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PUBH-D-20-05755
"An initiative of cooperation in Zika virus research: the experience of the ZIKABRA Study in Brazil"
Original Submission

Trias Mahmudiono, S.KM, MPH, Ph.D (Reviewer 2)

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Is the study design appropriate to answer the research question (including the use of appropriate controls), and are the conclusions supported by the evidence presented?	No
Are the methods sufficiently described to allow the study to be repeated?	No
Is the use of statistics and treatment of uncertainties appropriate?	No
Is the presentation of the work clear?	No
Are the images in this manuscript (including electrophoretic gels and blots) free from apparent manipulation?	Yes

Comments to Editor:

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This box is for confidential comments to the editors only.
Any comments included in this box will not be sent to the authors.
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Examples of points which might be included here and raised as confidential comments to the editors would be ethical concerns regarding any experiments, concerns regarding an undisclosed conflict of interest or concerns regarding plagiarism or publication ethics.

Comments to Author:

In the abstract section, perhaps the method on how the ZikaBra initiatives come into place could be described. Also, the conclusion in the abstract should be clearly stated.
In the introduction section line 88 to 94 perhaps it would be better to put it in the method section along with any ethic protocol for the study if available.
In the method section line 105 to 112 perhaps it would be better to described the prevalence of Zika in Brazil and how many of the partner health facilities made up from the total population.
The description of the collaboration could be better depicted with come sort of figures that showed the interrelation among members of the initiatives from local government to the international governing bodies.
Before the lesson learn I think it would be better to discuss the challenges and obstacle that was face during

the inception of the initiatives and how to overcome it so that we can understand better the context.
In the conclusion section, I think it was to long and could be made more condense and succinct.

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Author's Response To Reviewer Comments

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EDITOR COMMENTS:
1) Please prepare the manuscript based on BMC format and please include sufficient method since BMC Public Health usually not taking review paper.

Our response:
Thank you for pointing this out.

The manuscript was formatted based on BMC Public Health – Research in practice articles: BMC Public Health | Research in practice (biomedcentral.com).

In terms of the method, we made adjustments in the abstract and added a new method section in the paper.

Abstract section, line 44, page 3.

Main text section, Methods, line 103, page 6.

2) We operate a transparent peer review process for this journal where reviewer reports are published with the article, but the reviewers are not named (unless they opt in to include their name).
Our response:
Thank you for your remarks with which we completely agree.

REVIEWER 1:
This paper describes a research network for Zika led and coordinated by WHO. The title is misleading as it refers to a network in international cooperation in health, while this network was specifically created for Zika research, with a strong focus on Brazil, the country with most Zika cases.

Our response:
Thank you for pointing this out.

We added a new title accordingly.

An initiative of cooperation in Zika virus research: the experience of ZIKABRA Study in Brazil.

Title section, lines 1-2, page 1.

The paper describes the advantages of a network approach. The authors would need to include examples of other Zika research consortia, eg those funded by the European Commission:

ZikaPLAN: addressing the knowledge gaps and working towards a research preparedness network in the Americas. Glob Health Action. 2019;12(1):1666566. doi: 10.1080/16549716.2019.1666566. PMID: 31640505 Free PMC article

Zika Preparedness Latin American Network (ZikaPLAN) is a research consortium funded by the European Commission to address the research gaps in combating Zika and to establish a sustainable network with research capacity building in the Americas….

Our response:
Thank you for your pertinent suggestions. However, the ZIKABRA Study is not a research network along the lines of the networks mentioned below.

The ZIKV infection raised a relevant response from the major research centers in the affected countries, which are examples of the networks currently producing scientific evidence in a collaborative venture. The ZIKABRA Study is a cohort involving a technical cooperation among different institutions, that we consider as an example of successful initiative of the international cooperation.

It would also be important to highlight and quote the research protocols that were developed for Zika research, also coordinated by WHO:

BMJ Open 2019 Jun 18;9(6):e026092. doi: 10.1136/bmjopen-2018-026092.
Understanding the relation between Zika virus infection during pregnancy and adverse fetal, infant and child outcomes: a protocol for a systematic review and individual participant data meta-analysis of longitudinal studies of pregnant women

and their infants and children.

Our response:
We have addressed this point in the answer above. Thank you for your suggestion and please refer to our answer for similar points made before.

Another network is ZikAlliance that should be quoted:

BMC Infect Dis 2019 Dec 26;19(1):1081. doi: 10.1186/s12879-019-4685-9.
Study protocol for the multicentre cohorts of Zika virus infection in pregnant women, infants, and acute clinical cases in Latin America and the Caribbean: the ZIKAlliance consortium.

Our response:
We have addressed this point in the answer above. Thank you for your suggestion and please refer to our answer for similar points made before.

Such networks are incredibly useful, also as a spin-off for newly emerging infectious diseases, as now seen for COVID-19. For example, the ZIkaPLAN diagnostics evaluation platform for Zika diagnostics was rapidly used for the evaluation of COVID-19 diagnostics:

Need for sustainable biobanking networks for COVID-19 and other diseases of epidemic potential.
Peeling RW, Boeras D, Wilder-Smith A, Sall A, Nkengasong J. Lancet Infect Dis. 2020 Oct;20(10):e268-e273. doi: 10.1016/S1473-3099(20)30461-8. Epub 2020 Jul 24.

Our response:
We have addressed this point in the answer above. Thank you for your suggestion and please refer to our answer for similar points made before.

For all rare disease outcomes in Zika, it is important to network so that complications such as Guillain Barre Syndrome and other neurological complications, early postnatal symptomatic Zika infections, rare events such as thrombocytopenia etc. Even more frequent events such as attack rates and sexual transmission can be best documented in meta-analyses of large cohorts. The authors may want to highlight the IGOS network (for Guillain-Barre Syndrome) or GeoSentinel, a network of travel medicine providers that investigates returning travelers.

Guillain-Barré Syndrome Associated with Zika Virus Infection in Colombia.
Parra B, Lizarazo J, Jiménez-Arango JA, Zea-Vera AF, González-Manrique G, Vargas J, Angarita JA, Zuñiga G, Lopez-Gonzalez R, Beltran CL, Rizcala KH, Morales MT, Pacheco O, Ospina ML, Kumar A, Cornblath DR, Muñoz LS, Osorio L, Barreras P, Pardo CA. N Engl J Med. 2016 Oct 20;375(16):1513-1523. doi: 10.1056/NEJMoa1605564. Epub 2016 Oct 5. PMID: 27705091 Free article.

Postnatal symptomatic Zika virus infections in children and adolescents: A systematic review.

Ramond A, Lobkowicz L, Clemente NS, Vaughan A, Turchi MD, Wilder-Smith A, Brickley EB. PLoS Negl Trop Dis. 2020 Oct 2;14(10):e0008612. doi: 10.1371/journal.pntd.0008612. eCollection 2020 Oct. PMID: 33006989

Zika among international travellers presenting to GeoSentinel sites, 2012-2019: implications for clinical practice.
Angelo KM, Stoney RJ, Brun-Cottan G, Leder K, Grobusch MP, Hochberg N, Kuhn S, Bottieau E, Schlagenhauf P, Chen L, Hynes NA, Perez CP, Mockenhaupt FP, Molina I, Crespillo-Andújar C, Malvy D, Caumes E, Plourde P, Shaw M, McCarthy AE, Piper-Jenks N, Connor BA, Hamer DH, Wilder-Smith A. J Travel Med. 2020 Jul 14;27(4):taaa061. doi: 10.1093/jtm/taaa061. PMID: 32330261
INTRODUCTION: International travellers contribute to the rapid spread of Zika virus (ZIKV) and its sentinel identification globally.

Travel Surveillance and Genomics Uncover a Hidden Zika Outbreak during the Waning Epidemic.
Grubaugh ND, Saraf S, Gangavarapu K, Watts A, Tan AL, Oidtman RJ, Ladner JT, Oliveira G, Matteson NL, Kraemer MUG, Vogels CBF, Hentoff A, Bhatia D, Stanek D, Scott B, Landis V, Stryker I, Cone MR, Kopp EW 4th, Cannons AC, Heberlein-Larson L, White S, Gillis LD, Ricciardi MJ, Kwal J, Lichtenberger PK, Magnani DM, Watkins DI, Palacios G, Hamer DH; GeoSentinel Surveillance Network, Gardner LM, Perkins TA, Baele G, Khan K, Morrison A, Isern S, Michael SF, Andersen KG. Cell. 2019 Aug 22;178(5):1057-1071.e11. doi: 10.1016/j.cell.2019.07.018. PMID: 31442400 Free PMC article.

Our response:
We have addressed this point in the answer above. Thank you for your suggestion and please refer to our answer for similar points made before.

The authors highlight the advantages of research networks, but also need to highlight some disadvantages, such as the delays in creating networks, the bottlenecks such as obtaining memorandum of understanding, consensus on authorship issues, shipment of samples and sharing of samples and data banks. These delays that are well-known should be an impetus for proactively setting up research networks in inter-epidemic times, including cohorts (eg pregnant women cohort studies, community based cohorts, children cohorts).

Our response:
We have addressed this point in the answer above. Thank you for your suggestion and please refer to our answer for similar points made before.

REVIEWER 2:

1) In the abstract section, perhaps the method on how the ZikaBra initiatives come into place could be described. Also, the conclusion in the abstract should be clearly stated.

Our response:
Thank you for your appropriate suggestion. The abstract has been changed accordingly.

Abstract section, line 44, page 3.

2) In the introduction section line 88 to 94 perhaps it would be better to put it in the method section along with any ethic protocol for the study if available.

Our response:
We agree with this pertinent comment.

A new method section was created.

Main Text section, Methods, line 103, page 6, as suggested.

The text has been moved to the Main Text section, Methods, lines 104-110, page 6, as suggested.

3) In the method section line 105 to 112 perhaps it would be better to describe the prevalence of Zika in Brazil and how many of the partner health facilities made up from the total population.

Our response:
We would like to clarify that the participating health facilities are describe in the protocol. We intend to describe a successful the partnership between different international collaborating institutions. The health facilities involved was selected based on: local prevalence of the infection, high population density; strong community health network; laboratory facilities capable of carrying out the tests requested by the study protocol.
A paragraph has been added to the manuscript containing the information bellow.

Main text section, ZIKABRA Study: role of partners, lines 121-131, pages 7,8.

In 2016, at the time of the ZIKABRA Study development, 216,207 probable ZIKV cases were reported in Brazil [8]. From 2015 to 2016, 10,232 suspected cases and 2,202 confirmed cases of microcephaly were reported in the country [9]. The ZIKABRA study [7] is an observational cohort of men and non-pregnant women, aged 18 years or older, with infection confirmed by laboratory tests on blood or urine. Specimens of blood, semen, vaginal secretion, urine, rectal swab, sweat, saliva and breast milk were collected and tested for ZIKV RNA at the partner health facilities through 17 visits distributed over 12 months of follow-up [7]. The criteria used to select the recruitment sites were: high population density; high circulation of ZIKV; strong community health network; laboratory facilities capable of carrying out viral culture, ZIKV antigen assays, RT-PCR, IgM/IgG, neutralizing antibody test (specific for ZIKV, dengue and chikungunya) and virus genetic sequencing [7].

4) The description of the collaboration could be better depicted with some sort of figures that showed the interrelation among members of the initiatives from local government to the international governing bodies.

Our response:
The figure systematizing the ZIKABRA cooperation was added.

Fig.1. ZIKABRA International Scientific Cooperation Framework

Main Text section, ZIKABRA International Scientific Cooperation Framework, lines 269, page 15.

5) Before the lesson learn I think it would be better to discuss the challenges and obstacle that was face during the inception of the initiatives and how to overcome it so that we can understand better the context.

Our response:

Thank you for your suggestions they were accepted. The information regarding the challenges and obstacles was added in the Main Text section, ZIKABRA International Scientific Cooperation Framework, lines 270-295, pages 15,16.

6) In the conclusion section, I think it was too long and could be made more condense and succinct.

Our response:

Thank you for your remarks with which we completely agree.

The text of the Conclusions section has been modified to address the suggestions made by the reviewer Conclusions section, lines 337-359, pages 19,20, as requested.

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BMC Public Health

RESEARCH IN PRACTICE Open Access

An initiative of cooperation in Zika virus research: the experience of the ZIKABRA study in Brazil

Silvana Pereira Giozza[1*], Ximena Pamela Díaz Bermúdez[2], Edna Oliveira Kara[3], Guilherme Amaral Calvet[4], Ana Maria Bispo de Filippis[5], Marcus Vinícius Guimarães Lacerda[6,7], Camila Helena Aguiar Bôtto-Menezes[8], Marcia da Costa Castilho[7], Rafael Freitas Oliveira Franca[9], Armando Menezes Neto[9], Casey Storme[10], Noemia S. Lima[10], Kayvon Modjarrad[11], Maria Cristina Pimenta de Oliveira[1], Gerson Fernando Mendes Pereira[1], Nathalie Broutet[3] on behalf of ZIKABRA Study Team

Abstract

Background: The Zika virus outbreak has triggered a set of local and global actions for a rapid, effective, and timely public health response. A World Health Organization (WHO) initiative, supported by the Department of Chronic Condition Diseases and Sexually Transmitted Infections (DCCI) of the Health Surveillance Secretariat (SVS), Brazil Ministry of Health (MoH) and other public health funders, resulted in the start of the "Study on the persistence of Zika virus in body fluids of patients with ZIKV infection in Brazil – ZIKABRA study". The ZIKABRA study was designed to increase understanding of how long ZIKV persists in bodily fluids and informing best measures to prevent its transmission. Data collection began in July 2017 and the last follow up visit occurred in 06/26/2020.

Methods: A framework for the ZIKABRA Cooperation initiative is provided through a description and analysis of the mechanisms, strategies and the ethos that have guided the models of international governance and technical cooperation in health for scientific exchange in the context of a public health emergency. Among the methodological strategies, we included a review of the legal documents that supported the ZIKABRA Cooperation; weekly documents produced in the meetings and working sessions; technical reports; memorandum of understanding and the research protocol.

(Continued on next page)

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(Continued from previous page)
Conclusion: We highlight the importance of working in cooperation between different institutional actors to achieve more significant results than that obtained by each group working in isolation. In addition, we point out the advantages of training activities, ongoing supervision, the construction of local installed research capacity, training academic and non-academic human resources, improvement of laboratory equipment, knowledge transfer and the availability of the ZIKABRA study protocol for development of similar studies, favoring the collective construction of knowledge to provide public health emergency responses. Strategy harmonization; human resources and health services; timing and recruiting particularities and processing institutional clearance in the different sites can be mentioned as challenges in this type of initiative.

Keywords: Zika virus, Outbreak, Public health emergency, International cooperation in health

Background

The sudden emergence of the Zika virus (ZIKV) in Brazil, the wide geographical range of the mosquito vector of the virus and the link to microcephaly and other important disorders, triggered a call for a global response. On November 11, 2015, the Brazilian Ministry of Health (MoH) declared the ZIKV epidemic as a Public Health Emergency of National Importance [1, 2] and, in February 2016, the World Health Organization (WHO) declared the epidemic to be a Public Health Emergency of International Concern (PHEIC) [3]. Considering the extent of the infection in Brazil and its complications, urgent and coordinated public health strategies were developed in the areas of surveillance, by adopting vector control measures and strong risk communication strategies, in addition to research and development actions in the clinical, laboratory and social areas, summarized in the Brazilian Plan for Fighting *Aedes aegypti* and microcephaly [4]. In turn, the WHO has set out a strategic response plan, the *Strategic Response Framework* (SRF) [5], aiming to foster early responses to the ZIKV emergency, including implementation of clinical research studies, the design of study protocols for diagnosis, the development of studies with qualitative methodologies, the formulation of strategies to reduce the risks of exposure to the *Aedes* mosquito, the development of safe and effective therapies and vaccines and the identification of funding sources to support this extensive research and development agenda.

The "Study on the persistence of Zika virus (ZIKV) in body fluids of patients with ZIKV infection in Brazil – ZIKABRA Study" was conducted to address one of the research gaps identified by the WHO Research Agenda which comprises the characterization of the ZIKV infection [6]. The aim of the study was to assess the presence and duration of Zika virus and related markers in infected individuals and their symptomatic or asymptomatic household contacts [7]. The study was supported by the Department of Chronic Condition Diseases and Sexually Transmitted Infections (DCCI) of the Health Surveillance Secretariat (SVS), Ministry of Health (MoH).

The ZIKABRA study brought together the expertise of a multi-disciplinary group of scientists to meet the requirements of designing a research protocol with complex clinical, epidemiological, biomedical, virological, laboratory and public policy features. The national epidemiological situation and research capacity dictated the selection of the study sites, notably in three major capitals in the northern (Manaus), northeastern (Recife) and southeastern (Rio de Janeiro) regions of the country, with its reference laboratories at the Tropical Medicine Foundation Dr. Heitor Vieira Dourado (FMT-HVD), Oswaldo Cruz Foundation, in Rio de Janeiro (Fiocruz-RJ) and Brazil Oswaldo Cruz Foundation-Institute Aggeu Magalhães, Recife (CPqAM – Fiocruz - PE). On the international side, the United States Department of Defense laboratory, and the Walter Reed Army Institute of Research (WRAIR) provided technical, material and immunologic assay support.

This paper provides a framework for the ZIKABRA initiative through a description and analysis of the mechanisms, strategies and the ethos that have guided the models of international cooperation and scientific exchange between Brazil and international organizations in the context of a public health emergency.

Methods

The project was jointly funded by the MoH/SVS/DCCI (grant 837059/2016, Process SEI 25000162039201616); WHO (UNDP-UNFPA-UNICEF-WHO-World Bank Special Programme of Research, Development and Research Training in Human Reproduction); WRAIR - (0130602D16) - Cooperation Agreement (W81XWH-18-2-0040) between Henry M. Jackson Foundation for the Advancement of Military Medicine and US Department of the Army; Wellcome Trust (WT) (grant 206522/Z/17/Z); and National Institutes of Health (NIH) (Award Number R21AI139777), thus completing the ZIKABRA international cooperation.

This is a descriptive and documentary analytical study, based on primary data, produced by the ZIKABRA Cooperation. The documents produced by the research study group and technical reports were reviewed. Basically,

the weekly meetings fed the information about the management and governance information, once all the representatives of the participant institutions attend these calls.

Data underlying the study cannot be made publicly available due to ethical concerns, as data contain several personally identifiable information. Data are available from Oswaldo Cruz Foundation for researchers who meet the criteria for access to confidential data. To contact information, please report the Declarations section of the Manuscript, Availability data and materials.

ZIKABRA study: role of partners

The study design was developed in partnership by a team of scientists from WHO, Fiocruz-RJ, CPqAM - Fiocruz-PE, FMT-HVD, WRAIR and DCCI and marked the beginning of the formation of the network of collaborators from MoH, PAHO, WHO, WRAIR, Fiocruz, WT and NIH, which is named ZIKABRA Cooperation.

