correction for multiple testing to obtained the adjusted P-values.

AR: allergic rhinitis subjects; HC: healthy control subjects.

Altogether, despite the clear differences in the systemic total IgE levels between AR and HC subjects in both urban and rural groups, there were less prominent differences in peripheral blood immune cells known to have important role in the pathogenesis of AR. The only statistically significant difference was in the $CD163^+$ mDCs, which was upregulated in the rural AR individuals compared to HCs.

Nasal mucosal immune profiles of allergic rhinitis vs. healthy control subjects in urban and rural group

From 1.01 X 10^5 nasal immune cells included for unsupervised clustering and based on the expression of immune cell markers (**Fig. 2A and S7**), a total of 23 immune cell clusters were included for further statistical analysis (**Fig. 2B and 2C, Table S4**). Higher frequencies of cluster 06 (basophils), cluster 07 ($CD4^+CD25^{hi}CD127^-$), and cluster 10 (mast cells) were found in urban AR compared to HC, but not in rural subjects. A similar trend was also observed for an activated memory T cell cluster 08 ($CD4^+CD45RO^+CD38^+PD\text{-}1^{+)}$ (**Fig. 2D, Table S8**). Moreover, trends of decreased frequencies of cluster 05 ($HLA\text{-}DR^+CD11c^+CD16^+$) and the NK cluster 19 ($CD7^+CD56^+CD11c^+NKp46^+$) were seen in rural AR versus HC, but not in the urban group (**Fig. S8, Table S8**). Thus, more differences in the nasal mucosal immune cell clusters were observed in the urban than rural AR subjects when compared to the HCs.

Systemic and nasal mucosal pathogenic Th2A and $CD38^+$ Th2A in urban and rural allergic rhinitis subjects

Although CD4 Th2 cells have long been established as a major player in allergic rhinitis,[31,51] recent studies revealed a distinct subpopulation of Th2 cells, which was identified as pathogenic Th2A ($CD4^+CRTH2^+CD27^-CD161^+$), that might have a crucial role in the pathogenesis of allergic diseases [32,33]. Therefore, we manually gated this cell population within the WB mass cytometry dataset (**Fig. S3A**). We did not observe differences in the percentages of systemic CD4 Th2 cells in either urban or rural AR, in comparison to the HC subjects. However, a higher frequency of pathogenic Th2A was observed in rural AR subjects but not in the urban AR when compared with corresponding HC. In addition, the activation marker CD38 was similarly upregulated

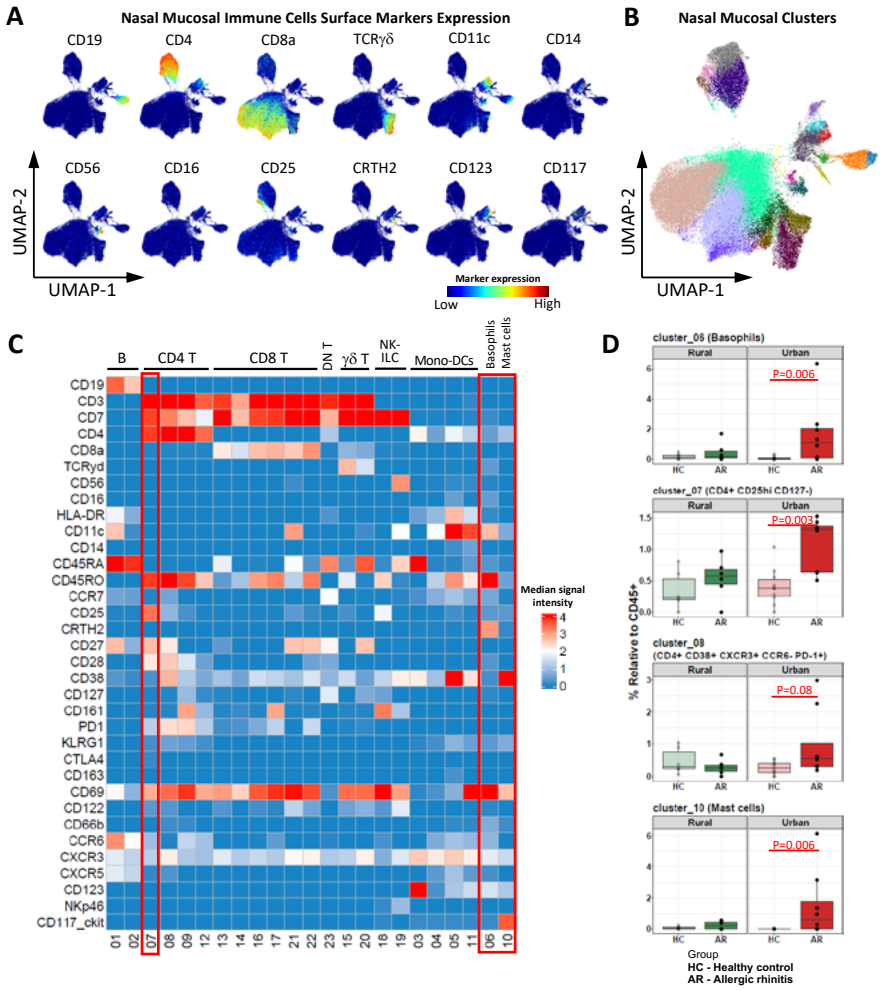

Figure 2. Higher frequency of nasal mucosal basophils, mast cells, and CD4+CD25hiCD127- were observed in urban allergic rhinitis compared to healthy control subjects but not in the rural group.

A. The expression of several immune cell surface markers obtained from mass-cytometry measurement, used for the clustering of nasal mucosal immune cell populations. **B**. Uniform Manifold Approximation and Projection (UMAP) of nasal mucosal immune cells clustering in mass-cytometry data based on the cell markers expression. **C**. A heatmap summary of median expression values of cell markers expressed by nasal mucosal immune cell clusters identified in hierarchical clustering. Red line box in the heatmap indicated the cluster with statistically significant differences (adjusted P-value <0.05) between AR vs. HC subjects. **D**. Box plots depicting the percentages of several systemic immune cell clusters (relative to $CD45^+$ cells) significantly different between AR vs. HC subjects. To evaluate the differences between AR vs. HC subjects for each rural and urban group, generalized linear mixed model test was performed for all identified clusters with Benjamini-Hochberg correction for multiple testing to obtained the adjusted P-values.
AR: allergic rhinitis subjects; HC: healthy control subjects.

on these Th2A cells in both urban and rural AR compared to their HC counterparts (**Fig. 3A**). These findings in the WB mass cytometry dataset were confirmed in a second cohort using flow cytometry (**Fig. S9A**). Furthermore, these Th2A and CD38$^+$ Th2A cells were positively correlated with the levels of activity disturbances, especially in the urban AR subjects (**Fig. S9B**).

Previous unsupervised clustering in the nasal mucosal immune cells did not identify the CD4 Th2 cells as a separate cluster. By performing manual gating, we could identify this population, as well as the pathogenic Th2A cells (**Fig. S3B**). We found elevated percentages of nasal mucosal CD4 Th2 and Th2A cells, as well as the upregulation of CD38 in the Th2A immune cells, only in the urban AR versus HC subjects, but not for rural group (**Fig. 3B**). Subsequently, positive correlations were observed for these frequencies of nasal mucosal CD4 Th2, Th2A, and CD38$^+$ Th2A cells with the levels of activity disturbances, also only in urban but not rural group (**Fig. S9C**).

Taken together, our study confirmed the role of peripheral blood pathogenic Th2A and/or CD38$^+$ Th2A in AR, but the presence of these cells in the nasal mucosa was only seen in urban AR, which might be related with the severity of AR clinical manifestation.

CCR3 expression on Th2 cells in urban and rural allergic rhinitis subjects

To explain the discrepancies in the findings between systemic and nasal mucosal compartments regarding the Th2A and CD38$^+$ Th2A, we evaluated the expression of CCR3 on these cells (**Fig. S3C**), a marker that characterizes immune cells from peripheral blood which migrate to the upper respiratory tract mucosa.[47] A higher frequency of Th2A CCR3$^+$ cells was observed in urban AR compared to HC. Moreover, upregulation of CD38$^+$ Th2A CCR3$^+$ cells was only found in the urban but not rural AR, in comparison to the HC subjects (**Fig. 4**). Thus, increased frequency of activated Th2A cells expressing homing marker (CCR3) in peripheral blood in the urban AR group, is in agreement with the increased presence of these cells in the nasal mucosa of urban individuals with AR.

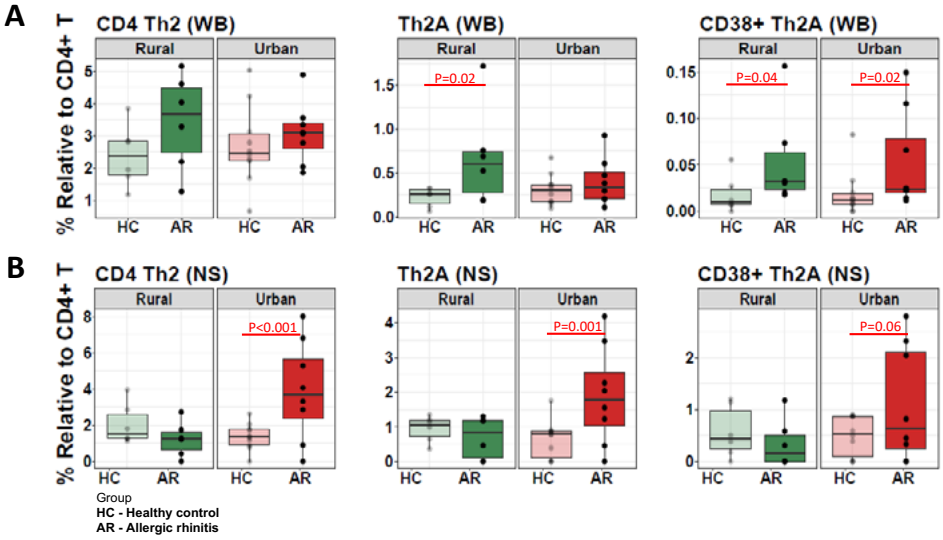

Figure 3. Discrepancies in the findings of CD4 Th2, Th2A, and CD38⁺ Th2A cell percentages between systemic and nasal mucosal compartments in urban and rural allergic rhinitis subjects compared to healthy controls.

A. Box plots depicting the differences of the percentages of systemic CD4 Th2, Th2A, and CD38⁺ Th2A cells (relative to CD4 T cells) between allergic rhinitis vs. healthy control subjects for each rural and urban group, in the whole-blood mass cytometry dataset. **B**. Similar box plots as (**A**) for the nasal mucosal mass cytometry dataset. To evaluate the differences of the percentages of the immune cell populations between allergic rhinitis vs. healthy control subjects for each rural and urban group, generalized linear mixed model test was performed.
AR: allergic rhinitis subjects; HC: healthy control subjects, WB: whole-blood, NS: nasal mucosa.

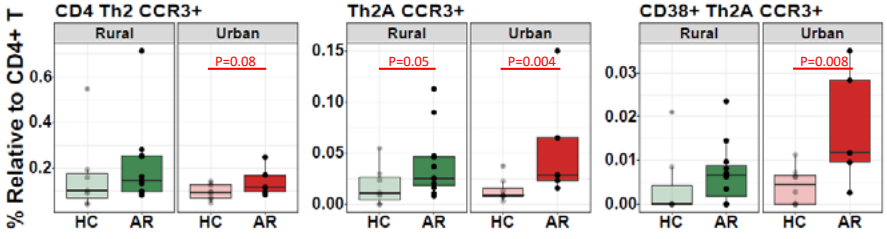

Figure 4. Higher frequency of systemic Th2A CCR3⁺ and CD38⁺ Th2A CCR3⁺ were observed in urban allergic rhinitis compared to healthy control subjects but not in the rural group.

Box plots depicting the differences of the percentages of systemic CD4 Th2 CCR3⁺, Th2A CCR3⁺ , and CD38⁺ Th2A CCR3⁺ between allergic rhinitis (AR) vs. healthy control (HC) subjects for each rural and urban group, in the confirmation study (flow-cytometry) dataset. To evaluate the differences between AR vs. HC subjects for each rural and urban group, generalized linear mixed model test was performed.
AR: allergic rhinitis subjects; HC: healthy control subjects.

DISCUSSION

Here, we performed high-dimensional immune profiling using mass-cytometry in urban and rural AR subjects evaluating both peripheral blood and nasal mucosal immune compartment. Systemic immune profiling revealed an increase of $CD163^+$ mDCs and Th2A in rural but not in urban AR subjects, while $CD38^+$ Th2A were upregulated in AR subjects from both rural and urban groups. At the same time, striking differences were observed for the nasal mucosal immune cells in the urban AR compared to HC subjects, but not for the rural group.

Although we did not observe higher numbers of CD4 Th2, ILC2, and basophils in peripheral blood of either urban or rural AR subjects when compared to their HC counterparts, the findings of elevated percentages of $CD38^+$ Th2A in both rural and urban AR confirmed a role for these cells in AR individuals.[33] Interestingly, these differences did not manifest in the nasal mucosal site for rural AR subjects. Indeed, more pronounced differences were seen in the nasal mucosal immune cells of urban AR subjects, including the upregulation of CD4 Th2, Th2A, and $CD38^+$ Th2A compared to the urban HCs, which was not observed in the rural group. Moreover, the positive correlations between the percentages of these effector cells in the nasal mucosa with the level of activity disturbances, suggest a higher disease activity in the urban AR.[52] Thus, our study findings support the importance of evaluating the immune system in AR not only in the peripheral blood, but importantly also in the nasal mucosal site as the effector organ.

