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## Is grip strength linked to body composition and cardiovascular risk markers in primary schoolchildren? Cross-sectional data from three African countries

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1 **Is grip strength linked to body composition and cardiovascular risk markers in primary**

2 **schoolchildren? Cross-sectional data from three African countries**

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4 *Brief running head:* Grip strength, body composition and cardiovascular risk

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31

32   **Abstract**

33   **Objectives:** Muscular strength represents a specific component of health-related fitness. Hand grip

34   strength (HGS) is used as an indicator for musculoskeletal fitness in children. HGS can also be used as

35   a marker of cardiometabolic risk, but most available HGS data are derived from Western high-income

36   countries. Therefore, this study examines whether HGS is associated with body composition and

37   markers of cardiovascular risk in children from three sub-Saharan African countries.

38   **Design:** Cross-sectional study.

39   **Setting:** Public primary schools (grade 1-4) in Taabo (Côte d'Ivoire), Port Elizabeth (South Africa) and

40   Ifakara (Tanzania).

41   **Participants:** Data from 467 children from Côte d'Ivoire (210 boys, 257 girls), 864 children from South

42   Africa (429 boys, 435 girls) and 695 children from Tanzania (334 boys, 361 girls) were analysed.

43   **Primary and secondary outcome measures:** Body composition was the primary outcome, which was

44   assessed via bioelectrical impedance analysis. Cardiovascular risk markers were considered as

45   secondary outcome. Blood pressure was measured with an oscillometric monitor, and blood markers

46   (cholesterol, triglycerides, glycated haemoglobin) via Afinion point-of-care testing. Grip strength

47   (independent variable) was assessed with a hydraulic hand dynamometer. Inferential statistics are

48   based on mixed linear regressions and analyses of covariance.

49   **Results:** Across all study sites, higher HGS was associated with lower body fat, higher muscle mass

50   and higher fat-free mass (p<0.001, 3.9-10.0% explained variance), both in boys and girls. No

51   consistent association was found between HGS and cardiovascular risk markers.

52   **Conclusions:** HGS assessment is popular due to its simplicity, feasibility, practical utility, and high

53   reliability of measurements. This is one of the first HGS studies with children from sub-Saharan

54   Africa. There is a great need for further studies to examine whether our findings can be replicated, to

55   establish links to other health outcomes, to explore longitudinal relationships, and to develop

56   reference values for African children.

57   **Trial registration:** https://doi.org/10.1186/1SRCTN29534081 (August 9, 2018).

**Article summary**

**Strengths and limitations of this study:**

Strengths:

- Hand grip strength can be used as a simple indicator to assess the maximum voluntary force of the hand, but is also associated with arm, trunk and leg strength.

- Assessments are quickly obtainable by a range of different health professionals.

- This is one of the first HGS studies with children from sub-Saharan Africa.

- This study assesses associations between grip strength, body composition and various cardiovascular risk markers.

- The same methods were used across three different study sites (Côte d'Ivoire, South Africa, Tanzania), which allows direct comparison of the findings.

Limitations:

- There is still no consensus on HGS measurement protocols.

- The children from the three study sites differed with regard to age, height and weight.

- The cross-sectional nature of the study precludes conclusions regarding cause and effect.

- Study sites were not representative for any of the countries involved.

- In all three study sites, children were recruited from rural and peri-urban areas and thus represent rather poor segments of the population, which limits the generalisability of our findings.

## INTRODUCTION

Muscular strength represents a specific component of health-related fitness and is defined as the ability to develop maximal muscle force.[1] Muscle strength plays an important role in child development as it is the basis for locomotive movement and, therefore, can facilitate play and social interaction with other children.[2]

Hand grip strength (HGS) is used as a simple indicator to assess the maximum voluntary force of the hand,[3] but HGS is also associated with arm, trunk and leg strength.[4] Research further shows that HGS is a good indicator for musculoskeletal fitness in children.[5] Accordingly, assessment of HGS is recommended as part of school-based fitness testing and as a screening tool for adequate levels of muscle strength and bone health.[6]

HGS is strongly dependent on age, sex and body mass.[7] The association with age is curvilinear. HGS increases through childhood and adolescence and peaks around the age of 40 years.[3] As HGS depends on children's maturation, HGS should be interpreted in terms of biological rather than chronological age.[8] Moreover, boys have higher HGS than girls, especially after puberty.[3, 7] Following puberty, there is an exponential progression in muscle strength among boys, which has been ascribed to the increase of testosterone and which in turn favours the growth of lean muscle mass.[9] In girls, an increase of total body fat was observed after puberty.[10] Research has also shown that HGS depends on body mass, and that HGS divided by body weight is more closely associated with health outcomes than absolute HGS values.[11]

Meanwhile, normative HGS reference values have been developed for children and adolescents in several countries.[8, 12] For instance, based on normalized HGS measurements from 1,326 sixth-grade students (10-12 years), Peterson et al.[13] developed thresholds for high metabolic risk and intermediate metabolic risk adolescents living in the United States. However, it is doubtful whether such HGS norms can be generalised across countries.[3] For instance, a recent meta-analysis showed that HGS scores are significantly higher in high-income compared to low-to-middle income countries (LMICs).[14]

Furthermore, skeletal muscle has an important role for health and disease because it is the primary protein store within the body and the primary tissue for glucose disposal.[15] In line with this notion, HGS has been associated with a variety of health outcomes among adults, including nutritional status,[16] chronic physical conditions such as cancer and cardiovascular diseases, as well as cardiovascular and all-cause mortality.[15, 17] These relationships have also been observed in people living in LMICs.[18] Interestingly, HGS seems to be a better predictor of mortality than muscle mass,[19] however, the underlying mechanisms are not yet well understood.[17, 20] The association might be mediated via cardiovascular risk factors such as hypertension, diabetes, metabolic syndrome, inflammatory markers, or risk of arrhythmia.[11, 21, 22] In line with this, a meta-analysis showed that cardiovascular risk factors indeed attenuated the relationship between HGS and mortality, but they did not fully explain the observed relationship.[17]

Prior research has corroborated that HGS is a relevant health outcome among children and adolescents. For instance, a study with 1,421 children (M=11.6 years) from the United States pointed towards a significant inverse relationship between HGS and blood pressure, cholesterol levels, and triglycerides.[23] Similarly, a study with 2,818 Norwegian children (9-15 years) reported that, after adjusting for age, sex and puberty, higher HGS was associated with lower waist circumference, higher high density lipoprotein (HDL) cholesterol, lower triglycerides concentration, and lower systolic blood pressure.[24] These relationships were confirmed in Colombian children (M=11.5 years) living in low socioeconomic background settings.[25] Finally, a prospective cohort study with more than a million Swedish participants showed that high grip strength in adolescence is associated with lower premature death later in life (median follow-up period of 24 years).[26]

In summary, these studies highlight that HGS has the potential to be used not only as an indicator of muscular strength, but also as a marker of cardiometabolic risk. However, most of today's data are derived from Western high-income countries, whereas research in sub-Saharan Africa, where rapid social, cultural, economic and urban developments are currently occurring, is lacking, particularly among children. Therefore, the purpose of this paper was to examine whether HGS is associated with body composition (body fat, muscle mass, fat free mass) and markers of cardiovascular risk (blood

pressure, cholesterol, triglycerides, blood glucose [HbA1c]) in primary schoolchildren from three

African countries (Côte d'Ivoire, South Africa, and Tanzania).

## METHODS

### Study design and setting

Data presented in this paper comes from the baseline data assessment of the KaziAfya

cluster randomized controlled trial, which was conducted in public schools in Taabo (Côte d'Ivoire),

Port Elizabeth (South Africa), and Ifakara (Tanzania). The goal of this trial was to implement and test

two school-based health interventions (physical activity promotion, multi-micronutrient

supplementation) over a period of two school years to improve children's health status. All study

sites are located in rural and peri-urban settings. The Ivorian public primary schools were selected

from Taabo city and village, located in the rural South-central Agnéby-Tiassa region (150 km

Northwest from Abidjan). Taabo's economy relies on agriculture (90% of the employees), and has

two companies in the agricultural sector and a power station (hydroelectric dam), which produces

2% of the country's energy. Our project schools in South Africa are located in peri-urban marginalized

communities in the Eastern Cape (Port Elizabeth region). Only schools with poor ratings with

reference to national poverty tables, income levels, dependency ratios and literacy rates were

involved. Our project schools in South-central Tanzania are situated in a rural town (Ifakara) in the

Kilombero District, Morogoro Region (413 km from Dar es Salaam). The main economic activity of the

local population is rice farming, but small businesses and animal husbandry are also conducted. The

procedures to select the schools for this study have been described in the study protocol.[27]

### Participants and procedures

Children were eligible if they attended grade 1–4, were no older than 12 years, did not participate in

any other research projects, and did not suffer from clinical conditions that prevent participation in

physical activity, as determined by qualified medical personnel. Written informed consent was

obtained from the legal guardians before study enrolment, after having provided detailed written

and oral information about the aims, procedures, risks and benefits of the study. The responsible

ethics committees in Côte d'Ivoire, South Africa, Tanzania and Switzerland and the local school

authorities approved the study. Children who suffered from severe medical conditions and/or

malnourishment (as diagnosed by a nurse, following national guidelines) were referred to local

clinics. The intervention study was registered at http://www.isrctn.com/ISRCTN29534081. The data

assessment took place between October and December 2018 in Côte d'Ivoire, between January and

April 2019 in South Africa, and between July and August 2019 in Tanzania.


**Measures**

Data assessments took place at children's schools before the start of the intervention. Saehan

hydraulic hand dynamometers (MSD Europe BVBA; Tisselt, Belgium) were used to assess HGS. The

children were instructed to sit with spine erect, relaxed, and with an arm position at a 90° angle.

Before the first data assessment, a field investigator briefly demonstrated how to hold the hand

dynamometer. Children were instructed to grip the hand dynamometer as hard as possible. Each

child performed six trials (three with the left, and three with the right hand), which were recorded to

the nearest 1 kg. After each trial and after a 30 sec resting period, the hand was changed. An overall

HGS score was constructed by calculating the mean across all six trials, which was then divided by the

child's body weight. Previous research with children showed that HGS is closely associated with total

muscle strength,[28] and that HGS measures generally have a good reliability.[29] Body composition was

assessed via bioelectrical impedance analysis (BIA) using a wireless body composition monitor (Tanita

MC-580; Tanita Corp., Tokyo, Japan). Children wearing only light clothing were asked to stand

barefoot on the metal plates of the device, being guided by the research assistant to ensure optimal

contact according to the manufacturer's instructions. The MC-580 was also used to assess body

weight to the nearest 0.1 kg. Body height was taken to the nearest 0.1 cm with each child standing

with his/her back erect and shoulders against a stadiometer. Blood pressure was assessed with a

validated oscillometric digital blood pressure monitor (Omron® M3; Hoofddorp, Netherlands). Three

measurements were performed, and the mean of the last two measurements were employed as

indicators of systolic and diastolic blood pressure. Capillary blood sampling was used to assess blood markers for cardiovascular risk, using the Afinion 2 analyser (Abbott Technologies; Abbott Park, USA). Total cholesterol, HDL cholesterol, low density lipoprotein (LDL) cholesterol, triglycerides and HbA1c were assessed using fasting capillary blood sampling.[30] Evidence of the clinical utility and accuracy of this finger prick technology has been described previously.[31]

**Statistical analyses**

Descriptive statistics for all study variables are reported separately for each study site (Table 1). Normality was tested via the Kolmogorov-Smirnov test. Univariate analyses of covariance (ANCOVAs) with Bonferroni post-hoc tests were calculated to examine differences between study sites. Mixed linear regression analyses with random intercepts for school classes to account for the nested nature of the data (learners assessed in classes) were performed separately for each study site to determine whether HGS is associated with body composition and single cardiovascular risk markers (Table 2). All regression analyses were controlled for sex and height. Weight and age were not considered as covariates to avoid over-adjustment and collinearity issues (e.g., weight is already considered in the body weight-adjusted HGS measure, age is highly correlated with height [$r$=0.71 to 0.77, $p$<0.001]). To compare children with high vs. low HGS scores, ANCOVAs (controlling for sex and height) with Bonferroni post-hoc tests were calculated to compare HGS quartiles (Table 3). All analyses were carried out with SPSS (version 26, IBM Corporation, Armonk, USA). The level of statistical significance was set at $p$<0.05 across all analyses.

**RESULTS**

**Sample description**

As shown in Table 1, in Côte d'Ivoire, 467 children presented with complete data (210 boys, 257 girls). In South Africa, 864 children were included (429 boys, 435 girls), whereas in Tanzania, the final sample consisted of 695 children (334 boys, 361 girls).

**Descriptive statistics and between-country differences**

Table 1 displays the descriptive statistics, separately for each study site. Although none of the

metric variables were normally distributed, no evidence for severe non-normality was found

(skewness and kurtosis values of ≥|2| and ≥|7|, respectively).[32] Therefore, no log-transformation of

the outcomes was needed. High internal consistency across the six HGS measurements was

confirmed in each study site (Cronbach's alpha: 0.95-0.98). As shown in Table 1, significant

differences between study sites were found in most of the variables, before and after controlling for

confounders. Children from the Tanzanian study site were oldest (M=9.34±1.74 years), followed by

peers from the South African (M=8.24±1.42 years) and Ivorian (M=7.86±2-63 years) study sites. In

line with this, children from Tanzania were tallest, heaviest, and had the highest body mass index

(BMI) scores. They also achieved the highest absolute and weight-adjusted HGS scores. Furthermore,

children from Tanzania had the highest scores for systolic/diastolic blood pressure, total cholesterol,

as well as HDL and LDL cholesterol. By contrast, children from the Ivorian study site had higher

triglyceride levels, whereas children from the South African study site had the highest HbA1c

concentrations. Differences in HGS, body composition and cardiovascular risk persisted after

controlling for sex, height and weight.

**HGS, body composition and cardiovascular risk markers**

Table 2 shows the results of the mixed linear regression models, separately for each study

site. The results consistently show that HGS is positively associated with relative muscle mass and

fat-free mass, whereas HGS is negatively associated with relative body fat. These associations

persisted after controlling for sex and height.

Regarding the association between HGS and cardiovascular risk markers, only a few

significant results were found, after adjusting for sex and height. More specifically, in children from

the Ivorian study site, those with higher HGS had higher total and LDL cholesterol levels. However, no

such relationships were found among children recruited from the South African and Tanzanian study

sites.

**Differences between HGS quartiles**

Table 3 shows the means and standard deviations for the different HGS quartiles. Consistently across all study sites, significant between-quartile differences were found for relative body fat, muscle mass and fat-free mass. Bonferroni post-hoc tests pointed towards a clear gradient, indicating that students in the higher HGS quartiles had lower body fat levels, higher muscle mass and higher fat-free mass. Using the example of muscle mass, Figure 1 shows that this gradient occurred in both girls and boys.

**DISCUSSION**

The key findings of this study are that in primary school children from Côte d'Ivoire, South Africa and Tanzania, higher HGS is associated with lower body fat, higher muscle mass and higher fat-free mass. A clear gradient appeared in both boys and girls. The level of explained variance (3.9 to 10.0%) points towards between-quartile differences of moderate magnitude. Unlike previous findings, no consistent association was found between HGS and further (single) cardiovascular risk markers (blood pressure, cholesterol, triglycerides, glycated haemoglobin). To the best of our knowledge, this is the first study carried out with children in sub-Sahara Africa and testing the relationship between HGS, body composition and cardiovascular risk markers. It is also one of the few studies carried out with younger schoolchildren.

The present study corroborates the notion that grip strength serves as a dynamic indicator of muscle mass and healthy body composition.[3, 4, 33] This also supports public health recommendations that children should regularly engage in muscle-strengthening activities.[34] Schools can play an important role in this process as they can reach all children, independent of their sex, ethnicity and socioeconomic background via school physical education. On the other hand, the association between muscle mass and HGS was of limited (moderate) magnitude. This indicates that other factors have an impact on children's HGS. Possible influences are overall dietary quality,[35] disease-related factors[33] and socioeconomic status.[36]

The present study also confirms prior research[3, 7, 8] showing that increasing age is positively associated with both absolute HGS (in this sample: $r$=0.50 to 0.70, $p$<.001, data not shown) and body weight-adjusted HGS ($r$=0.17 to 0.46, $p$<.001, data not shown). While chronological and biological age can differ,[8] similarly strong associations were found between children's height and absolute HGS (in this sample: $r$=0.54 to 0.74, $p$<.001, data not shown) and body weight-adjusted HGS ($r$=0.11 to 0.36, $p$<.01, data not shown). Our study also aligns with previous investigations showing that boys have higher HGS than girls.[3, 7, 9, 10] In the present sample, boys had higher absolute HGS (3.0 to 4.7% of explained variance, data not shown) and body weight-adjusted HGS (3.9 to 6.5% of explained variance, data not shown) than girls.

Regarding the relationship between HGS and cardiovascular risk markers, our findings are at odds with most previous studies with adult[11, 21, 22] and child/adolescent populations.[23-26] Whereas prior research with children and adolescents showed that higher HGS are associated with more favourable blood pressure, more favourable cholesterol profiles, lower triglyceride concentrations, and lower clustered cardiovascular risk, it is important to note that not all studies found such relationships. For instance, HGS was not associated with cardiovascular risk markers in a sample of 1,642 Australian children and adolescents (9-15 years).[37] The fact that in our study no clear association occurred in any of the study sites indicates that our results have a certain generalisability: however, we can only speculate about the underlying reasons. One assumption relates to the (low) age of the children and the fact that, on average, risk marker scores were relatively low in the present sample.[38] It is possible that the relationship between HGS and cardiovascular risk markers becomes more pronounced as muscle strength increases with age due to changes in muscle mass,[28] and as the prevalence of cardiovascular risk factors increases during later childhood and adolescence. In younger children, HGS may be more closely associated with other (e.g., subjective or mental) health outcomes.[39] This aspect deserves more attention in future investigations, especially as few studies have so far examined the relationship between HGS and subjective/mental health in children/adolescents. Among adults, the relevance of low HGS as a risk marker for cardiovascular health seems well established. In line with this, a UK Biobank based study with 403,199 adults

showed that obesity and lower grip strength are independent predictors of higher mortality risk. However, all-cause mortality risk was lower for obese but stronger individuals compared to non-obese, but weaker individuals,[34] which suggests that "improving muscle strength may be a more important public health priority than reducing adiposity in decreasing mortality risks, although excessive adiposity itself is a strong risk factor of mortality" (p. 780). Finally, the relationship between HGS and health outcomes may be different in children from low- versus middle-income countries.

**Strengths and Limitations**

The fact that there is still no consensus on HGS measurement protocols complicates between-study comparisons.[33] For instance, research showed that methodological aspects (e.g., dynamometer handle position) can have a significant influence on the HGS performance of children.[40] Therefore, the fact that we applied the same methods across three different study sites is an advantage, as it allows direct comparison of the findings. By contrast, the fact that children from the three study sites differed with regard to age, height and weight can be seen as a limitation. To address this issue, we focused on body weight-adjusted HGS, and considered sex and height (as a proxy for biological age) as covariates. We further acknowledge that due to the cross-sectional nature of our study, no conclusions are possible regarding cause and effect. Researchers have pointed to the issue of reversed causality and the fact that poor HGS could be considered as a marker of poor overall health.[17] We also acknowledge that differences between study sites should not be (mis)interpreted as between-country differences, since the study sites were not representative for any of the countries involved. In all three study sites, children were recruited from rural and peri-urban areas and thus represent rather poor segments of the population, which limits the generalisability of our findings.

**CONCLUSIONS**

HGS assessment is popular due to its simplicity, feasibility, practical utility, and high reliability of measurements. Assessments are quickly obtainable by a range of different health professionals. They

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can also be easily implemented as part of school-based fitness testing and as a screening tool for adequate levels of muscle strength and bone health.