In 2016, at the time of the ZIKABRA study development, 216,207 probable ZIKV cases were reported in Brazil [8]. From 2015 to 2016, 10,232 suspected cases and 2,202 confirmed cases of microcephaly were reported in the country [9]. The ZIKABRA study [7] is an observational cohort of men and non-pregnant women, aged 18 years or older, with infection confirmed by laboratory tests on blood or urine. Specimens of blood, semen, vaginal secretion, urine, rectal swab, sweat, saliva and breast milk were collected and tested for ZIKV RNA at the partner health facilities through 17 visits distributed over 12 months of follow-up [7]. The criteria used to select the recruitment sites were: high population density; high circulation of ZIKV; strong community health network; laboratory facilities capable of carrying out viral culture, ZIKV antigen assays, RT-PCR, IgM/IgG, neutralizing antibody test (specific for ZIKV, dengue and chikungunya) and virus genetic sequencing [7].

The laboratory component of the ZIKABRA study is a key part of the protocol, whose performance complexity requires mature operational and logistic support, essential to meet the requirements of all steps of the study, which included: recruitment; collection and transport of biological samples and specimens for laboratory testing at partner institutions in Brazil and WRAIR; follow-up of participants; data management and analysis; and production and progressive dissemination of knowledge generated within the scope of the project, [7, 10–12]. The main functions assumed by the institutions that make up the ZIKABRA Cooperation are briefly outlined below.

Ministry of Health (MoH)

The research agenda integrated into the Brazilian Plan for Fighting *Aedes aegypti* and Microcephaly [13] guided the building of researcher teams, consortia, agreements, and technical cooperation around a common theme [14], covering all fields of scientific knowledge. The SVS/MoH played a key role in this knowledge-building process by capturing and allocating the necessary financial resources to promote research related to ZIKV and microcephaly. The ZIKABRA study was included in the SVS list of priority studies, in the form of direct contracting [15]. It was left to the discretion of DCCI/SVS/MoH to lead the collaboration process with the WHO and national and international partner institutions. The General Coordination for the Development of Epidemiology in Services (CGDEP/SVS) monitors these studies and promotes the integration between scientific research and health surveillance management, the General Coordination of Public Health Laboratories (CGLAB/SVS) provides laboratory support and, together with DCCI/SVS, participate in the ZIKABRA Cooperation governance.

World Health Organization (WHO)

The WHO provided the initial funding, concept and planning for the investigation of ZIKV persistence in body fluids, and was responsible for bringing together funding partners, (WRAIR and WT), and providing the research protocol using the “Ebola RNA Persistence in Semen of Ebola Virus Disease Survivors” [16] as a reference template for the design of the ZIKABRA study. The role of the WHO was particularly crucial for scientific development and in responding to knowledge gaps around new health threats, in defining best practices in prevention and care and in the international dimension of initiatives, thus favoring the exchange and sharing of experiences on strategic health topics. It plays a key role in leveraging its ability to call on experts and high-level officials to collaboratively address these challenges. The WHO has been in charge of coordinating, organizing and keeping record of the group’s weekly meetings, as well as systematizing the advances and challenges presented in the different research sites.

Pan American health organization (PAHO)

PAHO/WHO in Brazil has had a central role in the technical conduct of the public health emergency by ZIKV, contributing to the MoH in the national response to this epidemic in Brazil and in the countries of the region, with the direct participation of its specialized teams in the field, mainly in the infection hotspots, supporting interventions in the areas of surveillance, epidemiological analysis and purchase of laboratory supplies. PAHO has facilitated the dialogue among several health managers in Latin America and contributed to the production of knowledge about ZIKV infection, both in Brazil and in the region. It has also played an important role in the management of funds earmarked for the development of strategic research activities [13].

Oswaldo Cruz Foundation (Fiocruz)

Fiocruz-RJ was responsible for coordinating the study and managing funds from international sources: Wellcome Trust, WHO and WRAIR. Additionally, it is in charge of carrying out laboratory tests together with FMT-HVD and Institute Aggeu Magalhães (CPqAM), in Fiocruz-PE, coordinating the laboratory management interface of the study with CGLAB/SVS. Fiocruz is a federal agency whose mission is to produce, disseminate and share knowledge and technologies aimed at contributing to the promotion of health and quality of life for the Brazilian population. Besides generating knowledge, Fiocruz is also responsible for the production of medicines, immunobiologicals and diagnostic tests [17], and works as a Collaborating Center for Global Health and South-South Cooperation, through its Center for International Relations in Health (CRIS Fiocruz) [18]. With the declaration of ZIKV as a Public Health Emergency of National Importance, Fiocruz prepared the "Fiocruz Plan" for coping with this public health emergency, presenting action strategies in several areas, including the national and international technical cooperation that supports the ZIKABRA study cooperation [17], a Zika social research network, among other initiatives.

Tropical Medicine Foundation Dr. Heitor Vieira Dourado (FMT-HVD)

FMT-HVD was the center responsible for the management of the national funding of the study. Patients from the city of Manaus, made up almost the entire sample of the study and the researchers were essential for the recruitment and follow-up of volunteer participants, laboratory analyses and transport of samples to the other sites in the national territory. As a national and world reference center for the treatment of tropical diseases, FMT-HVD plays an important role in clinical research and diagnosis and treatment of tropical diseases in the Amazon.

Wellcome Trust (WT)

The Wellcome Trust supports major projects in partnership with WHO and other institutions around the world to respond quickly to the global health threat caused by the ZIKV, including ZIKABRA. Its funding schemes offer grants across biomedical sciences, population health, medical innovation, humanities and social sciences, and public engagement [19].

The National Institutes of Health (NIH)

The NIH, more specifically the National Institute of Allergy and Infectious Diseases (NIAID), has partially funded the study through a grant, obtained via a bid notice. It promoted the research in areas such as the natural history of the disease, basic research on ZIKV, pathogenesis, rapid diagnostic tests, as well as treatments and vaccines [20].

Walter reed Army Institute of research (WRAIR)

In this cooperation, WRAIR added its experience by providing scientific resources as a center of excellence for immunology and the development of vaccines and medicines for diseases such as dengue, Zika, Ebola, coronaviruses, malaria, HIV/AIDS and others. WRAIR's contributions to the ZIKABRA study included co-funding, protocol review, laboratory testing, technology transfer, laboratory capacity building and production and dissemination of results.

Regulatory and governance context

The systematization and governance of the ZIKABRA Cooperation was based on the formalization of a memorandum of understanding (MoU) between the Ministry of Health, Fiocruz-RJ, WHO and WRAIR, which established the technical criteria for the cooperation, the allocation of responsibilities among the partners, the sharing of technical and scientific information and the commitment to national and international ethical and regulatory requirements [21]. In addition to ensuring sustainable training, the MoU was designed to create a local knowledge base and support future development of other programs with similar components [21]. It should be noted that the close collaboration among ZIKABRA scientists has led to the qualification of technicians at the post-doctoral, master's and other levels. The MoU is complemented by a Term of Reference containing guidelines for disseminating the results of the study [21]. A Steering Committee followed up the products derived from the study and supported the dissemination of data relevant to public health.

Within the scope of the ZIKABRA Cooperation governance, a weekly dialogue routine was established since the study protocol development phase, as proposed by WHO, through the use of virtual platforms, in order to enable the participation of those involved, with transparency and integration of the study management as the ethos that characterizes its governance. In these meetings, events that occurred at each site, advances in recruitment, logistical problems faced, and the solutions found, doubts, decentralized monitoring of the protocol follow-up and other topics are reviewed. Each of these meetings is documented by WHO in minutes that record the topics covered, the decisions and courses of action and are circulated to those involved on a weekly basis. This routine helped everyone in the process of following up the work on different areas and sites.

In short, the programmatic instruments supporting the study are analyzed in this locus, namely: the MoU [21]; the research protocol; the Term of Reference for

dissemination of the study scientific results; the mechanisms for planning and following up the study's actions; the research sites life memory documentation; the technical reports of follow-up of the participating institutions; the compliance with the ethical aspects of research with human beings; the strategies for reaching consensus in the collective decisions of the research team and the set of participating actors; and the scientific production.

Regulatory framework for ethics in research with human being

In Brazil, the National Commission for Research Ethics (CONEP) regulates research involving human beings and coordinates the institutions' Research Ethics Committee (CEP) network, forming the CEP/CONEP System. The current regulatory framework is the CNS/MoH Resolution 466/2012 [22]. After the ZIKV emergency, the CNS Resolution No. 580 [23] was published, to allow research protocols, that are strategic for SUS (Brazil's Unified Health System), to be processed as a matter of urgency, representing a considerable advancement for the agility of ethical evaluation in research, essential during health emergencies.

It is noteworthy that, in the ZIKV emergency context in Brazil, the CEP/CONEP System needed to adapt quickly to this reality [24] in order to appraise the large volume of research projects that have emerged, while attending to the relevant ethical and scientific foundations [22]. The ZIKABRA protocol was timely approved by CONEP and submitted for ethical evaluation at the local CEPs of the participating centers in order to comply with the double ethical evaluation required in the country. The process of ethical evaluation at the local and central level observed in the ZIKABRA's case indicated the need for improvement in the CEP/CONEP System's harmonization, considering that the CEPs of the participating institutions and services did not keep pace with CONEP to meet the requirements of the double ethical evaluation set out in the country.

The ZIKABRA study strictly followed the current regulations for the transportation, processing and use of human biological material, for the purpose of creating biorepositories and sending biological samples outside the country in accordance with national rules [25, 26]. For the internal management of the project, the MoU guarantees the sharing of human biological material stored in a biorepository and related information among partner institutions.

ZIKABRA international scientific cooperation framework

The ZIKABRA Cooperation (Fig. 1), is based on the partnership, support and promotion of public institutions of excellence, common funding for joint development and performance of their activities and future prospects for generating technological innovation such as the development of anti-ZIKV vaccines and specific diagnosis [21]. We consider that these features support a model of international scientific-technological cooperation [27].

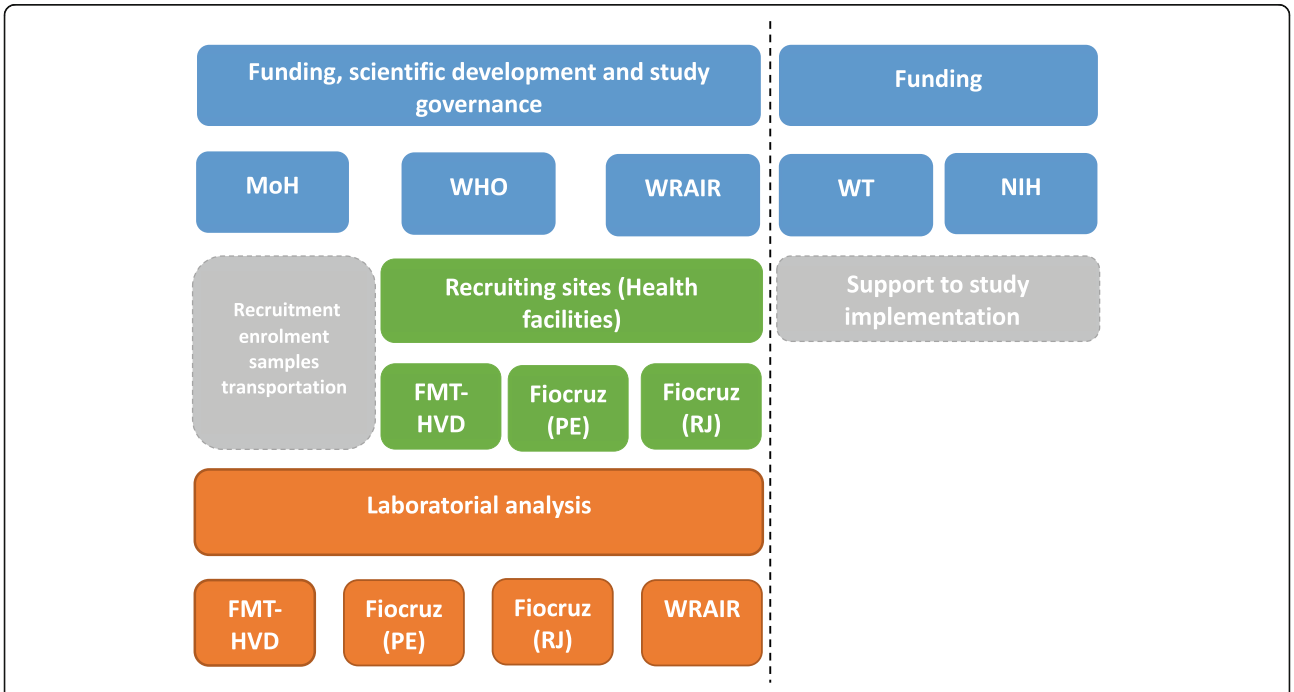

Fig. 1 ZIKABRA International Scientific Cooperation Framework. *Source:* SPG

This cooperation can be thought of as an example of global health governance, a process that makes up the type of collaboration between countries that work with common goals and interests. It can have a wide and flexible range of formal and informal joint activities that may include funding and coordination in health matters [28]. It can also operate at different levels of dialogue and foster the exchange of ideas on specific topics of expertise as one of the main challenges to be delivered.

The ZIKABRA Cooperation had a direct benefit for Brazil, but it also has potential applicability to other settings. The evidence generated by this cooperation could be used more broadly to address efforts regarding vulnerable and most at-risk population, such as those in countries with active transmission of the ZIKV or other emergent pathogens. It is, also, expected that the scientific information produced can support national and international guidelines for body fluid testing and contribute to the future health technology innovations [21].

However, we point out that one of the main barriers to researching in health emergency scenarios is the bureaucratic mechanisms and the slow chain procedures in conducting the rational use of financial resources in the implementation of the study. For instance, the delay in building the MoU, hiring qualified personnel, procurement and importing supplies, standardizing laboratory tests, transfer of funds to local sites, excessive delay in adjusting the work plan and to reach international quality standards.

It should be noted that the team of collaborators and supporters from the institutional partners played a leading role in the implementation of the ZIKABRA Study. The existence of this joint team of collaborators and supporters was key to overcome the barriers that came up during the throughout the execution of the study.

Working in cooperation in fact contributes to achieving more significant results than each group working in isolation. For this reason, careful structuring, relevant distribution of functions, well-coordinated collaboration and cooperation are pivotal. Cooperation also enables to provide consultation and coordination mechanisms so that this synergy takes place effectively [29].

Lessons learned from the ZIKABRA international cooperation

Brazilian practices of international cooperation in health have shown fruitful results in tackling public health problems with the consolidation of research and development actions. Other studies have been produced showing the importance of international collaborations for responding to the ZIKV public health emergency, including innovation, joint publications, and research networks [30].

Another Zika research consortia were installed by the European Commission, for instance the Zika Preparedness Latin American Network (ZikaPLAN) [31] to address the research gaps in combating Zika and to establish a sustainable network with research capacity building in the Americas. This network involves the participation of 25 multinational and interdisciplinary institutional partners from Europe, Latin America, North America, Africa, and Asia. Created in October 2016, this consortium initiative includes 15 work packages in different areas: virology, diagnostics, entomology and vector control, modelling to clinical cohort studies in pregnant women and neonates, as well as studies on the neurological complications of Zika infections in adolescents and adults. Also, the ZikaPLAN diagnostics evaluation platform was rapidly used for evaluation of COVID 19 diagnostics [32].

It's important to highlight the research protocols coordinated by WHO like the ZIKV Individual Participant Data (IPD) Consortium, that aims the development of systematic review to describe and gather data to IPD from longitudinal studies of pregnant women with ZIKV infection during pregnancy and fetal, infant or child outcomes, in the absence of a ZIKV vaccine or prophylactics [33, 34].

Another network initiative is supported by the European Commission (EC) Horizon 2020, was structured as a Consortium that comprises 53 partners organized in eight scientific and one management work packages (WPs), as well as three cross-cutting WPs, it's the ZIKAlliance Consortium. This is a multicentre research that covers Latin America and the Caribbean region. Among the goals is to describe the dynamic course of the ZIKV epidemic in pregnant women (PW), children (CH) and natural history (NH) cohorts. This Consortium also collaborates with the EC H2020-funded consortia: ZikaPlan and ZikAction. It works as a spin-off for newly emerging infectious diseases, as now seen for COVID-19 [35].

It is relevant to highlight that for all rare disease outcomes in ZIKV infection, networking has been a concrete response to share action regarding side events such as Guillain Barre Syndrome and other neurological complications, early postnatal symptomatic ZIKV infections and other rare events such as thrombocytopenia. Even more frequent events such as attack rates and sexual transmission [7] can be best documented in meta-analyses of large cohorts. For instance, the International Guillain-Barré syndrome Outcome Study (IGOS) network or GeoSentinel, a network of travel medicine providers that investigates returning travelers [36–39].

We understand that organizational (regarding global public health emergency response), administrative and regulatory processes must be sufficiently agile to meet the demands of scientific research in favor of the

benefits that science can bring to public health [40], without neglecting the good research practices.

In ZIKABRA study, groundwork was done in the health units of the selected sites, which involved the participation of health service coordinators, doctors, nurses and social workers, through prospecting visits and meetings with the presence of the researchers in charge and members of WHO, PAHO, WRAIR and MoH. During these visits, technical training on the protocol, good research practices, ethical respect for volunteer participants, structuring of services to avoid interruption in the routine care to regular patients, professionalization of non-academic professionals, among other activities, were promoted. The results were particularly enriching due to the experience shared between the site health teams and the ZIKABRA team, favoring the engagement of all actors involved for the good execution of the study.

One of the main bottlenecks in the operationalization of the ZIKABRA study was the change in ZIKV epidemiology, making it impossible to recruit participants in Rio de Janeiro, and very few participants in Recife. Additionally, there was a mismatch between the finalization of the ZIKABRA protocol, including all its regulatory and financial aspects completed in January 2017, and the end of the Rapid Action Strategy by SVS/MoH, on October 31, 2016, as well as the PHEIC closure by WHO, on November 18, 2016 [9]. This demanded an operational logistics rearrangement in the laboratories of the CPqAM - Fiocruz-PE, and Fiocruz-RJ, and in the support of the laboratory of the FMT-HVD.

On the other hand, the ZIKABRA Cooperation structure, with clearly defined roles in the operational instruments, has overcome the difficulties encountered, reaching the proposed goals, greater efficiency in fund management and strengthening research autonomy. The ZIKABRA study leaves as a legacy the interaction among the actors experienced in an arena of weekly discussions where decisions were made in full respect for the cooperative ethos among peers, the intertwining of institutional visions, in order to support the team of collaborators, aiming at the common interest and achieving excellence in results.

Based on this cooperation, it is suggested that there is greater flexibility and autonomy in the mechanisms of disbursement of funds and execution of the work plan, ease in hiring qualified human resources and in the purchase and import of laboratory supplies, standardization of diagnostic tests, among others. Table 1 summarizes the lessons learned from the ZIKABRA cooperation.

Conclusions

The legacy of the ZIKABRA Cooperation includes the construction of installed research capacity in the site, with improvement of laboratory equipment, laboratory and clinical data management platforms, training of academic and non-academic human resources, dissemination of generated knowledge, advancement of knowledge about the studied theme, knowledge transfer, availability of the ZIKABRA study protocol for development of similar studies, thus favoring the collective construction of knowledge to provide public health emergency responses. Additionally, the benefits arising from the study contribute to the strengthening of new partnerships with national and international researchers of recognized excellence in sciences, supported by public and government institutions in political, economic and technical terms [24].