The elevated $CD163^+$ mDCs in the systemic compartment of rural AR subjects might explain the lack of cellular perturbations, in particular, in the nasal mucosa of these subjects compared to the healthy controls. The $CD163^+$ subset of dendritic cells has been reported to have more anti-inflammatory and tolerogenic properties.[53-55] This systemic skewing of immune cells towards more regulatory condition in these rural AR subjects is also supported by the trends of higher numbers of Tregs and non-classical monocytes compared to HCs. Tregs have been shown to suppress Th2 cells and their activation and migration to the inflammatory sites, and induce IgG4 instead of IgE production by B cells.[56,57] In addition, non-classical monocytes could alter Th2 cells activation [58] and were upregulated after allergen immunotherapy.[59,60]

C-C chemokine receptor 3 (CCR3) has an essential role in the clinical manifestation of AR and is expressed by many effector cells associated with the pathogenesis of AR, such as eosinophils,[61] basophils,[62] mast cells,[63] and CD4 Th2.[61] Our study extended the role of CCR3 in AR, further to Th2A and CD38^{+} Th2A. The elevated numbers of the nasal mucosal CD4 Th2, Th2A, and CD38^{+} Th2A immune cell populations in the urban AR compared to HC subjects was supported by the similar findings of the CD4 Th2 CCR3$^{+,}$ Th2A CCR3$^{+,}$ and CD38^{+} Th2A CCR3^{+} percentages in the WB, suggesting more migration of these immune cells from the systemic to the effector site.

To our knowledge, this is the first study comprehensively comparing the immune profiles of both peripheral blood and nasal mucosal compartments in AR subjects from urban and rural sites, that evaluated the pathogenic Th2A cells and the migratory marker CCR3. Nevertheless, there are some limitations to this study. First, we did not include CCR3 in our mass cytometry antibody panel and the CCR3 expression was only measured in the flow cytometry dataset. However, as the clinical characteristics and the findings related to CD4 Th2, Th2A, and CD38+ Th2A were similar for both datasets, we could infer the results regarding the CCR3^{+} populations for mass cytometry dataset. The lack of functional study to confirm more regulatory states in the rural AR subjects and the antigen specificity of Th2A cells are another limitation of this study. Lastly, quantifications of cytokines both in WB and nasal mucosa would add valuable information to the differences of the immune profiles between urban and rural AR.

To summarize, we observed distinct immune profiles between Indonesian young adults with AR originating from rural and urban areas. Urban AR showed more differences in the nasal mucosal immune cells when compared to their HC counterparts, but this was not seen in the rural group. In addition, these alterations of immune cells in urban AR might be associated with more severe clinical manifestation, as reported previously [21,22]. Our study also confirmed the important role of Th2A and CD38^{+} Th2A cells in AR. Altogether, this study improves our understanding on the pathogenesis of AR and might be useful when considering clinical treatment, although further study are needed.

Conflict of Interest

All authors declare that they have no relevant conflicts of interest.

Author Contributions

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Data Availability Statement

The data that support the findings of this study are available from the corresponding author (F.K. and M.Y.) upon reasonable request.

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SUPPLEMENTARY MATERIALS

Table S1. Antibody panel mass-cytometry for nasal-mucosal and whole-blood.

Label	Specificity	Clone	Vendor	Catalogue number	Dilution
^{89}Y	CD45	HI30	Fluidigm[a]	3089003B	1/200
^{141}Pr	CD196 (CCR6)	G034E3	Fluidigm	3141003A	1/100
^{142}Nd	CD19	HIB19	Fluidigm	3142001B	1/200
^{143}Nd	CD117 (c-Kit)	104D2	BioLegend[b]	313223	1/100
^{144}Nd	CD66b	REA306	Miltenyi[c]	130-108-019	1/50
^{145}Nd	CD4	RPA-T4	Fluidigm	3145001B	1/100
^{146}Nd	CD8a	RPA-T8	Fluidigm	3146001B	1/200
^{147}Sm	CD183 (CXCR3)	G025H7	BioLegend	353733	1/100
^{148}Nd	CD14	M5E2	BioLegend	301843	1/100
^{149}Sm	CD25 (IL-2Ra)	2A3	Fluidigm	3149010B	1/100
^{150}Nd	CD185 (CXCR5)	J252D4	BioLegend	356902	1/100
^{151}Eu	CD123	6H6	Fluidigm	3151001B	1/100
^{152}Sm	TCRγδ	11F2	Fluidigm	3152008B	1/50
^{153}Eu	CD7	CD7-6B7	Fluidigm	3153014B	1/100
^{154}Sm	CD163	GHI/61	Fluidigm	3154007B	1/100
^{155}Gd	CD69	FN50	BioLegend	313502	1/200
^{156}Gd	CD294 (CRTH2)	BM16	BioLegend	350102	1/50
^{158}Gd	CD122 (IL-2Rb)	TU27	BioLegend	339015	1/100
^{159}Tb	CD197 (CCR7)	G043H7	Fluidigm	3159003A	1/100
^{160}Gd	Epcam	51.1	BioLegend	324229	1/50
^{161}Dy	KLRG1 (MAFA)	REA261	Miltenyi	Special order	1/100
^{162}Dy	CD11c	Bu15	Fluidigm	3162005B	1/200
^{163}Dy	CD152 (CTLA-4)	BNI3	BioLegend	369602	1/100
^{164}Dy	CD161	HP-3G10	Fluidigm	3164009B	1/100
^{165}Ho	CD127 (IL-7Ra)	AO19D5	Fluidigm	3165008B	1/200
^{167}Er	CD27	O323	Fluidigm	3167002B	1/200
^{168}Er	HLA-DR	L243	BioLegend	307651	1/200
^{169}Tm	CD45RA	HI100	Fluidigm	3169008B	1/100
^{170}Er	CD3	UCHT1	Fluidigm	3170001B	1/100
^{171}Yb	CD28	CD28.2	BioLegend	302937	1/100
^{172}Yb	CD38	HIT2	Fluidigm	3172007B	1/200
^{173}Yb	CD45RO	UCHL1	BioLegend	304239	1/100
^{174}Yb	CD335 (NKp46)	92E	BioLegend	331902	1/100
^{175}Lu	CD279 (PD-1)	EH 12.2H7	Fluidigm	3175008B	1/100
^{176}Yb	CD56	NCAM16.2	Fluidigm	3176008B	1/100
^{209}BI	CD16	3G8	Fluidigm	3209002B	1/400

[a]Fluidigm, South San Francisco, CA, USA. [b]Biolegend, San Diego, CA, USA. [c]Miltenyi Biotech, Bergisch Gladbach, Germany. CCR, C-C chemokine receptor. CD, cluster of differentiation. CRTH2, prostaglandin D2 receptor 2. CXCR, CXC chemokine receptor. EpCAM, epithelial cell adhesion molecule. HLA-DR, human leukocyte antigen-D-related. IL-2R, interleukin-2 receptor. IL-7Rα, interleukin-7 receptor α. KLRG1, killer cell lectin-like receptor subfamily G member 1. MAFA, mast cell function-associated antigen. PD-1, programmed cell death protein-1. TCR, T-cell receptor. All markers were stained on the cell surface.

Table S2. Barcoding panel for mass cytometry.

Label	Specificity	Clone	Vendor	Catalogue number	Dilution
^{106}Cd	B2M	2M2	BioLegend[a]	316302	1/50
^{110}Cd	B2M	2M2	BioLegend	316302	1/50
^{111}Cd	B2M	2M2	BioLegend	316302	1/50
^{112}Cd	B2M	2M2	BioLegend	316302	1/50
^{114}Cd	B2M	2M2	BioLegend	316302	1/50
^{116}Cd	B2M	2M2	BioLegend	316302	1/50
^{198}Pt	B2M	2M2	BioLegend	316302	1/50

[a]Biolegend, San Diego, CA, USA. B2M, Beta-2 microglobulin.

Table S3. Whole-blood clusters' identification.

Clusters' number	Clusters' identification
cluster_01	DN T cells (EM) CD27+ CD28+ CD38-
cluster_02	yd T cells (RO+ CD27+)
cluster_03	yd T cells (RA+ CD27- CD161-)
cluster_04	CD8 T cells (EMRA) CD27- CD127- KLRG1+
cluster_05	Plasma cells
cluster_06	B cells memory
cluster_07	B cells naïve
cluster_08	yd T cells (RA+ CD27- CD161+)
cluster_09	ILC3
cluster_10	CD8 T cells (EMRA) CD27+ KLRG1+ CXCR3-
cluster_11	B cells CD11c+
cluster_12	pDCs
cluster_13	CD4 T cells (EM) CD27- CD28+ CD38- CD127+ CD161+ KLRG1+
cluster_14	CD4 T cells (EM) CD27- CD28- CD38- CD127+ PD1+ KLRG1+
cluster_15	CD8 T cells (EM) CD27- CD28- CD127+ KLRG1+
cluster_16	CD8 NKT cells
cluster_17	Classical monocytes
cluster_18	CD4 T cells (EM) CD25+ CD27- CD28+ CD127+
cluster_19	CD4 Th2
cluster_20	CD4 T cells (EMRA)
cluster_21	DN T cells (EM) CD27lo CD28lo CD38-
cluster_22	CD4 T cells (EM) CD27+ CD28+ CD38- CD127+ CD161+ KLRG1+
cluster_23	CD4 T cells (EM) CD28+ CD127+
cluster_24	CD4 T cells (EM) CD27+ CD28+ CD38- CD161- PD1+
cluster_25	CD8 T cells (EM) CD27- CD28- CD127- KLRG1+
cluster_26	ILC2
cluster_27	Basophils
cluster_28	CD163+ mDCs
cluster_29	CD4 T cells (EM) CD25+ CD127- (Tregs)
cluster_30	CD4 T cells (EM) CD28+ CD127+
cluster_31	CD8 T cells (EM) CD27+ PD1+ KLRG1+
cluster_32	NK cells (NKp46+)
cluster_33	NK cells (CD16-)
cluster_34	NK cells (CD16+) CD38+ CD161+ KLRG-
cluster_35	Non-classical monocytes
cluster_36	CD4 T cells (CM) CD27+ CD28+ CD38+ CD161+ PD1+
cluster_37	CD8 T cells (EM) CD27+ CD28+ CD38+ PD1+
cluster_38	CD8 T cells (EM) CD27+ CD28+ CD127+ KLRG1+
cluster_39	CD8 T cells (naïve)
cluster_40	NK cells (CD16+) CD38+ CD161- KLRG+-
cluster_41	NK cells (CD16+) CD38+ CD161+ KLRG+
cluster_42	CD4 T cells (naïve) CD25- CD27+ CD38+ CD127+
cluster_43	DN T cells (naïve) CD38+
cluster_44	CD8 T cells (CM) CD27+ CD28+ CD38- CD127+
cluster_45	CD8 T cells (EMRA) CD27+ KLRG1- CXCR3+
cluster_46	CD4 T cells (naïve) CD25- CD27+ CD127+
cluster_47	CD4 T cells (naïve) CD25+ CD27+ CD38+ CD127+
cluster_48	yd T cells (RA+ CD27+ CD161-)
cluster_49	CD163- mDCs

Table S4. Nasal mucosal clusters' identification.

Clusters' number	Clusters' identification
cluster_01	B cells memory
cluster_02	B cells naïve
cluster_03	pDCs
cluster_04	HLA-DR+ CD11c+ (mDCs)
cluster_05	HLA-DR+ CD11c+ CD16+
cluster_06	Basophils
cluster_07	CD4+ CD25+ CD127- CD69+
cluster_08	CD4 T cells RO+ CD25lo CD69+ CD38+ CXCR3+ PD1+
cluster_09	CD4 T cells RO+ CD27- CD127+ CD69hi CD161+ PD1+
cluster_10	Mast cells
cluster_11	HLA-DR+ CD11c+ CD14+ (CD14+ monocytes)
cluster_12	CD4 T cells CD69+ CD27- CD127- CD161- PD1+
cluster_13	CD8 T cells RA+ CCR7- CD69+ CD27lo CD122lo
cluster_14	CD8 T cells CD27- CD69+ CD38- CD161- PD1-
cluster_15	yd T cells (RO+ CD27-)
cluster_16	CD8 T cells RO+ CD69+ CD27- CD38+ CD161- PD1+
cluster_17	CD8 T cells RO+ CD69+ CD27- CD38+ CD161+ PD1+
cluster_18	ILCs
cluster_19	CD7+ CD56+ CD11c+ NKp46+ (NK cells)
cluster_20	yd T cells (RA+ CD27+)
cluster_21	CD8 T cells RO+ CCR7- CD69+ CD27+ CD122+
cluster_22	CD8 T cells RO+ CD69+ CD27+ CD38+ CD161- PD1+
cluster_23	DN T cells

Table S5. Antibody panel flow-cytometry (confirmation study) for Th2A analysis in whole-blood.