Our study highlights that higher body weight-adjusted HGS is associated with healthy body composition (lower body fat, higher fat-free mass) and higher muscle mass. To the best of our knowledge, this is the first HGS study with children from sub-Saharan Africa. Hence, there is a great and continued need for further studies to examine whether our findings can be replicated (e.g., with older primary school students), to establish links to other health outcomes (e.g., nutritional status, mental health), to explore longitudinal relationships, and to develop reference values for African children and adolescents. It will also be important to understand whether HGS in these populations with low level of cardiovascular/metabolic risks will be of value in predicting long-term cardiovascular/metabolic disease risk.

**CONFLICT OF INTEREST**

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

**AUTHOR CONTRIBUTIONS**

MG, KZL, UP and JU were responsible for the conceptualization of the study and for initial funding acquisition. MG serves as principal investigator of the study, BB, HM and CW are the principal investigators in the three partner countries. CL is responsible for the overall administration of the study. SGT, MFF and SN are the local project coordinators. RDR, DD, KZL, FOO, NPH, UP, and PS served as project advisors and provide resources. SAA, JB, BK, BCG, SGT, JTC, MFF, GJM, EGM, SG, GJM, CL, IM, and SN contributed to the data assessment and curation. MG performed the statistical analysis and wrote the first draft of the manuscript. All authors contributed to manuscript revision, read, and approved the submitted version.

**Data sharing**

The raw datasets are available from the corresponding author on reasonable request.

**Ethical approval**

In Côte d'Ivoire, the study protocol was approved by the Institutional Review Board (IRB) of the Centre Suisse de Recherches Scientifiques en Côte d'Ivoire (CSRS; Abidjan, Côte d'Ivoire) and the Comité National d'Ethique des Sciences de la Vie et de la Santé (CNESVS; reference number: 100–18/MSHP/CVESVS-km). In South Africa, approval was granted by the research ethics committee of the Nelson Mandela University in Port Elizabeth (reference number: H18-HEA-HMS-006) and the Eastern Cape Departments of Education and Health. In Tanzania, the study protocol was approved by the responsible ethics committee at the Ifakara Health Institute (IHI–IRB; reference number: # IHI/IRB/No 39-2018), the National Institute for Medical Research (NIMR; reference number:

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NIMR/HQ/R.8a/Vol. IX/3137) and the Tanzania Food and Drugs Authority (TFDA; reference number:

TMDA0019/CTR/0016/05). Children who suffered from severe medical conditions and/or

malnourishment (as diagnosed by a nurse, following national guidelines) were referred to local

clinics. Ethical approval was also obtained from the 'Ethikkommission Nordwest- und Zentralschweiz'

in Switzerland (EKNZ; reference number: Req-2018-00608), and the intervention study was

registered in the ISRCTN registry (http://www.isrctn.com/ISRCTN29534081).

**Patient and public involvement statement**

Patients or the public were not involved in the design, conduct, reporting or dissemination plans of

our study.

**REFERENCES**

1.  American College of Sports Medicine. *ACSM's health-related physical fitness assessment*

    *manual*. Philadelphia: Lippincott Williams & Wilkins; 2013.

2.  Fredriksen PM, Mamen A, Hjelle OP, Lindberg M. Handgrip strength in 6–12-year-old children:

    The Health Oriented Pedagogical Project (HOPP). Scand J Public Health 2018;**46**:S54-S60.

3.  Wong SL. Grip strength reference values for Canadians aged 6 to 79: Canadian Health Measure

    Survey, 2007 to 2013. Health Reports 2016;**27**:3-10.

4.  Wang M, Leger AB, Dumas GA. Prediction of back strength using anthropometric and strength

    measurements in healthy females. Clin Biomech 2005;**20**:685-692.

5.  Ortega FB, Cadenas-Sánchez C, Sánchez-Delgado G, Mora-González J, Martínez-Téllez B, Artero

    EG, Castro-Pinero J, Labayen I, Chillon P, Löf M, Ruiz JR. Systematic review and proposal of a

    field-based physical fitness-test battery in preschool children: the PREFIT battery. Sports Med

    2015;**45**:533-555.

6.  Ruiz JR, Castro-Pinero J, Espana-Romero V, Artero EG, Ortega FB, Cuenca MM, Jimenez-Pavon D,

    Chillon P, Girela-Rejon MJ, Mora J, Gutierrez A, Suni J, Sjöström M, Castillo MJ. Field-based

fitness assessment in young people: the ALPHA health-related fitness test battery for children and adolescents. Br J Sports Med 2010;**45**:518-524.

7. Ploegmakers JJ, Hepping AM, Geertzen JH, Bulstra SK, Stevens M. Grip strength is strongly associated with height, weight and gender in childhood: a cross sectional study of 2241 children and adolescents providing reference values. J Physiother 2013;**59**:255-261.

8. Gomez-Campos R, Andruske CL, de Arruda M, Sulla-Torres J, Pacheco-Carrillo J, Urra-Albornoz C, Cossio-Bolanos M. Normative data for handgrip strength in children and adolescents in the Maule Region, Chile: Evaluation based on chronological and biological age. PLoS One 2018;**13**:e0201033.

9. Round JM, Jones DA, Honour JW, Nevill AM. Hormonal factors in the development of differences in strength between boys and girls during adolescence: a longitudinal study. Ann Hum Biol 1999;**26**:49-62.

10. Dore E, Martin R, Ratel S, Duche P, Bedu M, Van PE. Gender differences in peak muscle performance during growth. Int J Sports Med 2005;**26**:274-280.

11. Chun S-W, Kim W, Choi KH. Comparison between grip strength and grip strength divided by body weight in their relationship with metabolic syndrom and quality of life in the elderly. PLos One 2019;**14**:e0222040.

12. Häger-Ross C, Rösblad B. Norms for grip strength in children aged 4–16 years. Acta Paediatr 2002;**91**:617-625.

13. Peterson MD, Zhang P, Saltarelli WA, Visich PS, Gordon PM. Low muscle strength thresholds for the detection of cardiometabolic risk in adolescents. Am J Prev Med 2016;**50**:593-599.

14. Dodds RM, Syddall H, Cooper R, Kuh D, Cooper C, Sayer AA. Global variation in grip strength: A systematic review and meta-analysis of normative data. Age Ageing 2016;**45**:209-216.

15. Ho FKW, Celis-Morales CA, Petermann-Rocha F, Sillars A, Welsh P, Welsh C, Anderson J, Lyall DM, Mackay DF, Sattar N, Gill JMR, Pell JP, Gray SR. The association of grip strength with health outcomes does not differ if grip strength is used in absolute or relative terms: A prospective cohort study. Age Ageing 2019;**48**:683-691.

16. Norman K, Stobäus N, Gonzalez MC, Schulzke JD, Pirlich M. Hand grip strength: outcome predictor and marker of nutritional status. Clin Nutr 2011;**30**:135-142.

17. Wu Y, Wang W, Liu T, Zhang D. Association of grip strength with risk of all-cause mortality, cardiovascular diseases, and cancer in community-dwelling populations: A meta-analysis of prospective cohort studies. JAMDA 2017;**18**:551.e17-551e35.

18. Vancampfort D, Stubbs B, Firth J, Koyanagi A. Handgrip strength, chronic physical conditions and physical multimorbidity in middle-aged and older adults in six lo- and middle income countries. Eur J Int Med 2019;**61**:96-102.

19. Newman AB, Kupelian V, Visser M, Simonsick EM, Goodpaster BH, Kritchevsky SB, Tylavsky FA, Rubin SM, Harris TB. Strength, but not muscle mass, is associated with mortality in the health, aging and body composition study cohort. J Gerontol 2006;**61**:72-77.

20. Gubelmann C, Vollenweider P, Marques-Vidal P. Association of grip strength with cardiovascular risk markers. Eur J Prev Cardiol 2017;**24**:514-521.

21. Mainous AGI, Tanner RJ, Anton SD, Jo A. Grip strength as a marker of hypertension and diabetes in healthy weight adults. Am J Prev Med 2015;**49**:850-858.

22. Andersen K, Rasmussen F, Held C, Neovius M, Tynelius P, Sundström J. Exercise capacity and muscle strength and risk of vascular disease and arrhythmia in 1.1 million young Swedish men: cohort study. BMJ 2015;**351**:h4543.

23. Peterson MD, Saltarelli WA, Visich PS, Gordon PM. Strength capacity and cardiometabolic risk clustering in adolescents. Pediatrics 2014;**133**:e896-e903.

24. Steene-Johannessen J, Anderssen SA, Kolle E, Andersen LB. Low muscle fitness is associated with metabolic risk in youth. Med Sci Sports Exerc 2009;**41**:1361-1367.

25. Cohen DD, Gomez-Arbelaez D, Camacho PA, Pinzon S, Hormiga C, Trejos-Suarez J, Duperly J, Lopez-Jaramillo P. Low muscle strength is associated with metabolic risk factors in Colombian children: the ACFIES study. PLoS One 2014;**9**:e93150.

26. Ortega FB, Silventoinen K, Tynelius P, Rasmussen F. Muscular strength in male adolescents and premature death: cohort study of one million participants. BMJ 2012;**345**(e7279):doi: 10.1136/bmj.e7279.

27. Gerber M, Ayekoé SA, Beckmann J, Bonfoh B, Coulibaly JT, Daouda D, du Randt R, Finda L, Gall S, Mollel GJ, Lang C, Long KZ, Ludyga S, Masanja H, Müller I, Nqweniso S, Okumu F, Probst-Hensch N, Pühse U, Steinmann P, Traoré SG, Walter C, Utzinger J. Effects of school-based physical activity and multi-micronutrient supplementation intervention on growth, health and wellbeing of schoolchildren in three African countries: the KaziAfya cluster randomised controlled trial protocol using a 2x2 factorial design. Trials 2020;**21**:doi: 10.1186/s13063-019-3883-5.

28. Wind A, Takken T, Helders P, Engelbert R. Is grip strength a predictor for total muscle strength in healthy children, adolescents, and young adults? Eur J Pediatr 2010;**169**:281-287.

29. Ruiz JR, Castro-Pinero J, Espana-Romero V, Artero EG, Ortega FB, Cuenca MM, Jimenez-Pavon D, Chillon P, Girela-Rejon MJ, Mora J, Gutierrez A, Suni J, Sjöström M, M.J. C. Field-based fitness assessment in young people: the ALPHA health-related fitness test battery for children and adolescents. Br J Sports Med 2011;**45**(518-524).

30. In. *WHO Guidelines on Drawing Blood: Best Practices in Phlebotomy*. Geneva 2010.

31. Parikh P, Mochari H, Mosca L. Clinical utility of a fingerstick technology to identify individuals with abnormal blood lipids and high-sensitivity C-reactive protein levels. Am J Health Promot 2009;**23**(4):279-82.

32. West SG, Finch JF, Curran PJ. Structural equation models with nonnormal variables: Problems and remedies. In: Hoyle RH, (ed). *Structural equation modeling. Concepts, issues, and applications*. Thousand Oakes: Sage; 1995, 56-75.

33. Norman K, Stobäus N, Cristina Gonzalez M, Schulzke J-D, Pirlich M. Hand grip strength: Outcome predictor and marker of nutritional status. Clin Nutr 2011;**30**:135-142.

34. Kim Y, Wijndaele K, Lee D-C, Sharp SJ, Wareham N, Brage S. Independent and joint associations of grip strength and adiposity with all-cause and cardiovascular disease mortality in 403'199 adults: The UK Biobank study. Am J Clin Nutr 2017;**106**:773-782.

35. Kim HR, Kwon O. Higher diet quality is associated with lower odds of low hand grip strength in the Korean elderly population. Nutrients 2019;**11**:1487.

36. Thorpe RJJ, Simonsick E, Zonderman A, Evans MK. Association between race, poverty status and grip strength in middle to old age adults. Ethn Dis 2016;**26**:493-500.

37. Magnussen CG, Schmidt MD, Dwyer T, Venn A. Muscular fitness and clustered cardiovascular disease risk in Australian youth. Eur J Appl Physiol 1012;**112**:3167-3171.

38. Stavnsbo M, Resaland GK, Anderssen SA, Steene-Johannessen J, Domazet SL, Skrede T, Sardinha LB, Kriemler S, Ekelund U, Andersen LB, Aadland E. Reference values for cardiometabolic risk scores in children and adolescents: Suggesting a common standard. Atherosclerosis 2018;**278**:299-306.

39. Ashdown-Franks G, Stubbs B, Koyanagi A, Schuch FB, Firth J, Veronese N, Vancampfort D. Handgrip strength and depression among 34,129 adults aged 50 years and older in six low- and middle-income countries. J Affect Disord 2019;**243**:448-454.

40. Gasior JS, Pawlowski M, Williams CA, Dabrowski MJ, Rameckers EA. Assessment of maximal isometric hand grip strength in school-aged children. Open Med 2018;**13**:22-28.

**FIGURE LEGENDS**

Figure 1. Differences in muscle mass between HGS quartiles, separately for girls and boys and for

each study site

Table 1. Sample characteristics and descriptive statistics, separately for each study site

| | Côte d'Ivoire (n=467) | | South Africa (n=864) | | Tanzania (n=695) | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| Categorical variables | n | % | n | % | n | % | χ² | φ | | |
| Sex | | | | | | | | | | |
| Male | 210 | 45.0 | 429 | 49.7 | 334 | 48.1 | 2.67 | 0.036 | | |
| Female | 257 | 55.0 | 435 | 50.3 | 361 | 51.9 | | | | |
| | | | | | | | | | Uncontrolled | Controlled for sex, height and weight |
| Metric variables | M | SD | M | SD | M | SD | F | η² | F | η² |
| Age (in years) | 7.86ᵃᵇ | 1.69 | 8.24ᵃᶜ | 1.42 | 9.34ᵇᶜ | 1.74 | 113.15*** | 0.118 | 60.12*** | 0.067 |
| Height (in cm) | 123.53ᵃ | 9.44 | 124.01ᵇ | 8.95 | 128.67ᵃᵇ | 10.28 | 49.83*** | 0.056 | 20.21***² | 0.020 |
| Weight (in kg) | 23.09ᵃᵇ | 4.43 | 24.54ᵃᶜ | 5.49 | 26.77ᵇᶜ | 5.86 | 50.07*** | 0.056 | 29.91***³ | 0.029 |
| BMI (in kg/m²) | 15.00ᵃᵇ | 1.29 | 15.79ᵃ | 2.04 | 16.01ᵇ | 1.90 | 24.89*** | 0.029 | 32.79***⁴ | 0.042 |
| Grip strength | | | | | | | | | | |
| Absolute grip strength (in kg) | 8.47ᵃᵇ | 2.63 | 11.23ᵃᶜ | 4.38 | 12.43ᵇᶜ | 4.40 | 82.74*** | 0.089 | 56.19*** | 0.063 |
| Body weight adjusted grip strength¹ | 0.36ᵃᵇ | 0.09 | 0.46ᵃ | 0.17 | 0.46ᵇ | 0.11 | 52.92*** | 0.059 | 48.13*** | 0.065 |
| Body composition | | | | | | | | | | |
| Body fat (in %) | 19.50ᵃᵇ | 2.96 | 22.13ᵃᶜ | 4.37 | 20.23ᵇᶜ | 3.95 | 68.94*** | 0.076 | 113.16*** | 0.118 |
| Muscle mass (in %) | 76.01ᵃᵇ | 2.73 | 73.50ᵃᶜ | 4.04 | 75.38ᵇᶜ | 3.68 | 74.52*** | 0.081 | 115.17*** | 0.120 |
| Fat-free mass (in %) | 80.51ᵃᵇ | 2.95 | 77.88ᵃᶜ | 4.38 | 79.78ᵇᶜ | 3.96 | 68.94*** | 0.075 | 112.50*** | 0.118 |
| Cardiovascular risk factors | | | | | | | | | | |
| Systolic blood pressure (in mm Hg) | 99.47ᵃᵇ | 10.44 | 101.36ᵃᶜ | 11.10 | 104.66ᵇᶜ | 11.07 | 20.35*** | 0.024 | 6.49** | 0.008 |
| Diastolic blood pressure (in mm Hg) | 64.95ᵃᵇ | 9.46 | 62.88ᵃᶜ | 8.50 | 67.34ᵇᶜ | 8.32 | 38.44*** | 0.044 | 32.66*** | 0.037 |
| Total cholesterol (in mmol/L) | 3.57ᵃ | 0.67 | 3.63ᵇ | 0.60 | 3.78ᵃᵇ | 0.67 | 14.43*** | 0.017 | 11.16*** | 0.013 |
| HDL cholesterol (in mmol/L) | 1.99ᵃᵇ | 0.52 | 2.06ᵃᶜ | 0.50 | 2.15ᵇᶜ | 0.53 | 35.98*** | 0.041 | 29.32*** | 0.034 |
| LDL cholesterol (in mmol/L) | 1.05ᵃᵇ | 0.36 | 1.24ᵃᶜ | 0.29 | 1.28ᵇᶜ | 0.41 | 12.86*** | 0.015 | 13.05*** | 0.015 |
| Triglycerides (in mmol/L) | 1.16ᵃᵇ | 0.35 | 0.74ᵃ | 0.22 | 0.77ᵇ | 0.33 | 241.99*** | 0.223 | 252.92*** | 0.231 |
| Glycated hemoglobin (HbA1c, in %) | 4.94ᵃᵇ | 0.38 | 5.42ᵃᶜ | 0.25 | 5.22ᵇᶜ | 1.73 | 266.20*** | 0.240 | 266.02*** | 0.240 |

*Notes.* ¹Body weight adjusted grip strength is calculated with the following formula: Absolute grip strength / body weight. ²ANCOVA controlled for sex and weight. ³ANCOVA controlled for sex and height. ⁴ANCOVA controlled for sex. Mean scores with the same superscript letters are significantly different (*p*<0.05), based on Bonferroni post-hoc tests. Significant results are marked in bold font. *\*p*<0.05. *\*\*p*<0.01. *\*\*\*p*<0.001.