We highlight some steps towards the potential continuity of the ZIKABRA Cooperation initiative, such as the idea of WHO as a global health hub mobilizing experts and putting its tradition, prestige and knowledge to the services of technical responses achieving health goals and sharing understanding and capabilities about the actions, norms and procedures adopted by the set of participating institutions.

Another relevant lesson learned is the horizontal cooperation among Brazilian researchers and institutions all over the country. The ZIKABRA project provided [41] technical responses to clinical and laboratory

Table 1 Lessons learned

Topic	Challenges	Proposed solutions
Ethical evaluation in local and international partner institutions	Different times for ethical evaluation of the study in the different national and international ethical instances	Harmonize the CEP/CONEP System for urgent ethical evaluation in the different instances, respecting the Good Research Practices, in the national context.
Multi-institutional funding	Raising and making public funds available quickly by partner institutions	Fine-tune regulatory and decision-making processes at partner institutions and make funds available on an urgent basis.
Technology availability	Standardization of diagnostic tests throughout the study execution period	Promote the sustainability and continuity of technological innovation development activities, training of high-level human resources.
Formalization of operational instruments	Sharing of institutional and individual responsibilities among the different actors	Expedite the drafting and harmonization of the basic text of the agreement among the parties.

questions of the research and also generated indirect benefits in the arena of policy studies and health cooperation frameworks.

It would also be important to bear in mind the role of interaction among government bodies of the participating institutions that takes place in the joint follow-up of the sites and project performance. Field supervision of all stakeholders during the implementation of the project occurred periodically. This helped to share the level of knowledge of the site settings by all participants and to avoid institutional constraints.

Abbreviations

ZIKV: Zika virus; WHO: World Health Organization; DCCI: Department of Chronic Condition Diseases and Sexually Transmitted Infections; SVS: Health Surveillance Secretariat; MoH: Ministry of Health; ZIKABRA Study: Study on the persistence of Zika virus (ZIKV) in body fluids of patients with ZIKV infection in Brazil; PHEIC: Public Health Emergency of International Concern; FMT-HVD: Tropical Medicine Foundation Dr. Heitor Vieira Dourado; Fiocruz: Oswaldo Cruz Foundation, RJ; CPqAM – Fiocruz – PE: Institute Aggeu Magalhães/Oswaldo Cruz Foundation; WRAIR: United States Department of Defense laboratory, and Walter Reed Army Institute of Research; WT: Wellcome Trust; NIH: National Institutes of Health; PAHO: Pan American Health Organization; CGDEP/SVS: General Coordination for the Development of Epidemiology in Services/ Health Surveillance Secretariat; CGLAB/ SVS: General Coordination of Public Health Laboratories/ Health Surveillance Secretariat; CRIS/Fiocruz: Cooperation, through its Center for International Relations in Health/ Oswaldo Cruz Foundation; CONEP: National Commission for Research Ethics; CEP: Research Ethics Committee; CNS/MoH: National Health Council/ Ministry of Health; SUS: Unified Health System

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Authors' contributions

Authors SPG and XPDB participated in the conceptualization, writing original draft and editing the manuscript. EOK, GAC provided inputs and revision of the original draft. AMBF, MVGL, MCC, NL, EOK, GAC, KM, provided critical feedback and input. GAC, EOK, CHAB-M, MVGL, SPG, XPDB, GFMP, MCPO, NL, CS, KM, NB, AMBF, AM-Neto, RFOF performed data analysis and study supervision. NL, EOK, GAC revised the text. All authors provided critical feedback and participated in editing and reviewing the paper. KM and EOK provided the final revision of the manuscript. All authors read and approved the final manuscript.

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Availability of data and materials

Data underlying the study cannot be made publicly available due to ethical concerns, as data contain several personally identifiable information. Data are available from Oswaldo Cruz Foundation for researchers who meet the criteria for access to confidential data. Contact information: Institutional Ethics and Research Committee of the Evandro Chagas National Institute of Infectious Diseases, email: cep@ini.fiocruz.br or Guilherme Amaral Calvet; email: guilherme.calvet@ini.fiocruz.br.

Declarations

Ethics approval and consent to participate

The study protocol in which this paper is based was reviewed and approved by the Research Ethics Review Committee (WHO ERC), Protocol ID: ERC.0002786; Brazilian National Research Ethics Commission (CONEP)(CAAE: 62518016.6.1001.0008); Institutional Ethics and Research Committee of the Evandro Chagas National Institute of Infectious Diseases, Fiocruz, Rio de Janeiro (CAAE: 62518016.6.2002.5262), Ethics and Research Committee of the Rio de Janeiro's Municipal Secretary of Health (CAAE: 2518016.6.3001.5279); Institutional Ethics and Research Committee of the Aggeu Magalhães Research Center, Fiocruz, Recife (CAAE: 62518016.6.2001.5190) and Institutional Ethics and Research Committee of the Tropical Medicine Foundation, Manaus, Amazonas (CAAE: 62518016.6.2003.0005). Informed consent will be obtained from all participants prior to enrollment.

Consent for publication
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Competing interests
The authors declare that they have no competing interests.

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Publisher's Note

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"Scaled-Up Nutrition Education on Pulse-Cereal Complementary Food Practice in Ethiopia: A Cluster-Randomized Trial"
Original Submission

Trias Mahmudiono, S.KM, MPH, Ph.D (Reviewer 1)

Reviewer Recommendation Term:	Accept after minor essential revisions
Transfer Authorization	**Response**
If this submission is transferred to another publication, do we have your consent to include your identifying information?	Yes
If this submission is transferred to another publication, do we have your consent to include your original review?	Yes
If this submission is transferred to another publication with Open or Transparent Peer Review, do we have your consent to publish your original review in a pre-publication history?	Yes
Custom Review Question(s):	**Response**
Are the methods appropriate and well described? If not, please specify what is required in your comments to the authors.	No
Does the work include the necessary controls? If not, please specify which controls are required in your comments to the authors.	Yes
Are the conclusions drawn adequately supported by the data shown? If not, please explain in your comments to the authors.	Yes
Are you able to assess any statistics in the manuscript or would you recommend an additional statistical review? If an additional statistical review is recommended, please specify what aspects require further assessment in your comments to the editors.	I am able to assess the statistics
Quality of written English Please indicate the quality of language in the manuscript:	Acceptable
Declaration of competing interests Please complete a declaration of competing interests, considering the following questions: 1. Have you in the past five years received reimbursements, fees, funding, or salary from an organisation that may in any way gain or lose financially from the publication of this manuscript, either now or in the future? 2. Do you hold any stocks or shares in an organisation that may in any way gain or lose financially from the publication of this manuscript, either now or in the future? 3. Do you hold or are you currently applying for any patents relating to the content of the manuscript? 4. Have you received reimbursements, fees, funding, or salary from an organization that	I declare that I have no competing interests

holds or has applied for patents relating to the content of the manuscript? 5. Do you have any other financial competing interests? 6. Do you have any non-financial competing interests in relation to this paper? If you can answer no to all of the above, write 'I declare that I have no competing interests' below. If your reply is yes to any, please give details below.	
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Comments to Editor:

===
This box is for confidential comments to the editors only.
Any comments included in this box will not be sent to the authors.
===

Examples of points which might be included here and raised as confidential comments to the editors would be ethical concerns regarding any experiments, concerns regarding an undisclosed conflict of interest or concerns regarding plagiarism or publication ethics.

Comments to Author:

In the abstract, I think it would be better if the authors add description of the statistical analysis used to test the hypothesis
In the abstract Page 1 line 17 there was typo for repetitive sub-heading "Results"
In the Background page 3 line 60 to 64, I think it would be better if the authors described further about the pilot study. How it differs from the current study. Such as whether the length of nutrition education is similar, targeting different population or the channel of nutrition education was different from the HEWs? Or it is the sample size that are lacking or else.
Or just answer the question of why through the previous pilot study only little is known of the effectiveness and sustainability of the nutrition education program through HEWs?
In the method section. Please clarify whether HEWs in the control group was trained for general nutrition education or not? Did the author have the chance to measure the capacity between HEWs in the intervention group or in the control group? How would the authors designate the significant change in the intervention group was not due to higher motivation or higher knowledge and skills on performing nutrition education among the HEWs?
Upon measuring the DDS, did the study used only yes or no intake related to the food groups or also incorporating minimum intake such as 10 grams was deemed consuming the food while less than that was not?
Did the intervention and the control group participate in the study received pulses from the study or from the government program? If yes, I think the authors might need to describe those in the methods section.
In the middle of the study the researchers provided Haricot beans in the intervention to the intervention only group and not the control group until the end line data collection. How do you think it will affect the observed results of KAP change in the study? How did the authors control for these intended differences in the study and sorting out the effect to be solely due to nutrition education on pulses?
Were there any inclusion and exclusion criteria used in the study? If any please explain why they were used? If not please explained why not?
In the results section, I think the study participants should be written in the method section instead.
Also please stated the inclusion criteria in the method section.
I think making a kind of CONSORT diagram of the study will made easier for the reader to better understand the study.
In the results section, since there was change in the intervention group with the distribution of Haricot bean, Did the authors saw drastic change from midline to end-line among intervention group or it was similar pattern? I would love to see the graph of the KAP Score as well as nutritional status at baseline, midline and and-line to visually analyse the change between intervention group and control group
In the discussion section, do you think the difference in the HEWs capacity assigned to the intervention and

control group was not an issue? Why?

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Author's Response To Reviewer Comments

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2020-05-07
Responding to peer reviewers' comments
Dear reviewers,
Thank you for giving us the opportunity to submit a revised draft of our manuscript "Scaled-Up Nutrition Education on Pulse-Cereal Complementary Food in Ethiopia: A Cluster-Randomized Trial". We are grateful for your insightful comments. The changes are highlighted within the manuscript. Here is a point-by-point response to the comments and concerns.
Report from Reviewer 1
Comment 1. In the abstract, I think it would be better if the authors add description of the statistical analysis used to test the hypothesis
Response. Statistical analysis used in our study is included in the abstract as follows: ANOVA and descriptive statistics used for data analysis. [Abstract, page # 1, line 15].
Comment 2. In the abstract Page 1 line 17 there was typo for repetitive sub-heading "Results"
Response. The repeated word deleted [Abstract, page # 1, line 16].
Comment 3. In the Background page 3 line 60 to 64, I think it would be better if the authors described further about the pilot study. How it differs from the current study. Such as whether the length of nutrition education is similar, targeting different population or the channel of nutrition education was different from the HEWs? Or it is the sample size that are lacking or else.
Response. Page 3 line 60-64 the difference between the pilot study and our study explained.
In these pilot studies the nutrition education to the mothers was given through the student researcher, as proof of principle but unsustainable. For example, Mulualem, et al., [15] provided 6 months of nutrition education to 80 mothers (compared to 80 controls) to evaluate the pulse messages and found improved KAP in mothers and weight gain in children but no significant change in stunting. [Background, page # 3, line 63-70].

Comment 4. In the method section. Please clarify whether HEWs in the control group was trained for general nutrition education or not?
Response. Clarification added to the manuscript as follow: All HEWs are trained for 1 year before deploying for their services in their local community. They trained on Family Health as one of the training packages where a general nutrition education covered. For this study, a Training of Trainer (TOT) manual was used to provide additional training on pulses to HEWs in the treatment kebeles but not in control kebeles. [Method, page #4, line 110-114].
Comment 5. Did the author have the chance to measure the capacity between HEWs in the intervention group or in the control group? How would the authors designate the significant change in the intervention group was not due to higher motivation or higher knowledge and skills on performing nutrition education among the HEWs?
Response. Unfortunately, we did not measure the motivation of HEWs. We put that as limitation as follows: Another limitation of the study was not assessing whether there were differences in the motivation of the HEWs between treatment and control kebeles, which could affect the outcome of the study. [Discussion, page # 13, line 373-375].
We did assess the HEWs experience and education level which was reported in another publication cited as [40]. Even though we did not test for motivation, there were no apparent differences in the two groups of HEWs for KAP.
Comment 6. Upon measuring the DDS, did the study used only yes or no intake related to the food groups or also incorporating minimum intake such as 10 grams was deemed consuming the food while less than that was not?
Response. We did measure a both a yes or no for intake as well as estimated intake and considered both during analysis. In 2008, WHO document "Indicators for assessing infant and young child feeding practices. Part 1: Definitions" stated that to calculate DDS in children there is not a minimum quantity, which means consumption of any amount of food from each food group is sufficient to count. In this manuscript we did not report intake. But we have clarified the method further as follows: The response of mothers was recorded as "Yes" if they said the child eat the particular food and "No" if they said the child did not eat the food. The answer "Yes" recorded as 1 and "No" recorded as 0 and a sum of the total number of food groups consumed was calculated. The mothers also asked to estimate the amount of the food the child eats using locally used equipment for each child and the proportion of children consuming four or more food groups per day was determined. [Method, page #5, line 143-148].
Comment 7. Did the intervention and the control group participate in the study received pulses from the study or from the government program? If yes, I think the authors might need to describe those in the methods section.
Response. The study provided the seed and we improve our explanation that was included in the method section under "Haricot Beans for Women's Empowerment in Household Decision Making" by adding this additional sentence: The researchers decided, after the midline data collection to provide each of the mothers in the intervention group with a single gift of a two kg bag of quality haricot bean seed and a two kg bag of fertilizer to plant during the June-July planting season. Both intervention and control groups received pulse seeds and fertilizer for future planting from the study, with controls receiving seeds after the endline. [Method, page #7, line 187-189].
Comment 8. In the middle of the study the researchers provided Haricot beans in the intervention to the intervention only group and not the control group until the end line data collection. How do you think it will affect the observed results of KAP change in the study? How did the authors control for these intended differences in the study and sorting out the effect to be solely due to nutrition education on pulses?

Response. We agree the provision of seeds could have changed KAP but with drought and floods we had to provide this goodwill gesture. We explain this on page #11 as follow: Changes in knowledge and attitudes observed at the midline of the study were similar to endline. However, the practice change showed greater significance at the endline than at midline. We attribute this to the seed provision which may have motivated mothers to practice than nutrition education alone.
In addition, we put the provision of seed in the midline of the study as limitation. [Discussion, page # 11, line 316-319].

Comment 9. Were there any inclusion and exclusion criteria used in the study? If any please explain why they were used? If not please explained why not?
Response. Inclusion and exclusion criteria included in the method as follows: Mothers who have apparently healthy breastfeeding infants aged 6-15 months who were permanent residents in the area included in the study. Children who were receiving supplemental or those that were severely or moderately malnourished and had started therapeutic food were excluded from the study. Children who started therapeutic feeding excluded because their weight gain or improved situation would not show the effect of the intervention. [Method, page #4, line 90-95].
Comment 10. In the results section, I think the study participants should be written in the method section instead.
Response. Moved to method section as suggested (only the text moved) [Method, page #3, line 85-90].
Comment 11. Also please stated the inclusion criteria in the method section.
Response. Inclusion criteria included in the method section as stated on comment 9. [Method, page #4, line 90-95].
Comment 12. I think making a kind of CONSORT diagram of the study will made easier for the reader to better understand the study.
Response. Figure 1 shows the flow chart of the study design which is was made using CONSORT instructions. [Results, page #8, line 211].
Comment 13. In the results section, since there was change in the intervention group with the distribution of Haricot bean, Did the authors saw drastic change from midline to end-line among intervention group or it was similar pattern? I would love to see the graph of the KAP Score as well as nutritional status at baseline, midline and and-line to visually analyse the change between intervention group and control group
Response. For knowledge and practice the change was significant between midline and endline as well. However, attitude did not change much after midline which suggested no effect of giving seeds. [Discussion, page #11, line 316-319], [Figure 2 showed the pattern. Results, page #8, line 221].
Comment 14. In the discussion section, do you think the difference in the HEWs capacity assigned to the intervention and control group was not an issue? Why?
Response. We put the capacity issue as limitation as follows: We assessed the HEWs experience and education level as being similar which was reported in other publication [40]. Even though we did not test for motivation, there were no apparent differences in the two groups of HEWs for KAP regarding pulses and experience. However, we did not analyze how the motivational difference impacted the intervention, thus, we put it as limitation.
Report from Reviewer 2
Comment 1. The impact of nutrition education on improving mothers' knowledge, attitude, and practices towards incorporating pulses into their children's food intake is a valuable research topic.
Response. We appreciate for the suggestion on the title and modify the title as follows: Scaled-up nutrition education on pulse-cereal complementary food practices in Ethiopia: A cluster randomized trial. [Title page]
Comment 2. Under ABSTRACT: The acronyms DDS and CF need to be defined first before being used.
Response. Accepted and corrected [Abstract, page #1, line 19, 24].
Comment 3. Suggestion: This study aimed to assess the impact of a 9-month pulse-nutrition education program on improving mothers' knowledge, attitude, and practices (KAP) towards pulses, as well as its effect on children's dietary diversity and nutritional status
Response. Accepted and corrected [Abstract, page # 1, line 5-7].
Comment 4. Ref. #1 does not seem to reflect it
Response. Replaced with: Bhutta, A. Z., Das, K. D., Rizvi, A., Gaffey, F. M., Walker, N., Horton, S., & Maternal and Child Undernutrition Study Group. (2013). Evidence-based interventions for improvement of maternal and child nutrition: what can be done and at what cost? Lancet, 382 (9890), 452-477. Doi:10.1016/S0140-6736(13)60996-4. [Reference, page # 16, line 444-447].
Comment 5. Can you please expand on the previous pilot studies? How this study assessed sustainability?
Response. An example of previous study given on page # 3 and further explanation included as follows: Sustainability of a reasercher-led program is uncertain, and 6 months of intervention may not have been sufficient to have a sustainable impact on improved KAP in rural mothers. [Background, page #3, line 67-70].
Comment 6. Suggested edits for the above statements: line 47-48
Response. Accepted and corrected [Background, page # 2, line 46-48].
Comment 7. Line 141 Food Insecurity Access Scale," was used [25]. As suggested by [26], Please delete ref#26, and say as suggested by Ballard et al.,
Response. Accepted and corrected [Method, page #6, line 164].
Comment 8. Line 238: Nutritional Status of Children: 238 Anthropometry measurements: Please use add (ic): anthropometric
Response. Accepted and corrected [Results, page # 10, line 262].
Comment 9. I suggest that authors revise the conclusion and state that nutrition education helped in improving the maternal KAP and frequency of pulse consumption as well as the nutritional status of their children
Response. Accepted and suggestion included in the conclusion as follows: The pulse nutrition education given to mothers by HEWs for 9-month helped in improving the maternal KAP and frequency of pulse consumption as well as the nutritional status of their children. [Conclusion, page # 13-14, line 380-382].
Comment 10. How authors assessed that all trained local HEWs had the same level of experience as they delivered the intervention?

Response. We did assess the HEWs experience and education level which was reported in another publication [40]. Even though we did not test for motivation, there were no apparent differences in the two groups of HEWs for KAP. However, we did not analyze how the motivational difference impacted the intervention, thus, we put it as limitation as follow: Another limitation of the study was not assessing whether there were differences in the motivation of the HEWs between treatment and control kebeles, which could affect the outcome of the study. [Discussion, page # 13, line 373-375].
Comment 11. Please add a section on recommendations for future studies.
Response. Accepted and recommendation for future study now included as follows: Finally, we recommend a future study on cost-benefit analysis enhanced nutrition education that integrates pulse use into the HEWs information package and training. [Conclusion, page # 14, line 390-392].