Marker	Fluorochrome	Vendor	Catalogue number	Dilution
Live/dead				
CD3	BV510	Biolegend[a]	317332	1/200
CD4	SB550	Biolegend	344656	1/100
CD8	PE	In Vitro[b]	12-0088-42	1/200
CD161	pe-cy5	BD Biosciences[c]	551138	1/100
CD294 (CRTH2)	BV711	Biolegend	350124	1/50
CD27	FITC	BD Biosciences	555440	1/100
CD197 (CCR7)	pe-cy7	BD Biosciences	557648	1/200
CD38	apc-fire810	Biolegend	303550	1/100
CCR3	AF647	Biolegend	310709	1/100

[a]Biolegend, San Diego, CA, USA. [b]In Vitro Technologies, Mt Wellington, Auckland, New Zealand. [c]BD Biosciences Franklin Lakes, NJ, USA. CCR, C-C chemokine receptor. CD, cluster of differentiation. CRTH2, prostaglandin D2 receptor 2.

Table S6. Comparison between allergic rhinitis (AR) and healthy control (HC) subjects in whole study population urban and rural group.

Variables	Urban (N=88)		Rural (N=79)	
	SPT+RHI+ (AR) (n=36)	SPT-RHI- (HC) (n=52)	SPT+RHI+ (AR) (n=32)	SPT-RHI- (HC) (n=47)
Age (years old), mean (SD)	18.50 (0.70)	18.54 (0.70)	18.96 (0.95)	18.79 (0.60)
Sex, n male (%)	21 (58.3)	18 (34,6)*	21 (65.6)	16 (34.0)*
BMI (kg/m2), mean (SD)	22.08 (4.78)	21.71 (4.16)	21.98 (4.23)	20.47 (3.09)
Total IgE (IU/mL)#, median (25^{th}; 75^{th} percentile)	450 (283; 914)	54 (20; 119)*	364 (180; 715)	67 (33; 212)*
Eosinophil counts# (%), median (25^{th}; 75^{th} percentile)	3.0 (2.0; 5.0)	1.0 (0.5; 3.0)*	3.0 (1.8; 6.0)	2.5 (1.0; 4.0)
Activity disturbances, n positive (%)				
- No	8 (22.2)	NA	10 (31.3)	NA
- Mild	16 (44.4)		16 (50.0)	
- Moderate	12 (33.3)		5 (15.6)	
- Severe	0 (0)		1 (3.1)	

#non-normally distributed continuous variables, presented as median (25^{th}; 75^{th} percentile).

*statistically significant differences (P<0.05) between allergic rhinitis vs healthy control subjects for each urban and rural group.

SD: standard deviation; BMI: body mass index; SPT: skin prick test; SPT+RHI+: reactive SPT result combined with the presence of rhinitis symptoms; SPT-RHI-: negative SPT results without any rhinitis symptoms; AR: allergic rhinitis; HC: healthy control;

NA: not applicable.

Table S7. Statistical analysis for the comparison of whole-blood immune cell clusters between allergic rhinitis vs. healthy control subjects for each rural and urban group.

	Rural				Urban			
Clusters	**Intercept**	**Group_AR**	**p_group_AR**	**fdr_group_AR**	**Intercept**	**Group_AR**	**p_group_AR**	**fdr_group_AR**
cluster_01	-3,55767	-0,54261	0,148998	0,892306	-3,43947	-0,12626	0,586195	0,921133
cluster_02	-3,80563	0,061606	0,860665	0,983213	-3,44024	0,029917	0,902846	0,991261
cluster_03	-6,13988	0,947396	0,207174	0,892306	-4,57296	-0,60109	0,225303	0,710842
cluster_04	-3,34224	0,090367	0,821248	0,983213	-3,80633	0,482496	0,163887	0,710842
cluster_05	-6,00724	0,38784	0,386994	0,892306	-5,66692	-0,48564	0,346505	0,77176
cluster_06	-3,56128	-0,04103	0,86454	0,983213	-3,5361	-0,16292	0,471889	0,856392
cluster_07	-2,3516	-0,09744	0,70642	0,983213	-2,53146	-0,37527	0,025432	0,587003
cluster_08	-6,24144	0,289572	0,747326	0,983213	-5,74545	0,042371	0,943747	0,991261
cluster_09	-8,2815	0,179265	0,636861	0,983213	-7,801	-0,04422	0,871145	0,991261
cluster_10	-4,44398	-0,05421	0,882885	0,983213	-4,88498	0,259467	0,332654	0,77176
cluster_11	-4,83964	0,121549	0,508626	0,892306	-4,5138	-0,36429	0,090472	0,710842
cluster_12	-6,2091	0,194338	0,407058	0,892306	-5,9509	-0,00411	0,985367	0,991261
cluster_13	-4,49637	0,261459	0,473786	0,892306	-4,57402	-0,11206	0,541563	0,915054
cluster_14	-5,71435	0,529654	0,254048	0,892306	-5,43439	0,128427	0,820664	0,991261
cluster_15	-5,52616	0,06298	0,846384	0,983213	-5,5878	-0,16041	0,613914	0,921133
cluster_16	-5,5162	-0,69191	0,474596	0,892306	-6,47317	0,972854	0,169029	0,710842
cluster_17	-2,16845	0,054704	0,881916	0,983213	-2,01932	-0,16676	0,470223	0,856392
cluster_18	-4,52343	0,293236	0,156177	0,892306	-4,3603	0,054272	0,723326	0,957918
cluster_19	-5,42008	0,286091	0,359188	0,892306	-5,5394	0,460345	0,038857	0,587003
cluster_20	-7,22466	0,385508	0,715607	0,983213	-7,10484	0,331644	0,579428	0,921133
cluster_21	-4,78606	-0,42164	0,180375	0,892306	-4,76118	0,050428	0,785978	0,991261
cluster_22	-4,07875	0,299905	0,410331	0,892306	-4,10484	0,236415	0,146589	0,710842
cluster_23	-2,85693	0,121889	0,448565	0,892306	-2,81173	0,147479	0,232112	0,710842
cluster_24	-5,69943	0,239748	0,40576	0,892306	-5,64789	0,162749	0,331791	0,77176
cluster_25	-4,02079	0,00181	0,99597	0,99597	-4,5851	0,333127	0,375414	0,799796
cluster_26	-7,33508	0,566889	0,072137	0,88368	-7,13338	0,110324	0,630962	0,921133
cluster_27	-5,52127	0,474089	0,507684	0,892306	-5,21812	0,467054	0,188352	0,710842
cluster_28	-6,3888	1,020754	0,000136	0,006673	-5,56576	0,003016	0,991261	0,991261
cluster_29	-4,93722	0,289195	0,040764	0,665805	-4,68805	-0,0079	0,960711	0,991261
cluster_30	-5,11902	0,004029	0,985162	0,99597	-5,37382	0,307747	0,18961	0,710842
cluster_31	-4,36921	0,100603	0,714037	0,983213	-4,78184	0,606083	0,012563	0,587003
cluster_32	-5,62688	-0,22977	0,464158	0,892306	-5,67664	0,205092	0,20368	0,710842
cluster_33	-4,49609	0,092423	0,859997	0,983213	-4,74745	0,753349	0,047919	0,587003
cluster_34	-3,37854	-0,0306	0,909154	0,989968	-3,25156	-0,24557	0,328906	0,77176
cluster_35	-5,25582	0,647368	0,011728	0,287339	-4,72946	-0,16021	0,639154	0,921133
cluster_36	-5,73263	0,265968	0,311791	0,892306	-5,61862	0,07161	0,715287	0,957918
cluster_37	-5,37863	-0,3204	0,408213	0,892306	-5,73337	-0,15682	0,284609	0,77176
cluster_38	-5,25219	-0,0324	0,942546	0,99597	-5,18497	0,179423	0,218342	0,710842
cluster_39	-1,92976	-0,33882	0,17775	0,892306	-2,02111	-0,03786	0,838414	0,991261
cluster_40	-4,485	0,681045	0,367426	0,892306	-3,73559	-0,75799	0,162517	0,710842
cluster_41	-3,91048	0,095051	0,860189	0,983213	-3,35189	-0,07444	0,860331	0,991261
cluster_42	-4,20841	-0,09524	0,672113	0,983213	-4,16447	0,152812	0,409384	0,835826
cluster_43	-6,265	-0,03651	0,872472	0,983213	-6,2363	-0,01983	0,927223	0,991261
cluster_44	-4,7826	-0,0511	0,831218	0,983213	-4,43467	-0,11875	0,513301	0,898276
cluster_45	-5,99488	0,308045	0,221806	0,892306	-5,77229	0,056988	0,860456	0,991261
cluster_46	-2,04208	-0,34386	0,189401	0,892306	-2,4052	0,291022	0,063359	0,620914
cluster_47	-4,92859	0,008166	0,980784	0,99597	-4,83024	0,144953	0,341395	0,77176
cluster_48	-5,8641	0,241216	0,509889	0,892306	-5,60893	-0,19927	0,438414	0,856392
cluster_49	-5,51903	0,509481	0,171193	0,892306	-5,31237	0,100064	0,67469	0,944566

Generalized linear mixed model analyses were performed to compare the differences between AR vs HC subjects for each rural and urban group with adjusted p-values were obtained using fdr-correction based on Benjamini-Hochberg method to correct for multiple testing hypothesis. Cells highlighted in yellow: p-values <0.05 and cells highlighted in red: adjusted p-values <0,05. AR: allergic rhinitis; HC: healthy control; fdr: false discovery rate.

Table S8. Statistical analysis for the comparison of nasal mucosal immune cell clusters between allergic rhinitis vs. healthy control subjects for each rural and urban group.

	Rural				Urban			
Clusters	**Intercept**	**Group_AR**	**p_group_AR**	**fdr_group_AR**	**Intercept**	**Group_AR**	**p_group_AR**	**fdr_group_AR**
cluster_01	-5,3928	-0,50912	0,460365	0,663235	-5,11841	-0,30311	0,512115	0,867185
cluster_02	-3,59012	-0,45238	0,212557	0,618828	-4,01548	-0,28037	0,557409	0,867185
cluster_03	-6,25172	0,189693	0,576007	0,779303	-6,3758	0,520506	0,464525	0,867185
cluster_04	-3,33407	-0,52538	0,109615	0,618828	-3,39083	0,169373	0,713547	0,867185
cluster_05	-4,51813	-0,94199	0,009783	0,225005	-5,76668	0,117491	0,82238	0,873545
cluster_06	-7,34547	1,302471	0,136119	0,618828	-8,03599	2,892854	0,000882	0,006762
cluster_07	-5,66	0,424663	0,252465	0,618828	-5,73141	1,062497	0,000125	0,002884
cluster_08	-5,62415	-0,24126	0,628838	0,78375	-6,00514	0,983091	0,014363	0,082586
cluster_09	-2,94355	0,049845	0,81989	0,852554	-3,1563	0,142224	0,650318	0,867185
cluster_10	-7,74556	1,001738	0,319463	0,618828	-10,4792	4,706901	0,000851	0,006762
cluster_11	-5,58745	-0,69142	0,348952	0,618828	-6,38334	-0,42852	0,644717	0,867185
cluster_12	-2,90447	-0,34099	0,358128	0,618828	-2,93333	0,059714	0,754074	0,867185
cluster_13	-3,40142	0,266795	0,333751	0,618828	-3,11594	-0,2234	0,501584	0,867185
cluster_14	-1,41181	0,211995	0,10161	0,618828	-1,32219	0,059984	0,740542	0,867185
cluster_15	-3,3992	0,240711	0,681522	0,78375	-3,70424	-0,1355	0,748552	0,867185
cluster_16	-1,78845	0,321794	0,269493	0,618828	-1,87336	0,213849	0,40279	0,867185
cluster_17	-1,01411	-0,24341	0,461381	0,663235	-1,16387	-0,02899	0,929617	0,929617
cluster_18	-6,31481	0,1601	0,760529	0,83296	-6,47394	0,810459	0,046696	0,2148
cluster_19	-4,72208	-0,62348	0,047777	0,549439	-5,23858	0,044894	0,835565	0,873545
cluster_20	-3,74794	-0,20034	0,376678	0,618828	-4,25026	-0,18253	0,752166	0,867185
cluster_21	-5,86452	0,091562	0,852554	0,852554	-6,1574	0,208773	0,711679	0,867185
cluster_22	-4,32784	0,143384	0,675566	0,78375	-4,44161	0,454245	0,124943	0,47895
cluster_23	-6,07245	-1,24415	0,302388	0,618828	-7,65866	0,714969	0,156411	0,513921

Generalized linear mixed model analyses were performed to compare the differences between AR vs HC subjects for each rural and urban group with adjusted p-values were obtained using fdr-correction based on Benjamini-Hochberg method to correct for multiple testing hypothesis. Cells highlighted in yellow: p-values <0.05 and cells highlighted in red: adjusted p-values <0,05. AR: allergic rhinitis; HC: healthy control; fdr: false discovery rate.