Table 2. Association between weight-adjusted HGS, body composition and cardiometabolic risk markers, separately for each study site

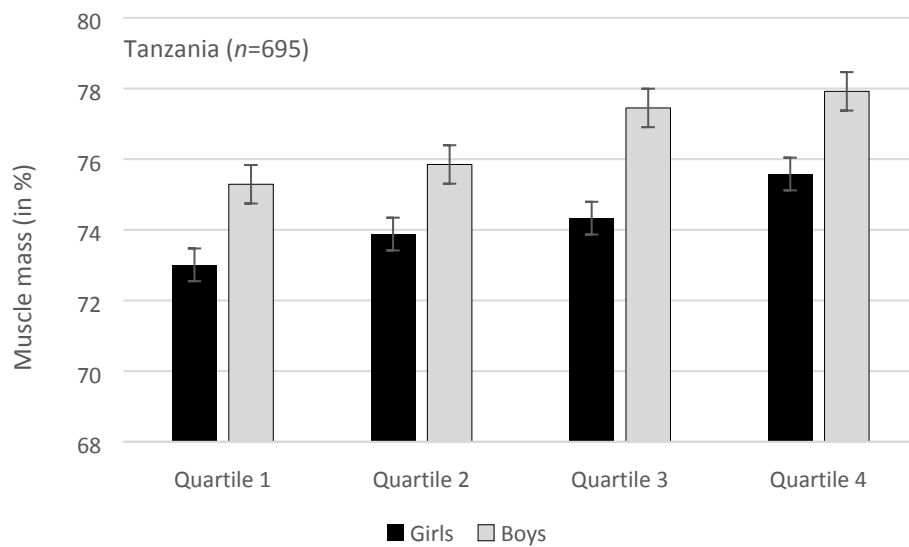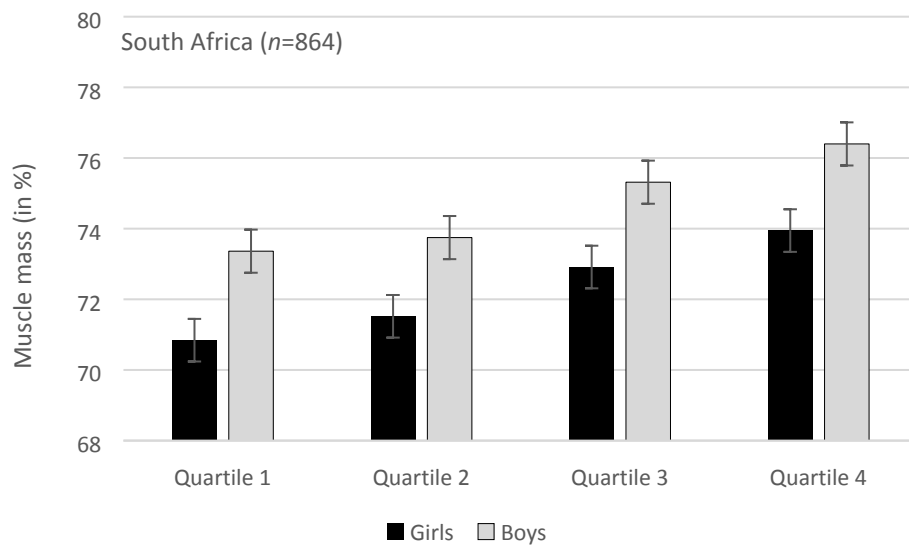| | Côte d'Ivoire (n=467) | | | South Africa (n=864) | | | Tanzania (n=695) | | |
|---|---|---|---|---|---|---|---|---|---|
| | Model 1: Uncontrolled | | | | | | | | |
| | B (SE) | 95% CI | p | B | 95% CI | p | B | 95% CI | p |
| Body composition | | | | | | | | | |
| Body fat | **-12.65 (1.48)** | **-15.55 to -9.74** | **<0.001** | **-7.69 (0.85)** | **-9.36 to -6.03** | **<0.001** | **-12.49 (1.19)** | **-14.83 to -10.16** | **<0.001** |
| Muscle mass | **11.66 (1.36)** | **8.99 to 14.34** | **<0.001** | **7.03 (0.79)** | **5.48 to 8.59** | **<0.001** | **11.70 (1.11)** | **9.53 to 13.87** | **<0.001** |
| Fat free mass | **12.64 (1.47)** | **9.75 to 15.53** | **<0.001** | **7.69 (0.85)** | **6.02 to 9.36** | **<0.001** | **12.47 (1.19)** | **10.14 to 14.81** | **<0.001** |
| Cardiovascular risk markers | | | | | | | | | |
| Systolic blood pressure | **13.98 (5.91)** | **2.37 to 25.59** | **<0.001** | 1.36 (2.15) | -2.87 to 5.58 | 0.529 | **10.05 (3.83)** | **2.54 to 17.56** | **0.009** |
| Diastolic blood pressure | 4.07 (5.09) | -5.93 to 14.06 | 0.425 | -1.25 (1.69) | -4.47 to 2.17 | 0.496 | 0.84 (2.79) | -4.63 to 6.32 | 0.762 |
| Total cholesterol | 0.66 (0.37) | -0.07 to 1.38 | 0.078 | 0.20 (0.12) | -0.04 to 0.44 | 0.098 | -0.27 (0.20) | -0.66 to 0.11 | 0.162 |
| HDL cholesterol | 0.33 (0.18) | -0.04 to 0.69 | 0.076 | 0.10 (0.06) | -0.01 to 0.22 | 0.075 | 0.19 (0.11) | -0.02 to 0.40 | 0.082 |
| LDL cholesterol | 0.48 (0.28) | -0.07 to 1.03 | 0.088 | 0.13 (0.10) | -0.07 to 0.34 | 0.189 | **-0.51 (0.17)** | **-0.85 to -0.18** | **0.003** |
| Triglycerides | -0.32 (0.20) | -0.71 to 0.08 | 0.113 | -0.07 (0.04) | -0.16 to 0.01 | 0.091 | 0.12 (0.09) | -0.05 to 0.29 | 0.169 |
| Glycated hemoglobin (HbA1c) | -0.16 (0.21) | -0.56 to 0.24 | 0.433 | 0.06 (0.05) | -0.04 to 0.16 | 0.229 | -0.04 (0.10) | -0.24 to 0.17 | 0.712 |
| | Model 2: Controlled for sex and height | | | | | | | | |
| | B | 95% CI | p | B | 95% CI | p | B | 95% CI | p |
| Body composition | | | | | | | | | |
| Body fat | **-8.30 (1.45)** | **-11.16 to -5.45** | **<0.001** | **-5.93 (0.77)** | **-7.45 to -4.42** | **<0.001** | **-7.02 (1.34)** | **-9.66 to -4.39** | **<0.001** |
| Muscle mass | **7.68 (1.39)** | **4.95 to 10.41** | **<0.001** | **5.53 (0.73)** | **4.10 to 6.96** | **<0.001** | **6.55 (1.26)** | **4.08 to 9.03** | **<0.001** |
| Fat free mass | **8.29 (1.45)** | **5.43 to 11.15** | **<0.001** | **5.93 (0.77)** | **4.41 to 7.45** | **<0.001** | **7.02 (1.34)** | **4.38 to 9.65** | **<0.001** |
| Cardiovascular risk markers | | | | | | | | | |
| Systolic blood pressure | 1.94 (5.97) | -9.80 to 13.68 | 0.746 | -0.65 (2.02) | -4.62 to 3.32 | 0.747 | 4.88 (1.17) | -3.28 to 13.04 | 0.241 |
| Diastolic blood pressure | -0.31 (5.41) | -10.95 to 10.32 | 0.954 | -1.06 (1.67) | -4.34 to 2.23 | 0.523 | 0.06 (3.04) | -5.91 to 6.03 | 0.984 |
| Total cholesterol | **0.83 (0.39)** | **0.06 to 1.59** | **0.035** | 0.21 (0.12) | -0.04 to 0.45 | 0.094 | -0.15 (0.23) | -0.59 to 0.30 | 0.519 |
| HDL cholesterol | 0.27 (0.20) | -0.13 to 0.67 | 0.178 | 0.06 (0.06) | -0.06 to 0.17 | 0.350 | 0.19 (0.12) | -0.05 to 0.43 | 0.125 |
| LDL cholesterol | **0.07 (0.30)** | **0.15 to 1.32** | **0.014** | 0.19 (0.11) | -0.02 to 0.39 | 0.079 | -0.35 (0.20) | -0.73 to 0.04 | 0.079 |
| Triglycerides | -0.39 (0.22) | -0.81 to 0.04 | 0.075 | -0.07 (0.04) | -0.16 to 0.02 | 0.119 | 0.04 (0.10) | -0.16 to 0.23 | 0.699 |
| Glycated hemoglobin (HbA1c) | -0.23 (0.22) | -0.66 to 0.20 | 0.297 | 0.04 (0.05) | -0.06 to 0.14 | 0.463 | -0.05 (0.11) | -0.26 to 0.17 | 0.669 |

*Notes.* Significant results are marked in bold font.

Table 3. Differences between HGS quartiles in body composition and cardiometabolic risk markers, separately for each study site

| | Côte d'Ivoire (n=467) | | | | | | | | | |
| | Quartile 1 (n=121) | | Quartile 2 (n=112) | | Quartile 3 (n=121) | | Quartile 4 (n=113) | | ANCOVA | |
| | M | SD | M | SD | M | SD | M | SD | F | η² |
| BF | **21.14[abc]** | **2.86** | **19.75[ade]** | **2.85** | **18.63[bdf]** | **2.47** | **18.42[cef]** | **2.82** | **13.33\*\*\*** | **0.080** |
| MM | **74.49[abc]** | **2.65** | **75.79[ade]** | **2.65** | **76.82[bd]** | **2.24** | **76.99[ce]** | **2.62** | **12.88\*\*\*** | **0.077** |
| FFM | **78.88[abc]** | **2.85** | **80.24[ade]** | **2.84** | **81.36[bd]** | **2.47** | **81.61[ce]** | **2.83** | **13.43\*\*\*** | **0.080** |
| SBP | 97.89 | 11.81 | 100.87 | 10.20 | 99.77 | 9.64 | 99.44 | 9.82 | 0.25 | 0.002 |
| DBP | 64.90 | 9.64 | 65.36 | 9.95 | 64.83 | 9.11 | 64.72 | 9.26 | 0.37 | 0.002 |
| TC | 3.59 | 0.72 | 3.47 | 0.61 | 3.56 | 0.69 | 3.64 | 0.67 | 1.32 | 0.009 |
| HDL-C | 1.04 | 0.35 | 1.01 | 0.37 | 1.06 | 0.37 | 1.10 | 0.37 | 1.34 | 0.009 |
| LDL-C | 2.00 | 0.55 | 1.93 | 0.48 | 1.98 | 0.53 | 2.04 | 0.50 | 0.89 | 0.006 |
| TRIG | 1.21 | 0.37 | 1.18 | 0.35 | 1.17 | 0.37 | 1.10 | 0.32 | 1.89 | 0.012 |
| HbA1c | 4.97 | 0.38 | 4.98 | 0.37 | 4.90 | 0.37 | 4.92 | 0.40 | 1.41 | 0.009 |
| | South Africa (n=864) | | | | | | | | | |
| | Quartile 1 (n=213) | | Quartile 2 (n=218) | | Quartile 3 (n=216) | | Quartile 4 (n=217) | | ANCOVA | |
| | M | SD | M | SD | M | SD | M | SD | F | η² |
| BF | **24.13[ab]** | **4.88** | **23.13[cd]** | **4.47** | **21.35[ace]** | **3.72** | **19.93[bde]** | **2.96** | **31.69\*\*\*** | **0.100** |
| MM | **71.64[ab]** | **4.50** | **72.58[cd]** | **4.14** | **74.24[ace]** | **3.44** | **75.52[bde]** | **2.73** | **31.58\*\*\*** | **0.099** |
| FFM | **75.88[ab]** | **4.89** | **76.88[cd]** | **4.48** | **78.66[ace]** | **3.73** | **80.08[bde]** | **2.97** | **31.58\*\*\*** | **0.099** |
| SBP | 99.79 | 11.99 | 102.06 | 10.37 | 101.57 | 11.46 | 101.98 | 10.43 | 0.60 | 0.002 |
| DBP | 62.32 | 8.79 | 63.63 | 8.64 | 62.64 | 8.15 | 62.93 | 8.40 | 0.90 | 0.003 |
| TC | 3.61 | 0.63 | 3.57 | 0.61 | 3.64 | 0.53 | 3.70 | 0.61 | 2.01 | 0.007 |
| HDL-C | 1.22 | 0.29 | 1.21 | 0.28 | 1.24 | 0.31 | 1.27 | 0.29 | 1.20 | 0.004 |
| LDL-C | 2.03 | 0.52 | 2.02 | 0.51 | 2.07 | 0.47 | 2.10 | 0.51 | 1.81 | 0.006 |
| TRIG | **0.78[a]** | **0.25** | **0.75** | **0.22** | **0.70[a]** | **0.20** | **0.73** | **0.22** | **4.58\*\*** | **0.016** |
| HbA1c | 5.49 | 0.25 | 5.43 | 0.26 | 5.44 | 0.24 | 0.42 | 0.26 | 0.06 | 0.002 |
| | Tanzania (n=695) | | | | | | | | | |
| | Quartile 1 | | Quartile 2 | | Quartile 3 | | Quartile 4 | | ANCOVA | |
| | M | SD | M | SD | M | SD | M | SD | F | η² |
| BF | **21.91[ab]** | **4.15** | **21.01[cd]** | **3.57** | **19.69[ace]** | **3.57** | **18.29[bde]** | **3.55** | **9.49\*\*\*** | **0.040** |
| MM | **73.78[ab]** | **3.86** | **74.67[cd]** | **3.32** | **75.91[ace]** | **3.28** | **77.16[bde]** | **3.31** | **9.40\*\*\*** | **0.039** |
| FFM | **78.09[ab]** | **4.16** | **79.01[cd]** | **3.56** | **80.30[ace]** | **3.57** | **81.71[bde]** | **3.56** | **9.34\*\*\*** | **0.039** |
| SBP | 103.36 | 10.38 | 104.28 | 10.38 | 104.85 | 11.75 | 106.16 | 11.59 | 0.44 | 0.002 |
| DBP | 67.20 | 8.05 | 67.07 | 8.49 | 67.61 | 8.49 | 67.47 | 7.84 | 0.09 | 0.000 |
| TC | 3.75 | 0.65 | 3.86 | 0.64 | 3.77 | 0.64 | 3.71 | 0.56 | 1.44 | 0.006 |
| HDL-C | 1.24 | 0.32 | 1.30 | 0.37 | 1.28 | 0.34 | 1.30 | 0.35 | 1.19 | 0.005 |
| LDL-C | 2.17 | 0.55 | 2.22 | 0.50 | 2.13 | 0.57 | 2.07 | 0.49 | 0.86 | 0.004 |
| TRIG | 0.72 | 0.23 | 0.79 | 0.31 | 0.79 | 0.36 | 0.76 | 0.31 | 1.02 | 0.004 |
| HbA1c | 5.17 | 0.31 | 5.15 | 0.32 | 5.14 | 0.32 | 5.16 | 0.33 | 0.20 | 0.001 |

*Notes.* BF = Body fat (in %). MM = Muscle mass (in %). FFM = Fat-free mass (in %). SBP = Systolic blood pressure (in mm Hg). DBP = Diastolic blood pressure (in mm Hg). TC = Total cholesterol (in mmol/L). HDL-C = HDL cholesterol (in mmol/L). LDL-C (in mmol/L). TRIG = Triglycerides (in mmol/L). HbA1c = Glycated hemoglobin (in %). ANCOVAs controlled for sex and height. Mean scores with the same superscript letters are significantly different (*p*<0.05), based on Bonferroni post-hoc tests. Significant results are marked in bold font.

\**p*<0.05. \*\**p*<0.01. \*\*\**p*<0.001.

## STROBE Statement—checklist of items that should be included in reports of observational studies

| | Item No | Recommendation |
|---|---|---|
| **Title and abstract** | 1 | (*a*) Indicate the study's design with a commonly used term in the title or the abstract (L1-2) |
| | | (*b*) Provide in the abstract an informative and balanced summary of what was done and what was found (L33-56) |
| **Introduction** | | |
| Background/rationale | 2 | Explain the scientific background and rationale for the investigation being reported (L84-135) |
| Objectives | 3 | State specific objectives, including any prespecified hypotheses (L135-138) |
| **Methods** | | |
| Study design | 4 | Present key elements of study design early in the paper (L142-157) |
| Setting | 5 | Describe the setting, locations, and relevant dates, including periods of recruitment, exposure, follow-up, and data collection (L142-157) |
| Participants | 6 | *Cross-sectional study*—Give the eligibility criteria, and the sources and methods of selection of participants (L159-170) |
| Variables | 7 | Clearly define all outcomes, exposures, predictors, potential confounders, and effect modifiers. Give diagnostic criteria, if applicable (L172-195) |
| Data sources/ measurement | 8* | For each variable of interest, give sources of data and details of methods of assessment (measurement). Describe comparability of assessment methods if there is more than one group (L172-195) |
| Bias | 9 | Describe any efforts to address potential sources of bias (L201-210) |
| Study size | 10 | Explain how the study size was arrived at (L156-157) |
| Quantitative variables | 11 | Explain how quantitative variables were handled in the analyses. If applicable, describe which groupings were chosen and why (L198-210) |
| Statistical methods | 12 | (*a*) Describe all statistical methods, including those used to control for confounding (L198-210) |
| | | (*b*) Describe any methods used to examine subgroups and interactions (L198-210) |
| | | (*c*) Explain how missing data were addressed (not applicable, all children had complete data, L214-216) |
| | | (*d*) *Cross-sectional study*—If applicable, describe analytical methods taking account of sampling strategy (L201-210) |
| | | (*e*) Describe any sensitivity analyses (not applicable) |

Continued on next page

**Results**

| | | |
|---|---|---|
| Participants | 13* | (a) Report numbers of individuals at each stage of study—eg numbers potentially eligible, examined for eligibility, confirmed eligible, included in the study, completing follow-up, and analysed (L214-216) |
| | | (b) Give reasons for non-participation at each stage (not applicable) |
| | | (c) Consider use of a flow diagram (not applicable) |
| Descriptive data | 14* | (a) Give characteristics of study participants (eg demographic, clinical, social) and information on exposures and potential confounders (L218-233, Table 1) |
| | | (b) Indicate number of participants with missing data for each variable of interest (not applicable) |
| Outcome data | 15* | *Cross-sectional study*—Report numbers of outcome events or summary measures (L235-244, Table 2) |
| Main results | 16 | (*a*) Give unadjusted estimates and, if applicable, confounder-adjusted estimates and their precision (eg, 95% confidence interval). Make clear which confounders were adjusted for and why they were included (L246-252, Table 3, Figure 1) |
| | | (*b*) Report category boundaries when continuous variables were categorized (Table 3) |
| | | (*c*) If relevant, consider translating estimates of relative risk into absolute risk for a meaningful time period (not applicable) |
| Other analyses | 17 | Report other analyses done—eg analyses of subgroups and interactions, and sensitivity analyses (not applicable) |

**Discussion**

| | | |
|---|---|---|
| Key results | 18 | Summarise key results with reference to study objectives (L255-263) |
| Limitations | 19 | Discuss limitations of the study, taking into account sources of potential bias or imprecision. Discuss both direction and magnitude of any potential bias (L306-320) |
| Interpretation | 20 | Give a cautious overall interpretation of results considering objectives, limitations, multiplicity of analyses, results from similar studies, and other relevant evidence (L264-305, L322-335) |
| Generalisability | 21 | Discuss the generalisability (external validity) of the study results (L287ff., L316-320) |

**Other information**

| | | |
|---|---|---|
| Funding | 22 | Give the source of funding and the role of the funders for the present study and, if applicable, for the original study on which the present article is based (L347-351) |

Trials

## STUDY PROTOCOL

**Open Access**

# Effects of school-based physical activity and multi-micronutrient supplementation intervention on growth, health and well-being of schoolchildren in three African countries: the *KaziAfya* cluster randomised controlled trial protocol with a 2 × 2 factorial design

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## Abstract

**Background:** In low- and middle-income countries, infectious diseases remain a key public health issue. Additionally, non-communicable diseases are a rapidly growing public health problem that impose a considerable burden on population health. One way to address this dual disease burden, is to incorporate (lifestyle) health promotion measures within the education sector. In the planned study, we will (i) assess and compare physical activity, physical fitness, micronutrient status, body composition, infections with soil-transmitted helminths, *Schistosoma mansoni*, malaria, inflammatory and cardiovascular health risk markers, cognitive function, health-related quality of life, and sleep in schoolchildren in Côte d'Ivoire, South Africa and Tanzania. We will (ii) determine the bi- and multivariate associations between these variables and (iii) examine the effects of a school-based health intervention that consists of physical activity, multi-micronutrient supplementation, or both.

**Methods:** Assuming that no interaction occurs between the two interventions (physical activity and multi-micronutrient supplementation), the study is designed as a cluster-randomised, placebo-controlled trial with a 2 × 2 factorial design. Data will be obtained at three time points: at baseline and at 9 months and 21 months after the baseline assessment. In each country, 1320 primary schoolchildren from grades 1–4 will be recruited. In each school, classes will be randomly assigned to one of four interventions: (i) physical activity; (ii) multi-micronutrient supplementation; (iii) physical activity plus multi-micronutrient supplementation; and (iv) no intervention, which will serve as the control. A placebo product will be given to all children who do not receive multi-micronutrient supplementation. After obtaining written informed consent from the parents/guardians, the children will be

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(Continued from previous page)
subjected to anthropometric, clinical, parasitological and physiological assessments. Additionally, fitness tests will be performed, and children will be invited to wear an accelerometer device for 7 days to objectively assess their physical activity. Children infected with *S. mansoni* and soil-transmitted helminths will receive deworming drugs according to national policies. Health and nutrition education will be provided to the whole study population independently of the study arm allocation.