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RESEARCH ARTICLE Open Access

Scaled-up nutrition education on pulse-cereal complementary food practice in Ethiopia: a cluster-randomized trial

Getenesh Berhanu Teshome[1*], Susan J. Whiting[2], Timothy J. Green[3], Demmelash Mulualem[1] and Carol J. Henry[2]

Abstract

Background: Improving children's weight status through nutrition education (NE) for mothers about using pulses in complementary feeding has been demonstrated in pilot studies, but no effect on stunting was reported. The aim of the study was to assess the impact of a 9-month pulse-nutrition education program on improving mothers' knowledge, attitude, and practices (KAP) towards pulses, as well as its effect on children's diet diversity, and nutritional status. The NE was delivered by Health Extension Workers (HEWs).

Methods: A cluster randomized study was employed for the community-based interventional study. Twelve randomly selected villages in Sidama Zone, Southern Ethiopia were included in the study. A total of 772 mother-child pairs involved in the study; where 386 mother-child pairs in the intervention group received additional messages about pulse-cereal complementary food, and 386 pairs (the control) received only routine health education for 9 months. A survey on mothers' KAP and anthropometric measurements of the children were taken at baseline, midpoint, and end point. ANOVA and descriptive statistics were used to analyzed data.

Results: At baseline and end point, maternal KAP and the dietary diversity score of the children (mean age at end point 18.8 ± 2.9 mo) were assessed. Intervention mothers' KAP improved ($p < 0.001$) at midpoint and end point compared to that of the control group, as did frequency of pulse consumption and Dietary Diversity Score (DDS) among children. At 9 months, the prevalence of stunting, wasting, and underweight was significantly reduced in the intervention group compared to the control group ($p = 0.001$).

Conclusions: NE delivered by HEWs improved KAP of mothers regarding pulse consumption and dietary diversity of children led to improved nutritional status of the children. Training HEWs on the use of pulses for complementary food may be an effective way to improve the health of children in Ethiopian communities.

Trial registration: Clinicaltrials.gov #NCT02638571.
Date of registration: 12/18/2015.
Prospectively registered.

Keywords: Complementary food, Dietary diversity, Germination, Health extension workers, Nutrition education, Pulses, Scale-up

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Background

Undernutrition in the first 1000 days is responsible for the annual death worldwide of over 3 million children under 5 years of age, and poor complementary feeding is one reason for undernutrition [1]. In Ethiopia, the prevalence of underweight, stunting, and wasting in children under 5 years of age is 24, 38, and 10% respectively [2]. Similar rates are found in the Southern Nations and Nationalities People Region (SNNPR) [2], the least urbanized region of the country (90% rural), where almost one in four live under the poverty line [3].

Exclusive breastfeeding is recommended for the first 6 months of a child's life, followed by the introduction of complementary foods with continued breast-feeding. If complementary foods are insufficient in quality and/or quantity, nutritional deficiencies are at high risk of developing during the second half of infancy [4]. In Ethiopia, most complementary foods are made from unfortified cereal-based gruels [2, 5, 6], which are low in energy and nutrient density, leading to inadequacies of many nutrients including protein, iron, and zinc [7]. Indeed, in a 2016 Ethiopian survey, only 14% of children aged 6–23 months were found to consumed food from four or more food groups, and only 45% were fed the minimum acceptable diet [2]. In SNNPR, these rates were 12.5 and 41.9%, respectively [2].

In 2004, the Government of Ethiopia introduced the Health Extension Program to improve primary health services. Four years later, the National Nutrition Program (NNP) was introduced to address the growing concern of malnutrition among children under 5 years of age was introduced. A second NNP launched in 2016 emphasized the involvement of Health Extension Workers (HEWs) in the implementation of the program. HEWs are female high school graduates from the local community who speak the local language. As part of their job description, HEWs are mandated to provide nutrition education for mothers about essential child feeding practices [8, 9].

Pulses are important crops, providing high quality protein when blended with cereal [10]. They are also good sources of iron, zinc, and other important micronutrients when processed appropriately, which also improves protein digestibility [11, 12]. However, in Ethiopia, only 26% of children aged 6–24 months consume pulses. In SNNPR, although pulse crops are locally grown and available, only 9% of the population consumes pulses [13]. One of the major problems encountered in addressing malnutrition is lack of knowledge among mothers, family members, and health workers about the benefits of pulses for young children [4]. Previous pilot studies have shown that nutrition education on pulses improved the knowledge, attitude, and practice (KAP) of mothers in child feeding, and that nutrient intake and some parameters of growth improved [14, 15]. In these pilot studies, mothers received nutrition education via student researcher for a short interventional period. As an example, Mulualem et al. [14] provided 6 months of nutrition education to 80 mothers (compared to 80 controls) to improve mothers' KAP and children's nutritional status. Results of this small study indicated that the pulse messages improved KAP in mothers and weight gain in children, but no significant change in stunting [14]. Sustainability of a researcher-led program is uncertain, and 6 months of intervention may not have been sufficient to have a sustainable impact on improved KAP in rural mothers. Thus, little is known about the effectiveness and sustainability of a nutrition education program conducted through the regular activities of the HEWs. The main objective of the study was to assess the effectiveness of nutrition education on pulse-incorporated complementary food to the wider rural community through the government health system to improve maternal KAP, dietary diversity, and the nutritional status of children (6 to 24 months). The hypothesis was that nutrition education would improve mothers' KAP of pulse-incorporated complementary food and subsequently would improve nutritional status, and dietary diversity of their young children.

Methods

Study setting and participants

This study was registered as Clinicaltrials.gov #NCT02638571, and the protocol, along with baseline results, have been published [16]. Briefly, this cluster-randomized intervention trial for community-based nutrition education was done in 12 kebeles/villages, selected from two districts of the Sidama Zone, Southern Ethiopia. Kebeles were randomly assigned to the intervention and control groups after stratification by districts using the lottery method, as the prevalence of child malnutrition and number of children was different in each district. A total of 772 mothers with children aged 6–15 months were recruited initially at the baseline. The total number of participants at baseline was 771 as one child was excluded due to not fulfilling the inclusion criteria. At the midpoint of data collection, the total number was 692 (354 in the intervention group and 338 in the control group), and at end point it was 621 (307 in the intervention group and 314 in the control group (Fig. 1). Mothers who have apparently healthy breastfeeding infants aged 6–15 months who were permanent residents in the area included in the study. Children who were receiving supplemental or those that were severely or moderately malnourished and had started therapeutic food were excluded from the study. Children who started therapeutic feeding excluded because their weight gain or improved situation would not show the

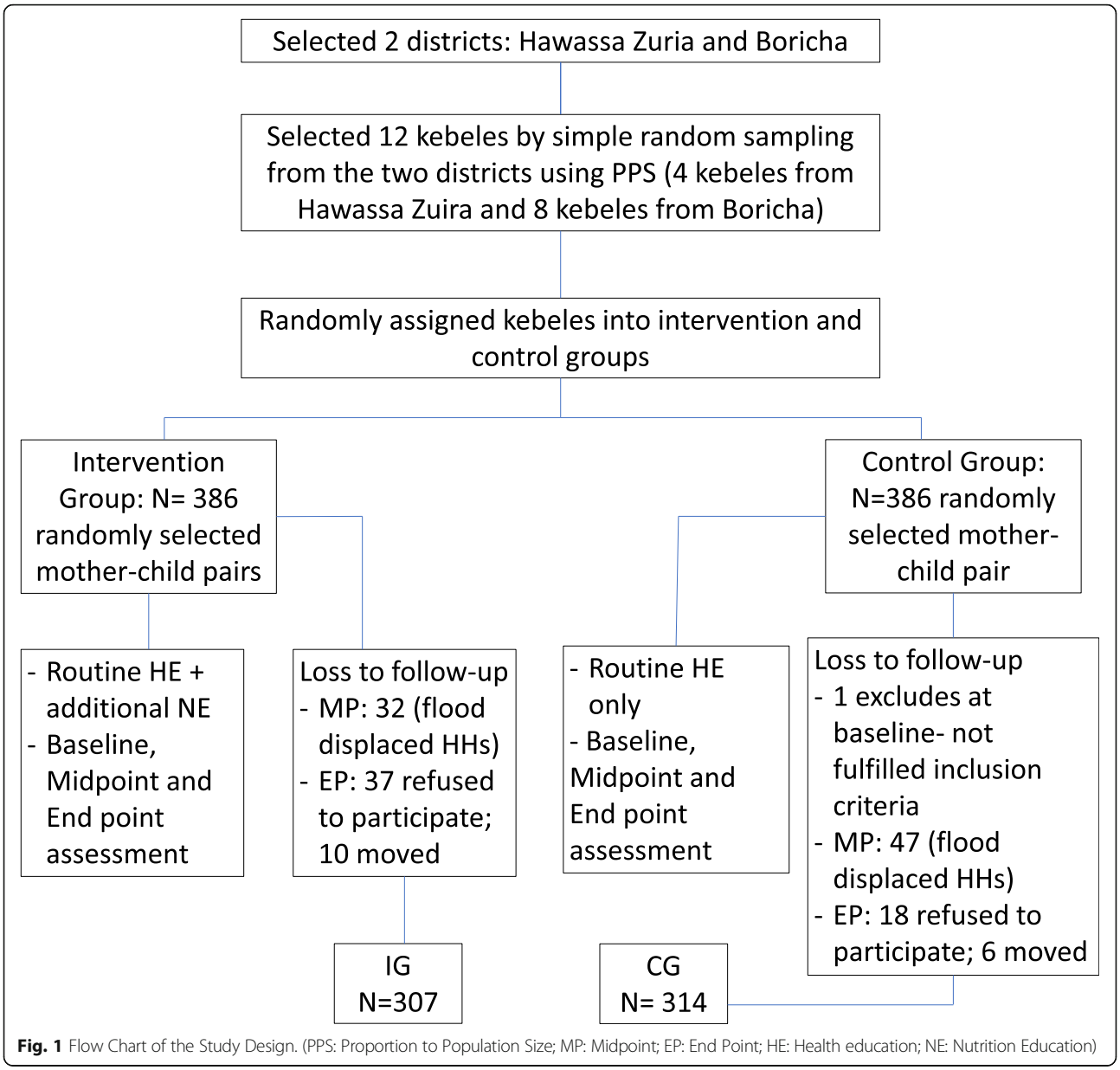

Fig. 1 Flow Chart of the Study Design. (PPS: Proportion to Population Size; MP: Midpoint; EP: End Point; HE: Health education; NE: Nutrition Education)

effect of the intervention. The study was not blinded, because the districts were far apart (it was not possible to walk between kebeles and back in 1 day), did not share markets, health centers, and health posts, and study personnel did not overlap between areas.

The intervention and education materials

Key messages were developed based on the Theory of Planned Behaviour (TPB) and Health Belief Model (HBM) principles [17, 18]. Health Extension Workers, two of whom were located in each kebele, were provided with 9 months of additional nutrition education, along with the usual health education. The HEWs provided the mothers in the intervention group with five main lessons. See the main lessons covered in intervention (Additional file 1: Table S1). An intervention with recipe demonstrations on preparation of porridge for complementary feeding using germinated pulse and cereal was given once a month and repeated again after midpoint (4.5 month) data collection. In addition, participating mothers in the intervention group were counseled by HEWs during house-to-house visits. The additional messages delivered to the intervention group were not included in the usual health education delivered to the control group. The control group received the usual health education provided in the area, which is mainly based on the essential nutrition action messages.

All HEWs are trained for 1 year before deploying for their services in their local community. They trained on Family Health as one of the training packages where a general nutrition education covered. For this study, a Training of Trainer (TOT) manual was used to provide additional training on pulses to HEWs in the treatment kebeles but not in control kebeles. This manual was developed and used by the Canadian International Food Security Research Fund (CIFSRF) for the "Scaling-up Pulse Innovations for Food and Nutrition Security" project [19]. Key messages included in the TOT manual were the importance of consuming food from all food groups and dietary diversification; the benefits of pulses; household pulse processing and preparation techniques, and the need to prepare and cook a variety of pulse-based dishes, including pulse-cereal mix complementary food. HEWs were trained for 3 days with demonstrations. At the same time, HEWs in the intervention group had refresher training in communication and counseling skills. In addition, HEWs were trained to use a quick guide when counseling mothers during house-to-house visits [20]. In the control sites, HEWs continued to provide routine health education. These HEWs had not been specially trained in using pulses in complementary food.

Before the intervention was introduced, the training material and counseling poster were pre-tested on purposively selected mothers to assess whether the content and format were realistic, understandable, culturally appropriate, visually appealing, and motivating. These mothers from the Hawassa Zuria district, who did not participate in the actual study, were provided with a half-day education and their understanding of the messages was assessed through discussion. Each picture on the poster was also assessed for its cultural acceptance.

The KAP of mothers regarding pulse consumption and feeding practices were collected at the baseline, midpoint, and end point of the intervention period. A standardized questionnaire was used to assess the mothers' intentions to use cereal-pulse incorporated complementary food. Theory of Planned Behavior [18] and the Health Belief Model (HBM) was used to frame questions to assess the KAP of mothers based on the guidelines of Macias and Glasauer [17].

Dietary diversity and growth assessment

Using a structured questionnaire, the mothers were asked about the type and number of meals consumed by their young children in the previous 24 h [21]. In addition, the Dietary Diversity Score (DDS) for each child was calculated based on the World Health Organizations (WHO) guidelines for measuring individual dietary diversity scores, using the following food groups to calculate the DDS: 1) grains, roots, and tubers; 2) legumes and nuts; 3) dairy products (milk, yogurt, cheese); 4) flesh foods (meat, fish, poultry, and liver/organ meats); 5) eggs; 6) vitamin-A rich fruits and vegetables; and 7) other fruits and vegetables [22]. The response of mothers was recorded as "Yes" if they said the child ate the particular food and "No" if they said the child did not eat the food. The answer "Yes" was recorded as 1 and "No" recorded as 0 and a sum of the total number of food groups consumed was calculated. The mothers also asked to estimate the amount of the food the child eats using locally used equipment for each child and the proportion of children consuming four or more food groups per day was determined. In addition to the number of meals, the frequency of the children's pulse consumption was assessed using a frequency questionnaire to evaluate monthly consumption of pulses.

The anthropometry of the children was taken at baseline, midpoint, and end point using standardized techniques [23]. In brief, weight was measured using an electronic scale (Seca 770), and the children were draped in a light cloth of known weight during the measurement. The recumbent length was measured to the nearest 0.1 cm using the Shorr measuring board. The Middle Upper Arm Circumference (MUAC) of the left arm of young children was measured using arm circumference insertion tape. All anthropometric measurements were entered and analyzed using WHOAnthro version 3.2.2.

Assessment of socio-demographic characteristics

Data on the socio-demographic characteristics of the participants, including those of the participants' household, such as age, gender, ethnicity, income, and KAP of mothers, were assessed using a standard questionnaire adopted from previous studies [14, 24] with modifications. To assess the food insecurity of the households in the study area, a standardized questionnaire adapted from Food and Nutrition Technical Assistance (FANTA), the "Household Food Insecurity Access Scale," was used [25]. As suggested by Ballard et al., 2011 [26], only the last three questions of the nine included to analyze food insecurity. These questions have been validated in low-income countries to measure household hunger. These three questions comprise the Household Hunger Scale (HHS). Food insecurity was assessed with a recall period of the last 4 weeks (30 days) prior to the data collection.

Household wealth status was measured by an asset-based (non-monetary) wealth index adopted from CSA [2]. During data collection, each participating household reported assets owned and other housing and sanitation-related characteristics. These included ownership of a radio, TV, mobile phone, TV, and bicycle, access to electricity, and quantity of livestock, land size, and level of income. Housing characteristics used in the wealth index calculation include the dwelling's structure, number of

rooms and bedrooms, and ownership (whether it is privately owned or rented). Each household received a score of 1 or 0 depending on whether it had the particular asset (1 = yes and 0 = No). Each binary variable was then weighted by the inverse of the proportion of households that owned the particular item or had the particular characteristics [27].

Haricot beans for women's empowerment in household decision making

Researchers associated with the larger project funded by CIFSRF attended an intervention nutrition education and demonstration session just prior to the midpoint data collection where mothers explained that although they understood the benefits of feeding their children pulses, they could not fully provide pulses as complementary food to their young children due to a shortage. At this time (late May and early June 2016), much of the population were affected by flooding that occurred due to an extended drought in the area. These climatic changes had prevented planting and/or reaping of pulses during the first harvest. The researchers decided, after the midpoint data collection to provide each of the mothers in the intervention group with a single gift of a two kg bag of quality haricot bean seed and a two kg bag of fertilizer to plant during the June–July planting season. Both intervention and control groups received pulse seeds and fertilizer for future planting from the study, with controls receiving seeds after the end point.

The women agreed to plant the seeds after a training session. Agriculture experts from Hawassa University's College of Agriculture (partner institution) trained 386 mothers for 1 day on techniques of planting, applying fertilizer, and weeding. The mothers in the control group were later provided with one kg of haricot bean seed at the end of end point data collection. The provision of a small amount of a new variety haricot bean seed was meant to enable smallholder female farmers to improve their wellbeing and that of their families.

Data analysis

Data were entered into SPSS version 20 software. Chi square and repeated measures Analysis of Variance (ANOVA) were used to investigate relationships between the pre- and post-intervention data on KAP of mothers, and growth and DDS of their children. ANOVA was used to compare means between the control and intervention groups, and when ANOVA was statistically significant, a post hoc test (Tukey HSD test) was used to determine the level of significance of values between and within groups. A value of $p < 0.05$ was considered as statistically significant.

Results

Study participants

The mean ± SD age of the mother was 25.5 ± 4.7 years: the majority (71%) were 21–30 years. About half the women were in primary school or had completed primary school. The mean ± SD age of young children was 9 ± 2.6 months, with about half of them between 6 and 8 months. A summary of the study participants' sociodemographic characteristics is presented in Table 1.

Knowledge, attitude, and practices of mothers

Both the intervention and control group mothers had low scores on KAP at baseline. At midpoint, KAP improved in the intervention group (Fig. 2). After the 9-month nutrition education, mothers' mean knowledge ($p = 0.001$) and practices ($p = 0.001$) significantly improved, but the attitude score remained the same as the midpoint score. There was a significant main effect of nutrition education on knowledge ($F = 488.498$; df = 2; $p = 0.001$), attitude ($F = 375.221$; df = 2; $p = 0.001$), and practices ($F = 201.431$; df = 2; $p = 0.001$) within groups. A similar significant effect was seen between groups on knowledge ($F = 3071.99$; df = 1; $p = 0.001$), attitude ($F = 1297.50$; df = 1; $p = 0.001$) and, practices ($F = 158.98$; df = 1; $p = 0.001$).

Household processing of pulses and preparation of complementary foods by the mothers

At baseline, none of the mothers from either the intervention or control group indicated having ever soaked and germinated pulse products. At midpoint, 132 (43%) from the intervention and 29 (9.2%) from the control group reported that they had soaked and germinated pulses. Only 13 (4.2%) from the intervention group could recall from memory all the steps in household processing techniques (sorting, soaking, draining and soaking for 48 h, sun drying, roasting in a warm pan, and milling), and no members of the control group could describe all of these steps. In addition, only five women (1.6%) from the intervention and three (1%) from the control group were able to recall the right proportion of cereal-pulse mix, which is 3/4th cereal and 1/4th pulse. At end point, 214 (69.7%) from the intervention and 37 (11.8%) from the control group reported that they soak and germinate pulse crops; only 23 (7.5%) from the intervention group could recall from memory all six steps of the household processing techniques, while none of the control group could. One hundred and forty-six (47.6%) from the intervention and 15 (4.8%) from the control group had learned the right proportion of cereal-pulse mix for preparing complementary food (Table 2).