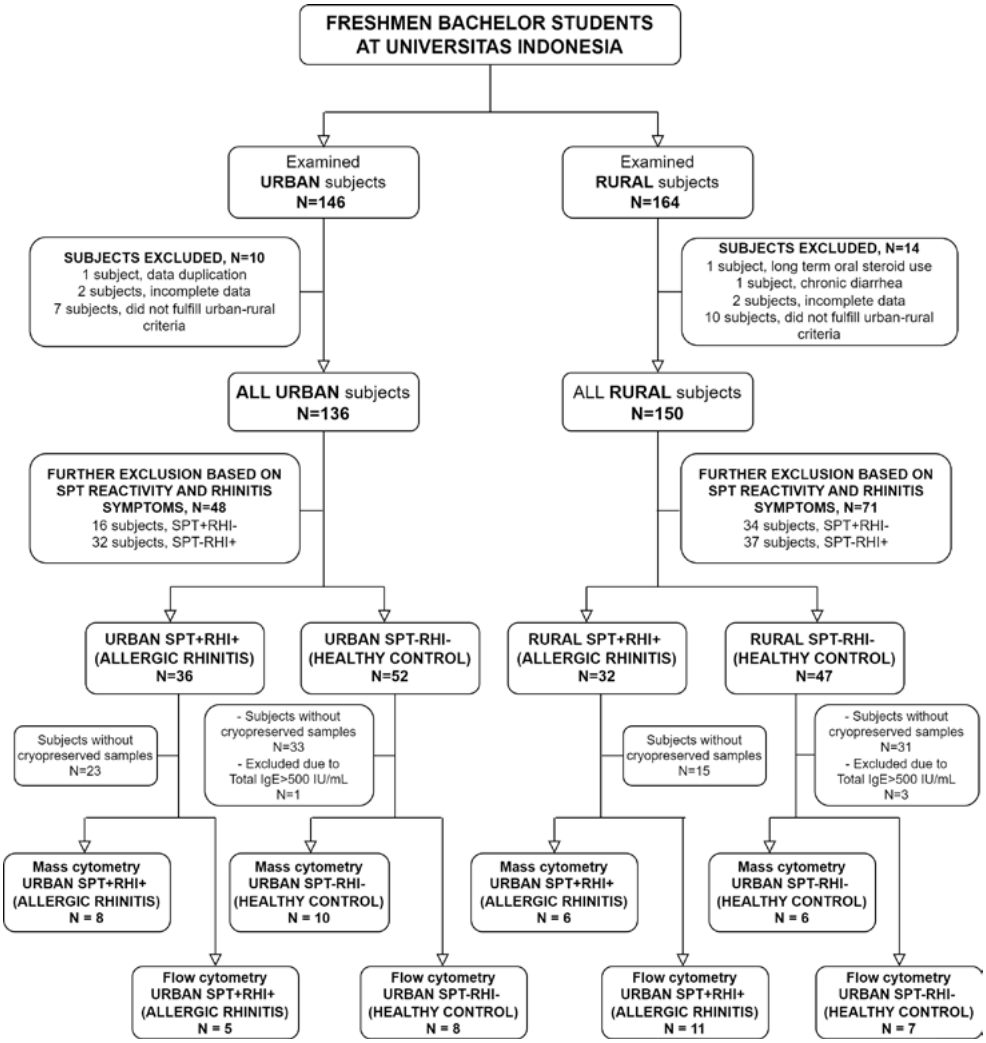

Figure S1. Flow chart for the inclusion of study participants.

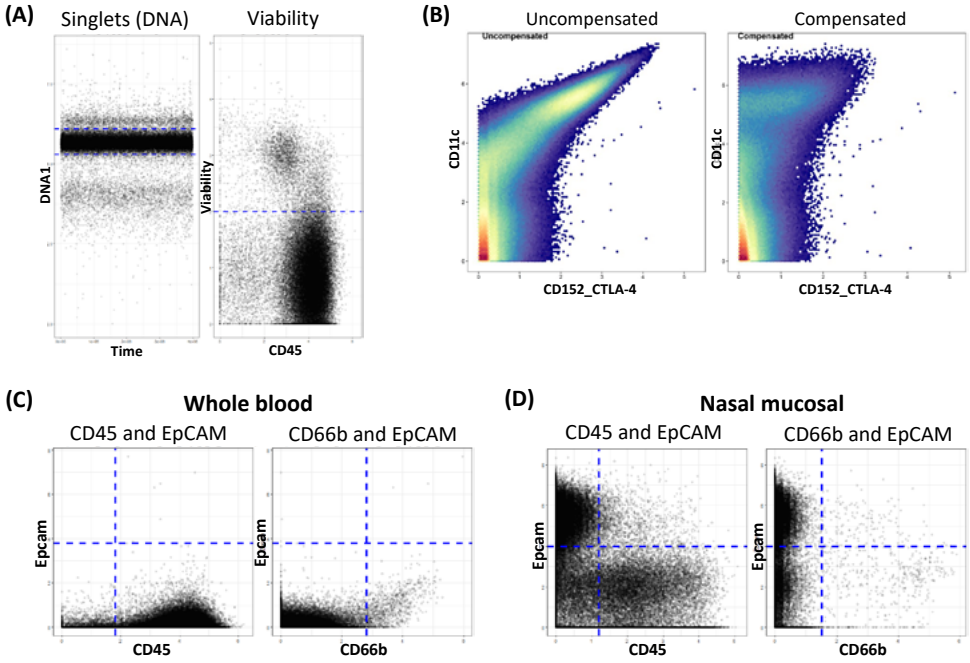

Figure S2. Pre-processing of mass cytometry data.
(A) Manual gating of the single and viable cells based on DNA dye (left) and viability dye (right). (B) Compensation of markers. Here, we showed the example of compensation between CD11c and CTLA4 markers before (left) and after (right) compensation. (C) Manual gating to obtain the two cell populations from whole blood samples, granulocytes ($EpCAM^-CD66b^+$) and other immune cells ($EpCAM^-CD45^+CD66b^-$). (D) Manual gating to obtain the three cell populations from nasal mucosal samples, epithelial cells ($EpCAM^+CD45^-$), granulocytes ($EpCAM^-CD66b^+$) and other immune cells ($EpCAM^-CD45^+CD66b^-$).

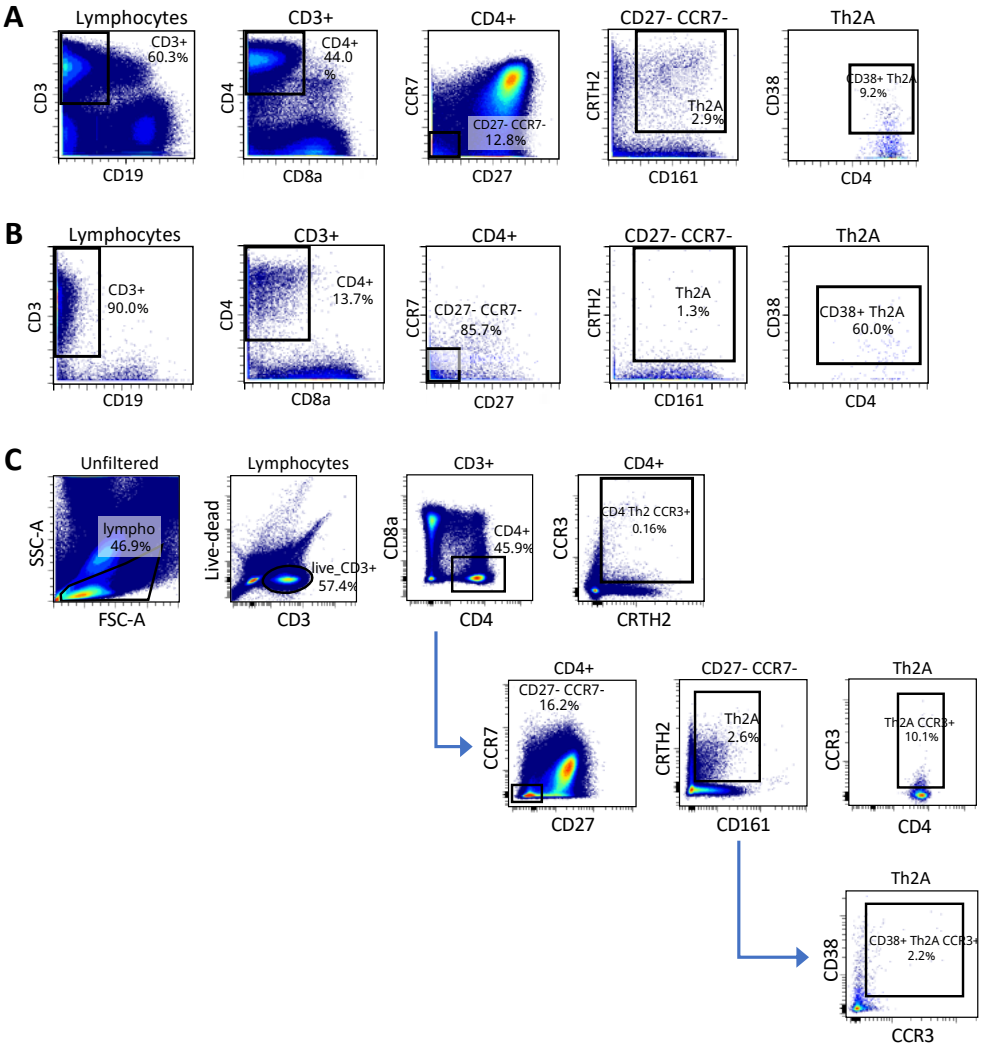

Figure S3. Gating strategy.

A. For the identification of Th2A and CD38$^+$ Th2A immune cell populations from whole blood mass cytometry data. **B**. For the identification of Th2A and CD38$^+$ Th2A immune cell populations from nasal mucosal mass cytometry data. **C**. For the identification of CD4 Th2 CCR3$^+$, Th2A, Th2A CCR3$^+$, CD38$^+$ Th2A, CD38$^+$ Th2A CCR3$^+$ from whole blood flow cytometry data.

Pathogenic Th2A immune cells population was identified as CD4$^+$CD27$^-$CCR7$^-$CRTH2$^+$CD161$^+$.

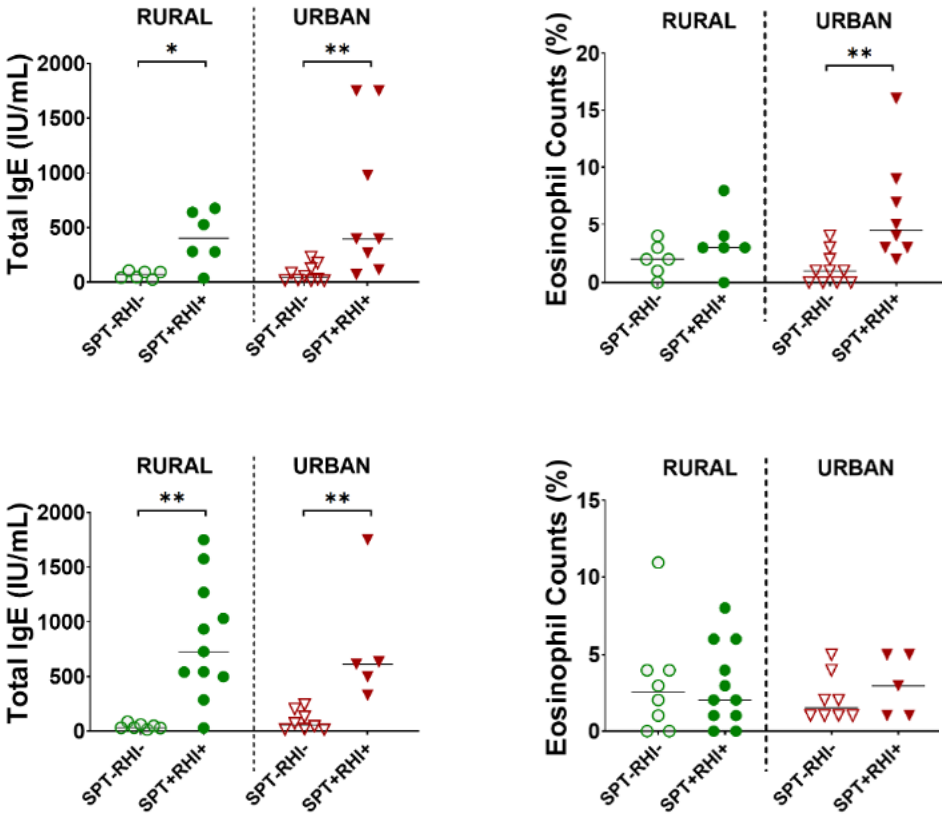

Figure S4. Comparison of total IgE levels and eosinophil counts between allergic rhinitis (SPT+RHI+) and healthy control (SPT-RHI-) subjects for urban and rural group in mass cytometry (A) and flow-cytometry (B) cohorts.