**Discussion:** The study builds on the experience and lessons of a previous study conducted in South Africa. It involves three African countries with different social-ecological contexts to investigate whether results are generalisable across the continent.

**Trial registration:** The study was registered on August 9, 2018, with ISRCTN. https://doi.org/10.1186/ISRCTN29534081.

**Keywords:** Children, Côte d'Ivoire, Dual disease burden, Health, Multi-micronutrient supplementation, Placebo, Physical activity, South Africa, Tanzania, Well-being

## Background

Ensuring healthy lives and promoting well-being among children is a complex and challenging endeavour. Indeed, children's health depends on cultural, environmental, genetic and socioeconomic factors as well as current living conditions and social and community networks [1]. In low- and middle-income countries (LMICs), infectious diseases remain a key public health issue, negatively impacting children's physical and cognitive development [2]. For example, more than a billion people are infected with parasitic worms (helminths) [3]. Helminth infections can cause abdominal pain, anaemia and (bloody) diarrhoea and might impair cognitive and physical development [4], resulting in reduced fitness and work productivity [5]. Moreover, helminth infections can have a negative impact on a child's nutritional status [6]. A deprived socioeconomic status (SES) of parents can put children at risk of malnutrition and growth retardation. Malnutrition has been found to be associated with stunting and poor cognitive development, thereby resulting in low IQ, cognitive delays and problems with motor development. This, in turn, can cause problems with a child's ability to concentrate, process information and focus on school work [7]. Children from low SES families are also less likely to have access to health care or health insurance and are more prone to be absent from school, which may have negative consequences on their academic performance. These deficiencies can prevent school-aged children from realising their full potential and perpetuate a vicious cycle of poverty and poor health.

Non-communicable diseases (NCDs) are a rapidly growing public health problem that impose a considerable burden on population health [8]. New research revealed that African populations have moved towards a disease profile similar to Western countries, with increasing proportions of deaths attributed to chronic, lifestyle-related diseases [9] and overweight, replacing undernutrition as a risk factor [2, 10]. Consequently, children are at an increased risk of compromised health due to a dual burden of diseases, which may hamper their development and well-being [8, 11]. The drivers of this dual burden may relate to the shift in dietary consumption patterns and energy expenditure as these countries pass through rapid nutritional and epidemiological changes. This trend might also result from the effect that underlying micronutrient deficiencies have on childhood stunting and changes in body composition, leading to greater adiposity and possibly contributing to long-term risks of obesity [12–15]. This dual burden constitutes a challenge for health systems in Africa and elsewhere. Although many children are still affected by infectious diseases, at a young age, they may already have developed risk factors predisposing them to NCDs in early adulthood [16, 17].

Given that (i) childhood physical inactivity is an independent risk factor for NCDs, which can lead to poor health outcomes in later life [18–20] and that (ii) micronutrient status influences health and body composition and subsequently the development of obesity and obesity-related conditions [21], one plausible strategy is to focus on the promotion of physical activity and multi-micronutrient supplementation through school-based health promotion programmes. School-based physical activity interventions are worthwhile because a considerable amount of children's daily physical activity is acquired during school hours [22]. Moreover, school-based physical activity programmes are generally effective in increasing physical activity and physical fitness in children and adolescents aged 6–18 years [23]. For instance, a randomised controlled trial with Swiss primary schoolchildren (first and fifth graders) showed that physical activity and fitness could be significantly improved with a 1-year, school-based, physical activity intervention, whereas adiposity could be decreased [24].

An attempt to increase health literacy in South African children from disadvantaged schools was undertaken in

the 'Disease, Activity and Schoolchildren's Health' (DASH) project [3]. The study primarily focused on the development of healthy school environments by implementing a series of clearly defined and standardised intramural measures. The developed intervention toolkit was pilot-tested among fourth graders in disadvantaged primary schools in Port Elizabeth, South Africa. The preliminary findings suggest that (i) the prevalence of parasitic worm infection was high in several schools [25]; (ii) children infected with soil-transmitted helminths had lower maximal oxygen uptake compared to their non-infected peers [26]; (iii) helminth infections and low physical fitness were significant predictors of low selective attention and poor academic achievement [27]; (iv) increased levels of physical activity were associated with a higher health-related quality of life (HRQoL) [28]; (v) increased physical activity levels were associated with lower risks of obesity and hypertension, but increased risk for soil-transmitted helminth infections [29]; and (vi) the DASH physical activity component resulted in small but significant decreases in the body mass index (BMI) of the children [30] and had a positive impact on academic performance within one year [31]. The intervention teaching material used in the DASH study was further elaborated and adapted and then pilot-tested among schoolchildren in grades 4–7 from two disadvantaged primary schools in Port Elizabeth. After the pilot-testing, final adaptations were made according to the feedback of school teachers, teacher-coaches and students.

The present study will build on and expand DASH by implementing this school-based health promotion programme in two other African countries and by providing multi-micronutrient supplementation. Placing an additional emphasis on multi-micronutrient supplementation is justified on multiple grounds. First, a recent meta-analysis concluded that helminth infections and micronutrient deficiencies are highly prevalent in LMICs. Second, a strong relationship exists between helminth infections and serum retinol in school-aged children. Third, micronutrient-supplementation randomised controlled trials (RCTs) showed a modest, but significant protective effect on helminth infection and reinfection rates [32]. Fourth, periodic deworming has not translated into the expected health gains according to recent systematic reviews and meta-analyses [33, 34]. One reason for this may be the concurrent problem of malnutrition and underlying micronutrient deficiencies, which makes it difficult for children to compensate for delays in growth and development that resulted from infectious diseases. Thus, multi-micronutrient supplementation might make deworming more effective in terms of a positive health impact. Fifth, as shown in previous studies [35, 36], multi-micronutrient supplementation might

result in a decrease in fat mass and increased lean body mass.

## Specific aims of the study

There are three interrelated specific aims that will be addressed in the planned study:

- To assess and compare physical activity, physical fitness, micronutrient status, body composition, infections with soil-transmitted helminths, *S. mansoni*, malaria, inflammatory and cardiovascular health risk markers, cognitive function, HRQoL, and sleep in schoolchildren in Côte d'Ivoire, South Africa and Tanzania.
- To determine the bi- and multivariate associations that exist among physical activity, physical fitness, micronutrient status, body composition, infections with soil-transmitted helminths, *S. mansoni*, malaria, inflammatory and cardiovascular health risk markers, cognitive function, HRQoL, and sleep in schoolchildren in the three study countries.
- To examine the effects of a school-based health intervention (physical activity, multi-micronutrient supplementation or both) on physical activity, physical fitness, micronutrient status, body composition, infections with soil-transmitted helminths, *S. mansoni*, malaria, inflammatory and cardiovascular health risk markers, cognitive function, HRQoL, and sleep in schoolchildren in the three study countries.

Additionally, secondary aims are to determine how the intervention is perceived by the school principals, teachers, parents and children, and whether or not the teachers can be empowered to implement the teaching material successfully by themselves. The study also has the potential for highlighting how micronutrient deficiencies may contribute to the observed increases in obesity prevalence, which is on the rise in some African countries.

Specific hypotheses for each of the outcome variables were formulated, hereby taking into account existing evidence from previous studies, mostly carried out with children living in Western societies. These hypotheses are described in detail in the supplementary material (see Additional file 1).

## Methods/Design
### Study design

We assume that no interaction occurs between the two interventions (physical activity and multi-micronutrient supplementation). The study is designed as a cluster randomised, placebo-controlled trial [37] using a $2 \times 2$ factorial design to assess the effect of physical activity and multi-micronutrient supplementation on children's

growth, health and well-being (Fig. 1). Data will be assessed at three time points (baseline and 9 months and 21 months after the baseline assessment). At baseline, children from grades 1–4 (most of them aged 6–10 years) will be recruited. The intervention will span two school years. The project officially started in January 2018. After 9 months of preparation and obtaining ethical approval from the relevant authorities, interventions have been launched in 2019. The project will last until December 2021 (see SPIRIT Flow Chart in Additional file 2).

The study will be carried out in primary schools in the area of Taabo in south-central Côte d'Ivoire, in Port Elizabeth in the Eastern Cape Province of South Africa, and in Ifakara in the Kilombero district of Tanzania. To obtain an adequate sample size for overweight/obese children, the intervention will not take place in the most remote areas but rather in villages that are located in rural (Côte d'Ivoire and Tanzania) or peri-urban (South Africa) settings in the three study countries.

In each country, we aim to recruit 1320 children from public school classes. Classes will be randomly assigned to one of four interventions (for more details, see the next section). To ensure allocation concealment, the four treatment arms will be determined by a computer-generated code after the baseline assessment. The four intervention arms are (i) physical activity; (ii) multi-micronutrient supplementation; (iii) physical activity plus multi-micronutrient supplementation; and (iv) no specific interventions, which will serve as the control. Based on the four intervention arms, the main and inter-action effects of the two intervention components (physical activity and multi-micronutrient supplementation) will be examined.

We will provide a placebo product to all children who do not receive multi-micronutrient supplementation. For physical activity, controlling with a placebo is not feasible. Thus, classes not involved in the physical activity intervention will follow their routine lesson plans so that, during the intervention phase, all students will have similar amounts of contact with schoolmates and teachers. To minimise subjective biases, teachers and local study personnel will be blinded with regard to the multi-micronutrient supplementation or placebo tablets.

In case of missing values (e.g. when children drop out during the intervention), all analyses will be performed



**Fig. 1** Overview of the planned study design (*Note*. MMNS = multi-micronutrient supplementation; PA = physical activity; STH = soil-transmitted helminth; T2 = 9-month follow-up; T3 = 21-month follow-up). All children receive health and nutritional education during the 2-year study period 4

with and without intention-to-treat [38]. After a thorough dropout analysis, a decision will be made on the most suitable method to use to analyse the intention-to-treat effects (e.g. imputation of missing values) [39].

### Participants and procedures

All children will be recruited in the schools involved in the project. School authorities will be contacted first. Contact with schools is made through the school principals. School principals will be informed about the objectives, procedures and potential risks and benefits of the study. Based on this information, the principals can state their interest in being part of the project.

To achieve at least small effects (f = 0.10) in the primary outcomes (physical activity and micronutrient deficiency) and to take into account the children's weight status (underweight, normal weight, or overweight/obese), power calculations indicate that a total sample of 1096 children is needed per study site (calculations based on G*power 3.1: alpha error probability = 0.05, power = 0.80, number of groups = 12 (2x2x3: physical activity intervention: yes/no, multi-micronutrient supplementation: yes/no; weight status: underweight, normal weight, or overweight), number of measurements = 3). Assuming a yearly dropout-rate of 10%, the targeted sample size is 1320 children per country at baseline. Thus, in each country, approximately 330 students will be assigned to one of the four intervention arms.

After having identified suitable schools, we will proceed in two steps to allocate classes to the four intervention arms. First, we develop a stratification table to ensure that each intervention arm will be represented in every grade across all schools. We then randomly assign the schools to the different strata, as shown in Fig. 2. If a school has several classes per grade, the classes will be randomly selected. Given that our intervention takes place in a school setting, is implemented during official class hours, is integrated in the current curriculum, and is provided by the class teachers, we have no choice other than to randomise the participants in clusters

(classes). A division of classes (in order to allow individual randomisation) would affect the everyday school life in a negative way and would be considered interruptive by the teacher staff. As a consequence, our intervention would not be acceptable to the school principals and education authorities.

Before baseline data assessment, written informed consent will be sought from the parents/guardians of the children. In line with the Ottawa Statement [40], despite cluster randomisation, we will ask each research participant and his/her parents guardians for informed consent before the children and their parents guardians know to which cluster they will be allocated. Research assistants will explain the purpose and procedures of the study, the expected duration, potential risks and benefits and any discomfort it may entail for the children. The parents/guardians will be provided with a participant information sheet and a consent form describing the study and providing sufficient information to make an informed decision about whether or not to participate. For illiterate parents, the information sheet will be read aloud and, if necessary, an oral translation of the information sheet into any of the local languages will be provided. Parents/guardians will be informed that participation is voluntary, data will be handled confidentially, and withdrawal from the study can occur anytime without further obligation, and that withdrawal of consent will not have any negative consequences except forgoing the potential benefits of the allocated intervention. After having obtained written informed consent, the parents/guardians will be asked a few specific questions regarding the families' SES, and the dietary intake information, sleep and physical activity behaviour of the children. Additionally, oral assent will be sought from children before the start of the study.

### Criteria for inclusion/exclusion

School eligibility criteria include (i) public schools from disadvantaged areas; (ii) facilities for the implementation of physical education lessons (free space: lawn, sand or



| | School 1 | School 2 | School 3 | School 4 |
|---|---|---|---|---|
| **Grade 1** | PA | Control | PA + MMNS | MMNS |
| **Grade 2** | MMNS | PA | Control | PA + MMNS |
| **Grade 3** | PA + MMNS | MMNS | PA | Control |
| **Grade 4** | Control | PA + MMNS | MMNS | PA |

**Fig. 2** Assignment of classes to conditions via stratification to ensure that each intervention arm is represented at each grade across all schools (*Note*. PA = physical activity; MMNS = multi-micronutrient supplementation)

concrete ground); and (iii) not engaged in any other re-search project or clinical trial or located in areas where governmental nutrition interventions take place.

To be included in the data assessment, children must accomplish the following inclusion criteria: (i) attend grades 1–4 at baseline; (ii) aged 6–12 years at baseline; (iii) have written informed consent from their parents/guardians; (iv) not participating in other research pro-jects or clinical trials; (v) not participating in any food/nutritional programme; and (vi) not suffering from clin-ical conditions that prevent participation in physical ac-tivity, as determined by qualified medical personnel.

Children will be excluded from data analyses if (i) they have a congenital or acquired alteration of the gastro-intestinal tract, which could impair absorption of the multi-micronutrient supplements; (ii) they participated in food/nutritional programmes in the past 6 months, and hence, received regular vitamin and mineral supple-ments; (iii) their parents/guardians did not provide writ-ten informed consent; and (iv) the children denied oral assent.

Since some parts of the intervention are integrated in the compulsory school curriculum, children will be auto-matically exposed to physical activity and health/nutri-tion education intervention. In fact, physical education is foreseen in the curriculum in all three study countries, not as a separate subject but as part of other (broader) subjects. For instance, in South Africa, physical educa-tion is part of the 'life orientation' subject. Nevertheless, physical education is not implemented as it should be in any of the involved countries. Typically, this slot is used to increase learning time for academic subjects. There-fore, our intervention aims to ensure that the time allo-cated to physical education in the curriculum is used appropriately and filled with meaningful content.

In the event that qualified medical personnel identify children during the baseline data assessment as having a clinical condition that prevents their participation in physical activity, we will contact the school principal and suggest that these children be removed from the physical activity intervention. Moreover, as multi-micronutrient supplementation and deworming are additional compo-nents, children and their parents/guardians who did not provide informed consent can decide whether or not the child should participate in the multi-micronutrient sup-plementation and/or deworming. Finally, all children have the possibility to withdraw from the study anytime without consequences from these two additional components.

### Intervention and control conditions

Participants allocated to the physical activity interven-tion will receive the so-called *KaziKidz* physical activity component (see: www.kazibantu.org). Of note, regular physical activity opportunities are incorporated into the main school curriculum, including daily in-class activity breaks as well as one weekly 40-min playful physical education lesson and one 40-min moving-to-music lesson. These measures are designed towards improving children's physical activity levels and positively affecting their school satisfaction and psychosocial well-being. The intervention materials presented above were pilot-tested in 2015 and 2016 in the DASH study with fourth grade children from disadvantaged schools in Port Eliza-beth, South Africa. Qualitative data revealed that the physical activity materials were well received at the pilot schools.

Participants allocated to the multi-micronutrient sup-plementation condition will receive a daily chewable tablet containing vitamins and trace elements. The multi-micronutrient supplement is provided free of charge by DSM Nutritional Products Ltd. (Basel, Switzerland; see: www.dsm.com). The exact composition of the multi-micronutrient supplement is summarised in Table 1. During school days, the supplement is taken at schools under the direct supervision of a teacher. To avoid the risk that supplements are exchanged between students or given to other family members during week-ends or public holidays, no supplements will be provided on non-school days.

Participants allocated to the physical activity plus multi-micronutrient supplementation condition will re-ceive both intervention measures, as described above. Children who do not receive multi-micronutrient sup-plementation (physical activity only and control condi-tions) will receive a placebo product. Thus, during school days, they will receive a daily chewing tablet, similar in taste and appearance to the multi-micronutrient supplementation and administered to the children from identical packages. The tablet will not contain any macronutrients or micronutrients, except sugar, citric acid, water and artificial flavour (orange) to mask the taste and to ensure similar appearance.

### Complementary interventions for all participating children

Independently of the study arm allocation, children diag-nosed with helminth infections will receive deworming medication after each data assessment. The treatment strategy chosen for each school will follow national and international guidelines, including recommendations by the World Health Organization (WHO) [41]. In brief, in schools where the infection prevalence of soil-transmitted helminths is below 20%, infected children will be treated individually; in schools where the infec-tion prevalence of soil-transmitted helminths is between 20% and 50%, all children will be treated once a year; and, finally, in schools were the prevalence of soil-

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**Table 1** Composition of the multi-micronutrient supplement

| No. | Nutrient | Average per 1 tablet |
| --- | --- | --- |
| 1 | β-carotene (as BetTab 20%S) | 3.6 mg |
| 2 | Vitamin D | 400 IU/10 mcg |
| 3 | Vitamin E | 9 mg TE |
| 4 | Vitamin K | 30 mcg |
| 5 | Vitamin C | 60 mg |
| 6 | Vitamin B1 Thiamine | 1.1 mg |
| 7 | Vitamin B2 Riboflavin | 1.3 mg |
| 8 | Vitamin B6 Pyriodoxine | 0.5 mg |
| 9 | Vitamin B12 | 1.2 mcg |
| 10 | Folic acid | 200 mcg |
| 11 | Niacinamide | 8 mg |
| 12 | Iron (added as Fe-EDTA) | 8 mg |
| 13 | Zinc (added as zinc oxide) | 5 mg |
| 14 | Selenium (added as sodium selenite anhydrous) | 20 mcg |
| 15 | Iodine (added as potassium iodate) | 100 mcg |

*Notes.* Ingredients 1–15 are nutrients and will be produced with an overage to ensure required amounts during the shelf life
Inactive ingredients: sugar, citric acid, sorbitol, non-nutritive sweetener, flavour
Placebo tablets do not contain nutrients, but colorants will be added
Dosage and directions: one tablet daily during the first school lesson
The tablets will be stored in a climate-controlled storage room at the respective research institution and will be provided to school on a fortnightly basis. At school, the tablets will be stored in locked cupboards in the teachers' room

transmitted helminth infection is 50% and above, mass treatment will be carried out twice a year. We will either administer a single 400-mg oral dose of albendazole or a single 500-mg oral dose of mebendazole. Additionally, children infected with *S. mansoni* will receive praziquantel (single 40 mg/kg oral dose).

All children will additionally benefit from health and nutritional education lessons. The developed *KaziKidz* teaching material includes a series of classroom-based health education lessons to increase awareness of helminth infections, how to prevent helminth infections (e.g. hygiene behaviour, adequate sanitation habits and the importance of consuming clean water and healthy food). Likewise, nutritional education lessons aim at highlighting the importance of healthy nutrition.

### Education and training of the teachers
The initial experiences in the DASH study showed that teachers needed considerable support for the implementation of the physical education lessons. In the planned study, during the initial phase, the intervention will be carried out in close collaboration among the teacher-coach, teachers, and school staff to allow sufficient time for capacity building and to empower schools to incorporate the programme in their given structures and to implement the programme with their own resources. Hence, teachers will be assisted by a teacher-coach during the first year of the intervention, whereas during the second year, the physical activity intervention will be

carried out by the teachers themselves without external assistance.