Table 1 Socio-Demographic Characteristics of Study Participants at Baseline, Comparison of the Intervention Group (IG) and Control Group (CG), Southern Ethiopia, 2016 ($n = 771$)

Socio-demographic characteristics	IG n (%)	CG n (%)	*P*
Age of the mother (years)			
< 24	146 (37.8)	175 (45.5)	0.59
25–34	227 (58.8)	207 (53.8)	
> 35	13 (3.4)	3 (0.8)	
Marital status			
Married	365 (94.6)	379 (98.4)	**0.004**
Divorced	14 (3.6)	4 (1.0)	
Widowed	7 (1.8)	2 (0.5)	
Educational status			
Illiterate	150 (38.9)	147 (38.2)	0.58
Read and write	236 (61.2)	238 (61.8)	
In charge of food purchase			
Yes	150 (38.9)	158 (59.0)	0.54
No	236 (61.1)	158 (41.0)	
Source of income generating activities by woman			
Yes (petty trade, day labor)	62 (16.1)	49 (12.7)	0.19
No	324 (83.9)	336 (87.3)	
Household size			
Low (1–4 family members)	97 (25.1)	124 (32.2)	
Medium (5–10 family members)	222 (57.5)	190 (49.4)	0.45
Large (> 10 family members)	67 (17.4)	71 (18.4)	
Age of the children (months)			
6–8	172 (44.6)	197 (51.2)	0.19
9–11	123 (31.9)	110 (28.6)	
12–15	91 (23.6)	78 (20.3)	
Sex of children			
Male	211 (54.7)	201 (52.2)	0.50
Female	175 (45.3)	184 (47.8)	
Wealth Index			
Poor	255 (66.1)	229 (59.5)	0.55
Better	131 (33.9)	156 (40.5)	
Household hunger			
No household hunger	77 (19.9)	140 (36.4)	0.10
Mild household hunger	46 (11.9)	27 (7.0)	
Moderate household hunger	125 (32.4)	84 (21.8)	
Severe household hunger	138 (35.8)	134 (34.8)	

P is significant at < 0.05

Pulse consumption, dietary diversity, meal frequency, and nutrient intake

Haricot bean was the most commonly used pulse crop in the study area. Mothers who reported using pulses once or more than once per day in complementary food increased from 11.1% at baseline to 80.1% at end point in the intervention group and from 15.3% at baseline to 58.6% at end point in the control group. At baseline, there was no significant difference between the intervention and control groups in children's consumption of pulses ($p = 0.47$). At midpoint and end point, there was a significant difference in consumption of pulses between the control and intervention groups ($p = 0.001$). Figure 3 shows the frequency of pulse consumption by young children in both the intervention and control groups.

Mean dietary diversity at baseline was similar for the intervention children (2.1 ± 1.0) and controls (2.2 ± 0.8); at midpoint, values were 2.5 ± 0.8 and 2.2 ± 0.9, respectively and, at end point, 3.7 ± 1.4 and 3.2 ± 1.4, respectively. At baseline, only 23 (7.5%) from the intervention and 12 (3.8%) from the control group had consumed food from four or more food groups in the last 24 h prior to data collection. At midpoint, 27 (8.8%) from the intervention and 26 (8.3%) from the control group had consumed food from four or more food groups in the same period. At end point, 158 (51.5%) from the intervention and 136 (43.3%) from the control had consumed food from four or more food groups. There was a statistically significant difference between baseline and midpoint diet diversity ($p = 0.001$) and between midpoint and end point ($p = 0.001$) between and within groups, which indicated change overtime in both groups. Table 3 summarizes the food groups consumed by young children in the 24 h prior to data collection at baseline, midpoint, and end point.

Nutritional status of children

Anthropometric measurements were measured in the study children at baseline, midpoint, and end point. At baseline, there was no significant difference between the intervention and control groups in all anthropometry measurements and anthropometry indices. At end point, the mean age of male children was 18.8 ± 2.9 mo and female children was18.7 ± 2.9 mo. After intervention there was a significant difference ($p < 0.05$) in all anthropometric indices in the intervention group compared to the control group, as well as differences over time (Table 4). At baseline, low height and weight measurements were reflected in the high prevalence of stunting, wasting, and underweight. At midpoint and end point measurements, there were increases in the prevalence of stunting in both intervention and control groups; however, wasting and underweight improved only in the intervention group.

Differences between the intervention and control groups were largely seen in all three measures: stunting, wasting, and underweight during the course of the study. At end point, the prevalence of stunting increased more in the control group than in the intervention group, and the difference was significant ($p = 0.02$). At baseline, 14.4% in the intervention group and 25.8% in the control

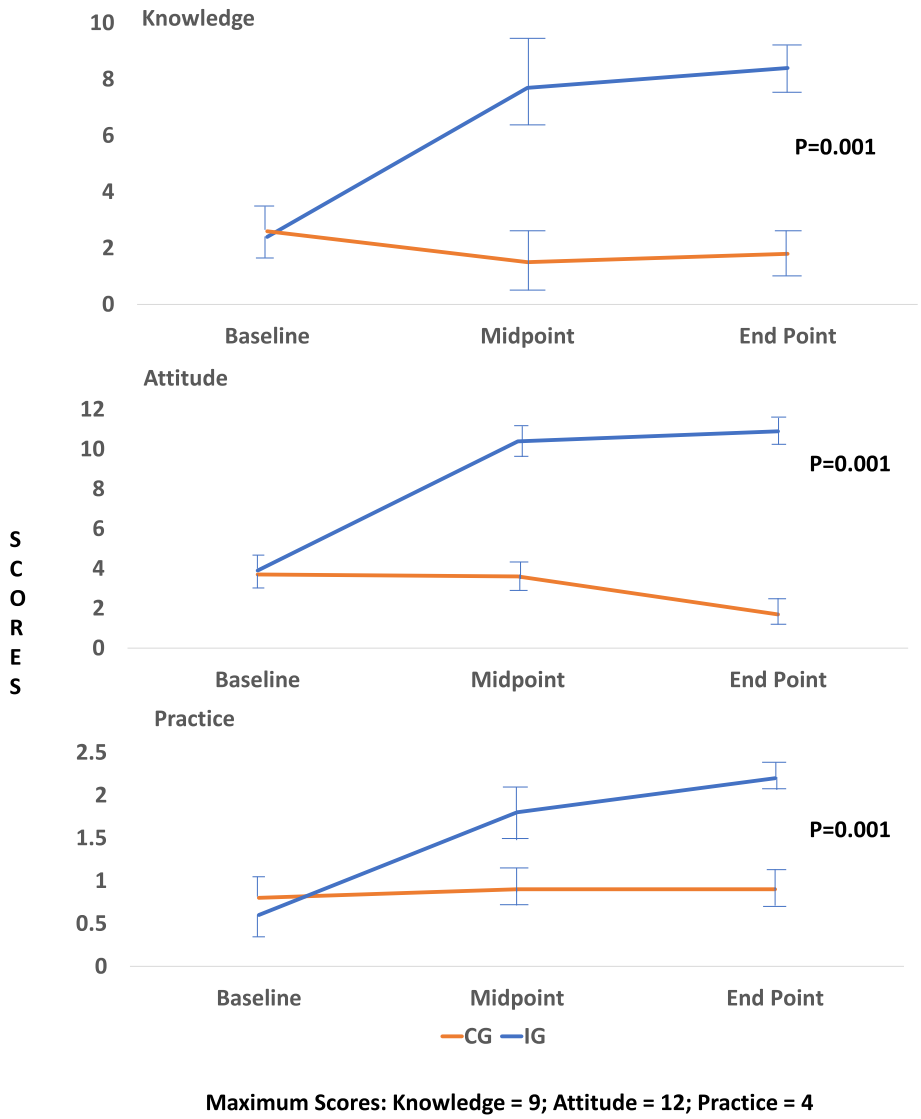

Fig. 2 Mean Knowledge, Attitude, and Practice of Mothers about Pulse-Incorporated Complementary Foods, Sidama Zone, 2016 ($N = 621$)

group were wasted, and prevalence did not change much at midpoint (15.3 and 28.1%, respectively); at end point, the prevalence of wasting had significantly increased ($p = 0.001$) in the control group (29.7%) over time, while the intervention group (11.8%) had declined from baseline, showing a significant positive effect of the intervention. At baseline, 32.9% of children in the intervention group and 34% of children in the control group were underweight; at midpoint only the control group showed a worsening (47.4% of children). Both groups had a decreased prevalence of underweight (11.7 and 29.3%, respectively) at end point, but more children were underweight in the control group ($p = 0.001$). These changes in the children in the two groups were reflected in MUAC measurements. At baseline, 35% of children from the intervention group and 37% of children from the control group had lower MUACZ-scores. Although the number of children who had lower MUACZ scores increased in both the intervention (40%) and control group (54%), at midpoint, more children in the control group had lower MUACZ-scores compared to the intervention group. At end point, the number of children with low MUACZ scores had decreased in both the intervention (28%) and control (38.6%) groups compared to midpoint. However, at end point the number of children who had low MUACZ-scores was greater in the control group than in the intervention group ($p = 0.001$) (Table 4).

Women's empowerment in household decision making

All participating mothers ($n = 386$) in the intervention group reported that they had planted the haricot bean

Table 2 Percentage of Selected Practice Variables of Intervention Group (IG) and Control Group (CG), Southern Ethiopia, 2016

Variables	Midpoint		X^2	End point		X^2
	N	%		N	%	
Prepare pulse incorporating CF						
IG	289	94	**0.001**	302	98.4	**0.001**
CG	211	67.2		213	67.7	
Soak and germinate pulse crops						
IG	132	43.0	**0.001**	414	69.7	**0.001**
CG	29	9.2		37	11.8	
Show 3 or more steps of household processing techniques						
IG	88	28.6	**0.001**	117	38	**0.001**
CG	1	0.3		3	0.9	
Mix cereal and pulse crops in the right proportions						
IG	5	1.6	**0.001**	146	47.6	**0.001**
CG	3	1.0		15	4.8	

Midpoint: 4.5 months; End point: 9.0 months. IG $n = 307$; CG $n = 314$

seed provided to them after the midpoint data collection. The seeds were later harvested following the end point data collection. Several visits to the field were carried out by the agriculture agents and the project team to a selected group of women; however, we did not collect information on how much they harvested, how much they kept for consumption, or how much they sold during end point data collection.

Discussion

The first 2 years of life are critical to reducing problems related to malnutrition [28]. The current study scaled-up this strategy by implementing a community-based nutrition education intervention entirely delivered through HEWs. The results of the study showed that mothers' KAP regarding pulse-incorporated complementary food was low at baseline in both the intervention and control groups. After 4 months of education (i.e., at midpoint), mothers in the intervention group improved KAP, which continued through the 9-month period. Almost all mothers in the intervention group had good knowledge about the benefits of pulses, household food processing techniques, and methods of preparation; they also started preparing complementary food using pulse crops. This current study findings are similar to other large intervention studies in other counties such as China [29, 30] and Kenya [31], which showed improved mothers' knowledge about feeding practices when health service providers were used to provide nutrition education.

Improvements in the intervention group were seen in many measures. Frequency of pulse consumption significantly increased in the intervention group as most mothers started using pulses as a complementary food more than once per day. Frequency of pulse consumption improved in the intervention group after midpoint. Changes in knowledge and attitudes observed at the midpoint of the study were similar to the endpoint. However, the practice change was more significant at the end of the study than at midpoint. This finding may be attributed to the seed provision, which has motivated mothers to practice rather than nutrition education alone. Furthermore, there was a significant difference in mean dietary diversity between the intervention and control groups. At the end of the intervention, consumption of pulses and nuts group improved significantly in the intervention group. Although the mean diet diversity in both groups improved at the midpoint and end point, the intervention group had a slightly higher mean diet diversity score than the control group. However, at end point, both groups were preparing foods from fewer food groups per day than recommended (i.e., less than four) [22]. Meal frequency in young children was significantly higher in the intervention group at both midpoint and end point. This study results were similar to those of a Chinese study, which was designed to improve the feeding practices of young children and found improved diet diversity as well as meal frequency in the intervention group [30].

Stunting increased with age in both groups, but by end point, less children were stunted in the intervention group than in the control group, which could reflect the positive impact of the intervention. The study findings were consistent with those of similar studies in Peru and China. A Peruvian study found a significant difference in stunting between the intervention and control groups and a reduced rate of stunting in the intervention group at the end of the study [32]. A Chinese study found a marginal significant difference in stunting between treatment and comparison groups [29]. Other similar studies have also observed a decreased rate of stunting and improvement in linear growth at the end of intervention, although these studies found no statistically significant difference between the treatment and comparison groups [33–36]. However, other studies have found neither improvement nor significant differences in stunting between the treatment and comparison group [14, 31]. The range of the nutrition education period in most studies ranged from six to 18 months, a short time to find a significant change in stunting. In the current study, the prevalence of stunting increased with age, consistent with previous studies [33–36]. This increase in stunting with age could occur because of factors such as infectious disease, poor maternal malnutrition during pregnancy, poor hygiene, and poverty [31], all of which limit the impact of an intervention over a relatively short period. In the same way, in the current study, underweight also increased with age in both intervention and

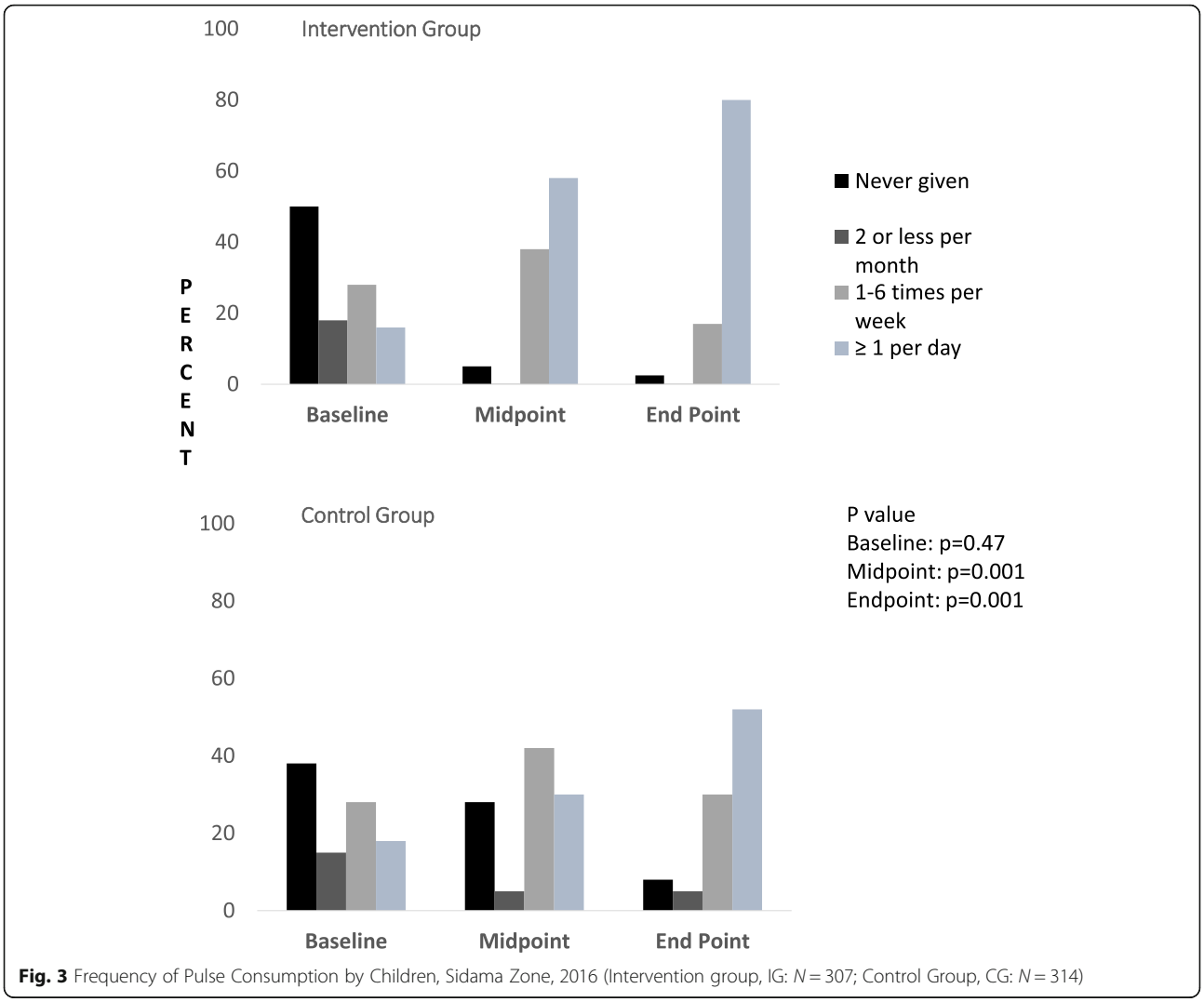

Fig. 3 Frequency of Pulse Consumption by Children, Sidama Zone, 2016 (Intervention group, IG: *N* = 307; Control Group, CG: *N* = 314)

Table 3 Food Groups of Young Children in the last 24 Hours Prior to Data Collection Date at Baseline, Midpoint and End point, Southern Ethiopia, 2016

Food group	Baseline N (%)		Midpoint N (%)		End point N (%)	
	IG	CG	IG	CG	IG	CG
	Yes	Yes	Yes	Yes	Yes	Yes
Cereal & root crops	236 (76.9)	273 (86.9)	281 (91.5)	281 (89.5)	304 (99)	312 (99.4)
Pulse & nuts	96 (31.3)	121 (38.5)	189 (61.1)	135 (43)	264 (86)	81 (25.8)
Milk & milk group	27 (8.8)	12 (3.8)	31 (10.1)	10 (3.2)	134 (43.6)	128 (40.8)
Meat & organ meat	0	0	0	0	4 (1.3)	6 (1.9)
Egg	27 (8.8)	12 (3.8)	42 (13.7)	64 (20.4)	147 (47.9)	168 (53.5)
Vitamin A rich fruits and vegetables	0	0	105 (34.2)	74 (23.6)	192 (62.5)	198 (63.1)
Other fruits and vegetables	265 (86.3)	283 (90.1)	116 (37.8)	133 (42.4)	106 (34.5)	99 (31.5)
Mean Diet Diversity Score	**2.1 ± 1.0**	**2.2 ± 0.8**	**2.5 ± 0.8**	**2.2 ± 0.9**	**3.7 ± 1.4**	**3.2 ± 1.4**

IG Intervention Group (*n* = 307), *CG* Control Group (*n* = 314)

Table 4 Anthropometric Status of Children at Baseline, Midpoint and End point at Sidama Zone, Southern Ethiopia, 2016

Anthropometry measurements	Mean (SD) (***N*** = 771)	Mean (SD) (***N*** = 621)	
	Baseline	Midpoint	End point
Weight (kg)			
Intervention	8.3 (±1.3)[a]	9.3[b,x]	10.2[c,x]
Control	7.3 (±1.2)[a]	8.7[b,y]	9.4[c,y]
Length (cm)			
Intervention	69.3 (± 4.7)[a]	74.3[b,x]	78.1[c,x]
Control	68.9 (±1.1)	73.6[b,x]	77.1[c,y]
MUAC (cm)			
Intervention	13. 6 (±1.1)[a]	13.8[b,x]	14.4[c,x]
Control	13.6 (±1.0)[a]	13.4[b,y]	13.9[c,y]
Anthropometric indices			
Wasting (weight-for-length z score)			
Intervention	−0.44 (±1.2)[a]	0.06[b,x]	0.28[c,x]
Control	−0.73 (±1.1)[a]	−0.5[b,y]	−0.43[c,y]
Stunting (length-for-age z score)			
Intervention	−1.05 (±1.4)[a]	−1.2[b,x]	−1.5[c,x]
Control	−1.10 (±1.2)[a]	1.3[b,x]	−1.7[c,y]
Underweight (weight-for-age z score)			
Intervention	0.23 (±1.2)[a]	−0.5[b,x]	− 0.5[c,x]
Control	−0.13 (±1.1)[a]	−1.3[b,y]	− 1.2[c,y]
MUAC z score			
Intervention	−0.68 (±1.0)[a]	−0.7[b,x]	− 0.4[c,x]
Control	−0.61 (±1.0)[a]	−1.0[b,y]	− 0.7[c,y]

SD Standard Deviation; Values in rows are significantly different ($p < 0.05$) from the baseline if they have the letter "b" or "c"; values with the letter "c" are significantly different from values having "b." Values for intervention and control in the midpoint and end point columns are significantly different if they have the letter "x" or "y"; values with the letter "x" are significantly different from values of "y" ($p < 0.05$). MUAC: mid-upper arm circumference. IG = Intervention Group ($n = 307$); *CG* Control group ($n = 314$)

control groups. However, the prevalence of wasting decreased over time in the intervention group, whereas the number of children in the control group who were wasted increased at the end point.