All parameters were presented as its median values and individual values for each subject. For the comparison between allergic rhinitis vs healthy control subjects, Mann-Whitney test was performed. SPT+RHI+: reactive SPT result combined with the presence of rhinitis symptoms, allergic rhinitis subjects; SPT-RHI-: negative SPT result without any rhinitis symptoms, healthy control subjects;
*P<0.05; **P<0.01

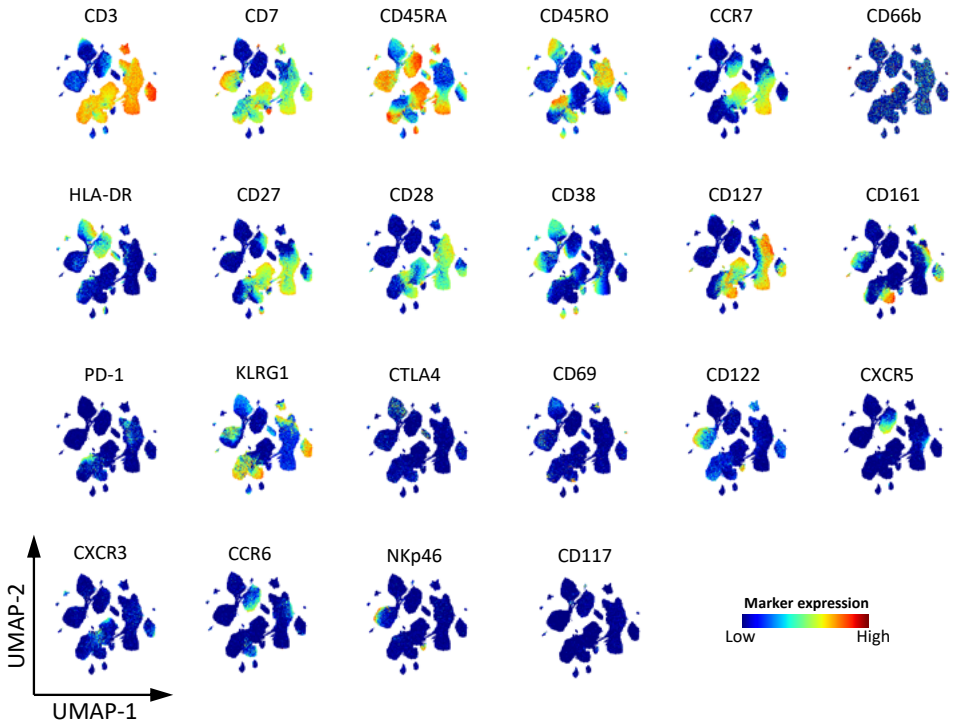

Figure S5. The expression of several immune cell surface markers obtained from mass-cytometry measurement, used for the clustering of whole-blood immune cell populations.

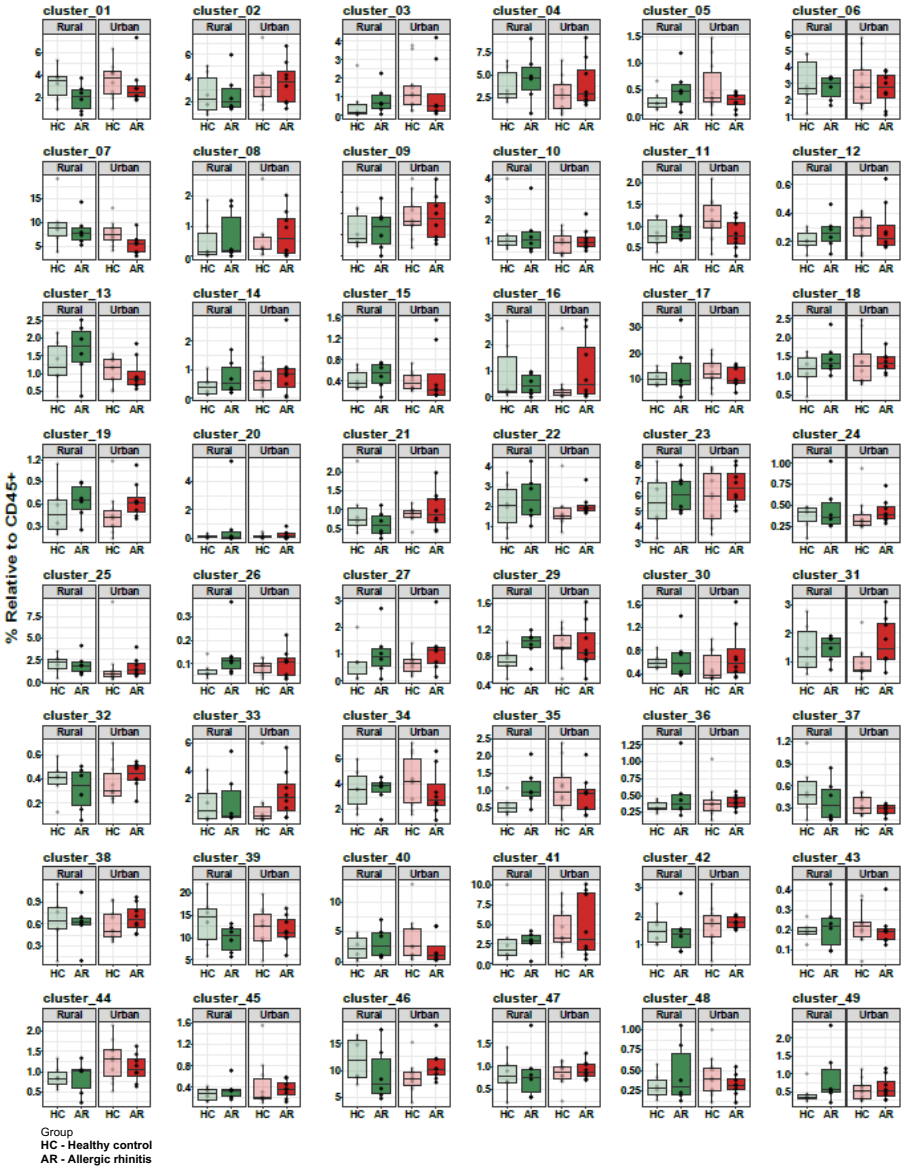

Figure S6. Comparison of the percentages (relative to CD45⁺ cells) of the remaining non-statistically significant whole-blood immune cell clusters between allergic rhinitis (AR) vs. healthy control (HC) subjects for each rural and urban group.

Individuals and boxplots with median percentage are depicted. Generalized linear mixed model test was performed with Benjamini-Hochberg correction for multiple testing hypothesis to obtain the adjusted P-values.

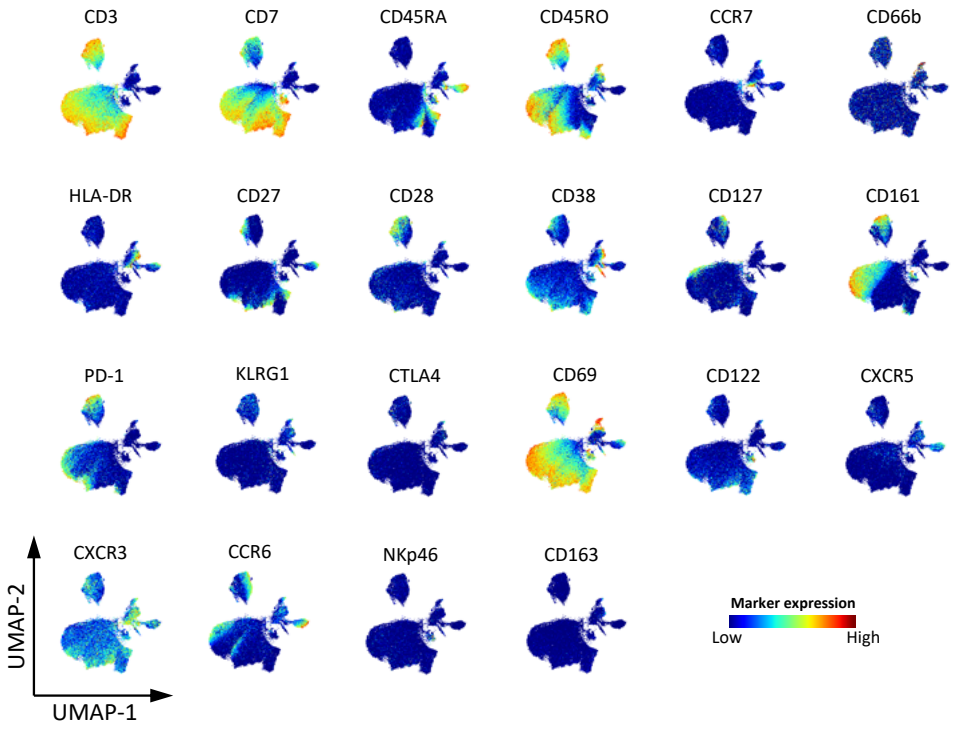

Figure S7. The expression of several immune cell surface markers obtained from mass-cytometry measurement, used for the clustering of nasal mucosal immune cell populations.

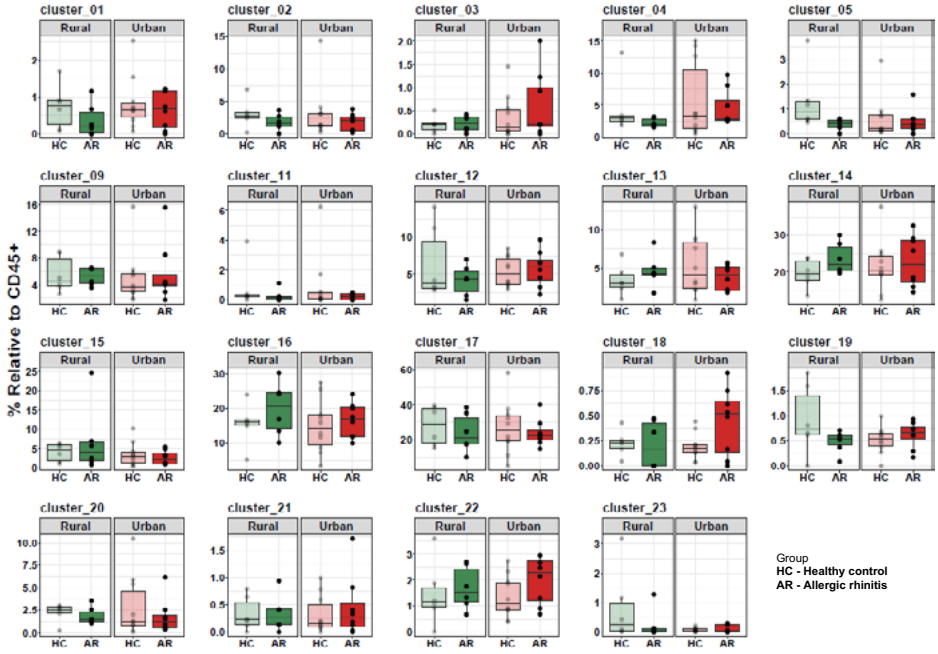

Figure S8. Comparison of the percentages (relative to CD45⁺ cells) of the remaining non-statistically significant nasal mucosal immune cell clusters between allergic rhinitis (AR) vs. healthy control (HC) subjects for each rural and urban group.

Individuals and boxplots with median percentage are depicted. Generalized linear mixed model test was performed with Benjamini-Hochberg correction for multiple testing hypothesis to obtain the adjusted P-values.

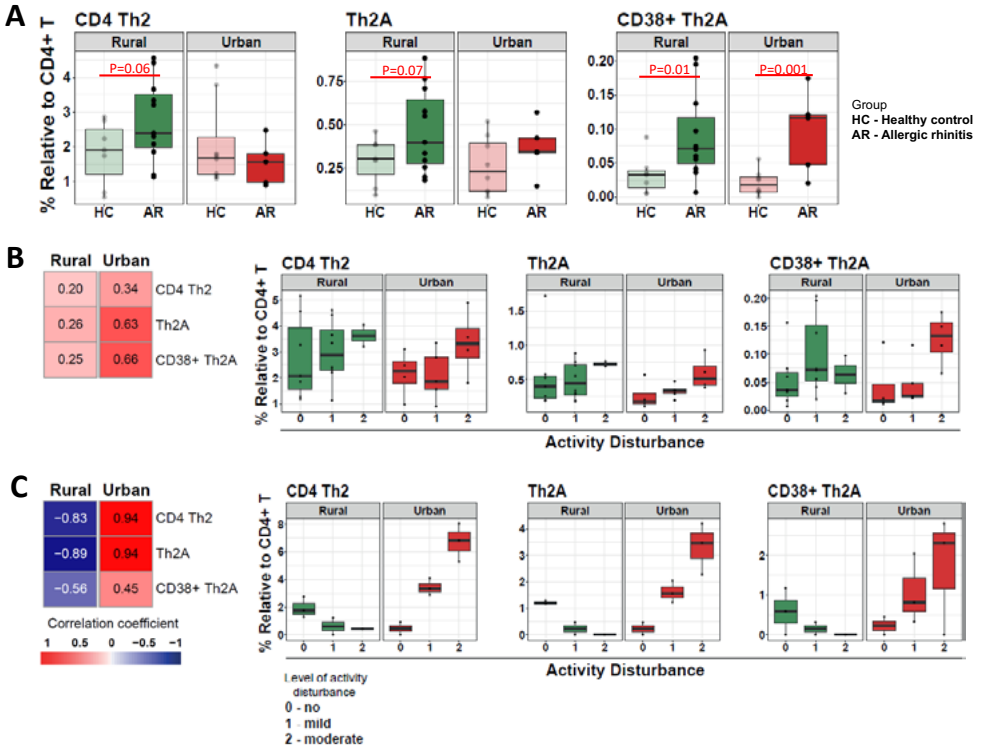

Figure S9. A. The percentages of systemic CD4 Th2, Th2A, and CD38⁺ Th2A cells (as relative to CD4⁺T cells) between allergic rhinitis (AR) vs. healthy control (HC) subjects for each rural and urban group, in the flow cytometry cohort. B. Correlation between the levels of activity disturbances with the percentages (relative to CD4⁺ T cells) of CD4 Th2, Th2A, and CD38+ Th2A in allergic rhinitis (AR) subjects for each rural and urban group in the combined whole blood mass and flow cytometry cohort, shown as correlation heatmap and individuals boxplots with median percentage. C. Correlation between the levels of activity disturbances with the percentages (relative to CD4⁺ T cells) of CD4 Th2, Th2A, and CD38⁺ Th2A in allergic rhinitis (AR) subjects for each rural and urban group in the nasal mucosal mass cytometry cohort, shown as correlation heatmap and individuals boxplots with median percentage.