### Monitoring of compliance with intervention implementation
One of the specific aims of the study is to obtain new insights regarding the possibility to empower schools to implement sustainable health-promotion measures, including an appraisal of the implementation quality of health-promotion measures, if carried out under the direction of the schools themselves. Using both quantitative and qualitative research methods, such as the systematic observation of lessons; (semi-)structured interviews with school principals and parents; and focus group discussions (FGDs) with teachers, teaching staff members, and children, the study will shed light on the quality of the implementation and sustainability of the proposed health promotion measures, as well as factors affecting the quality of the implementation and the sustainability of the programme. Thereof, important insights will result with regard to the training of the teachers and school staff, as well as the support needed by the schools to incorporate health-promotion measures in the existing structures.

### Data collection and measures
Data collection will take place in the schools. Data assessment procedures will be based on a series of standardised, validated and quality-controlled tools. The same

methods will be employed at each measuring occasion (except for the parent questionnaires, which will only take place during the baseline assessment). The collected data will be double-entered and validated using EpiData (version 3.1) and merged into a single database.

The following parameters are defined as equivalent primary outcomes: (i) physical activity (7-day accelerometry), and (ii) multi-micronutrient status (vitamin A, vitamin D, transferrin and zinc). In our study, physical activity is assessed via self-reports and 7-day accelerometry. Although both methods have advantages and disadvantages [42, 43], many researchers consider accelerometer-derived data as the most appropriate way to validly assess physical activity [44]. This particularly applies to children, where the assessment of self-reported physical activity is complicated by the fact that some cognitive functions are not yet fully developed. Multi-micronutrient status, per definition, is a multi-dimensional construct. Given that our supplement contains several (nutritional) ingredients, one can expect that the product will be effective in terms of multiple outcomes. As highlighted by Bailey et al. [45], iron, iodine, folate, vitamin A and zinc deficiencies are the most widespread multi-micronutrient deficiencies and are common contributors towards poor growth, intellectual impairment, and increased risk of morbidity. Making a decision about whether one nutritional indicator is more important than another is arbitrary and cannot be convincingly substantiated. Therefore, we consider vitamin A, vitamin D, transferrin and zinc as equivalent primary outcomes.

Table 2 provides an overview of all types of data to be collected and the specific parameters that will be assessed either as primary outcomes, secondary outcomes, moderators or control variables.

### Clinical examination

A research assistant will assess detailed disease history in a face-to-face interview. Features of disease history will focus on fevers, abdominal pain, change in bowel movements, diabetes, and psychosomatic symptoms. Additionally, a qualified nurse will conduct an abdominal examination.

For the detection of hypertension, the blood pressure of each child will be taken three times after the child has been resting for approximately 5 min, with a 1-min rest in between the assessments. An Omron M3® digital blood pressure monitor (Omron Healthcare Europe; Hoofddorp, The Netherlands) will be used. A cuff size appropriate to the arm circumference of the child will be chosen.

### Blood testing

Capillary blood will be collected for haematological analyses. The child's finger will be pricked once (or if

**Table 2** Overview of parameters assessed in the planned study

Clinical examinations
  Disease history of children and parents/guardians
  Subjective health complaints (15 items)
  Blood pressure (SBP, DBP)
Blood testing
  Haemoglobin concentration (Hb)
  Blood lipids (TC, HDL-C, LDL-C, TG, Non-HDL, C-HDL ratio)
  Blood glucose (HbA1c)
  Micronutrient status (vitamin A, vitamin D, zinc, transferrin)
  Cytokines (IL-6)
  Leptin
Anthropometric measurements
  Body weight and height
  Body composition (body fat)
  Waist-to-hip ratio
  Body mass index
Parasitological examinations
  Soil-transmitted helminths (*Ascaris lumbricoides*, hookworm, *Trichuris trichiura*)
  *Schistosoma mansoni*
Cognitive function and academic performance
  Flanker task (executive function)
  School grades
Student survey
  Socio-demographic background (sex, ethnicity, home language)
  Self-reported physical activity
  Health-related quality of life (KIDSCREEN-10)
  Perceived stress (1 item)
  School satisfaction (1 item)
  Perceived academic competence (1 item)
  Sleep (6 items)
Objective assessment of physical activity
  7-day actigraphy
Fitness testing
  20-m shuttle run test (cardiorespiratory fitness)
  Grip strength test (upper body strength)
Parental survey
  Family socioeconomic status
  Dietary intake information (food frequency questionnaire)
  Food insecurity
  Sleep
Other variables
  Country, school, grade, class, distance/traveling to/from school

necessary twice) to collect approximately 10 blood drops. Haemoglobin (Hb), blood lipid and blood glucose analyses will be performed on the spot (further details are provided below), with rapid finger prick malaria tests being done in Côte d'Ivoire and Tanzania. All tests will be performed with the same measurement devices and are carried out by trained research assistants. Testing cassettes will be disposed immediately after completion of the analyses.

**Haemoglobin** For the detection of anaemia, Hb concentration will be measured once with a HemoCue® Hb 301 system according to the manufacturer's instructions (HemoCue AB; Ängelholm, Sweden).

**Blood glucose** For the measurement of glycated haemoglobin (HbA1c) level, a point-of-care (POC) instrument employing the Afinion test (Alere Technologies, Abbott; Wädenswil, Switzerland) will be used. Notably, the HbA1c level reflects the average plasma glucose concentration levels over the previous 8–12 weeks before measurement with no prior fasting required.

**Blood lipids** For the assessment of blood lipid profiles (total cholesterol (TC), low-density-lipoprotein cholesterol (LDL-C), high-density-lipoprotein cholesterol (HDL-C) and triglycerides (TG), capillary samples for blood lipid will be analysed by the Afinion test (Alere Technologies, Abbott; Wädenswil, Switzerland). One drop of blood will be taken up by the test strip and read by the machine. Children will be instructed to fast during the 3 h prior to the data assessment.

**Micronutrient status, inflammatory cytokines and leptin** The finger prick technique will also be used to prepare dried blood spots to assess children's micronutrient status/deficiencies (concentrations of vitamin A, vitamin D, zinc and transferrin) and to determine the inflammatory cytokine (IL-6) and leptin concentrations. Blood drops will be collected on a filter paper for further examination in a specialised laboratory. Dried blood spot samples on the filter paper will be shipped to the Global Clinical and Viral Laboratory (Durban, South Africa). The laboratory was involved in the WHO SAGE study [46] and is a partner institution of the Global Health Biomarker Lab at the University of Oregon, USA.

### Anthropometric measurements

**Body weight, body height and body composition** Body composition will be assessed via bioelectrical impedance analysis (BIA) with a wireless body composition monitor (Tanita MC-580, Tanita Corp.; Tokyo, Japan). The participants will be asked to fast for 3 h before the data

assessment, to void their bladder immediately before the assessment, and to wear only light sport clothing (≤1 kg). Participants will be asked to stand barefoot on the metal plates of the machine, while being guided by the research assistant to ensure optimal contact according to the device manufacturer's instructions. The MC-580 is also able to assess body weight, which will be measured to the nearest 0.1 kg. With shoes off, each child will stand against a stadiometer with the back erect and shoulders relaxed. Body height will be taken to the nearest 0.1 cm. Sex-specific height or length-for-age and weight-for-age $z$ scores will be computed from the CDC/WHO growth reference data [47].

### Parasitological examinations

For parasitological examinations, a researcher will visit the schools and distribute pre-labelled plastic containers to each class for the children to take home and use to collect a stool sample. These plastic containers will be returned to the research assistant in the morning of the following day. Further visits may be required to catch up with absent children. To reduce discomfort, paper bags are provided along with the containers. All stool samples will be processed on the day of collection at the study site.

Parasitic infections to be detected with the Kato-Katz technique [48] include the three main soil-transmitted helminths (*Ascaris lumbricoides*, hookworm and *Trichuris trichiura*), and *Schistosoma mansoni*. In brief, stool samples (at least 10–15 g) will first be visually examined for the presence of blood, mucus and diarrhoea. Second, duplicate 41.7 mg Kato-Katz thick smears will be prepared from each stool sample [48]. For quality control, a random sample of 10% of the Kato-Katz slides will be re-examined by a senior technician. In case of discordant results, the slides will be read a third time, and the results will be discussed among the technicians until agreement has been reached [49, 50]. Parasitological status will be established in terms of prevalence and intensity of infection with individual helminth species, and the extent of multiparasitism will be determined.

### Cognitive function and academic performance

**Executive function** Inhibitory control is a core component of executive function and will be assessed with a computer-based version of the Flanker task [51], a standardised psychological test [52] suitable for repeated measures [53]. The task requires participants to respond to the direction of a centrally presented target stimulus, while flanking stimuli are facing in the same (congruent trials) or opposite direction (incongruent trials). Performance is assessed by calculating the mean reaction

time for correct responses as well as mean accuracy separately for different trial types. Congruent trials are a measure of basic processing speed and attention, whereas incongruent trials assess selective attention and inhibitory control.

**Academic performance** In cooperation with the schools, the end of year marks will be obtained from the following subjects: school/home language, first additional language, mathematics and life orientation. The sum-score of the four subjects will be used to estimate a child's overall academic achievement.

### Student survey

**Self-reported physical activity** Two self-report instruments will be used to assess physical activity in the present study [42, 54]. The first instrument is a single-item tool taken from the HBSC survey. The exact wording of this item is as follows: 'Physical activity is any activity that increases your heart rate and makes you get out of breath some of the time. Over the past 7 days, on how many days did you engage in such activity?' Answering options range from 1 to 7 days [55]. A similar item has been used in previous research enrolling children [56–58], including the DASH study in South Africa [28].

The second instrument is the Physical Activity Questionnaire for Children (PAQ-C), a 9-item instrument specifically designed for school-aged children [59]. Due to the limited age of our sample and time constraints, only five items will be included. The PAQ-C consists of a 7-day recall that provides a summary physical activity score derived from several items, which are each scored on a 5-point Likert-scale (from 1 to 5). Items included in the present study refer to physical activity accumulated during physical education, recess, after school, in the evening and on weekends. Previous research has shown that the PAQ-C has acceptable reliability and convergent validity [60–62]. Furthermore, cut-off values have been established which are suitable to distinguish between those children who accomplish the recommended levels of physical activity (≥60 min of moderate-to-vigorous physical activity (MVPA) per day) and children who do not meet these criteria [63].

**Health-related quality of life** The KIDSCREEN-10 will be used to assess children's HRQoL. The KIDSCREEN-10 proved to be a valid instrument to assess the psychosocial health of children aged 8–18 years in different countries [64]. The construct validity of the KIDSCREEN instrument has been documented in an African context [65]. Moreover, we have used the KIDSCREEN in the DASH study, where we found satisfactory

psychometric properties of this instrument [28]. The KIDSCREEN-10 consists of 10 items, which can be used to build an overall HRQoL index. Following recommended procedures, item scores first will be summed up to obtain raw scores and then will be transformed into Rasch person parameter estimates using the available SPSS software version syntax for each dimension [66]. These steps will result in T-values with a scale mean of 50 and a standard deviation (SD) of 10. Higher mean scores generally reflect higher HRQoL. To be classified as 'normal', the threshold chosen by the KIDSCREEN developers was the mean, plus or minus half a SD. The KIDSCREEN scores can be compared with the norm scores of an international survey sample of 5754 European children, stratified by sex.

**Stress, school satisfaction and perceived academic competence** Perceived school-related stress, satisfaction with school and perceived academic competence will be assessed with three items from the HBSC survey. The stress measure has been used previously to show evidence for the stress-buffering effects of physical activity in European youngsters [58]. To assess school-related stress, students are asked how pressured they feel by the schoolwork they must pursue. Possible answers are as follows: not at all, a little, some, and a lot. To measure school satisfaction, students respond to the question of how they feel about school at present. Possible answers are as follows: 'I like it a lot', 'I like it a bit', 'I don't like it very much', and 'I don't like it at all'. Finally, perceived academic performance is assessed with the following question: 'In your opinion, what does your class teacher(s) think about your school performance compared to your classmates?' Possible answers are as follows: 'much better than classmates', 'better than classmates', 'similar/same as classmates', and 'worse than classmates'.

**Sleep** To assess sleep quality, questions from the Pittsburgh Sleep Quality Index (PSQI) [67] will be adapted. To screen for sleep disturbances, the three items of the Insomnia Severity Index [68] will ask about difficulty falling asleep, staying asleep and waking up too early in the morning. Evidence for the reliability and validity of this measure has been provided previously [69]. To assess further information about sleep quality and daytime functioning, children will be invited to rate their overall sleep quality and to report how restored they feel in the morning, how tired they feel during the day and how exhausted they feel in the evening. Children will also provide basic information about their sleep environment (e.g. room, type of bed, and people sleeping in the same room/bed).

*Actigraphy*

Objective physical activity will be assessed with an accelerometer device (Actigraph wGT3x-BT; Shalimar, FL, USA). The devices will be worn around the hip for 7 consecutive days to assess a full week, with a sampling epoch of 15 s [70]. Time per day spent in moderate physical activity (MPA; ≥3 metabolic equivalents of task (MET)) and vigorous physical activity (VPA; ≥6 MET) will be determined based on the raw accelerometry counts and the ActiLife® computer software (Actigraph; Shalimar, FL, USA), with cut-off values derived from Freedson et al. [71]. Of note, the ActiGraph accelerometers have been validated for children [72, 73].

*Fitness testing*

**Cardiorespiratory fitness** The children's cardiorespiratory fitness will be measured with the 20-m shuttle run test [74], which is part of the Eurofit fitness testing battery [75]. Before the start of the test, all children will be told to indicate any body discomfort and anyone who feels unwell or uncomfortable will not take part in the test. The pre-recorded sound signals will be played to the children, and they will be able to do a trial run of 2 intervals (40 m) under the supervision of a research staff member. Once children are familiar with the test procedures, they will be asked to run back and forth on the 20-m flat course (marked with colour-coded cones) in groups of 10–15 children, following the pace of the sound signals. Starting with a running speed of 8.5 km/h, the frequency of the signal increases every minute by 0.5 km/h. When a child fails to follow the pace in two consecutive intervals, the stage and the distance completed fully will be recorded. The age of the participating child and the speed at which the child stopped running will be converted into $VO_2$ max estimates.

**Upper body strength** Upper body strength will be determined with the grip strength test, with both right and left hands. The Saehan hydraulic hand dynamometer (MSD Europe BVBA; Tisselt, Belgium) will be used for this test. Before the start of the test, the hand span (distance from the tip of the thumb to the tip of the little finger) of the child's dominant hand will be measured (to the nearest 0.5 cm), and the grip span on the dynamometer will be adjusted accordingly [76, 77]. The outdoor staff will explain to the child how to hold the dynamometer correctly. The child, while sitting in an upright position, should grip the dynamometer with the arm fully extended. During this time, no other parts of the body should touch the dynamometer, and the arm being tested may not be squeezed against the body. Each child will then have three tries, or six tries in total (with a 30-s rest in between) to grip the dynamometer as hard

as possible with alternating hands. The maximum reading, measured to the nearest 1 kg, will be recorded.

*Parental survey*

**Dietary intake information** Information on dietary intake will be obtained from the parents/guardians to determine the adequacy of child's intake of macro- and micronutrients. Dietary intake of children will be assessed using a culturally sensitive food frequency questionnaire (FFQ) for each country [78, 79]. The FFQ will be administered to the child's carer at baseline.

**Food insecurity** USAID (www.usaid.gov) defines food security as a situation in which all people at all times have physical and economic access to sufficient food to meet their dietary needs for a productive and healthy life. For the purpose of this study, food insecurity will be measured with a questionnaire based on the Household Food Insecurity Access Scale [80], which has been validated in industrialized countries and LMICs. For example, the study by Knueppel et al. [81] showed satisfactory validity and reliability among poor households in rural Tanzania.

**Sleep** To gather information about children's sleep duration, parents/guardians will report the time at which their child goes to bed and wakes up in the morning on school nights and school days.

**Socioeconomic status** To estimate the SES, parents/guardians will be asked to answer nine items, covering household-level living standards, such as infrastructure and housing characteristics (house type, number of bedrooms, type of toilet and access to indoor water, indoor toilet/bathroom and electricity) and questions related to the ownership of three durable assets (presence of a working refrigerator, washing machine and car). The dichotomized items (0 = poor quality, not available; 1 = high quality, available) will be summed up to build an overall SES index, with higher scores reflecting higher SES. The validity of similar measures was established in previous research [82].

**Distance from home to school** Distance from home to school and mode of traveling to/from school will be taken into consideration as a confounding variable. Therefore, we will ask the parents/guardians about their living place (township, village and area). Using Google maps, we will determine the distance between the residence and the school location.

## Statistical analyses

To assess the effects of the intervention, changes in outcome variables over the three time points will be analysed using repeated measures analyses of variances (ANCOVAs), with three between-subject factor groups (physical activity intervention: yes/no; multi-micronutrient intervention: yes/no; and weight status: underweight, normal weight, or overweight/obese) and a within-subject factor time (baseline, 9 months, and 21 months), after controlling for relevant confounders. To take into account the non-independence of the children within a class, we will examine between-cluster differences in potentially relevant confounders, which might include characteristics of the children (e.g. sex, socioeconomic status, and ethnicity), class (e.g. class size) and the intervention (e.g. compliance with intervention). If we find systematic and substantial differences between the clusters for these variables, the factors will be controlled as covariates. Moreover, we will screen the data for univariate and multivariate outliers before performing the main analyses in order to exclude students with unrealistically high or low values. In case of missing values (e.g. when participants drop out), all analyses will be performed with and without an intention-to-treat [38]. After a thorough dropout analysis, a decision will be reached regarding the best suited method to analyse intention-to-treat effects (e.g. imputation of missing values) [39]. If significant group or time interactions are present, Bonferroni-adjusted post-hoc tests will be performed to identify individual differences. Statistical significance level will be defined at an alpha level of 0.05. Effect size will be calculated according to the recommendations of Cohen [83], with $0.49 \geq d \geq 0.20$ indicating small (negligible practical importance), $0.79 \geq d \geq 0.50$ indicating medium (moderate practical importance) and $d \geq 0.80$ indicating large effects (crucial practical importance). Additionally, bivariate and multivariate relationships between study variables will be tested using correlational analyses, regression analyses or structural equation modelling.

## Ethical considerations

### Ethical approval and trial registration

The study will be carried out in accordance with the protocol and with principles in the current version of the Declaration of Helsinki and the guidelines of Good Clinical Practice (GCP) issued by the International Conference of Harmonisation (ICH).

The study can only begin once approval from the required authorities has been received. Any additional requirements imposed by the authorities shall be implemented. Ethical approval has been obtained from the 'Ethikkommission Nordwest- und Zentralschweiz' in Switzerland (EKNZ; reference number: Req-2018-00608). The intervention study has been registered in the ISRCTN registry (http://www.isrctn.com/ISRCTN29534081).

The responsible investigator at each site ensures that ethical approval from an appropriately constituted competent ethics committee (CEC) is sought for the clinical study. The study protocol has been approved in Côte d'Ivoire by the Institutional Review Board (IRB) of the Centre Suisse de Recherches Scientifiques en Côte d'Ivoire (CSRS; Abidjan, Côte d'Ivoire) and the Comité National d'Ethique et de la Recherche (CNER), reference number: 100–18/MSHP/CVESVS-km. Approval has also been obtained in South Africa from the research ethics committee of the Nelson Mandela University in Port Elizabeth (reference number: H18-HEA-HMS-006) and the Department of Education of the Eastern Cape Province. Moreover, the study protocol has been approved by the responsible ethics committee in Tanzania (Ifakara Health Institute (IHI–IRB), the National Institute for Medical Research (NIMR) and the Tanzania Food and Drugs Authority (TFDA).

The principal investigator (MG) and local project leaders (BB, CW, and HM) are allowed to amend the protocol or to provide suggestions for a protocol amendment. Should amendments to the approved protocol be required during the project, these would only be implemented after receiving the approval of the CEC.