In our study, we found household processing practices such as germination to be difficult tasks for mothers to adopt. The number of mothers who were using soaked and germinated pulse crops for complementary food was low. Only 43 and 69.7% of mothers in the intervention group reported soaking and germination pulses at midpoint and end point, respectively. However, the results showed that more mothers performed household processing techniques in the intervention group than in the control group. The number of mothers who adopted practices for soaking and germination of pulses in this study was higher than that in a similar study in Malawi, where only 25% of participants adopted the practices from the message [37]. This difference could be due to the length of intervention, which was shorter than this current intervention. In general, the effect of nutrition education on mothers' knowledge and practice was high at both the midpoint and end point of this study, but, attitude did not change after the midpoint, possibly indicating that an intervention of approximately 5 months could be sufficient to change mothers' attitude.

The strength of this study was the involvement of trained local HEWs who can speak the local language in delivering the nutrition messages. The intervention contributed to women's empowerment by using HEWs and training mothers in their own communities. A peer mentoring approach can have positive impacts on the knowledge, confidence, and attitudes of participants [38]. We have described training the HEWs elsewhere [39]. In addition, multiple educational theories, namely Theory of Planned Behavior (TPB) and Health Belief Model (HBM) were used in designing the messages. TPB helped to identify factors that may influence pulse consumption in feeding practices through a combination of attitudes towards consumption of pulses, household processing techniques, cultural influences, and behavioral control that results in the formation of an intention [18]. HBM helped in addressing the problem behaviour, in this case the risk of inappropriate feeding practice and low consumption of pulses leading to undernutrition [17]. The limitations of the study included the potential for information contamination between intervention and control sites. Another limitation of the study was not assessing whether there were differences in the motivation of the HEWs between treatment and control kebeles, which could affect the outcome of the study. The distribution of seed after midpoint was an unexpected addition to the study; however, harvesting of these planted seeds had not occurred before end point, and thus this additional variable did not impact the results at 9 months.

Conclusions

This study found that nutrition education delivered through HEWs was successful in improving young children's consumption of pulses and their nutritional status. The pulse nutrition education given to mothers by HEWs for 9-month helped in improving the maternal KAP and frequency of pulse consumption as well as the nutritional status of their children. The study showed that training local HEWs is an effective way of promoting locally available nutritious foods and improving the health of local communities, particularly in low-income countries. Specifically, we showed soaking and germination and to incorporating germinated pulse product into complementary food were skills that needed to be effectively taught. The HEW's network was important to

bring changes in maternal KAP. It is necessary to strength the training of HEWs on germination particularly as the skills were not easily adopted in the communities. It is also showed that scaled-up the messages using established health education networks is possible at the national level. Finally, we recommend a future study on cost-benefit analysis of enhanced nutrition education that integrates pulse use into the HEWs information package and training.

Supplementary information

Supplementary information accompanies this paper at https://doi.org/10.1186/s12889-020-09262-8.

Additional file 1: Table S1. Lesson Plan for Mothers' Pulse Education. This is a content of nutrition education module covered during the intervention to teach mothers in the intervention group.

Abbreviations

ANOVA: Analysis of variance; CIFSRF: Canadian International Food Security Research Fund; CSA: Central Statistics Agency; DDS: Dietary Diversity Score; FANTA: Food and Nutrition Technical Assistance; HBM: Health Belief Model; HEW: Health Extension Worker; HHS: Household Hunger Scale; KAP: Knowledge, Attitude, Practice; MUAC: Mid-Upper Arm Circumference; NNP: National Nutrition Program; SNNPR: Southern Nations and Nationalities People's Region; TPB: Theory of Planned Behavior; TOT: Training of Trainers; WHO: World Health Organization

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Authors' contributions

GBT, SJW, TJG and CJH participated in the conception, design, and analyses of the study. Acquisition of data was by GBT and DM. The first draft of the work was by GBT. All authors revised the manuscript critically for important intellectual content. All authors reviewed the manuscript and approved the final version to be published.

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Availability of data and materials

The dataset generated and/or analyzed during the current study available from the corresponding author on reasonable request.

Ethics approval and consent to participate

The study was approved by University of Saskatchewan, Biomedical Research Ethics board (ID Number 15–272) and Hawassa University, Institutional Review Board approved the study (Ref. No: IRB/055/08). There were no invasive procedures and no private information were collected that could affect their privacy and harm the study participants. Thus, informed verbal consent was obtained from the mothers before data collection and intervention. The use of verbal consent approved by Institutional Review Board of Hawassa University as well as Biomedical Research Ethics Board of University of Saskatchewan. All study participants consented to participate in the study and the study was approved by the ethical review committee of both institutions.

Consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interest.

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Publisher's Note

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"The association of dietary patterns and adherence to WHO healthy diet with Metabolic Syndrome in children and adolescents: Tehran Lipid and Glucose Study"
Original Submission

Trias Mahmudiono, S.KM, MPH, Ph.D (Reviewer 2)

Reviewer Recommendation Term:	Accept after minor essential revisions
Transfer Authorization	**Response**
If this submission is transferred to another publication, do we have your consent to include your identifying information?	Yes
If this submission is transferred to another publication, do we have your consent to include your original review?	Yes
If this submission is transferred to another publication with Open or Transparent Peer Review, do we have your consent to publish your original review in a pre-publication history?	Yes
Custom Review Question(s):	**Response**
Are the methods appropriate and well described? If not, please specify what is required in your comments to the authors.	No
Does the work include the necessary controls? If not, please specify which controls are required in your comments to the authors.	No
Are the conclusions drawn adequately supported by the data shown? If not, please explain in your comments to the authors.	Yes
Are you able to assess any statistics in the manuscript or would you recommend an additional statistical review? If an additional statistical review is recommended, please specify what aspects require further assessment in your comments to the editors.	I am able to assess the statistics
Quality of written English Please indicate the quality of language in the manuscript:	Acceptable
Declaration of competing interests Please complete a declaration of competing interests, considering the following questions: 1. Have you in the past five years received reimbursements, fees, funding, or salary from an organisation that may in any way gain or lose financially from the publication of this manuscript, either now or in the future? 2. Do you hold any stocks or shares in an organisation that may in any way gain or lose financially from the publication of this manuscript, either now or in the future? 3. Do you hold or are you currently applying for any patents relating to the content of the manuscript?	I declare that I have no competing interests

4. Have you received reimbursements, fees, funding, or salary from an organization that holds or has applied for patents relating to the content of the manuscript? 5. Do you have any other financial competing interests? 6. Do you have any non-financial competing interests in relation to this paper? If you can answer no to all of the above, write 'I declare that I have no competing interests' below. If your reply is yes to any, please give details below.	
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Comments to Editor:

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This box is for confidential comments to the editors only.
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Examples of points which might be included here and raised as confidential comments to the editors would be ethical concerns regarding any experiments, concerns regarding an undisclosed conflict of interest or concerns regarding plagiarism or publication ethics.

Comments to Author:

In the abstract in the methods, I thing it would be better if the authors describe explicitly the design of the study. For example, "This was cross sectional study involving 424 subjects….". If it was analysis of secondary data from previous survey it should be stated
In the background in page 3 line 29, what does it mean by "the optimal dietary pattern for reducing Mets?". Is it the existing dietary guidelines? Or whether the existing dietary guidelines were failing hence the Mets increased?
In the background page 3 line 30-31 perhaps it needs a bridge sentences from talking about the guidelines that is not "optimal" for reducing Mets to the rise of dietary saturated fatty acids (SFA).
In the background if the authors could add more insight on the shift in the diet of Iranian children and adolescent related to the fat sugar and salt intake would make more stronger case for the study.
In the method section page 4 line 43-44 the authors stated that multistage cluster sampling was used. Please explain it further and perhaps a flow diagram of the sampling technique would made easier for the reader to understand it.
Please describe who performed the SQ FFQ, whether they were hospital staff or trained research assistant.
Also for each of the Mets measurement should be described in details who made the measurement or diagnosis, is it from the clinician in the hospital, or trained personnel?
In page 4 line 50 to 51, since the age group are different between subject, why would the author choose to use BMI for anthropometric measurement of nutritional status, as usually BMI only applicable to adults (>18 year old)? Please add justification for these. If not, perhaps clustered analysis of the nutritional status based on age group such as BMI for adults and BMIZ for adolescents. Or since the age were 6-19 using BMIZ might give more accurate depiction of nutritional status
In Table 3 the unadjusted MUFA was significant for the 3rd quartile dietary intake to Mets, and in the adjusted model it was not statistically significant. It could be easier to reader to put a bracket of the significance and also narrated this finding in the results and discuss it to what is the possible moderator for this association between the unadjusted and adjusted model.
In Table 5, the unadjusted unhealthy food was not significant for the 4rd quartile dietary intake to Mets, and in the adjusted model it was statistically significant. It could be easier to reader to put a bracket of the significance and also narrated this finding in the results and discuss it to what is the possible adjustment that resulted in this observed significant finding?
In the results I think it would be better if the author showed the scree plot of the FA results.
In the discussion section, did the authors think that the observed association of SFA to Mets in the Q2 and Q3 but nit in Q1 and Q4 was just a statistical artefact or it could means something? What was the known mechanism of SFA to be protective of Mets?

Why did lost to follow up was not seen as part of the limitation of the study? If not perhaps the author could explain the percentage and distribution of those lost to follow up. Whether its was at random or not.

Close

Author's Response To Reviewer Comments

Close

Editor Comments:

Please can you address the comments of reviewer 2. Thank you

Agreed and corrected. Figure 2, pages 6, 18 and 28.

Trias Mahmudiono, S.KM, MPH, Ph.D (Reviewer 2): Thank you for accepting my suggestion and explaining some of the argument made in your manuscript. However, I still think that making a kind of flow diagram to the sampling procedures as well as making it over follow up will be helping the reader to grasp the sampling technique and lost to follow up.

Agreed and corrected. Figure 2, pages 18 and 28.

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BMC Public Health

RESEARCH ARTICLE Open Access

The association of dietary patterns and adherence to WHO healthy diet with metabolic syndrome in children and adolescents: Tehran lipid and glucose study

Parvin Mirmiran[1*], Maryam Ziadlou[1], Sara Karimi[1], Firoozeh Hosseini-Esfahani[1*] and Fereidoun Azizi[2]

Abstract

Background: The optimal dietary pattern for reducing the extent of metabolic syndrome (MetS) has not been well established yet. The aim of this study was to evaluate dietary patterns and adherence to WHO healthy diet in children and adolescents and their associations with MetS.

Methods: Subjects of this cohort study were selected from among children and adolescents of the Tehran Lipid and Glucose Study participants, aged 6–18 years ($n = 424$). Dietary measurements were collected using a valid and reliable food frequency questionnaire. MetS was defined as the existence of at least 3 risk factors according to the Cook criteria. Diet was assessed based on dietary components of the WHO healthy diet. Dietary patterns were defined by principal component analysis.

Results: The mean ± SD age of participants (42% boys and 57% girls) was 13.5 ± 3.7 years. The most consistency with the WHO healthy diet was observed for cholesterol, free sugar and protein consumption in both genders, and the least was for n-3 poly-unsaturated fatty acid, trans-fatty acid and salt. Intake of SFA up to 12% of energy intake (third quartile) reduced the risk of MetS, compared to the first quartile. Subjects in the third quartile of n-6 poly-unsaturated fatty acid intake (6.2% of energy) showed the lowest odds ratio of MetS compared to the first quartile (OR: 0.18, CI: 0.04–0.66). In the adjusted model, the risk of MetS reduced across quartiles of MUFA intake by 60% (OR: 1, 0.40, 0.40, 0.42; P trend = 0.05). No significant trends were observed in the risk of MetS components across quartiles of the WHO healthy diet components. Three major dietary patterns were identified, the healthy, unhealthy and cereal/meat. An increased risk of MetS was observed in the highest quartile of unhealthy dietary pattern score compared to the lowest quartile (OR: 1, 0.81, 0.93, 2.49; P trend = 0.03).

Conclusions: Our results demonstrated that the majority of our population did not meet some components of WHO healthy diet recommendations. The quality and quantity of fatty acid intakes were associated with risk of MetS. Adherence to unhealthy dietary pattern was associated with two-fold increase in MetS risk.

Keywords: Dietary pattern, Healthy diet, World health organization, Metabolic syndrome

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Background

A collection of multifactorial metabolic disorders, including abdominal obesity, dyslipidemia, hypertension and hyperglycemia is called Metabolic Syndrome (MetS) [1]. The prevalence of MetS is increasing in children and adolescents of many countries, including Iran [2, 3]. MetS has been associated with a higher risk of cardiovascular disease and type 2 diabetes in adulthood, thus, becoming a major public health concern worldwide [4]. Diet plays a very important role in the growth and development of adolescents, during which the formation of healthy eating habits is important [5]. Unhealthy dietary patterns and lifestyle habits established during childhood and adolescence are significantly tracked into adulthood [6]. Dietary pattern is one of the risk factors reported to have a significant association with MetS and its related metabolic disorders [7]; however, the optimal local dietary guidelines for reducing MetS has not been well established yet [3]. Also dietary habits are largely influenced by nutrition transition which taking place in Iran in the context of demographic and socioeconomic changes. People are now consuming more foods high in energy, fats, free sugars or salt/sodium, and many do not eat enough dietary fibers including fruit, vegetable and whole grains [8]. Dietary saturated fatty acids (SFA) were positively associated with the prevalence of MetS in Iranian adults, independent of total dietary fat, mono-unsaturated fatty acid (MUFA) and poly-unsaturated fatty acid (PUFA) intakes [9]. Energy-dense nutrient-poor snacks, both salty and sweet, had undesirable effects on the incidence of MetS in Iranian children and adolescents [7, 10]. The World Health Organization (WHO) has issued dietary recommendations and guidelines on nutrition to help reduce the risk of chronic diseases and promote good health [11, 12], one of which is the Healthy Diet fact sheet [8] that includes practical advices on maintaining a healthy diet; recommending a shift in fat consumption away from SFAs to un-saturated fats and limiting total fat to less than 30% of total energy intake. Also this factsheet indicates elimination of free sugars to less than 10% of total energy intake and industrial trans-fats. A further reduction of free sugars to less than 5% of total energy intake is suggested for additional health benefits; moreover, reducing salt intake to less than 5 g per day is recommended for preventing hypertension and reducing the risk of heart disease and stroke in the adult population [8]. To our knowledge, limited data are available on the association of WHO healthy diet with metabolic abnormalities [7, 10]. In this study, we aimed to investigate the association of dietary patterns and adherence to WHO healthy diet and their association with MetS in Iranian children and adolescents of 6–18 years during 3.6 years of follow up.

Methods

Subjects

This population-based cohort study was done in the context of the Tehran Lipid and Glucose Study (TLGS), on residents of district no.13 of Tehran, the capital of Iran. These subjects, aged ≥3 years, were chosen by multistage cluster sampling, whose age and socio-economic status are illustrative of Tehran's overall population [13]. This study aimed to determine the prevalence of non-communicable disease risk factors. The first survey was initiated in 1999–2001, and the second (2002–2005), the third (2006–2008), and the fourth (2009–2011) were follow-up surveys.

Of total subjects who entered in the third survey (n = 12,523), 3462 subjects were chosen randomly for dietary assessment; including 621 children and adolescents aged 6–18 years. Participants were followed for a mean of 3.6 years at the fourth examination survey to evaluate the development of MetS and its components. Subjects who were lost to follow up (n = 123) (response rate: 80%) and those with energy intake < 800 or ≥ 4200 kcal/day (n = 6) were excluded. Those who had MetS (n = 68), hypertension (n = 52), high triglycerides (TGs) (n = 145), low high density lipoprotein cholesterol (HDL-C) (n = 202), high fasting blood sugar (FBS) (n = 20), or abdominal obesity (n = 141) at baseline were also excluded for individual analysis of MetS and its components' incidence. The final sample sizes varied by outcomes as follows: MetS (n = 424), abdominal obesity (n = 351), high TGs (n = 347), hypertension (n = 440), low HDL-C (n = 290), and high FBS (n = 472) (Fig. 1).

Measurements

Weight was measured, using digital scales, and recorded to the nearest 100 g (Seca 707; Seca Corporation, Hanover, Maryland; range, 0.1–150) kg while participants were minimally clothed without shoes. Height was measured to the nearest o.1 cm using a tape meter (model 208 Portable Body Meter Measuring Device; Seca) with subjects in standing position and without shoes. Body mass index (BMI) was calculated as weight (kg) divided by square of height (m^2).

Waist circumference (WC) was recorded to the nearest 0.1 cm, using a measuring tape at the umbilicus and without any pressure on body surface. The anthropometric measurement were carried out by one examiner for women and one for men to avoid subjective errors.

Before measuring blood pressure (BP), the participants remained seated for 15 min, and then using a standard mercury sphygmomanometer with the cuff placed on the right arm, blood pressure was measured twice by a clinician and mean values were documented [13].