Generalized linear mixed model test was performed for evaluating the differences between AR vs HC subjects in each urban and rural group for figure A. Spearman rank correlation test was performed to obtain the correlation coefficients in the heatmaps for figure B and C

Chapter 7

SUMMARIZING DISCUSSION

This thesis presents several clinical, epidemiological, and immunological aspects of urbanization in Indonesia in the context of metabolic health and allergic diseases. To this end, studies have been done in rural and urban areas of Indonesia. A cluster-randomized placebo-controlled clinical trial of anthelmintic treatment was conducted in an Indonesian rural population living in a soil-transmitted helminth (STH)-endemic area (**Chapter 2**) to assess how chronic helminth infections in rural areas affect metabolic health. A short-term high-fat high-calorie diet intervention was performed in both urban and rural Indonesian adults to evaluate how lifelong residence in an urban versus rural area responds to a strong metabolic perturbation (**Chapter 3**). The question of how metabolic health changes over increasing years of residence in an urban area, was also addressed in Chapter 3 examining the metabolic health of individuals who had moved from rural areas to reside for varying lengths of time in an urban area. Looking at the same question prospectively, a cohort study evaluated the effect of living in an urban area on the metabolic profiles in young Indonesian adults of urban- and rural-origin (**Chapter 4**). At the national level, secondary data from a large Indonesian Basic Health survey conducted in 2018 assessed the determinant factors for diabetes in urban and rural populations of Indonesia (**Chapter 5**). Lastly, the application of mass cytometry to evaluate the differences in the systemic and nasal mucosal immune profiles between individuals with and without allergic rhinitis in urban and rural areas, made a dive into how cellular immune responses might be affected by urbanization (**Chapter 6**).

SUMMARY OF MAIN FINDINGS

One characteristic that distinguishes urban and rural populations is the exposure to parasitic infections, in particular, soil-transmitted helminths. In **Chapter 2**, we showed that in adults, STH infection was associated with lower levels of serum free IGF-1, a metabolic hormone essential for human anabolic functions.[1] The lower adiposity (BMI) and fasting insulin levels observed in STH-infected subjects, partially mediated the lower free IGF-1 levels found, in agreement with previous reports.[2,3] Interestingly, there is an inverse correlation between the number of different helminth infections with the levels of free IGF-1. This might suggest a possible impact of higher infection burden on BMI and fasting insulin levels,[4] thereby resulting in further lowering of the free IGF-1 levels.

Anthelmintic treatment with albendazole increased the levels of free IGF-1, although not specifically in STH-infected subjects.[1] This indicates that albendazole treatment might have broader effects than its action on STH. Albendazole might affect the gut microbiome[5] and the intestinal protozoa,[6] potentially affecting the levels of free IGF-1.[7]

Based on the outcome on the clinical trials that show increasing adiposity following anthelmintic treatment,[8] the question arises as to what are the long-term metabolic effect of helminth eradication in rural areas endemic for STH. While the absorption of nutrients would be expected to be improved, thus lowering the risk of undernutrition and stunting in children,[9,10] the increasing adiposity and body mass index and their adverse impact on metabolic health also need to be considered for preparing appropriate policies. Furthermore, in the context of urbanization, rural people often migrate to urban areas and adopt urban lifestyles. The effect of these urbanization-related changes on the metabolic health of such rural populations, with or without previous helminth infection, is another important question that requires further research. We need to understand the precise changes that occur upon urbanization, in order to create a knowledge base to devise approaches for mitigation of any adverse impact of urbanization on health.

Our study described in **Chapter 3** attempted to answer the question of how migration to an urban area affects metabolic health. Indonesians with similar genetic backgrounds living in urban and rural environments were compared. We observed higher adiposity indices (body mass index and waist circumference), whole-body insulin resistance (IR), and leptin levels in the urban compared to rural subjects. Interestingly, in the individuals living in an urban area, the time spent in an urban environment was positively associated with increasing body mass index (BMI) and waist circumference (WC).[11] This suggests that a higher degree of acculturation in terms of urban lifestyle, could lead to a positive energy balance, hence increasing adiposity over time.[12]

We also showed that past or current exposure to STH, as assessed by total IgE, albeit relatively small, could contribute to the differences in adiposity, whole-body IR, and

leptin levels between individuals living in urban and rural areas. However, living in rural areas or having STH infections, could not protect against increasing IR induced by short-term high-fat diet (HFD) intervention. Nevertheless, increased liver inflammation, as assessed by cholesteryl ester transfer protein (CETP) level,[13] was observed after HFD intervention, in subjects living in urban, but not in rural areas. This might be explained by the higher pre-HFD intervention CETP levels in the individuals living in rural areas, thus precluding further increase. As CETP plays an important role in lipoprotein metabolism,[14] the effect of this relatively high CETP levels in individuals living in rural areas on the long-term risk of developing metabolic diseases, such as metabolic syndrome and dyslipidemia, warrants further studies. An important question is also what causes the higher CETP levels in those living in rural areas.

In **Chapter 3**, we only investigated short-term HFD as an urban-associated lifestyle that could influence metabolic health, in this case IR. Nonetheless, in real life, urbanization is associated with long-term adaptation to urban lifestyle and environmental exposures. Thus, the acute induction of IR by short-term HFD intervention in individuals living in urban and rural areas might not truly reflect the metabolic health effects of urbanization. This we attempted to address in **Chapter 4**.

Our prospective cohort study described in **Chapter 4**, confirmed overall better metabolic profiles in Indonesian young adults with lifelong residence in rural areas who had just recently migrated to an urban area in comparison to their urban counterparts. The better metabolic health was reflected in lower mean BMI and proportion of overweight/obese subjects, as well as lower whole-body IR and leptin/adiponectin (L/A) ratio. Despite these findings at baseline, after 1-year of living in an urban area, these rural subjects experienced almost double the increase in BMI and three times higher increase of L/A ratio, compared to subjects residing their whole life in urban areas.[15] These findings, once again, suggest that living in a rural area does not protect individuals from adverse alterations in their metabolic profile upon urbanization, and might even result in more unfavorable changes.

In the same study, we revealed the role of fat intake as the major driver of the increase in BMI for both groups originating from rural and urban areas. In addition, although the

rural group consumed almost twice as much protein compared to the urban group, this could not explain the enhanced gain in BMI of the rural subjects. Furthermore, the incorporation of physical activity, another important factor related to urbanization, could also not explain the differences in the BMI increase between the two groups after one year. This implies the possible role of changes in other factors, among others, epigenetics[16] and/or gut microbiome.[17]

Previous studies have shown the association between BMI and L/A ratio with whole-body IR.[18-20] Interestingly, despite significant changes in BMI and L/A ratio found in our study after one year, no changes in HOMA-IR was observed. The preserved pancreatic beta-cell function in young adults[21] and relatively short follow-up period might explain this finding. A previous longitudinal study with follow-up time of more than twenty years that reported greater adherence to Westernized diet, characterized by high-fat high calories, refined carbohydrates, and processed foods, to be associated with higher risk of metabolic syndrome and IR.[22] Thus, a longer prospective cohort study is needed to understand more clearly the effect of urbanization, and most important factors associated with it, on metabolic profiles, especially IR.

Our studies in chapters 3 and 4, similarly observed relatively better metabolic profiles in rural compared to urban Indonesians. Difference in the dietary intake pattern was also seen between these two populations. Diabetes as a metabolic-related disease, is increasing in prevalence in Indonesia, alongside rapidly growing socio-economic development and urbanization. Hence, in **Chapter 5**, we assessed the differences in the association between lifestyle, as well as clinical factors, with diabetes prevalence in the Indonesian urban and rural populations using secondary data from the 2018 Indonesian Basic Health Survey.

Our results confirm that the rural population has a more healthy metabolic profile than the urban population in Indonesia. Nevertheless, there were no differences in the prevalence of diabetes between rural and urban populations. Strikingly, the majority of individuals with diabetes were undiagnosed and untreated, in particular in the rural population. Despite the better metabolic profiles in the rural population, there were

no differences in the associations of lifestyle and clinical factors with the prevalence of diabetes between the two populations.

When considering immunological changes related to urbanization, **Chapter 6** focused on allergic rhinitis and its relationship to local and peripheral immune responses. We found that individuals with allergic rhinitis (AR) from urban areas in Indonesia had stronger inflammatory immune responses in the nasal mucosa compared to their rural counterparts, as shown by the upregulation of several immune cells known to play an important role in the AR pathogenesis, such as: basophils, mast cells, CD4 Th2, and pathogenic Th2A cells.[23-25] Moreover, these immune cells were positively correlated with the severity of activity disturbances due to AR symptoms, only in urban but not in rural subjects. Interestingly, systemic immune profiles in rural AR subjects showed a skewing towards more regulatory state with the upregulation of $CD163^{+}$ dendritic cells, regulatory T cells, and non-classical monocytes which are known to have anti-inflammatory and tolerogenic properties as reported previously,[26-28] and might dampen the expression of severe debilitating symptoms. Although these findings still need further confirmation by functional studies, they might explain the reports of less severe AR seen in populations living in rural areas.[29,30]

DIRECTIONS FOR FUTURE RESEARCH

Study of the adipose tissue

Although our studies provide us with information regarding the effects of urbanization on the metabolic health, the observed findings were mostly obtained from measurements in the peripheral blood due to ease of accessibility. It is important to note that BMI and waist circumference, consistently showed higher adiposity profiles in Indonesians living in urban rather than rural areas. Moreover, the findings of leptin, adiponectin, and L/A ratio are interlinked with adipose tissue as the major source of these adipokines. Thus, direct evaluation of the adipose tissue should be incorporated in future studies. Previous studies have utilized minimally invasive procedures under local anesthesia, such as 14G needle aspiration [31] or small liposuction cannula,[32] to obtain the subcutaneous adipose tissue (SAT) samples. For the visceral adipose tissue (VAT), samples could be obtained from patients with obesity that undergo

bariatric procedures.[33] Alternatively, elective abdominal surgery can be the source for SAT and VAT samples both for obese and lean patients.[32,34] From these adipose tissue samples, studies on the immune cells and the gene expression of adipokines, cytokines, or chemokines can be performed and correlated with the findings from systemic compartment.[31-34]

Study of the microbiome

Numerous studies have shown the differences in the gut microbiome between urban and rural populations, which could be influenced by many factors.[35-39] Moreover, certain gut microbiota composition and functionality were associated with metabolic markers. *Prevotella* genus was enriched in individuals with high consumption of vegetables[40] and a shift towards Bacteroides dominance was seen upon adaptation of Westernized diet,[41] while *Faecallibacterium* genus was positively correlated with the duration of exercise habits.[42] Gut microbiota dysbiosis and a lower relative abundance of short-chain fatty acid (SCFA) producing bacteria from *Prevotella, Faecallibacterioum, Roseburia, Bifidobacterium,* and *Ruminococcus genera*, were reported in patients with obesity and type 2 diabetes (T2D) compared to healthy subjects.[43,44] These SCFAs were known to have essential role for maintaining intestinal integrity, energy homeostasis and body weight regulation, improving insulin sensitivity, and anti-inflammatory properties.[45] In addition, several interventions taking into consideration urbanization-related factors, such as dietary modification and exercise training, showed significant alteration in the gut microbiome that correlated well with changes in metabolic and inflammatory parameters.[46-48] Hence, assessment of gut microbiome, with the focus on the presence of gut dysbiosis and health-promoting bacteria, as well as their association with changes in the adiposity profiles, dietary intake, and physical activity, is essential in studies evaluating the effect of urbanization on human metabolic homeostasis.

In addition, the differences of nasal microbiome between urban and rural populations have been observed,[49,50] and many studies have found that microbiome differs significantly in the airway of patients with allergic diseases, such as AR and asthma.[51-53] Since there is significant interaction between microbiome and host immune

system in the nose,[54,55] further studies incorporating the evaluation of these two aspects in the urban and rural populations will provide better understanding of the impact of urbanization on allergy, especially AR.

Epigenetic studies

Although individuals might have a genetic predisposition to certain cardiometabolic diseases,[56,57] the rapidly increasing prevalence of non-communicable diseases (NCDs) points to the importance of environmental, social, and behavioral determinants of health. Furthermore, significant interplay between genetic and environmental factors, which can result in the modifications of gene expression patterns without changing the DNA sequence, known as epigenetic changes, has been observed, and linked to the pathogenesis of NCDs.[58] Previous studies reported a negative association between DNA methylation levels in leptin (LEP) gene and BMI,[59,60] whereas DNA methylation levels in the adiponectin (ADIPOQ) gene were positively associated with BMI.[59] Urbanization is inseparable from environmental and social alteration that could potentially induce epigenetic changes. Obesogenic diet could induce alteration in DNA methylation of pro-opiomelanocortin (POMC) gene in the arcuate nucleus of hypothalamus, an important gene for regulating satiety and energy homeostasis. This gene has been shown to be associated with raised leptin and insulin levels and the development of obesity.[61] Altogether, further studies that incorporate measurements of DNA methylation levels as a representative of epigenetic changes, such as in the LEP and ADIPOQ genes from blood and adipose tissue, are needed. Such changes can then be correlated with the changes in metabolic profiles, dietary intake, physical activity, and adipokines, to provide a better understanding of the complex interaction between urbanization, epigenetic changes, and NCDs.