### Right to privacy and confidentiality

The principal investigator and local project leaders affirm and uphold the principle of the participants' rights to privacy and that they will comply with applicable privacy laws. Anonymity of the participants will be guaranteed when presenting the data at national or international conferences or publishing key findings in the peer-reviewed literature. Individual participant medical information obtained as a result of this study will be considered confidential, and disclosure to third parties is prohibited. Participant confidentiality will be further ensured by utilizing participant identification code numbers to correspond to treatment data in the computer files. For data verification purposes, authorised representatives of the EKNZ and the respective Human Ethics Committee at site may require direct access to parts of the clinical records relevant to the study, including participants' medical histories.

### Referral to local clinics

Children who suffer from severe medical conditions and/or malnourishment (as diagnosed by a nurse, following national guidelines) will be referred to local clinics. If children are infected with malaria at the time of the data assessment, immediate treatment will be offered to the children.

### Incentive for schools

As an incentive for schools, schools will be equipped with basic sports equipment and a music centre that allows for the implementation of moving-to-music lessons. Moreover, in South Africa, physical activity-friendly school environments will be developed through the implementation of simple painted games to encourage the free play of children.

### Data processing and archiving

Data will be double-entered, checked, and merged into a single SPSS file. Survey data obtained from the paper and pencil questionnaire will be scanned and entered automatically (using EvaSys software). Data analysis will be performed with established software packages (e.g. SPSS, STATA and Mplus). SPSS syntax files will be documented to assure transparency of the conducted data analysis and to assure GCP.

Data will be saved electronically. Backup files will be stored regularly on the external cloud *Switchdrive@Universität Basel*. The personal data of the participants will be encrypted, and all the data obtained (e.g. handwritten paper questionnaires and the case report forms (CRFs)) will be used exclusively for scientific research. The local study leader will keep records in locked cupboards, and after 10 years, these records will be destroyed. Completed paper sheets will be locked and stored at the respective home institution. Electronic data files will be archived on the database *Switchdrive@Universität Basel*. Only authorised investigators will have access to data files. However, in line with the guidelines and open access policies of nationally and internationally recognised foundations and institutions, the published data from our project will be made publicly available (as Additional file 1).

### Storage of biological material

Dried blood spots will be collected on filter paper and stored in a freezer at the home institution. Due to the complexity of assaying cytokines in dried blood spot samples, all samples will be shipped to the Global Clinical and Viral Laboratory in Durban, South Africa. Thus, biological samples from Côte d'Ivoire and Tanzania will leave the country conditional to material transfer agreements. Dried blood spot analyses will be carried out soon thereafter at the laboratory. Samples will be destroyed upon completion of all required assays.

### Safety

Research assistants shall report all serious adverse reactions and adverse events (AEs) that might occur after deworming, finger pricking or multi-micronutrient supplementation immediately to the local study leader and principal investigator of the clinical trial. More specifically, during the entire duration of the study, all AEs and all serious adverse events (SAEs) will be collected, fully investigated and documented in source documents and CRFs. The study duration encompasses the time from when the first participant signs the informed consent until the last protocol-specific procedure has been completed, including a safety follow-up period. In case of any SAE, the participant's well-being will be followed up until he or she shows normal laboratory values or vital signs below alert. Project leaders at the site are obliged to document and report the process, independently of the participant's termination of study. Each research institution will ensure that the required insurance coverage is in place for the trial under applicable laws. In case of AEs or SAEs, unblinding of the participant is permissible.

### Data monitoring and publication of data

The trial steering committee (composed of the sponsor-investigator (MG), the director of the Swiss Tropical and Public Health Institute (JU), and the principal investigators of the three study countries (BB, CW, HM)) will coordinate data monitoring, interim analysis, and dissemination of the study results through presentations at national and international conferences and publications in primarily open-access peer-reviewed journals. The trial steering committee will decide which researchers (beyond those listed as co-authors in the current study protocol) will have access to the final trial dataset. In agreement with the other members of the trial steering committee, the sponsor–investigator has the right to terminate the study prematurely according to certain circumstances, including ethical concerns, insufficient participant recruitment, and safety issues. Additional reasons for study termination include alterations in accepted clinical practice that render the continuation of the trial unwise and evidence of benefit or harm of the experimental intervention. On request of the local ethical review boards, an independent data monitoring committee will be appointed. The data monitoring board will have access to the unblinded interim data and can make recommendations to the trial steering committee. Trial auditing is carried out at the end of each year, whereas annual financial reports will be audited by an external auditor in each study country.

At the end of the study, the results will be communicated to the respective health and education authorities in the study countries and to the involved schools. All intervention materials will be made available to the respective control groups, so that the whole community can benefit from this project. Workshops will be offered to all teachers at the involved schools to assist with the

implementation of the school-based health promotion programme.

### Schedule and milestones

Recruitment of participants started in Côte d'Ivoire on October 18, 2018. Follow-up assessments will be complete in March 2021. The project will end in December 2021. An approximate schedule is presented in Table 3.

### Discussion

This investigation has scientific relevance because it is among the first to explore the associations between objectively assessed physical activity/fitness, micronutrient deficiencies, body composition, infectious diseases, inflammatory and cardiovascular health risk markers and sleep indicators in schoolchildren in three African settings. Moreover, our study will contribute to a deeper understanding of the effects of a school-based intervention, combining physical activity and multi-micronutrient supplementation. The study will provide new insights in how schools can be empowered to implement school-based health promotion measures. Finally, as happens in many African countries, physical education and sport activities are not implemented as foreseen because academic subjects are considered more important, and this project could help to maintain or even increase the time allowed for physical activities in the schools. Time allocation is crucial along with empowerment of the actors.

Additionally, the study will provide important information about factors that may negatively affect the quality of the implementation of the intervention. Our study will also highlight the opportunities and challenges associated with capacity building in disadvantaged schools, to empower them to carry out health promotion measures with their own limited resources. Using accelerometer-based physical

activity data will allow a more valid estimate of the level of physical activity of African schoolchildren inside and outside the school and will provide new insights into how a physical activity intervention impacts schoolchildren's physical activity behaviour.

In addition, our research will provide a comprehensive update on the status of micronutrient deficiencies, inflammatory and cardiovascular health risk markers and communicable diseases in the selected communities in Port Elizabeth (South Africa), Ifakara (Tanzania), and Taabo (Côte d'Ivoire) that are at different stages of an epidemiological and nutrition transition. By linking these factors with cognitive and academic performance, children's subjective health perceptions, and their psychosocial well-being, this wealth of information will reveal the true health consequences associated with the potential dual burden of diseases and will provide guidance for further health interventions to be implemented among school-aged children in these settings and elsewhere.

Thus far, only a few trials have investigated the potential of multi-micronutrient supplementations in African children. While physical activity was offered as part of our initial DASH study, the data assessment was limited to urban townships in South Africa. Hence, the current study will provide additional relevant information on whether or not the findings can be generalized to rural or peri-urban areas and to other African countries.

### Trial status

The study protocol corresponds to the first version of the protocol, as submitted to the EKNZ on July 19, 2018. The recruitment will start on October 18, 2018, in Côte d'Ivoire, in January 2019 in South Africa and in July 2019 in Tanzania. Data assessment should be complete in June 2020 in Côte d'Ivoire,

**Table 3** Planned schedule and milestones

| Due date | Milestone title |
| --- | --- |
| April 30, 2018 | Establishing contacts with authorities and schools |
| June 30, 2018 | Development of intervention materials |
| December 31, 2018 | Completion of initial teacher training in all countries |
| March 31, 2019 | Completion of baseline (T1) data assessment in all countries |
| November 30, 2019 | Completion of T2 follow-up data assessment in all countries |
| November 30, 2019 | Completion of first year of intervention in all countries |
| November 30, 2020 | Completion of T3 follow-up data assessment in all countries |
| March 31, 2021 | Completion of second year of intervention in all countries |
| November 30, 2021 | Completion of presentation of results to local authorities |
| December 31, 2021 | Final report |

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in December 2020 in South Africa, and in March 2021 in Tanzania. Ethical approval has been obtained from the relevant review boards in Switzerland, Côte d'Ivoire, South Africa and Tanzania.

## Supplementary information

**Supplementary information** accompanies this paper at https://doi.org/10.1186/s13063-019-3883-5.

> **Additional file 1:** Hypotheses associated with each of the study's outcomes.
>
> **Additional file 2:** Spirit flow chart.

### Abbreviations
AE: adverse event; ANCOVA: analysis of covariance; BIA: bioelectrical impedance analysis; BMI: body mass index; CDC: Centers for Disease Control and Prevention; CEC: competent ethics committee; CNER: Comité National d'Ethique et de la Recherche in Côte d'Ivoire; CSRS: Centre Suisse de Recherches Scientifiques en Côte d'Ivoire; DASH: Disease, Activity and Schoolchildren's Health project; EKNZ: Ethikkommission Nordwest- und Zentralschweiz; FFQ: food frequency questionnaire; FGD: focus group discussion; GCP: good clinical practice; Hb: haemoglobin; HbA1c: glycated haemoglobin; HDL-C: high-density lipoprotein; HRQoL: health-related quality of life; ICH: International Conference of Harmonisation; IHI-IRB: Institutional Review Board of the Ifakara Health Institute; IL-6: human interleukin 6; ISRCTN: International Standard Randomized Controlled Trial Number; KIDSCREEN-10: 10-item instrument to assess health-related quality of life among children; LDL-C: low-density lipoprotein; LMICs: low- and middle-income countries; NCDs: non-communicable diseases; PAQ-C: physical activity questionnaire for children; POC: point-of-care; PSQI: Pittsburgh sleep quality index; RCT: randomized controlled trial; SAE: serious adverse event; SD: standard deviation; SES: socioeconomic status; SPSS: Statistical Package for the Social Sciences; STATA: statistics and data software; TC: total cholesterol; TFDA: Tanzania Food and Drugs Authority; TG: triglycerides; USAID: US Agency for International Development; WHO: World Health Organization

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### References
1. Dahlgren G, Whitehead M. Policies and strategies to promote social equity in health. Stockholm: Institute for Future Studies; 1991.
2. Murray CJL, Vos T, Lozano R, Naghavi M, Flagman AD, Michaud C, et al. Disability-adjusted life years (DALYs) for 291 diseases and injuries in 21 regions, 1990-2010: a systematic analysis for the Global Burden of Disease Study 2010. Lancet. 2012;380:2197–223.
3. Yap P, Müller I, Walter C, Seelig H, Gerber M, Steinmann P, et al. Disease, Activity and Schoolchildren's Health (DASH) in Port Elizabeth, South Africa: a study protocol. BMC Public Health. 2015;15:1285. https://doi.org/10.1186/s12889-015-2636-y.
4. Utzinger J, Becker SL, Knopp S, Blum J, Neumayr AL, Keiser J, et al. Neglected tropical diseases: diagnosis, clinical management, treatment and control. Swiss Med Wkly. 2012;142:w13727.
5. Yap P, Du ZW CR, Zhang LP, Wu FW, Wang J, Wang XZ, Zhou H, Zhou XN, Utzinger J, Steinmann P. Soil-transmitted helminth infections and physical fitness in school-aged Bulang children in southwest China: results from a cross-sectional survey. Parasit Vectors. 2012;5:50. https://doi.org/10.1186/756-3305-5-50.
6. Hürlimann E, Houngbedji CA, N'Dri PB, Bänninger D, Coulibaly JT, Yap P, et al. Effect of deworming on school-aged children's physical fitness, cognition and clinical parameters in a malaria-helminth co-endemic area of Côte d'Ivoire. BMC Infect Dis. 2014;14:411. https://doi.org/10.1186/471-2334-14-411.

7.  Bloom B, Dey AN, Freeman G. Summary health statistics for U.S. children: National Health Interview Survey, 2005. Vit Health Stat. 2006;231:1–84.

8.  Marshall SJ. Developing countries face double burden of disease. Bull World Health Organ. 2004;82:556.

9.  Steyn K, Damasceno A. Lifestyle and related risk factors for chronic diseases. In: Jamison DT, Feachem RG, Makgoba MW, Bos ER, Baingana FK, Hofman KJ, et al., editors. Disease and mortality in sub-Saharan Africa. Washington: World Bank; 2006.

10. Lim SS, Vos T, Flaxman AD, Danaei G, Shibuya K, Adair-Rohani H, et al. A comparative risk assessment of burden of disease and injury attributable to 67 risk factors and risk factor clusters in 21 regions, 1990-2010: a systematic analysis for the Global Burden of Disease Study 2010. Lancet. 2012;380: 2224–60.

11. Santosa A, Wall S, Fottrell E, Hogberg U, Byass P. The development and experience of epidemiological transition theory over four decades: a systematic review. Glob Health Act. 2014;7:23574. https://doi.org/10.3402/gha.v7.23574.

12. Popkin BM, Richards MK, Montiero CA. Stunting is associated with overweight in children of four nations that are undergoing the nutrition transition. J Nutr. 1996;126:3009–16.

13. Brown KH, Peerson JM, Rivera J, Allen LH. Effect of supplemental zinc on the growth and serum zinc concentrations of prepubertal children: a meta-analysis of randomized controlled trials. Am J Clin Nutr. 2002;75:1062–71.

14. Gilbert-Diamond D, Baylin A, Mora-Plazas M, Marin C, Arsenault JE, Hughes MD. Vitamin D deficiency and anthropometric indicators of adiposity in school-age children: a prospective study. Am J Clin Nutr. 2010;92:1446–51.

15. Gunanti IR, Marks GC, Mamun AA, Long KZ. Low serum concentrations of carotenoids and vitamin E are associated with high adiposity in Mexican-American children. J Nutr. 2014;144:489–95.

16. Herman KM, Craig CL, Gauvin L, Katzmarzyk PT. Tracking of obesity and physical activity from childhood to adulthood: the Physical Activity Longitudinal Study. Int J Pediatr Obes. 2009;4:281–8.

17. The NCD Alliance. A focus on children and non-communicable diseases (NCDs). 2016. https://ncdalliance.org/sites/default/files/rfiles/20110627_A_Focus_on_Children_&_NCDs_FINAL_2.pdf. Accessed 14 May 2016.

18. Dwyer T, Magnussen CG, Schmidt MD, Ukoumunne OC, Ponsonby AL, Raitakari OT, et al. Decline in physical fitness from childhood to adulthood associated with increased obesity and insulin resistance in adults. Diabetes Care. 2009;32:683–7.

19. Gerber M, Börjesson M, Ljung T, Lindwall M, Jonsdottir I. Fitness moderates the relationship between stress and cardiovascular risk factors. Med Sci Sports Exerc. 2016;48:2075–81.

20. Walter CM, Du Randt R, Venter DJL. The physical activity and health status of two generations of black South African professional women. Health SA Gesondheid. 2011;16:538.

21. Garcia OP, Long KZ, Rosado JL. Impact of micronutrient deficiencies on obesity. Nutr Rev. 2009;67:559–72.

22. Meyer U, Roth R, Zahner L, Gerber M, Puder J, Hebestreit H, et al. Contribution of physical education to overall physical activity. Scand J Med Sci Sports. 2013;23:600–6.

23. Dobbins M, Husson H, DeCorby K, LaRocca RL. School-based physical activity programs for promoting physical activity and fitness in children and adolescents aged 6 to 18. Cochrane Database Syst Rev. 2013;2. https://doi.org/10.1002/14651858.CD007651.pub2.

24. Kriemler S, Zahner L, Schindler C, Meyer U, Hartmann T, Hebestreit H, et al. Effect of school based physical activity programme (KISS) on fitness and adiposity in primary schoolchildren: cluster randomised controlled trial. BMJ. 2010;340:c785.

25. Müller I, Gall S, Beyleveld L, Gerber M, Pühse U, du Randt R, et al. Shrinking risk profiles after deworming of children in Port Elizabeth, South Africa, with special reference to *Ascaris lumbricoides* and *Trichuris trichiura*. Geospat Health. 2017;12:601. https://doi.org/10.4081/gh.2017.601.

26. Müller I, Yap P, Steinmann P, Damons BP, Schindler C, Seelig H, et al. Intestinal parasites, growth and physical fitness of schoolchildren in poor neighbourhoods of Port Elizabeth, South Africa: a cross-sectional survey. Parasit Vectors. 2016;9:488. https://doi.org/10.1186/s13071-016-1761-5 .

27. Gall S, Müller I, Walter C, Seelig H, Steenkamp L, Pühse U, et al. Associations between selective attention and soil-transmitted helminth infections, socioeconomic status and physical fitness in disadvantaged children in Port Elizabeth, South Africa: an observational study. PLoS Negl Trop Dis. 2017;11: e0005573. https://doi.org/10.1371/journal.pntd.0005573.

28. Salvini M, Gall S, Müller I, Walter C, Du Randt R, Steinmann P, et al. Physical activity and health-related quality of life among schoolchildren from disadvantaged neighbourhoods in Port Elizabeth, South Africa. Qual Life Res. 2018;27:205–16.

29. Gerber M, Müller I, Walter C, Du Randt R, Adams L, Gall S, et al. Physical activity and dual disease burden among South African primary schoolchildren from disadvantaged neighbourhoods. Prev Med. 2018;112: 104–10.

30. Müller I, Schindler C, Adams L, Endes K, Gall S, Gerber M, et al. Effect of a multidimensional fitness education and hygiene intervention programme on physical fitness and body-mass-index in disadvantaged primary schoolchildren (DASH) in Port Elizabeth, South Africa: a longitudinal study. Int J Environ Res Public Health. 2019;16. https://doi.org/10.3390/ijerph16020232.

31. Gall S, Adams L, Joubert N, Ludyga S, Müller I, Nqweniso S, et al. Effect of a 20-week physical activity intervention on selective attention and academic performance in children living in disadvantaged neighborhoods in Port Elizabeth, South Africa. PLoS One. 2018;13:e0206908. https://doi.org/10.1371/journal.pone.0206908.

32. de Gier B, Campos Ponce M, van de Bor M, Doak CM, Polman K. Helminth infections and micronutrients in school-age children: a systematic review and meta-analysis. Am J Clin Nutr. 2014;99:1499–509.

33. Taylor-Robinson DC, Maayan N, Soares-Weiser K, Donegan S, Garner P. Deworming drugs for soil-transmitted intestinal worms in children: effects on nutritional indicators, haemoglobin, and school performance. Cochrane Database Syst Rev. 2015;7. https://doi.org/10.1002/14651858.CD000371.pub6

34. Welch VA, Ghogomu E, Hossain A, Awasthi S, Bhutta ZA, Cumberbatch C, et al. Mass deworming to improve developmental health and wellbeing of children in low-income and middle-income countries: a systematic review and network meta-analysis. Lancet Glob Health. 2017;5:40–50.

35. Li Y, Wang C, Zhu K, Feng RN, Sun CH. Effects of multivitamin and mineral supplementation on adiposity, energy expenditure and lipid profiles in obese Chinese women. Int J Obes. 2010;34:1070–7.

36. Arsenault JE, López de Romaña D, Penny ME, Van Loan MD, Brown KH. Additional zinc delivered in a liquid supplement, but not in a fortified porridge, increased fat-free mass accrual among young Peruvian children with mild-to-moderate stunting. J Nutr. 2008;138:108–14.

37. Campbell MK, Piaggio G, Elbourne DR, Altman DG. Consort 2010 statement: extension to cluster randomised trials. BMJ. 2012;345:e5661. https://doi.org/10.1136/bmj.e5661.

38. Gupta SK. Intention-to-treat concept: a review. Perspect Clin Res. 2011;2: 109–12.

39. White IR, Horton NJ, Carpenter J, Pocock SJ. Strategy for intention to treat analysis in randomised trials with missing outcome data. BMJ. 2011;342. https://doi.org/10.1136/bmj.d40.

40. Taljaard M, Weijer C, Grimshaw JM, Eccles MP. The Ottawa Statement on the ethical design and conduct of cluster randomised trials: précis for researchers and research ethics committees. BMJ. 2013;346. https://doi.org/10.1136/bmj.f2838.

41. WHO. Preventive chemotherapy in human helminthiasis: coordinated use of anthelminthic drugs in control interventions: a manual for health professionals and programme managers. Geneva: World Health Organization; 2006.