To measure blood glucose and lipid levels including TGs and HDL-C, blood samples, were obtained by a trained staff, from all subjects at baseline and follow-up,

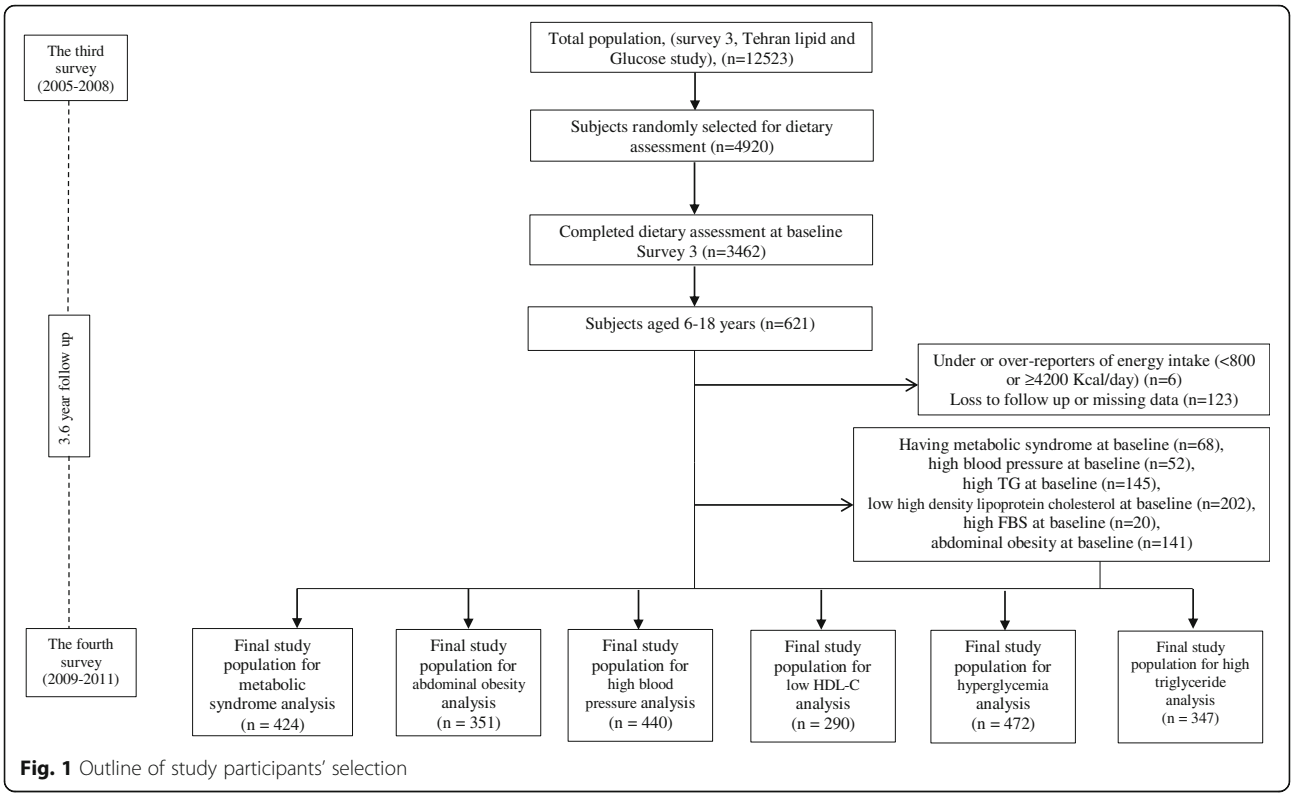

Fig. 1 Outline of study participants' selection

between 7 and 9 am after 12–14 h of fasting. All tests were done at the TLGS research laboratory on the day of blood collection. The enzymatic colorimetric method was used for FBS measurement. Serum concentration of TGs was estimated using commercially available enzymatic reagent (Pars Azmoon, Tehran, Iran) adapted to the Selectra auto analyzer. HDL-C was estimated after precipitation of the apolipoprotein B with a solution of phosphotungstic acid. Inter- and intra assay coefficients of variations were both 2.2% for FBS, 2.0 and 0.5% for HDL-C and 1.6 and 0.6% for TGs, respectively [2].

Dietary assessment

Dietary intakes were collected by trained research nutritionist, using a valid and reliable 168-items semi-quantitative food frequency questionnaire (FFQ) [14, 15]. Participants were asked (face to face), to report their consumption frequency during the previous year; when children were unable to recall, their mothers were asked about the type and quantity of meals and snacks. Portion sizes of food intakes, reported in household measures, were converted to daily intakes (gr/day). As the Iranian food composition table (FCT) is incomplete, the US Department of Agriculture FCT was used for analyzing energy and nutrient contents; for traditional foods not listed in the US Department of Agriculture FCT, the Iranian FCT was used [16, 17].

Physical activity assessment

Information on physical activity was collected using the modifiable activity questionnaire (MAQ); high reliability (97%) and moderate validity (49%) have been ascertained previously for the Persian translated MAQ in adolescents. Metabolic equivalent (MET) task (minutes per week) was calculated [18, 19].

Definitions

Metabolic syndrome (MetS)

In children and adolescents, Cook's criteria was used for definition of MetS, proposed as having ≥3 of the following [20]: fasting TGs ≥110 mg/dl; HDL-C < 40 mg/dl; WC ≥ 90th percentile for age and sex, based on the national reference curves [21]; systolic BP and diastolic BP ≥ 90th percentile for sex, age and height, according to cut points of the National Heart, Lung and Blood Institute [22], and FPG ≥ 100 mg/dl, based on the recommendations of American Diabetes Association.

Nutrient intake goals of the WHO healthy diet

The components of the WHO healthy diet include taking cholesterol < 300 mg per day, total fat less than 30%, SFAs less than 10%, and trans-fatty acids (TFAs) less than 1% of total energy intake, and replacing them with unsaturated fats including PUFAs (6–10% of total energy intake), n-6 PUFA (5–8% of total energy intake) and n-3 PUFA (1–2% of total energy intake); also, eating at least

400 g or 5 portions of fruits and vegetables per day to prevent non-communicable diseases and help ensure an adequate intake of dietary fiber (> 25 g). Consuming protein 10–15%, carbohydrates 55–75% and free sugars less than 10% of total energy and salt less than 5 g are other parts of WHO healthy diet [8]. Participants who met the component of the WHO healthy diet recommendations are complier of that component.

Statistical analyses

For analyzing data, the SPSS V.20 (SPSS Inc., Chicago, Illinois) was used. Continues variables were reported as mean ± SD and the categorical as percentages. A comparison of qualitative and quantitative variables among MetS and non-MetS subjects was done using the student T and Chi-square tests, respectively. Dietary patterns were determined using factor analysis with varimax rotation, based on 21 food groups. The patterns were extracted based on the eigenvalues (> 1), scree plot and factor interpretability (Fig. 2). Logistic regression analysis was used to estimate the odds ratio (OR) of MetS according to quartiles of WHO healthy diet components and dietary pattern scores after applying an adjustment for baseline age, sex, energy intake, family history of diabetes (yes or no) and BMI. Physical activity level (continuous) did not change the results, so this variable was excluded from the model. *P* value for trend was determined by logistic regression models using the median of each quartile of dietary factors as a continuous variable.

Results

The mean ± SD age of participants (42% boys and 57% girls) was 13.5 ± 3.71 years. There was no significant difference in physical activity level between MetS and non-MetS subjects. Also WC and TG level of MetS individuals were higher than non-MetS subjects. HDL-C level was lower in MetS than non-MetS subjects (Table 1).

The cumulative incidence of MetS was 11.1% after 3.6 years of follow up.

Table 2 shows the percentage of children and adolescents complying with WHO Healthy Diet by sex. The highest compliance was for cholesterol (100%), free sugar (> 80%) and protein intake (> 75%) in both genders, and the least was observed for n-3 PUFAs (< 2%), TFAs (< 7%) and salt (< 16%), without a significant difference between boys and girls. In this population, the mean intake of total fat, SFAs and TFAs were higher than WHO recommendations; the most inconsistency was for TFAs which was received 2 fold higher than recommended. The mean intakes of fruits and vegetables were higher than WHO Healthy Diet recommendations, and the compliance of fruit and vegetable with WHO was > 60% in both genders.

The risk of MetS incidence according to quartiles of WHO healthy diet components is shown in Table 3**.** In the adjusted model, intake of SFA up to 12% of energy intake (third quartile) reduced the risk of MetS, compared to the first quartile. In the crude model, the risk of MetS reduced across quartiles (Q) of MUFA intake (ORs from Q1 to Q4: 1, 0.62, 0.39, 0.45; P trend = 0.03); in the

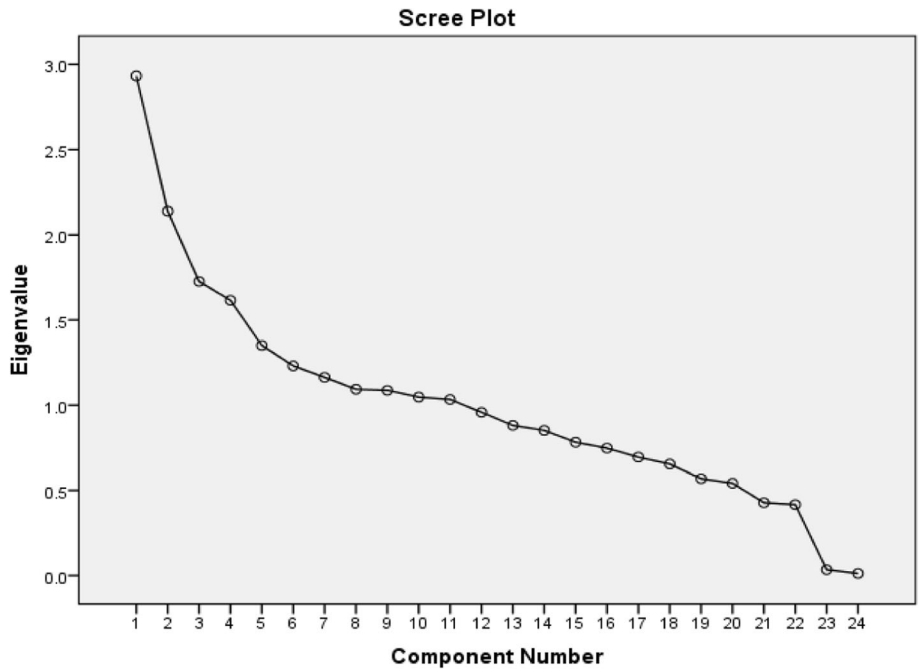

Fig. 2 The scree-plot of principal component analysis for extracting major dietary patterns

Table 1 Baseline characteristics of children and adolescents (6–19 years) participating in the Tehran Lipid and Glucose Study, 2005–8

	Total ($n = 424$)	Non-MetS subjects ($n = 376$)	MetS subjects ($n = 47$)	P value [†]
Age (year)	13.5 ± 3.71[*]	13.7 ± 3.7	12.7 ± 3.6	0.10
Family history of diabetes (%)	5.2	5.6	2.2	0.51
Waist circumference (cm)	68.8 ± 10.4	68.0 ± 10.1	75.5 ± 11.2	< 0.01
Systolic blood pressure (mmHg)	97.9 ± 11.9	97.6 ± 11.8	101 ± 10.2	0.07
Diastolic blood pressure (mmHg)	64.3 ± 10.0	64.5 ± 9.7	65.3 ± 9.5	0.64
Fasting plasma glucose (mg/dl)	84.9 ± 6.15	84.7 ± 6.12	87.2 ± 5.9	0.008
High density lipoprotein cholesterol (mg/dl)	45.8 ± 10.5	46.5 ± 10.8	40.7 ± 6.8	< 0.01
Triglycerides (mg/dl)	89.1 ± 42.2	79.0 ± 1.4	106 ± 1.5	< 0.01
Physical activity (Met/hour/week)	14.9 ± 14.7	15.1 ± 15.4	13.3 ± 7.8	0.41

[*] Values are reported as mean ± SD or percentages, using independent t-test for quantitative variables and chi square for qualitative variables
[†] *P* value: shown as difference between MetS and non-MetS subjects at baseline

adjusted model, the risk of MetS decreased by 60% (ORs from Q1 to Q4: 1, 0.40, 0.40, 0.42; P trend = 0.05). In both crude and adjusted models, subjects in the third quartile of n-6 PUFA intake (6.2% of energy) showed the lowest odds ratio of MetS compared to the first quartile (OR for Q3: 0.18, CI: 0.04–0.66). No significant trends were observed in the risk of MetS components across quartiles of the WHO healthy diet components.

Three major dietary patterns were identified; the healthy dietary pattern was highly loaded on vegetables, fruit and fruit juices, nuts, seeds, dairy and liquid oils; the unhealthy dietary pattern.

composed of fast foods, soft drinks, mayonnaise, salty snacks, tea, coffee, solid oils, sweets and sugar; the third dietary pattern, cereal/meat, was loaded densely on red and organ meat, fish and poultry, legumes, egg and refined grains (Table 4).

The odds ratios of developing MetS across quartiles of dietary pattern scores are presented in Table 5. There was no significant decreasing trend in the risk of MetS across quartiles of healthy and cereal/meat dietary patterns. A significant increased risk of MetS was observed in the highest quartile of unhealthy dietary pattern score compared to the lowest quartile after adjustment for confounding factors [OR: Q4:2.49 (CI:1.00–6.19); P trend = 0.03]; this association was not observed in the crude model. There was not any significant correlation between MetS components and three dietary pattern scores.

Table 2 Dietary intake and percentage of children and adolescents complying with WHO healthy diet in the Tehran Lipid and Glucose Study

Dietary intakes	Recommendation	Compliers[*] (%)		Intake (mean ± SD)		P[**]
		boys	girls	boys	girls	
Total fat (% of Energy)	15–30	42	36.6	32.1 ± 6.38	32.4 ± 7.39	0.71
SFA (% of Energy)	< 10	34.3	41.2	11.2 ± 3.06	11.0 ± 3.07	0.38
PUFAs (% of Energy)	6–10	51.9	50.6	6.52 ± 1.99	6.68 ± 2.40	0.47
n-6 PUFAs (% of Energy)	5–8	48.6	49	5.73 ± 1.88	5.83 ± 2.23	0.61
n-3 PUFAs (% of Energy)	1–2	1.1	1.2	0.49 ± 0.17	0.52 ± 0.32	0.34
Trans fatty acid (% of Energy)	< 1	6.1	5.8	2.26 ± 1.03	2.20 ± 1.18	0.54
Cholesterol (mg/day)	< 300	100	100	151 ± 26.4	154 ± 27.4	0.25
Total carbohydrate (% of Energy)	55–75	65.7	60.5	57.2 ± 6.58	56.8 ± 7.76	0.60
Fruits & vegetables (g/day)	≥400	64.6	67.1	606 ± 429	595 ± 361	0.75
Total dietary fiber (g/day)	> 25	46.4	42.4	27.5 ± 15.2	25.3 ± 13.1	0.10
Free sugars (% of Energy)	< 10	81.2	84.4	7.05 ± 3.93	6.84 ± 4.74	0.62
Protein (% of Energy)	10–15	78.5	76.1	12.9 ± 2.07	13.2 ± 2.37	0.16
Salt (g/day)	< 5	12.2	15.2	11.9 ± 7.69	12.2 ± 9.9	0.32

SFA: Saturated fatty acid, PUFA: Poly unsaturated fatty acids
[*]Participants who met each component of the WHO healthy diet recommendations are complier of that component
[**]*P* value: shown as difference of dietary intakes between genders using t-test

Table 3 Multivariable-adjusted odds ratio (95% CIs) for incident MetS according to quartiles of WHO healthy diet components among 424 children and adolescents

	Dietary intakes				
	Q1	Q2	Q3	Q4	P_{trend}
Total fat (% of Energy)	24.7	29.8	34.3	39.7	
Model 1	1.00	0.60 (0.26–1.36)	0.54 (0.23–1.25)	0.48 (0.20–1.14)	0.86
Model 2	1.00	0.51 (0.21–1.24)	0.51 (0.20–1.28)	0.40 (0.15–1.05)	0.06
SFA (% of Energy)	7.61	10.0	11.8	14.7	
Model 1	1.00	0.30(0.12–0.75)	0.21(0.07–0.59)	0.70(0.34–1.47)	0.34
Model 2	1.00	0.26(0.09–0.37)	0.18(0.06–0.55)	0.57(0.25–1.32)	0.16
PUFAs (% of Energy)	4.27	5.70	7.10	8.85	
Model 1	1.00	0.86(0.40–1.83)	0.20(0.06–0.63)	0.60(0.26–1.36)	0.06
Model 2	1.00	0.66(0.28–1.55)	0.23(0.07–0.78)	0.52(0.21–1.29)	0.08
n-6 PUFAs (% of Energy)	3.64	4.90	6.20	7.90	
Model 1	1.00	0.86(0.40–1.83)	0.15(0.04–0.53)	0.66(0.30–1.44)	0.09
Model 2	1.00	0.68(0.29–1.58)	0.18(0.04–0.66)	0.55(0.22–1.33)	0.09
n-3 PUFAs (% of Energy)	0.30	0.42	0.54	0.70	
Model 1	1.00	1.53(0.67–3.48)	1.00(0.41–2.41)	0.80(0.31–2.02)	0.42
Model 2	1.00	1.38(0.56–3.42)	0.97(0.37–2.56)	0.74(0.27–2.02)	0.42
MUFA (% of Energy)	8.20	10.2	12.0	13.8	
Model 1	1.00	0.62(0.28–1.37)	[0.39(0.16–0.96)]	0.45(0.19–1.06)	0.03
Model 2	1.00	0.40(0.16–0.98)	0.40(0.15–1.08)	0.42(0.17–1.05)	0.05
Trans fatty acid (% of Energy)	1.04	1.72	2.43	3.50	
Model 1	1.00	0.38(0.15–0.96)	0.75(0.34–1.65)	0.65(0.28–1.47)	0.57
Model 2	1.00	0.42(0.15–1.18)	0.75(0.30–1.85)	0.76(0.30–1.94)	0.89
Cholesterol (mg/day)	124	143	158	188	
Model 1	1.00	3.25(1.13–9.31)	2.88(0.99–8.41)	3.07(1.06–8.86)	0.10
Model 2	1.00	2.96(0.94–9.29)	2.42(0.76–7.72)	3.39(1.04–10.9)	0.08
Total carbohydrate (% of Energy)	49.0	55.4	59.2	64.7	
Model 1	1.00	0.55(0.20–1.46)	0.90(0.38–2.15)	1.49(0.67–3.30)	0.23
Model 2	1.00	0.63(0.22–1.83)	0.83(0.31–2.22)	1.66(0.69–4.01)	0.20
Free sugars (% of Energy)	3.00	5.14	7.22	10.9	
Model 1	1.00	0.49(0.20–1.22)	1.15(0.54–2.46)	0.42(0.16–1.09)	0.21
Model 2	1.00	0.39(0.13–1.11)	1.33(0.57–3.13)	0.39(0.14–1.08)	0.24
Protein (% of Energy)	10.7	12.2	13.5	15.4	
Model 1	1.00	1.00(0.38–2.62)	1.91(0.80–4.55)	1.50(0.61–3.69)	0.22

Table 3 Multivariable-adjusted odds ratio (95% CIs) for incident MetS according to quartiles of WHO healthy diet components among 424 children and adolescents *(Continued)*

	Dietary intakes				
	Q1	Q2	Q3	Q4	P_{trend}
Model 2	1.00	0.94(0.33–2.67)	1.67(0.65–4.28)	1.45(0.55–3.81)	0.31
Salt $_{(g/day)}$	4.91	7.70	11.83	20.1	
Model 1	1.00	0.74(0.3–1.78)	1.08(0.48–2.44)	0.74(0.31–1.78)	0.67
Model 2	1.00	0.71(0.27–1.89)	0.96(0.36–2.55)	1.21(0.43–3.38)	0.51
Fruits and Vegetables $_{(g/day)}$	236	420	624	999	
Model 1	1.00	1.20(0.51–2.83)	1.00(0.41–2.41)	1.10(0.46–2.62)	0.94
Model 2	1.00	1.10(0.43–2.84)	1.22(0.46–3.27)	1.39(0.46–4.13)	0.53
Total dietary fiber $_{(g/day)}$	6.94	9.04	10.9	14.4	
Model 1	1.00	1.86(0.74–4.65)	1.56(0.61–3.99)	1.71(0.67–4.32)	0.39
Model 2	1.00	1.52(0.57–4.01)	1.17(0.41–3.27)	1.70(0.63–4.58)	0.37

SFA: Saturated fatty acid, PUFA: Poly unsaturated fatty acids
Model 1: Crude
Model 2: Adjusted for baseline age, sex, total energy intake, family history of diabetes, and BMI

Table 4 Dietary patterns identified in children and adolescents participating in the Tehran Lipid and Glucose Study

Food groups	Dietary patterns		
	Healthy	Unhealthy	Cereal/meat
Fast foods		0.43	
Soft drinks		0.55	
Mayonnaise	0.27	0.53	
Salty snacks	0.29	0.43	
Tea/coffee		0.49	
Solid oils		0.26	
Sweets and Sugar		0.20	
Red/organ meats	0.25	0.23	0.47
Fish and Poultry			0.26
Legumes			0.45
Egg			0.32
Potato			0.44
Whole grains			0.43
Refined grains			0.20
Liquid oils	0.35		0.21
Vegetables	0.51		0.22
Fruits	0.68		
Fruit juice	0.55		
Nuts and seeds	0.34		
High fat dairy	0.31		
Low fat dairy	0.23		
Variance (%)	9.96	8.22	7.45

Values are factor loadings of dietary patterns measured by factor analysis. Factor loadings below ±0.2 are not shown in the table for simplicity, eigenvalues> 1, Kaiser-Meyer-Olkin (KMO): 0.53

Discussion

In our study, dietary patterns of Tehranian children and adolescents aged 6–19 years were identified and their dietary intakes were compared with WHO healthy diet recommendations. Also, the association of dietary adherence to WHO healthy diet components and dietary patterns with the risk of MetS and its components were assessed through 3.6 years of follow-up.