Immunological studies

Although we have included immunological evaluations in this thesis to assess differences between urban and rural AR, a lot more needs to be done. The functional assays and antigen/allergen specificity of the effector cells that were observed to be upregulated in either urban or rural AR, as well as the measurements of cytokines and chemokines in the nasal mucosa, will provide more comprehensive information to understand the differences between urban and rural AR individuals.

Furthermore, the fact that the pathogenesis of cardiometabolic diseases, such as T2D and atherosclerotic cardiovascular diseases, are closely related with systemic and vascular inflammation,[62,63] justifies the assessment of the immune system in such studies in Indonesia. In addition, it would be even more important to identify the immunological changes before the onset of these diseases. As the increasing prevalence of NCDs occurs concurrently and could be attributed to rapid urbanization,[64] the study on the effect of urbanization and its associated factors on the immune system and metabolic homeostasis is essential. For example, we need to identify specific subsets of T cells, dendritic cells, macrophages or other immune cells present in the peripheral blood and tissues, such as adipose tissue, with their functional capacities, and associate them with changes in metabolic profiles, dietary intake, and adipokines upon urbanization.

In the urbanization and AR study, we confirmed the importance of immunological evaluation not only in the systemic compartment but also at the effector site. With regards to metabolic homeostasis, the evaluation of the immune system in adipose tissue, intestinal tract, and endothelial layer would complement and add valuable information apart from the systemic immune profiles. As an example, it will allow the study of macrophages, immune cells that are absent in the peripheral blood, but have shown to play a key role in metabolic homeostasis.[65,66]

Studies of social aspects

Urbanization could greatly impact human social factors. Living in an urban area can be associated with higher levels of chronic stress due to factors like noise, pollution, crowding, and social pressures.[67] Prolonged stress might cause a disruption in the metabolic homeostasis.[68] Moreover, urbanization often leads to changes in social structures and support networks.[69] These changes could potentially affect dietary choices,[70] physical activity,[71] and the prevalence of social isolation or psychological stress,[72] all of which might influence metabolic health. All of our studies described in this thesis focus more on the biological (environmental and behavioral) aspects of urbanization, thus lacking the social aspects. Collaboration with social scientist is needed to examine more deeply the impact of these social aspects of urbanization on metabolic health and the biological pathways involved.

Longitudinal study: longer follow-up time

Our prospective cohort study with 1-year follow-up has provided us with valuable information on the unfavorable effects of urbanization on metabolic profiles, especially in individuals from rural areas who migrate to urban centers. Nevertheless, this one-year period is too short as most rural people who migrate to urban areas will stay for longer periods or even become permanent residents. Hence, a longitudinal study with longer follow-up period that incorporates suitable tools to assess dietary intake, physical activity, and psychological stress, as well as the evaluation of changes in microbiome, epigenetics, and the immune system, will be the ideal set up to obtain better understanding of the cumulative effects of urbanization over time, on metabolic health. Similar design should also be applied to studies on allergic diseases. However, such studies are very expensive and need strong financial commitment.

The urban wealthy versus the urban slum resident

The concept of rural and urban areas date as far back as classical Roman times and have acquired particular environmental, cultural, and social associations.[73] However, this dichotomy has been considered inadequate in many disciplines, due to several reasons: heterogeneity within categories, multidimensionality, changing dynamics, and interconnectedness with blurred boundaries.[73,74] Even within one urban setting, there can be some areas with high socio-economic status/SES (urban wealthy) and others with low SES (urban slum), which associate with different microbiome[75] and immune profiles.[76] Several urbanicity scales have been developed to quantify urbanization as a continuous variable, which can outperform the rural-urban dichotomy in terms of association with health.[77-79] It would be beneficial to adopt these urbanicity indexes in Indonesia and correlate it with the effects on metabolic health.

DIRECTIONS FOR FUTURE HEALTHCARE POLICY

Although the members of rural populations pose a relatively healthier metabolic profile, our studies observed that living in rural areas does not protect the inhabitants from the deleterious metabolic changes when adopting urban lifestyle. Thus, in terms of health policy, appropriate and adequate education and knowledge have to be

provided to prevent and monitor the development of NCDs, such as obesity and T2D.

Additionally, urbanization is not only related with negative impacts towards human health. There are many beneficial effects of urbanization, such as decreased burden of infectious diseases due to improved hygiene and sanitation, better access to health care facilities, and improved knowledge regarding health due to better education. This notion was supported by one of our study results using secondary data from the 2018 Indonesian National Health Survey showing a twice higher diagnosed diabetes in urban compared to rural populations. However, the prevalence of undiagnosed and untreated diabetes in Indonesia is still high and the number has not yet declined compared to the data reported in 2007. This problem requires special attention from all related stakeholders. It is important to develop locally or nationally, practical and sensitive diagnostic tools to detect the presence of NCDs, such as T2D, especially applicable to many resource-limited rural areas of Indonesia. Moreover, improvement of health care access and facilities, especially in many rural areas, is needed to better diagnose NCDs at an early stage to prevent further complications and to provide higher quality treatment.

As urbanization is unavoidable, it is essential to create an urban environment that can support good health, which is in line with the United Nations Sustainable Development Goals (SDG) 11: Sustainable Cities and Communities. Since urbanization is closely related to economic growth, creating adequate employment opportunities could lead to increased household incomes. Higher income levels can positively influence health by providing individuals and families with better access to nutritious food, healthcare, and improved living conditions. Providing enhanced transportation infrastructure in the urban environment can be another way to optimize positive urbanization impact on human health. Efficient transportation can increase accessibility to healthcare facilities, reduce air pollution by promoting the use of public transport, and encourage physical activity through active transportation options, like walking or cycling. Additionally, the establishment of recreational facilities, such as parks, sports complexes, and fitness centers, could promote physical activity and encourage a healthier lifestyle, reducing the risk of chronic diseases associated with sedentary behavior.

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191

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Appendix

SUMMARY

In many low-middle income countries, including Indonesia, socio-economic development goes hand in hand with rapid urbanization. Urbanization, the process of migration of people from rural to urban areas, as well as the process of transforming rural into an urban population, leads to alterations in the social, environmental, and lifestyle aspects of human lives. This causes an epidemiological transition of disease profiles, a decreasing prevalence of infectious disease on the one hand, but increasing prevalence of non-communicable diseases (NCDs) on the other. Studies have suggested that urbanization could also potentially affect disease outcome. In this thesis, we report the impact of urbanization in Indonesia on non-communicable diseases, in particular metabolic diseases and allergies.

Chapter 1

In this chapter, we described the potential social, environmental, and lifestyle changes associated with urbanization, such as dietary intake, physical activity and sedentary behavior, exposure towards microorganisms and parasites, biodiversity, farming, pollution, as well as social stress. These alterations could influence the human microbiome, epigenome, and immune system. Thus, potentially affect the disease prevalence and outcome. The increasing prevalence of NCDs and potentially worse outcomes related to urbanization causes significant burden on the health system. Therefore, more research is needed. Due to the multidimensionality of urbanization, studies incorporating many aspects of scientific investigation are important to evaluate the effect of urbanization on NCDs. In this thesis, we used different approaches to address the influence of urbanization on metabolic and allergic diseases. We conducted intervention studies, a prospective cohort study, and used a large dataset from Indonesian national health survey, but also incorporated an in depth immunological study, to evaluate the impact of urbanization on specific health outcomes.

Chapter 2

This chapter described the effect of soil-transmitted helminths (STH), a common feature of rural areas, on the serum levels of free IGF-1, a metabolic hormone, essential for

human anabolic functions. Serum samples were analyzed from a cluster-randomized double-blind placebo-controlled trial of albendazole treatment in an area endemic for STH. We observed lower levels of serum free IGF-1 in STH-infected subjects, which was partially mediated by the lower BMI and fasting insulin levels. Interestingly, with increasing number of different helminth infections, often also associated with higher burden of these parasitic infections, a further lowering of free IGF-1 levels was seen. Furthermore, the levels of this metabolic hormone were increased after four rounds of three-monthly albendazole (400 mg) treatment for three consecutive days, although not exclusively in STH-infected subjects. The metabolic parameter changes associated with STH and albendazole treatment warrant further research on the long term impact of deworming on metabolic health of rural populations living in STH-endemic areas.

Chapter 3

In this chapter we assessed the contribution of the living environment on metabolic profile. We compared people living in rural areas and their counterparts, who were individuals with similar genetic background that had migrated to an urban area, in terms of metabolic health and metabolic response. We found higher adiposity indexes (BMI and waist circumference), whole-body insulin resistance (IR), and leptin levels in residents of urban compared to rural areas. Increasing time spent in the urban area was positively correlated with higher adipose tissue mass, indicating a higher degree of acculturation in terms of urban lifestyle, which can lead to a positive energy balance, hence increasing adiposity over time. Additionally, acute intervention with a 5-days high-fat diet (HFD), induced a similar increase of whole-body IR in both groups living in urban and rural areas, as well as in rural subjects with and without helminth infection. These results show that living in rural areas or having current STH infection, was not protective against the induction of IR after short-term HFD intervention.

Chapter 4

Here we investigated the metabolic profiles of Indonesian young adults starting their university studies in an urban area. Metabolic parameters were compared between students originating from a rural area who had recently migrated to the urban center, and their counterparts originating from urban areas. We observed an overall better

metabolic profiles, reflected as lower BMI, whole-body IR, and leptin/adiponectin (L/A) ratio in those who originated from rural compared to those from urban areas. Moreover, after 1-year of living in an urban area, the rural subjects experienced almost double the increase in BMI and three times higher increase in L/A ratio, compared to subjects residing their whole life in urban area. Once again, we found that previously living in rural areas does not protect individuals from the negative changes in metabolic profiles upon migration to an urban area and adoption of urban lifestyle.

Chapter 5

Using large-scale nationally representative data of the 2018 Indonesian Basic Health Survey, we reported the differences in lifestyle and clinical factors and their association with diabetes in populations of urban and rural Indonesia. Here, we confirmed that Indonesian rural population has healthier lifestyle and metabolic profiles compared to their urban counterparts. Nevertheless, the prevalence of diabetes was similar between the two populations. Strikingly, the majority of individuals with diabetes were undiagnosed and untreated, particularly in the rural areas. Additionally, despite the better metabolic profiles in the rural population, there were no differences in the associations of lifestyle and clinical factors with the prevalence of diabetes between urban and rural populations. These findings indicate that living in a rural environment does not protect against metabolic disease, such as diabetes, in the Indonesian population.

Chapter 6

Urbanization could potentially affect disease prevalence and outcome. In this chapter we described how the immune system might contribute to the differences in clinical manifestation of allergic rhinitis (AR) between urban and rural populations in Indonesia. We observed that urban Indonesian young adults with AR have stronger inflammatory immune responses in the nasal mucosa compared to their rural counterparts, as shown by the upregulation of basophils, mast cells, CD4 Th2, and pathogenic Th2A cells. These immune cells showed positive correlation with the severity of activity disturbances due to AR, only in subjects from urban but not from rural areas. Additionally, systemic immune profiles in AR subjects from rural areas,

showed a skewing towards more regulatory state with the upregulation of CD163+ dendritic cells, regulatory T cells, and non-classical monocytes which are known to have anti-inflammatory and tolerogenic properties, and might dampen the expression of severe debilitating symptoms. These findings might explain the reports of less severe AR manifestation observed in populations living in rural areas, although further confirmation by functional studies are needed.

Chapter 7

This chapter summarized and discussed the main findings of this thesis. By incorporating multiple aspect of different scientific areas in our studies, we provided a better understanding of how urbanization could impact non-communicable diseases, in particular metabolic diseases and allergic rhinitis, in Indonesian population. First, we confirmed that helminth infections and anthelmintic treatment could influence metabolic health through their effect on metabolic hormones, although long term implications warrant further research. Second, our studies showed that Indonesian rural population generally have more favorable metabolic profiles compared to their urban counterparts, as shown by the lower adiposity indices, proportion of overweight/obesity, proportion of dyslipidemia, whole-body IR, and L/A ratio levels. Third, living in rural areas does not protect individuals from the negative consequences of urbanization and adoption of urban lifestyles on the metabolic health, even it might result in more unfavorable changes. Lastly, contrary to the finding related with metabolic health, rural living might associate with less severe clinical manifestation of allergic disease, as indicated in the findings of the systemic and nasal mucosal immune profiles. Altogether, urbanization could have major impacts on human health, although more research is needed to gain a more comprehensive understanding of the pathogenesis, and this thesis can be a valuable foundation for further studies. Additionally, healthcare and health-associated policies should take into consideration our reported impacts of urbanization on human health to improve overall health of the Indonesian population.