42. Biddle SJH, Gorely T, Pearson N, Bull FC. An assessment of self-reported physical activity instruments in young people for population surveillance: Project ALPHA. Int J Behav Nutr Phys Act. 2011;8:1. https://doi.org/10.1186/479-5868-8-1.

43. Hidding LM, Chinapaw MJM, van Poppel MNM, Mokkink LB, Altenburg TM. An updated systematic review of childhood physical activity questionnaires. Sports Med. 2018;48:2797–842.

44. Ekelund U, Sjöström M, Yngve A, Poortvliet E, Nilsson A, Froberg K, et al. Physical activity assessed by activity monitor and doubly labeled water in children. Med Sci Sports Exerc. 2001;33:275–81.

45. Bailey RL, West KP, Black RE. The epidemiology of global micronutrient deficiencies. Ann Nutr Metab. 2015;66:22–33.

46. McDade TW, Williams S, Snodgrass JJ. What a drop can do: dried blood spots as a minimally invasive method for integrating biomarkers into population-based research. Demography. 2007;44:899–925.

47. Ogden CL, Flegal KM, Carroll MD, Johnson CL. Prevalence and trends in overweight among US children and adolescents, 1999-2000. JAMA. 2002; 288:1728–32.

48. Katz N, Chaves A, Pellegrino J. A simple device for quantitative stool thick-smear technique in schistosomiasis mansoni. Rev Inst Med Trop Sao Paulo. 1972;14:397–400.

49. Sherkhonov T, Yap P, Mammadov S, Sayfuddin K, Martinez P, Amoss WP, et al. National intestinal helminth survey among schoolchildren in Tajikistan: prevalences, risk factors and perceptions. Acta Trop. 2013;126:93–8.

50. Knopp S, Mgeni AF, Khamis IS, Steinmann P, Stothard JR, Rollinson D, et al. Diagnosis of soil-transmitted helminths in the era of preventive chemotherapy: effect of multiple stool sampling and use of different diagnostic techniques. PLoS Negl Trop Dis. 2008;2:e331. https://doi.org/10.1371/journal.pntd.0000331..

51. Eriksen BA, Eriksen CW. Effects of noise letters upon the identification of a target letter in a nonsearch task. Percept Psychophys. 1974;16:143–9.

52. Diamond A. Executive functions. Ann Rev Psychol. 2013;64:135–68.

53. Wöstmann NM, Aichert DS, Costa A, Rubia K, Möller H-J, Ettinger U. Reliability and plasticity of response inhibition and interference control. Brain Cogn. 2013;81:82–94.

54. Bauman A, Chau J, van der Ploeg H, Hardy L. Physical activity measures for children and adolescents – recommendations on population surveillance: a rapid review. Sydney: Sax Institute; 2010.

55. Aït-Khaled N, Pearce N, Anderson HR, Ellwood P, Montefort S, Shah J, et al. Prevalence of symptoms of asthma, rhinitis and eczema in 13- to 14-year-old children in Africa: the ISAAC Phase III. Allergy. 2009;62:247–58.

56. Kjonniksen L, Torsheim T, Wold B. Tracking of leisure-time physical activity during adolescence and young adulthood: a 10-year longitudinal study. Int J Behav Nutr Phys Act. 2008;5:69.

57. Samdal O, Tynjala J, Roberts C, Sallis JF, Villberg J, Wold B. Trends in vigorous physical activity and TV watching of adolescents from 1986 to 2002 in seven European Countries. Eur J Pub Health. 2007;17:242–8.

58. Haugland S, Wold B, Torsheim T. Relieving the pressure? The role of physical activity in the relationship between school-related stress and adolescent health complaints. Res Quart Exerc Sport. 2003;74:127–35.

59. Crocker PRE, Bailey DA, Faulkner RA, Kowalski KC, McGrath R. Measuring general levels of physical activity: preliminary evidence for the physical activity questionnaire for older children. Med Sci Sports Exerc. 1997;29:1344–9.

60. Bervoets L, Van Noten C, Van Roosbroeck S, Hansen D, Van Hoorenbeeck K, Verheyen E, et al. Reliability and validity of the Dutch Physical Activity Questionnaires for Children (PAQ-C) and Adolescents (PAQ-A). Arch Public Health. 2014;72:47. https://doi.org/10.1186/2049-3258-72-47.

61. Kowalski KC, Crocker PRE, Faulkner RA. Validation of the physical activity questionnaire for older children. Ped Exerc Sci. 1997;9:174–86.

62. Wang JJ, Baranowski T, Lau PW, Chen TA, Pitkethly AJ. Validation of the Physical Activity Questionnaire for Older Children (PAQ-C) among Chinese children. Biomed Environ Sci. 2016;29:177–86.

63. Benitez-Porres J, Alvero-Cruz JR, Sardinha LB, Lopez-Fernandez I, Carnero EA. Cut-off values for classifying active children and adolescents using the Physical Activity Questionnaire: PAQ-C and PAQ-A. Nutr Hosp. 2016;33:1036–44.

64. Ravens-Sieberer U, Gosch A, Rajmil L, Erhart M, Bruil J, Power M, et al. The KIDSCREEN-52 quality of life measure for children and adolescents: psychometric results from a cross-cultural survey in 13 European countries. Value Health. 2008;11:645–58.

65. Taliep N, Florence M. Evaluating the construct validity of the KIDSCREEN-52 Quality of Life questionnaire within a South African context. S Afr J Psychol. 2012;42:255–69.

66. The Kidscreen Group E. The KIDSCREEN questionnaires: quality of life questionnaires for children and adolescents; 2006. p. 231.

67. Buysse DJ, Reynolds CF, Monk TH, Berman SR, Kupfer DJ. The Pittsburgh Sleep Quality Index: new instrument for psychiatric practice and research. Psychiatry Rev. 1989;28:193–213.

68. Morin CM, Belleville G, Belanger L, Ivers H. The Insomnia Severity Index: psychometric indicators to detect insomnia cases and evaluate treatment response. Sleep. 2011;34:601–8.

69. Gerber M, Lang C, Lemola S, Colledge F, Kalak N, Holsboer-Trachsler E, et al. Validation of the German version of the Insomnia Severity Index in adolescents, young adults and adult workers: results from three cross-sectional studies. BMC Psychiatry. 2016;16:174. https://doi.org/10.1186/s12888-016-0876-8.

70. Rowlands AV. Accelerometer assessment of physical activity in children: an update. Pediatr Exerc Sci. 2007;19:252–66.

71. Freedson PS, Melanson E, Sirard J. Calibration of the computer science and applications, Inc accelerometer. Med Sci Sports Exerc. 1998;30:777–81.

72. Crouter SE, Horton M, Bassett DR. Validity of ActiGraph child-specific equations during various physical activities. Med Sci Sports Exerc. 2013;45:1403–9.

73. Hänggi JM, Phillips LR, Rowlands AV. Validation of the GT3X ActiGraph in children and comparison with the GT1M ActiGraph. J Sci Med Sport. 2013;16:40–4.

74. Leger LA, Mercier D, Gadoury C, Lambert J. The multistage 20 metre shuttle run test for aerobic fitness. J Sports Sci. 1988;6:93–101.

75. Adam C, Klissouras V, Ravazzolo M, Renson R, Tuxworth W. Eurofit: European test of physical fitness. Rome: Council of European Committee for Development of Sport; 1988.

76. Espana-Romero V, Artero EG, Santaliestra-Pasias AM, Gutierrez A, Castillo MJ, Ruiz JR. Hand span influences optimal grip span in boys and girls aged 6 to 12 years. J Hand Surg Am. 2008;33:378–84.

77. Ruiz JR, Espana-Romero V, Ortega FB, Sjöström M, Castillo MJ, Gutierrez A. Hand span influences optimal grip span in male and female teenagers. J Hand Surg Am. 2008;31:1367–72.

78. Wentzel-Viljoen E, Laubscher R, Kruger A. Using different approaches to assess the reproducibility of a culturally sensitive quantified food frequency questionnaire. S Afr J Clin Nutr. 2011;24:143–8.

79. Zack RM, Irema K, Kazonda P, Leyna GH. Validity of a FFQ to measure nutrient and food intake in Tanzania. Public Health Nutr. 2018;21:2211–20.

80. Salvador Castell G, Perez Rodrigo C, Ngo de la Cruz J, Aranceta Bartrina J. Household food insecurity access scale (HFIAS). Nutr Hosp. 2015;31:272–8.

81. Knueppel D, Demment M, Kaiser L. Validation of the household food insecurity access scale in rural Tanzania. Public Heath Nutr. 2010;13:360–7.

82. Filmer D, Pritchett LH. Estimating wealth effects without expenditure data or tears: An application to educational enrollments in states of India. Demography. 2001;38:115–32.

83. Cohen J. Statistical power analysis for the behavioral sciences. Mahwah: Erlbaum; 1988.

## Publisher's Note

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## Is grip strength linked to body composition and cardiovascular risk markers in primary schoolchildren? Cross-sectional data from three African countries

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**Is grip strength linked to body composition and cardiovascular risk markers in primary schoolchildren? Cross-sectional data from three African countries**

*Brief running head:* Grip strength, body composition and cardiovascular risk

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**Abstract**

**Objectives:** Muscular strength represents a specific component of health-related fitness. Hand grip strength (HGS) is used as an indicator for musculoskeletal fitness in children. HGS can also be used as a marker of cardiometabolic risk, but most available HGS data are derived from Western high-income countries. Therefore, this study examines whether HGS is associated with body composition and markers of cardiovascular risk in children from three sub-Saharan African countries.

**Design:** Cross-sectional study.

**Setting:** Public primary schools (grade 1-4) in Taabo (Côte d'Ivoire), Gqeberha (South Africa) and Ifakara (Tanzania).

**Participants:** Data from 467 children from Côte d'Ivoire (210 boys, 257 girls), 864 children from South Africa (429 boys, 435 girls) and 695 children from Tanzania (334 boys, 361 girls) were analysed.

**Primary and secondary outcome measures:** Body composition (assessed via bioelectrical impedance analysis) was the primary outcome. Cardiovascular risk markers were considered as secondary outcome. Blood pressure was measured with an oscillometric monitor, and blood markers (cholesterol, triglycerides, glycated haemoglobin) via Afinion point-of-care testing. HGS (independent variable) was assessed with a hydraulic hand dynamometer. Inferential statistics are based on mixed linear regressions and analyses of covariance.

**Results:** Across all study sites, higher HGS was associated with lower body fat, higher muscle mass and higher fat-free mass (p<0.001, 3.9-10.0% explained variance), both in boys and girls. No consistent association was found between HGS and cardiovascular risk markers.

**Conclusions:** HGS assessment is popular due to its simplicity, feasibility, practical utility, and high reliability of measurements. This is one of the first HGS studies with children from sub-Saharan Africa. There is a great need for further studies to examine whether our findings can be replicated, to develop reference values for African children, to establish links to other health outcomes, and to explore whether HGS is associated with later development of cardiovascular risk markers.

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**Article summary**

**Strengths and limitations of this study:**

- This is one of the first HGS studies with children from sub-Saharan Africa.

- The same methods were used across three different study sites (Côte d'Ivoire, South Africa, Tanzania).

- There is still no consensus on HGS measurement protocols.

- The children from the three study sites differed with regard to age, height and weight, and the samples were not representative for any of the countries involved.

- Future studies should use longitudinal designs to explore whether grip strength in childhood is associated with later development of CV risk factors.

*Keywords:* blood pressure; body fat; cholesterol; glycated haemoglobin; muscle mass; triglycerides

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**INTRODUCTION**

Muscular strength represents a specific component of health-related fitness and is defined as the ability to develop maximal muscle force.[1] Muscle strength plays an important role in child development as it is the basis for locomotive movement and, therefore, can facilitate play and social interaction with other children.[2]

Hand grip strength (HGS) is used as a simple indicator to assess the maximum voluntary force of the hand,[3] but HGS is also associated with arm, trunk and leg strength.[4] Research further shows that HGS is a good indicator for musculoskeletal fitness in children.[5] Accordingly, assessment of HGS is recommended as part of school-based fitness testing and as a screening tool for adequate levels of muscle strength and bone health.[6]

HGS is strongly dependent on age, sex and body mass.[7] The association with age is curvilinear. HGS increases through childhood and adolescence and peaks around the age of 40 years.[3,8] Moreover, boys have higher HGS than girls, especially after puberty.[3, 7] Following puberty, there is an exponential progression in muscle strength among boys, which has been ascribed to the increase of testosterone and which in turn favours the growth of lean muscle mass.[9] In girls, an increase of total body fat was observed after puberty.[10] Research has also shown that HGS depends on body mass, and that HGS divided by body weight is more closely associated with health outcomes than absolute HGS values.[11]

Meanwhile, normative HGS reference values have been developed for children and adolescents in several countries.[8, 12] For instance, based on normalized HGS measurements from 1,326 sixth-grade students (10-12 years), Peterson et al.[13] developed thresholds for high metabolic risk and intermediate metabolic risk adolescents living in the United States. However, it is doubtful whether such HGS norms can be generalised across countries.[3] For instance, a recent meta-analysis showed that HGS scores are significantly higher in high-income compared to low-to-middle income countries (LMICs).[14]

Furthermore, skeletal muscle has an important role for health and disease because it is the primary protein store within the body and the primary tissue for glucose disposal.[15] In line with this

notion, HGS has been associated with a variety of health outcomes among adults, including nutritional status,[16] chronic physical conditions such as cancer and cardiovascular diseases, as well as cardiovascular and all-cause mortality.[15, 17] These relationships have also been observed in people living in LMICs.[18] Interestingly, HGS seems to be a better predictor of mortality than muscle mass,[19] however, the underlying mechanisms are not yet well understood.[17, 20] The association might be mediated via cardiovascular risk factors such as hypertension, diabetes, metabolic syndrome, inflammatory markers, or risk of arrhythmia.[11, 21, 22] In line with this, a meta-analysis showed that cardiovascular risk factors indeed attenuated the relationship between HGS and mortality, but they did not fully explain the observed relationship.[17]

Prior research has corroborated that HGS is a relevant health outcome among children and adolescents. For instance, a study with 1,421 children (M=11.6 years) from the United States pointed towards a significant inverse relationship between HGS and blood pressure, cholesterol levels, and triglycerides.[23] Similarly, a study with 2,818 Norwegian children (9-15 years) reported that, after adjusting for age, sex and puberty, higher HGS was associated with lower waist circumference, higher high density lipoprotein (HDL) cholesterol, lower triglycerides concentration, and lower systolic blood pressure.[24] These relationships were confirmed in Colombian children (M=11.5 years) living in low socioeconomic background settings.[25] Finally, a prospective cohort study with more than a million Swedish participants showed that high grip strength in adolescence is associated with lower premature death later in life (median follow-up period of 24 years).[26]

In summary, these studies highlight that HGS has the potential to be used not only as an indicator of muscular strength, but also as a marker of cardiometabolic risk. However, most of today's data are derived from Western high-income countries, whereas research in sub-Saharan Africa, where rapid social, cultural, economic and urban developments are currently occurring, is lacking, particularly among children. Therefore, the purpose of this paper was to examine whether HGS is associated with body composition (body fat, muscle mass, fat free mass) and markers of cardiovascular risk (blood pressure, cholesterol, triglycerides, blood glucose [HbA1c]) in primary schoolchildren from three African countries (Côte d'Ivoire, South Africa, and Tanzania).

**METHODS**

**Study design and setting**

Data presented in this paper comes from the baseline data assessment of the KaziAfya cluster randomized controlled trial, which was conducted in public schools in Taabo (Côte d'Ivoire), Gqeberha (South Africa), and Ifakara (Tanzania). The goal of this trial was to implement and test two school-based health interventions (physical activity promotion, multi-micronutrient supplementation) over a period of two school years to improve children's health status. All study sites are located in rural and peri-urban settings. The Ivorian public primary schools were selected from Taabo city and village, located in the rural South-central Agnéby-Tiassa region (150 km Northwest from Abidjan). Taabo's economy relies on agriculture (90% of the employees), and has two companies in the agricultural sector and a power station (hydroelectric dam), which produces 2% of the country's energy. Our project schools in South Africa are located in peri-urban marginalized communities in the Eastern Cape (Gqeberha region). Only schools with poor ratings with reference to national poverty tables, income levels, dependency ratios and literacy rates were involved. Our project schools in South-central Tanzania are situated in a rural town (Ifakara) in the Kilombero District, Morogoro Region (413 km from Dar es Salaam). The main economic activity of the local population is rice farming, but small businesses and animal husbandry are also conducted. As described in the study protocol, education authorities were contacted first, before contact was made with principals of schools that met inclusion criteria. Detailed information was given to school principals about the objectives, procedures and potential risks and benefits of the study. Based on this information, school principals could state their interest in being part of the project. Schools were eligible if they were public schools, had facilities to implement physical education lessons (free space: lawn, sand or concrete), and did not participate in any other research project or clinical trial.[27]

**Participants and procedures**

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Children were eligible if they attended grade 1–4, were no older than 12 years, did not participate in any other research projects, and did not suffer from clinical conditions that prevent participation in physical activity, as determined by qualified medical personnel. Written informed consent was obtained from the legal guardians before study enrolment, after having provided detailed written and oral information about the aims, procedures, risks and benefits of the study. The responsible ethics committees in Côte d'Ivoire, South Africa, Tanzania and Switzerland and the local school authorities approved the study. Children who suffered from severe medical conditions and/or malnourishment (as diagnosed by a nurse, following national guidelines) were referred to local clinics. The intervention study was registered at http://www.isrctn.com/ISRCTN29534081. The data assessment took place between October and December 2018 in Côte d'Ivoire, between January and April 2019 in South Africa, and between July and August 2019 in Tanzania.

**Measures**

Data assessments took place at children's schools before the start of the intervention. Saehan hydraulic hand dynamometers (MSD Europe BVBA; Tisselt, Belgium) were used to assess HGS. The children were instructed to sit with spine erect, relaxed, and with an arm position at a 90° angle. Before the first data assessment, a field investigator briefly demonstrated how to hold the hand dynamometer. Children were instructed to grip the hand dynamometer as hard as possible. Each child performed six trials (three with the left, and three with the right hand), which were recorded to the nearest 1 kg. After each trial and after a 30 sec resting period, the hand was changed. An overall HGS score was constructed by calculating the mean across all six trials, which was then divided by the child's body weight. Previous research with children showed that HGS is closely associated with total muscle strength,[28] and that HGS measures generally have a good reliability.[29] Body composition was assessed via bioelectrical impedance analysis (BIA) using a wireless body composition monitor (Tanita MC-580; Tanita Corp., Tokyo, Japan). Children wearing only light clothing were asked to stand barefoot on the metal plates of the device, being guided by the research assistant to ensure optimal contact according to the manufacturer's instructions. The MC-580 was also used to assess body

weight to the nearest 0.1 kg. Body height was taken to the nearest 0.1 cm with each child standing

with his/her back erect and shoulders against a stadiometer. Blood pressure was assessed with a

validated oscillometric digital blood pressure monitor (Omron® M3; Hoofddorp, Netherlands). Three

measurements were performed, and the mean of the last two measurements were employed as

indicators of systolic and diastolic blood pressure. Capillary blood sampling was used to assess blood

markers for cardiovascular risk, using the Afinion 2 analyser (Abbott Technologies; Abbott Park, USA).