Compared with WHO recommendations, Iranian children and adolescents showed the highest adherence with cholesterol, free sugars, protein, total carbohydrate, fruit and vegetable, respectively; whereas, the lowest was observed for n-3 PUFAs, TFAs, SFAs and salt.

The mean dietary intake of total fat was higher than the upper limit of WHO healthy diet recommendation in both genders (32% vs 30% of energy); however, no correlation was seen between the risks of MetS across quartiles of total fat intake.

In our study population, n-3 PUFAs was consumed much lower than WHO recommendations. Previous studies showed that, dietary n-3 PUFA had a protective effect against MetS and low-grade inflammation among children and adolescents [23]; this was in contrast to our results, which seems to be due to the lower consumption of foods containing n-3 PUFA in the Iranian society.

Dietary SFAs up to 12% of energy showed a protective effect on MetS up to 80%; which disappeared in higher consumption of SFA. Although we could not find a study on children and adolescent regarding the correlation of SFA and Mets, but the present study was in line with Dehghan et al's study which reported an inverse association between SFA intake and the risk of stroke in adults [24]. Replacing carbohydrate by SFA is also associated with a decreased risk of stroke by 20% among the Asian population. Higher carbohydrate, especially from refined sources and lower fat consumption is more

Table 5 Multivariable-adjusted odds ratio (95% CIs) for incident MetS according to quartiles of dietary pattern scores among 424 children and adolescents

	Quartile of Dietary pattern score				
	Q1	Q2	Q3	Q4	P_{trend}*
Healthy (n)	106	106	106	106	
Model 1	1.00	0.37(0.14–0.93)	0.54(0.23–1.25)	0.73(0.33–1.59)	0.61
Model 2	1.00	0.36(0.13–0.99)	0.41(0.15–1.06)	0.70(0.28–1.72)	0.43
Unhealthy (n)	107	105	106	106	
Model 1	1.00	0.92(0.39–2.20)	0.64(0.25–1.65)	1.40(0.63–3.13)	0.42
Model 2	1.00	0.81(0.30–2.20)	0.93(0.33–2.57)	[2.49(1.00–6.19)]	0.03
Cereal/meat (n)	106	106	106	106	
Model 1	1.00	2.17(0.88–5.33)	1.71(0.67–4.32)	1.27(0.48–3.37)	0.88
Model 2	1.00	2.32(0.86–6.22)	1.54(0.56–4.28)	1.28(0.44–3.66)	0.91

Model 1: Crude
Model 2: Adjusted for baseline age, sex, total energy intake, family history of diabetes, and BMI
*Based on logistic regression model using median scores of dietary patterns in each quartile as a continuous variable

common in low and middle income countries which have been shown to increase the metabolic risk factors. Individuals with high carbohydrate intake might benefit from a reduction in carbohydrate intake and increase in the consumption of fats [24, 25]. Thus the effect of SFA on MetS may also be affected by the remaining components of dietary macronutrients [26, 27].

In the present study, there was no correlation between higher intake of TFA (2 fold higher than WHO recommendation) and the risk of MetS or its components among children and adolescents. There were no studies showing the correlation between TFA and Mets among children and adolescent. Our results are inconsistent with the recent study claiming that there was a positive correlation between TFA and MetS among US adults [28].

In the present study, total dietary fiber, fruits and vegetables were received more than WHO recommendations, and may be considered as a factor in suppressing the adverse effects of SFA and TFA rich foods. There are limited data about the association of fruit and vegetable consumption with Mets among children and adolescents. Based on a previous study on 131 Latino children, an inverse association with total dietary fiber, particularly soluble fiber and MetS was found [29]. One study showed that the frequency of vegetable consumption in childhood is inversely associated with MetS in adulthood [30].

Salt intake among Iranian children and adolescents was 2 fold higher than WHO recommendation; however, there was no correlation between salt intake and the risk of MetS or hypertension in our study population. There are limited studies that have directly assessed the relationship between salt intake and the risk of MetS among children; but, a recent Korean study of 1738 boys aged 10–18 years revealed that high sodium intake may be independently associated with MetS [31]. In addition, previous studies showed that sodium intake more than WHO recommendations is positively associated with systolic BP and risk of pre-high BP and high BP, and this association may be stronger among those who are overweight or obese [32, 33]. There is strong evidence on the association between salt intake and high BP in children, adolescents and adults; however, some studies among children did not find an association between salt intake and hypertension, which was in line with our findings. These studies hypothesized that salt intake in childhood might lead to high BP in adulthood and older ages [33, 34]. Nonetheless, such a high rate of children and adolescents consuming salt more than recommended is worrisome, and individuals should minimize their salt intake by reducing processed foods and salty snacks, thus, contributing to healthier dietary patterns.

The results of our study can be interpreted in several aspects, MUFA intake showed a negative correlation with the risk of MetS up to 10–12% of energy intake. This association was weaker after adjustment of confounding factors. Further analysis in age and sex subgroups is needed to find out its moderators; however, total dietary fat may modulate this association, which emphasize that both the quality and quantity of dietary fats are relevant with Mets [9].

High intake of MUFA may suppress the adverse effect of expression of inflammatory genes that induce insulin resistance and secretion of inflammatory cytokines due to the rich SFA and TFA rich foods [35, 36]. To confirm this hypothesis, previous studies reported that there was negative association between the Mediterranean diet, rich in MUFA, and MetS among children and adolescents [37, 38].

Furthermore, in our study cholesterol and free sugar intake had the highest consistency with WHO recommendations. A study on 151 white girls showed that, sweetened beverages were the only dietary component related to MetS [39]. Another study of 424 subjects aged 6–18 years showed that high intakes of carbonated beverages increased the risk of MetS [7]. A recent CASPIAN-V study on 3843 Iranian children and adolescents showed that sweet dietary pattern increased the risk of MetS and some components [40]. Also, in the Framingham Heart Study of 6842 metabolically healthy adults, individuals consuming sugar sweetened beverages were more likely to display metabolic abnormalities compared to those who did not [41]. Therefore, high consistency of free sugar intake (> 80%) with WHO recommendations may be considered as a reason for preventing the incidence of MetS, despite high intake of SFA and TFA.

Due to in vivo interaction of macronutrients on body health, dietary components alone cannot be a good indicator for evaluating the incidence of MetS. Focusing on dietary patterns and considering all aspects of an individual's dietary intake provides more precision to determine the risk of MetS among children and adolescents. The present study supports the positive correlation between unhealthy diet and MetS. Based on previous studies, the 'Western' dietary pattern was associated with a greater risk for MetS among children and adolescent [35, 42, 43]. Also there was no significant association between unhealthy dietary pattern and MetS in the crude model due to different relationships in age and sex groups or BMI and energy intake levels with MetS; after controlling these confounding variables, this association became significant.

Our study has its limitations; using an FFQ can estimate the usual intake of participants not actual intake, however, this FFQ can rank individuals accurately based on their intakes. The FFQ relies on recalling of food consumption, which is difficult in children; although, using experienced interviewers helps subjects or their parents to remind what they eat regarding the quality and quantity of food items, which decrease memory limitation. Lack of information on puberty stage and Carotid intima-media thickness (IMT) are other limitations of our study. It is evident that puberty stage can affect

MetS components. IMT helps in predicting childhood and adolescent MetS more precisely [44]. Also socio-economic status of the child/adolescent's family may play a role in the risk of MetS, which was not assessed in our study. Loss to follow up occurred in 20% of our study participants which may raise the selection bias. Moreover, lack of a significant relationship between WHO healthy diet components and MetS may be due to the limited number of MetS subjects in each quartile of these food or nutrient items.

Our study has major strengths too, to our knowledge, this is the first study assessing the adherence to nutritional intake based on WHO healthy diet, in a large nationally representative sample of Iranian children and adolescents aged 6–19 years; also, it has a prospective design with an appropriate follow-up duration for assessing the incidence of MetS. In addition, national cut-off points were used for assessing abdominal obesity in children and adolescents. Measurement of confounding factors is the other strength of our study.

Conclusion

Our results demonstrated that the majority of our study population did not meet WHO healthy diet recommendations on n-3 PUFAs, TFAs and salt intake; also, WHO healthy diet alone cannot predict the risk of MetS and its components in Tehranian children and adolescents. Adherence to unhealthy dietary pattern was associated with two-fold increase in MetS risk; thus, reducing the consumption of unhealthy food items including fast foods, sweetened beverages, salty snacks, sweets and high fat red meats may reduce the risk of MetS in children and adolescents.

Abbreviations

BMI: Body mass index; BP: Blood Pressure; FBS: Fasting blood sugar; FCT: Food composition table; FFQ: Food frequency questionnaire; HDL-C: High density lipoprotein cholesterol; IMT: Carotid intima-media thickness; MAQ: Modifiable activity questionnaire; MET: Metabolic equivalent; MetS: Metabolic syndrome; MUFA: Mono-unsaturated fatty acid; PUFA: Poly-unsaturated fatty acid; SFA: Saturated fatty acids; TFA: Trans-fatty acids; TG: Triglycerides; TLGS: Tehran Lipid and Glucose Study; WC: Waist circumference; WHO: World Health Organization

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Authors' contributions

Authors' contributions are as follows: P.M., F.H.E., S.K., and M.Z. designed the study, analyzed and interpreted the data, and drafted the manuscript. P.M. supervised the study, and F.A. critically revised the manuscript for important intellectual content and final approval of the version to be published. All authors have read and approved the manuscript.

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Availability of data and materials

The datasets generated and/or analyzed during the current study are not publicly available, but are available from the corresponding author on reasonable request.

Ethics approval and consent to participate

The study proposal was approved by the Research Council of the Research Institute for Endocrine Sciences, Shahid Beheshti University of Medical Sciences, and an informed written consent was obtained from the parents of each participant.

Consent for publication

"Not applicable".

Competing interests

The authors declare that they have no competing interests.

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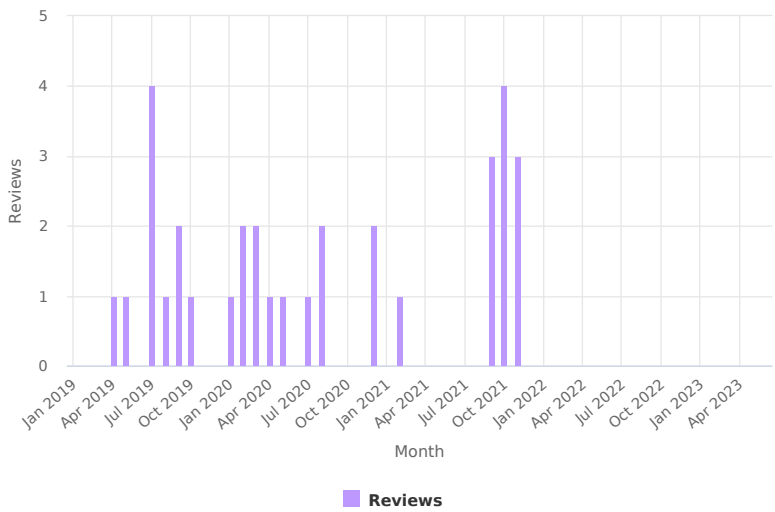

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(4) Nutrition Journal	(3) Asia Pacific Journal of Public Health
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(2) The American Journal of Clinical Nutrition	(1) Revista de Saude Publica

(1) Malawi Medical Journal

(1) Frontiers in Public Health

(1) Malaysian Journal of Nutrition

(1) Journal of Nutrition and Metabolism

(1) Nutrition and Metabolic Insights

(1) Journal of Nutritional Science

(1) Media Gizi Indonesia

33 REVIEWS OF 28 MANUSCRIPTS

For manuscripts published from date range December 2018 - June 2023

Naturally Acquired Lactic Acid Bacteria from Fermented Cassava Improves Nutrient and Anti-dysbiosis Activity of Soy Tempeh
Reviewed: Nov 2021 for Open Access Macedonian Journal of Medical Sciences

STUNTING IS THE DOMINANT FACTOR ASSOCIATED WITH IRON DEFICIENCY IN ELEMENTARY SCHOOL CHILDREN
Reviewed: Nov 2021 for Open Access Macedonian Journal of Medical Sciences

Double Burden of Malnutrition as a Risk Factor for Overweight and Obesity
Reviewed: Nov 2021 for Revista de Saude Publica

Household food security during the COVID-19 pandemic in urban and semi-urban areas in Indonesia
2 rounds from Oct 2021 to Oct 2021 for Journal of Health, Population and Nutrition

Socio-demographic characteristics of children and young adults with varied asthma control- does it make a difference?
Reviewed: Oct 2021 for Malawi Medical Journal

The Primary Prevention Intervention Of Stunting In Pasaman Barat Regency West Sumatera Indonesia 2019
Reviewed: Oct 2021 for Open Access Macedonian Journal of Medical Sciences

Body Fat Composition Related to Fatty Snack Habit Consumption of Office Workers in Urban Area
Reviewed: Sep 2021 for Open Access Macedonian Journal of Medical Sciences

An Analysis Of Baby Stunting Prevalence Causing Factors In The Pandemy Era Covid-19
Reviewed: Sep 2021 for Open Access Macedonian Journal of Medical Sciences

The Improving the performance of children meal using meal report at Kindergarten School in Indonesia
Reviewed: Sep 2021 for Open Access Macedonian Journal of Medical Sciences

Association Between Food Choices Motivators, Physical Activity in Body Image (Dis)satisfaction in Portuguese Adolescents
Reviewed: Feb 2021 for Frontiers in Public Health

Zikabra: A Networking Initiative for International Cooperation in Health
Reviewed: Dec 2020 for BMC Public Health

An initiative of cooperation in Zika virus research: the experience of the ZIKABRA study in Brazil
Reviewed: Dec 2020 for BMC Public Health

Energy, Protein, and Vitamin C Intakes Are Associated to Stunting Among Preschool Children in Central Jakarta, Indonesia: a Case-control Study
Reviewed: Aug 2020 for Malaysian Journal of Nutrition

Prevalence Stunting Among Preschool Children (1-5Years) in Bushenyi District Southwestern Uganda- a Health Center Based Study
Reviewed: Aug 2020 for Journal of Nutrition and Metabolism

Geographical, Socioeconomical and Behavioral Factors Related to Obesity Among Adults in the Republic of Palau Based on the WHO STEPwise Approach to Surveillance 2011–2013
Reviewed: Jul 2020 for Asia Pacific Journal of Public Health

Social circumstances and cultural beliefs influence maternal nutrition, breastfeeding and child feeding practices in South Africa
2 rounds from Mar 2020 to May 2020 for Nutrition Journal

Intra-individual Double Burden of Overweight Or Obesity and Micronutrient Deficiencies Or Anemia Among Women of Reproductive Age in 17 Population-based Surveys
2 rounds from Jan 2020 to Apr 2020 for The American Journal of Clinical Nutrition

Scaled-Up Nutrition Education on Pulse-Cereal Complementary Food in Ethiopia: A Cluster-Randomized Trial
Reviewed: Mar 2020 for BMC Public Health

Overfed-Undernourished: The Nutritional Dual Burden of Malnutrition
Reviewed: Feb 2020 for Nutrition and Metabolic Insights

The Effectiveness of Nutritional Educational Comics on Increasing Haemoglobin Levels in Anaemic Adolescent Girls
Reviewed: Feb 2020 for Public Health Nutrition

Nutrient Adequacy of Japanese Schoolchildren By Household Income: Comparison Between Days with and Without School Lunch
Reviewed: Oct 2019 for Asia Pacific Journal of Public Health

Video Animasi Dan Pengetahuan Gizi Remaja
Reviewed: Sep 2019 for Media Gizi Indonesia

The association of dietary patterns and adherence to WHO healthy diet with metabolic syndrome in children and adolescents: Tehran lipid and glucose study
2 rounds from Jul 2019 to Sep 2019 for BMC Public Health

Nutrient adequacy of Japanese schoolchildren on days with and without a school lunch by household income
Reviewed: Aug 2019 for Asia Pacific Journal of Public Health

Coping through a drought: the association between child nutritional status and household food insecurity in the district of iLembe, South Africa
Reviewed: Jul 2019 for Public Health Nutrition

Parent–child cooking meal together may alleviate parental concerns about the diets of their toddlers and preschoolers: a cross-sectional analysis in Japan
2 rounds from May 2019 to Jul 2019 for Nutrition Journal

The double burden of overnutrition and undernutrition in mother-child dyads in Kenya: demographic and health survey data, 2014
Reviewed: Jul 2019 for Journal of Nutritional Science

Coping Through a Drought: The Association Between Child Nutrition and Household Food Insecurity in the District of iLembe, South Africa
Reviewed: Apr 2019 for Public Health Nutrition