NEDERLANDSE SAMENVATTING

In veel lage-middeninkomenslanden, waaronder Indonesië, gaat sociaaleconomische ontwikkeling hand in hand met snelle verstedelijking. Verstedelijking, het proces van migratie van mensen van het platteland naar stedelijke gebieden, evenals het proces van transformatie van een plattelandsbevolking naar een stedelijke bevolking, leidt tot veranderingen in de sociale, ecologische en levensstijlaspecten van het menselijk leven. Dit veroorzaakt een epidemiologische transitie van ziekteprofielen, met enerzijds een dalende prevalentie van infectieziekten, maar anderzijds een stijgende prevalentie van niet-overdraagbare ziekten. Studies hebben gesuggereerd dat verstedelijking mogelijk ook de uitkomst van ziekten kan beïnvloeden. In dit proefschrift beschrijven we de impact van verstedelijking in Indonesië op niet-overdraagbare ziekten, in het bijzonder stofwisselingsziekten en allergieën.

Hoofdstuk 1

In dit hoofdstuk hebben we de mogelijke sociale, ecologische en levensstijlveranderingen beschreven die samenhangen met verstedelijking, zoals voedselinname, fysieke activiteit en sedentair gedrag, blootstelling aan micro-organismen en parasieten, biodiversiteit, landbouw, vervuiling en sociale stress. Deze veranderingen kunnen het menselijke microbioom, epigenoom en immuunsysteem beïnvloeden en zijn dus mogelijk van invloed op de prevalentie en uitkomst van de ziekten. De toenemende prevalentie van niet-overdraagbare aandoeningen en mogelijk slechtere gezondheidsuitkomsten in verband met verstedelijking resulteren in een aanzienlijke belasting van het gezondheidssysteem. Daarom is er meer onderzoek nodig. Vanwege de multidimensionaliteit van verstedelijking zijn studies waarin veel aspecten van wetenschappelijk onderzoek zijn opgenomen belangrijk om het effect van verstedelijking op niet-overdraagbare ziekten te evalueren. In dit proefschrift hebben we verschillende benaderingen gebruikt om de invloed van verstedelijking op metabole en allergische ziekten aan te pakken. We voerden interventiestudies en een prospectieve cohortstudie uit en gebruikten een grote dataset van de Indonesische nationale gezondheidsenquête, maar namen ook een diepgaande immunologische studie op om de impact van verstedelijking op specifieke gezondheidsuitkomsten te evalueren.

Hoofdstuk 2

Dit hoofdstuk beschrijft het effect van geohelminthen, een gemeenschappelijk kenmerk van plattelandsgebieden, op de serumspiegels van vrij IGF-1, een metabool hormoon, essentieel voor menselijke anabole functies. Serummonsters werden geanalyseerd van een cluster-gerandomiseerde, dubbelblind, placebo-gecontroleerde studie naar behandeling met albendazol in een gebied dat endemisch is voor geohelminthen. We zagen lagere niveaus van serumvrij IGF-1 bij met wormen geïnfecteerde proefpersonen, wat gedeeltelijk werd gemedieerd door de lagere BMI en nuchtere insulinespiegel. Interessant is dat met een toenemend aantal verschillende worminfecties, dat vaak ook geassocieerd met een hogere last van deze parasitaire infecties, een verdere verlaging van vrije IGF-1-niveaus werd gezien. Bovendien waren de waardes van dit metabolische hormoon verhoogd na vier rondes van driemaandelijkse behandeling met albendazol (400 mg) gedurende drie opeenvolgende dagen, echter niet alléén bij met wormen geïnfecteerde proefpersonen. De veranderingen in metabole markers geassocieerd met geohelminthen en albendazol behandeling rechtvaardigen verder onderzoek naar de langetermijnimpact van ontwormen op de metabolische gezondheid van plattelandsbevolking die in gebieden leeft die endemisch zijn voor geohelminthen.

Hoofdstuk 3

In dit hoofdstuk hebben we de bijdrage van de leefomgeving aan het metabool profiel onderzocht. We vergeleken de metabole gezondheid en metabole reacties van mensen die op het platteland wonen en hun tegenhangers, individuen met een vergelijkbare genetische achtergrond die naar een stedelijk gebied waren gemigreerd. We vonden hogere adipositas-indexen (BMI en middelomtrek), insulineresistentie (IR) en leptinewaarden bij inwoners van stedelijke gebieden in vergelijking met plattelandsgebieden. De tijd doorgebracht in het stedelijk gebied was positief gecorreleerd met een hogere vetweefselmassa, wat wijst op een hogere mate van acculturatie van de stedelijke levensstijl, wat kan leiden tot een positieve energiebalans, en dus toenemende adipositas in de loop van de tijd. Bovendien veroorzaakte een acute interventie met een 5-daags vetrijk dieet een vergelijkbare toename van IR in beide groepen die in stedelijke en landelijke gebieden woonden,

evenals bij proefpersonen op het platteland met en zonder worminfectie. Deze resultaten tonen aan dat het leven op het platteland of het hebben van een huidige worminfectie niet beschermend was tegen de inductie van IR na een kort interventie met een vetrijkdieet.

Hoofdstuk 4

Hier onderzochten we de metabolische profielen van Indonesische jongvolwassenen die hun universitaire studie in een stedelijk gebied begonnen. Metabole parameters werden vergeleken tussen studenten afkomstig uit een plattelandsgebied die recent naar de stad waren gemigreerd, en hun tegenhangers afkomstig uit stedelijke gebieden. We zagen over het algemeen betere metabole profielen, weerspiegeld in een lagere BMI, IR en leptine/adiponectine (L/A)-ratio bij degenen die afkomstig waren van het platteland in vergelijking met degenen uit stedelijke gebieden. Bovendien ervoeren de proefpersonen op het platteland na 1 jaar in een stedelijk gebied te hebben gewoond bijna een verdubbelde toename van de BMI en een driemaal hogere toename van de L/A-ratio, in vergelijking met proefpersonen die hun hele leven in een stedelijk gebied woonden. Opnieuw ontdekten we dat het eerder wonen op het platteland individuen niet beschermt tegen de negatieve veranderingen in metabole profielen bij migratie naar een stedelijk gebied en het overnemen van een stedelijke levensstijl.

Hoofdstuk 5

Met behulp van grootschalige, nationaal representatieve gegevens van de Indonesische gezondheidsenquête 2018, rapporteerden we de verschillen in levensstijl en klinische factoren en hun relatie tot diabetes in populaties uit steden en het platteland van Indonesië. Hier hebben we bevestigd dat de Indonesische plattelandsbevolking een gezondere levensstijl en metabole profielen hebben in vergelijking met hun stedelijke tegenhangers. Desalniettemin was de prevalentie van diabetes vergelijkbaar tussen de twee populaties. Opvallend was dat de meerderheid van de mensen met diabetes ongediagnosticeerd en onbehandeld was, vooral in de plattelandsgebieden. Bovendien waren er, ondanks de betere metabole profielen in de plattelandsbevolking, geen verschillen in de associaties van levensstijl en klinische

factoren met de prevalentie van diabetes tussen stedelijke en plattelandsbevolking. Deze bevindingen geven aan dat het leven in een landelijke omgeving de Indonesische bevolking niet beschermt tegen stofwisselingsziekten, zoals diabetes.

Hoofdstuk 6

Verstedelijking kan mogelijk de prevalentie en uitkomst van ziekten beïnvloeden. In dit hoofdstuk hebben we beschreven hoe het immuunsysteem zou kunnen bijdragen aan de verschillen in klinische manifestatie van allergische rhinitis (AR) tussen de stedelijke en plattelandsbevolking in Indonesië. We hebben waargenomen dat stedelijke Indonesische jongvolwassenen met AR sterkere inflammatoire immuunresponsen in het neusslijmvlies hebben in vergelijking met hun tegenhangers op het platteland, zoals blijkt uit de opregulatie van basofielen, mestcellen, CD4 Th2 en pathogene Th2A-cellen. Deze immuuncellen lieten een positieve correlatie zien met een verstoring van de dagelijkse activiteiten als gevolg van AR bij alleen proefpersonen uit stedelijke maar niet uit plattelandsgebieden. Bovendien vertoonden systemische immuunprofielen bij AR-proefpersonen uit plattelandsgebieden een neiging tot meer regulerende toestand met de opwaartse regulatie van CD163+ dendritische cellen, regulerende T-cellen en niet-klassieke monocyten waarvan bekend is dat ze ontstekingsremmende en tolerogene eigenschappen hebben, en die de expressie van ernstig beperkende symptomen kunnen dempen. Deze bevindingen zouden kunnen verklaren waarom minder ernstige AR-manifestaties worden waargenomen bij populaties die in plattelandsgebieden wonen, hoewel verdere bevestiging door functionele studies nodig is.

Hoofdstuk 7

In dit hoofdstuk zijn de belangrijkste bevindingen van dit proefschrift samengevat en besproken. Door meerdere aspecten van verschillende wetenschappelijke gebieden in onze studies op te nemen, hebben we een beter begrip gekregen van de invloed van verstedelijking op niet-overdraagbare ziekten, met name stofwisselingsziekten en allergische rhinitis, bij de Indonesische bevolking. Ten eerste hebben we bevestigd dat worminfecties en behandeling met anthelmintica de metabole gezondheid kunnen beïnvloeden door hun effect op metabolische hormonen, hoewel implicaties

op de lange termijn verder onderzoek rechtvaardigen. Ten tweede toonden onze studies aan dat de Indonesische plattelandsbevolking over het algemeen gunstigere metabole profielen heeft in vergelijking met hun stedelijke tegenhangers, zoals blijkt uit de lagere adipositas-indices, het aandeel overgewicht/obesitas, het aandeel dyslipidemie, de IR en de L/A-ratio. Ten derde beschermt het leven op het platteland individuen niet tegen de negatieve gevolgen van verstedelijking en het aannemen van een stedelijke levensstijl op de metabole gezondheid, zelfs als dit kan resulteren in meer ongunstige veranderingen. Tot slot, in tegenstelling tot de bevinding met betrekking tot de metabole gezondheid, zou het leven op het platteland geassocieerd kunnen worden met minder ernstige klinische manifestaties van allergische aandoeningen, zoals geobserveerd in de systemische en nasale mucosale immuunprofielen. Al met al zou verstedelijking grote gevolgen kunnen hebben voor de gezondheid van mensen, hoewel er meer onderzoek nodig is om de pathogenese beter te begrijpen. Dit proefschrift kan een waardevolle basis vormen voor verder onderzoek. Bovendien moeten gezondheidszorg- en gezondheidsgerelateerde beleidsmaatregelen rekening houden met onze gerapporteerde gevolgen van verstedelijking op de menselijke gezondheid om de algehele gezondheid van de Indonesische bevolking te verbeteren.

CURRICULUM VITAE

Farid Kurniawan was born on the 6th of January 1984 in Sidoarjo, East Java, Indonesia, where he also completed his primary and secondary education. In 2001, he moved to Jakarta to start his university education and obtained his medical doctor (MD) degree in 2007 from the Faculty of Medicine, Universitas Indonesia (FKUI). In 2015, he completed his training as an internal medicine specialist at the same university. Afterward, he began working as academic medical staff in the Division of Endocrinology, Metabolism, and Diabetes, Department of Internal Medicine FKUI and Dr. Cipto Mangunkusumo National General Hospital.

In 2016, Farid received a six months Erasmus+ travel grant to join the Half minor program at Leiden University Medical Center (LUMC) in the Netherlands, followed by a research internship in the Department of Parasitology under the supervision of Prof. Maria Yazdanbakhsh, Dr. Erliyani Sartono, and Dr. Dicky L. Tahapary. During this period, he became interested in learning more about the impact of urbanization on metabolic health of Indonesians in relation to varying degrees of exposure to microorganisms and parasites.

After being appointed as one of the internal medicine doctors for the 2017 Indonesian Hajj Health Mission and becoming a civil servant in 2018 under the Ministry of Health Republic of Indonesia, Farid won a highly competitive scholarship from the Indonesian Endowment Funds for Education (Lembaga Pengelola Dana Pendidikan/LPDP) to start his PhD under the supervision of Prof. Maria Yazdanbakhsh, Dr. Erliyani Sartono, and Dr. Dicky L. Tahapary in April 2019. During his PhD, Farid concentrated on two research projects in Indonesia. First, a prospective cohort study in young Indonesian adults to evaluate the impact of urbanization on metabolic health and allergic diseases. Secondly, a longitudinal study incorporating clinical and immunological assessments to better understand the pathogenesis of COVID-19 in obesity and diabetes. He also collaborated with Dr. Renee de Mutsert from the Department of Clinical Epidemiology of LUMC on analyzing a large dataset from the Indonesian national health survey. During his PhD trajectory, he joined a four months clinical observership at the Department of Endocrinology LUMC under the supervision of Prof. Nienke R. Biermasz.

After finishing his PhD, Farid will return to FKUI and Dr. Cipto Mangunkusumo National General Hospital to complete his endocrinology subspecialty residency program, combined with clinical work and teaching. He will continue with his research activities in the Cluster of Metabolic Disorders, Cardiovascular, and Aging of the Indonesian Medical Education and Research Institute (IMERI)-FKUI. He intends to focus on the immunological and gut ecosystem aspects of diabetes, obesity, and endocrinology.

LIST OF PUBLICATIONS

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