Total cholesterol, HDL cholesterol, low density lipoprotein (LDL) cholesterol, triglycerides and HbA1c

were assessed using fasting capillary blood sampling.[30] Evidence of the clinical utility and accuracy of

this finger prick technology has been described previously.[31]

**Statistical analyses**

Descriptive statistics for all study variables are reported separately for each study site (Table

1). Normality was tested via the Kolmogorov-Smirnov test. Univariate analyses of covariance

(ANCOVAs) with Bonferroni post-hoc tests were calculated to examine differences between study

sites. Mixed linear regression analyses with random intercepts for school classes to account for the

nested nature of the data (learners assessed in classes) were performed separately for each study

site to determine whether HGS is associated with body composition and single cardiovascular risk

markers (Table 2). All regression analyses were controlled for sex and height (Table 2). Weight and

age were not considered as covariates to avoid over-adjustment and collinearity issues (e.g., weight

is already considered in the body weight-adjusted HGS measure, age is highly correlated with height

[$r$=0.71 to 0.77, $p$<0.001]). Objectively assessed physical activity, cardiorespiratory fitness (VO₂max),

dietary diversity, and socioeconomic status were considered as additional covariates (Supplementary

Online Material, Table S1). To compare children with high vs. low HGS scores, ANCOVAs (controlling

for sex and height) with Bonferroni post-hoc tests were calculated to compare HGS quartiles (Table

3). Analyses controlled for further covariates are presented as Supplementary Online Material (Table

S2). All analyses were carried out with SPSS (version 26, IBM Corporation, Armonk, USA). The level of

statistical significance was set at $p$<0.05 across all analyses.

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**Patient and public involvement statement**

Patients or the public were not involved in the design, conduct, reporting or dissemination plans of our study.

**RESULTS**

**Sample description**

As shown in Table 1, in Côte d'Ivoire, 467 children presented with complete data (210 boys, 257 girls) across all study variables. In South Africa, 864 children were included (429 boys, 435 girls), whereas in Tanzania, the final sample consisted of 695 children (334 boys, 361 girls). A participant flow chart is provided in Figure 1. Because the blood tests and fitness tests took place on different days, because of the limited time allocated by schools for data collection, and due to a beginning national teacher strike in Côte d'Ivoire, a considerable number of students of the original sample had missing values and could not be included in the analyses.

**Descriptive statistics and between-country differences**

Table 1 displays the descriptive statistics, separately for each study site. Although none of the metric variables were normally distributed, no evidence for severe non-normality was found (skewness and kurtosis values of ≥|2| and ≥|7|, respectively).[32] Therefore, no log-transformation of the outcomes was needed. High internal consistency across the six HGS measurements was confirmed in each study site (Cronbach's alpha: 0.95-0.98). As shown in Table 1, significant differences between study sites were found in most of the variables, before and after controlling for confounders. Children from the Tanzanian study site were oldest (M=9.34±1.74 years), followed by peers from the South African (M=8.24±1.42 years) and Ivorian (M=7.86±2-63 years) study sites. In line with this, children from Tanzania were tallest, heaviest, and had the highest body mass index (BMI) scores. They also achieved the highest absolute and weight-adjusted HGS scores. Furthermore, children from Tanzania had the highest scores for systolic/diastolic blood pressure, total cholesterol,

as well as HDL and LDL cholesterol. By contrast, children from the Ivorian study site had higher

triglyceride levels, whereas children from the South African study site had the highest HbA1c

concentrations. Differences in HGS, body composition and cardiovascular risk persisted after

controlling for sex, height and weight.

**HGS, body composition and cardiovascular risk markers**

Table 2 shows the results of the mixed linear regression models, separately for each study

site. The results consistently show that HGS is positively associated with relative muscle mass and

fat-free mass, whereas HGS is negatively associated with relative body fat. These associations

persisted after controlling for sex and height. The associations also persisted after controlling for

further confounders (see Supplementary Online Material, Table S1).

Regarding the association between HGS and cardiovascular risk markers, only a few

significant results were found, after adjusting for sex and height. More specifically, in children from

the Ivorian study site, those with higher HGS had higher total and LDL cholesterol levels. However, no

such relationships were found among children recruited from the South African and Tanzanian study

sites.

**Differences between HGS quartiles**

Table 3 shows the means and standard deviations for the different HGS quartiles.

Consistently across all study sites, significant between-quartile differences were found for relative

body fat, muscle mass and fat-free mass. Differences remained after controlling for sex and height

(Table 3) and further confounders (Supplementary Online Material, Table S2). Bonferroni post-hoc

tests pointed towards a clear gradient, indicating that students in the higher HGS quartiles had lower

body fat levels, higher muscle mass and higher fat-free mass. Using the example of muscle mass,

Figure 2 shows that this gradient occurred in both girls and boys.

**DISCUSSION**

The key findings of this study are that in primary school children from Côte d'Ivoire, South Africa and Tanzania, higher HGS is associated with lower body fat, higher muscle mass and higher fat-free mass. A clear gradient appeared in both boys and girls. The level of explained variance (3.9 to 10.0%) points towards between-quartile differences of moderate magnitude. Unlike previous findings, no consistent association was found between HGS and further (single) cardiovascular risk markers (blood pressure, cholesterol, triglycerides, glycated haemoglobin). To the best of our knowledge, this is the first study carried out with children in sub-Sahara Africa and testing the relationship between HGS, body composition and cardiovascular risk markers. It is also one of the few studies carried out with younger schoolchildren.

The present study corroborates the notion that grip strength serves as a dynamic indicator of muscle mass and healthy body composition.[3, 4, 33] This also supports public health recommendations that children should regularly engage in muscle-strengthening activities.[34] Schools can play an important role in this process as they can reach all children, independent of their sex, ethnicity and socioeconomic background via school physical education. On the other hand, the association between muscle mass and HGS was of limited (moderate) magnitude. This indicates that other factors have an impact on children's HGS. Possible influences are overall dietary quality,[35] disease-related factors[33] and socioeconomic status.[36] However, in the present study, the association between HGS and muscle mass persisted after controlling for physical activity, cardiorespiratory fitness, dietary diversity and socioeconomic status.

The present study also confirms prior research[3, 7, 8] showing that increasing age is positively associated with both absolute HGS (in this sample: $r$=0.50 to 0.70, $p$<.001, data not shown) and body weight-adjusted HGS ($r$=0.17 to 0.46, $p$<.001, data not shown). While chronological and biological age can differ,[8] similarly strong associations were found between children's height and absolute HGS (in this sample: $r$=0.54 to 0.74, $p$<.001, data not shown) and body weight-adjusted HGS ($r$=0.11 to 0.36, $p$<.01, data not shown). Our study also aligns with previous investigations showing that boys have higher HGS than girls.[3, 7, 9, 10] In the present sample, boys had higher absolute HGS (3.0 to 4.7%

of explained variance, data not shown) and body weight-adjusted HGS (3.9 to 6.5% of explained variance, data not shown) than girls.

Regarding the relationship between HGS and cardiovascular risk markers, our findings are at odds with most previous studies with adult[11, 21, 22] and child/adolescent populations.[23-26] Whereas prior research with children and adolescents showed that higher HGS are associated with more favourable blood pressure, more favourable cholesterol profiles, lower triglyceride concentrations, and lower clustered cardiovascular risk, it is important to note that not all studies found such relationships. For instance, HGS was not associated with cardiovascular risk markers in a sample of 1,642 Australian children and adolescents (9-15 years).[37] The fact that in our study no clear association occurred in any of the study sites indicates that our results have a certain generalisability: however, we can only speculate about the underlying reasons. One assumption relates to the (low) age of the children and the fact that, on average, risk marker scores were relatively low in the present sample.[38] It is possible that the relationship between HGS and cardiovascular risk markers becomes more pronounced as muscle strength increases with age due to changes in muscle mass,[28] and as the prevalence of cardiovascular risk factors increases during later childhood and adolescence. In younger children, HGS may be more closely associated with other (e.g., subjective or mental) health outcomes.[39] This aspect deserves more attention in future investigations, especially as few studies have so far examined the relationship between HGS and subjective/mental health in children/adolescents. Among adults, the relevance of low HGS as a risk marker for cardiovascular health seems well established. In line with this, a UK Biobank based study with 403,199 adults showed that obesity and lower grip strength are independent predictors of higher mortality risk. However, all-cause mortality risk was lower for obese but stronger individuals compared to non-obese, but weaker individuals,[34] which suggests that "improving muscle strength may be a more important public health priority than reducing adiposity in decreasing mortality risks, although excessive adiposity itself is a strong risk factor of mortality" (p. 780). Finally, the relationship between HGS and health outcomes may be different in children from low- versus middle-income countries.

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**Strengths and Limitations**

The fact that there is still no consensus on HGS measurement protocols complicates between-study comparisons.[33] For instance, research showed that methodological aspects (e.g., dynamometer handle position) can have a significant influence on the HGS performance of children.[40] Therefore, the fact that we applied the same methods across three different study sites is an advantage, as it allows direct comparison of the findings. By contrast, the fact that children from the three study sites differed with regard to age, height and weight can be seen as a limitation. To address this issue, we focused on body weight-adjusted HGS, and considered sex and height (as a proxy for biological age) as covariates. Nevertheless, some questions remain unanswered. For instance, it is not entirely clear why – after controlling for sex, height and weight – children from Côte d'Ivoire had lower weight-adjusted HGS scores than peers from South Africa and Tanzania. We further acknowledge that due to the cross-sectional nature of our study, no conclusions are possible regarding cause and effect. Researchers have pointed to the issue of reversed causality and the fact that poor HGS could be considered as a marker of poor overall health.[17] For future studies, the important question is whether grip strength in childhood is associated longitudinally with later development of CV risk factors. We also acknowledge that differences between study sites should not be (mis)interpreted as between-country differences, since the study sites were not representative for any of the countries involved. In all three study sites, children were recruited from rural and peri-urban areas and thus represent rather poor segments of the population, which limits the generalisability of our findings.

**CONCLUSIONS**

HGS assessment is popular due to its simplicity, feasibility, practical utility, and high reliability of measurements. Assessments are quickly obtainable by a range of different health professionals. They can also be easily implemented as part of school-based fitness testing and as a screening tool for adequate levels of muscle strength and bone health.

Our study highlights that higher body weight-adjusted HGS is associated with healthy body composition (lower body fat, higher fat-free mass) and higher muscle mass. To the best of our

knowledge, this is the first HGS study with children from sub-Saharan Africa. Hence, there is a great and continued need for further studies to examine whether our findings can be replicated (e.g., with older primary school students), to establish links to other health outcomes (e.g., nutritional status, mental health), to explore longitudinal relationships, and to develop reference values for African children and adolescents. It will also be important to understand whether HGS is a predictor of the level of cardiovascular/metabolic risks in specific populations in different countries of sub-Saharan Africa, and to consider how cardiovascular/metabolic risk can be improved in these populations in the longer term.

**CONFLICT OF INTEREST**

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

**AUTHOR CONTRIBUTIONS**

MG, KZL, UP and JU were responsible for the conceptualization of the study and for initial funding acquisition. MG serves as principal investigator of the study, BB, HM and CW are the principal investigators in the three partner countries. CL is responsible for the overall administration of the study. SGT, MFF and SN are the local project coordinators. RDR, DD, KZL, FOO, NPH, UP, and PS served as project advisors and provide resources. SAA, JB, BK, BCG, SGT, JTC, MFF, GJM, EGM, SG, GJM, CL, IM, and SN contributed to the data assessment and curation. MG performed the statistical analysis and wrote the first draft of the manuscript. All authors contributed to manuscript revision, read, and approved the submitted version.

**Data sharing**

The raw datasets are available from the corresponding author on reasonable request.

**Ethical approval**

In Côte d'Ivoire, the study protocol was approved by the Institutional Review Board (IRB) of the Centre Suisse de Recherches Scientifiques en Côte d'Ivoire (CSRS; Abidjan, Côte d'Ivoire) and the Comité National d'Ethique des Sciences de la Vie et de la Santé (CNESVS; reference number: 100–18/MSHP/CVESVS-km). In South Africa, approval was granted by the research ethics committee of the Nelson Mandela University in Port Elizabeth (reference number: H18-HEA-HMS-006) and the Eastern Cape Departments of Education and Health. In Tanzania, the study protocol was approved by the responsible ethics committee at the Ifakara Health Institute (IHI–IRB; reference number: # IHI/IRB/No 39-2018), the National Institute for Medical Research (NIMR; reference number: NIMR/HQ/R.8a/Vol. IX/3137) and the Tanzania Food and Drugs Authority (TFDA; reference number: TMDA0019/CTR/0016/05). Children who suffered from severe medical conditions and/or

malnourishment (as diagnosed by a nurse, following national guidelines) were referred to local

clinics. Ethical approval was also obtained from the 'Ethikkommission Nordwest- und Zentralschweiz'

in Switzerland (EKNZ; reference number: Req-2018-00608), and the intervention study was

registered in the ISRCTN registry (http://www.isrctn.com/ISRCTN29534081).

**REFERENCES**

1. American College of Sports Medicine. *ACSM's health-related physical fitness assessment manual*. Philadelphia: Lippincott Williams & Wilkins; 2013.

2. Fredriksen PM, Mamen A, Hjelle OP, Lindberg M. Handgrip strength in 6–12-year-old children: The Health Oriented Pedagogical Project (HOPP). Scand J Public Health 2018;**46**:S54-S60.

3. Wong SL. Grip strength reference values for Canadians aged 6 to 79: Canadian Health Measure Survey, 2007 to 2013. Health Reports 2016;**27**:3-10.

4. Wang M, Leger AB, Dumas GA. Prediction of back strength using anthropometric and strength measurements in healthy females. Clin Biomech 2005;**20**:685-692.

5. Ortega FB, Cadenas-Sánchez C, Sánchez-Delgado G, Mora-González J, Martínez-Téllez B, Artero EG, Castro-Pinero J, Labayen I, Chillon P, Löf M, Ruiz JR. Systematic review and proposal of a field-based physical fitness-test battery in preschool children: the PREFIT battery. Sports Med 2015;**45**:533-555.

6. Ruiz JR, Castro-Pinero J, Espana-Romero V, Artero EG, Ortega FB, Cuenca MM, Jimenez-Pavon D, Chillon P, Girela-Rejon MJ, Mora J, Gutierrez A, Suni J, Sjöström M, Castillo MJ. Field-based fitness assessment in young people: the ALPHA health-related fitness test battery for children and adolescents. Br J Sports Med 2010;**45**:518-524.

7. Ploegmakers JJ, Hepping AM, Geertzen JH, Bulstra SK, Stevens M. Grip strength is strongly associated with height, weight and gender in childhood: a cross sectional study of 2241 children and adolescents providing reference values. J Physiother 2013;**59**:255-261.

8.  Gomez-Campos R, Andruske CL, de Arruda M, Sulla-Torres J, Pacheco-Carrillo J, Urra-Albornoz C, Cossio-Bolanos M. Normative data for handgrip strength in children and adolescents in the Maule Region, Chile: Evaluation based on chronological and biological age. PLoS One 2018;**13**:e0201033.

9.  Round JM, Jones DA, Honour JW, Nevill AM. Hormonal factors in the development of differences in strength between boys and girls during adolescence: a longitudinal study. Ann Hum Biol 1999;**26**:49-62.

10. Dore E, Martin R, Ratel S, Duche P, Bedu M, Van PE. Gender differences in peak muscle performance during growth. Int J Sports Med 2005;**26**:274-280.

11. Chun S-W, Kim W, Choi KH. Comparison between grip strength and grip strength divided by body weight in their relationship with metabolic syndrom and quality of life in the elderly. PLos One 2019;**14**:e0222040.

12. Häger-Ross C, Rösblad B. Norms for grip strength in children aged 4–16 years. Acta Paediatr 2002;**91**:617-625.

13. Peterson MD, Zhang P, Saltarelli WA, Visich PS, Gordon PM. Low muscle strength thresholds for the detection of cardiometabolic risk in adolescents. Am J Prev Med 2016;**50**:593-599.

14. Dodds RM, Syddall H, Cooper R, Kuh D, Cooper C, Sayer AA. Global variation in grip strength: A systematic review and meta-analysis of normative data. Age Ageing 2016;**45**:209-216.

15. Ho FKW, Celis-Morales CA, Petermann-Rocha F, Sillars A, Welsh P, Welsh C, Anderson J, Lyall DM, Mackay DF, Sattar N, Gill JMR, Pell JP, Gray SR. The association of grip strength with health outcomes does not differ if grip strength is used in absolute or relative terms: A prospective cohort study. Age Ageing 2019;**48**:683-691.

16. Norman K, Stobäus N, Gonzalez MC, Schulzke JD, Pirlich M. Hand grip strength: outcome predictor and marker of nutritional status. Clin Nutr 2011;**30**:135-142.

17. Wu Y, Wang W, Liu T, Zhang D. Association of grip strength with risk of all-cause mortality, cardiovascular diseases, and cancer in community-dwelling populations: A meta-analysis of prospective cohort studies. JAMDA 2017;**18**:551.e17-551e35.

18. Vancampfort D, Stubbs B, Firth J, Koyanagi A. Handgrip strength, chronic physical conditions and physical multimorbidity in middle-aged and older adults in six lo- and middle income countries. Eur J Int Med 2019;**61**:96-102.

19. Newman AB, Kupelian V, Visser M, Simonsick EM, Goodpaster BH, Kritchevsky SB, Tylavsky FA, Rubin SM, Harris TB. Strength, but not muscle mass, is associated with mortality in the health, aging and body composition study cohort. J Gerontol 2006;**61**:72-77.

20. Gubelmann C, Vollenweider P, Marques-Vidal P. Association of grip strength with cardiovascular risk markers. Eur J Prev Cardiol 2017;**24**:514-521.

21. Mainous AGI, Tanner RJ, Anton SD, Jo A. Grip strength as a marker of hypertension and diabetes in healthy weight adults. Am J Prev Med 2015;**49**:850-858.

22. Andersen K, Rasmussen F, Held C, Neovius M, Tynelius P, Sundström J. Exercise capacity and muscle strength and risk of vascular disease and arrhythmia in 1.1 million young Swedish men: cohort study. BMJ 2015;**351**:h4543.

23. Peterson MD, Saltarelli WA, Visich PS, Gordon PM. Strength capacity and cardiometabolic risk clustering in adolescents. Pediatrics 2014;**133**:e896-e903.

24. Steene-Johannessen J, Anderssen SA, Kolle E, Andersen LB. Low muscle fitness is associated with metabolic risk in youth. Med Sci Sports Exerc 2009;**41**:1361-1367.

25. Cohen DD, Gomez-Arbelaez D, Camacho PA, Pinzon S, Hormiga C, Trejos-Suarez J, Duperly J, Lopez-Jaramillo P. Low muscle strength is associated with metabolic risk factors in Colombian children: the ACFIES study. PLoS One 2014;**9**:e93150.

26. Ortega FB, Silventoinen K, Tynelius P, Rasmussen F. Muscular strength in male adolescents and premature death: cohort study of one million participants. BMJ 2012;**345**(e7279):doi: 10.1136/bmj.e7279.

27. Gerber M, Ayekoé SA, Beckmann J, Bonfoh B, Coulibaly JT, Daouda D, du Randt R, Finda L, Gall S, Mollel GJ, Lang C, Long KZ, Ludyga S, Masanja H, Müller I, Nqweniso S, Okumu F, Probst-Hensch N, Pühse U, Steinmann P, Traoré SG, Walter C, Utzinger J. Effects of school-based physical activity and multi-micronutrient supplementation intervention on growth, health and wellbeing

of schoolchildren in three African countries: the KaziAfya cluster randomised controlled trial protocol using a 2x2 factorial design. Trials 2020;**21**:doi: 10.1186/s13063-019-3883-5.

28. Wind A, Takken T, Helders P, Engelbert R. Is grip strength a predictor for total muscle strength in healthy children, adolescents, and young adults? Eur J Pediatr 2010;**169**:281-287.

29. Ruiz JR, Castro-Pinero J, Espana-Romero V, Artero EG, Ortega FB, Cuenca MM, Jimenez-Pavon D, Chillon P, Girela-Rejon MJ, Mora J, Gutierrez A, Suni J, Sjöström M, M.J. C. Field-based fitness assessment in young people: the ALPHA health-related fitness test battery for children and adolescents. Br J Sports Med 2011;**45**(518-524).

30. In. *WHO Guidelines on Drawing Blood: Best Practices in Phlebotomy*. Geneva 2010.

31. Parikh P, Mochari H, Mosca L. Clinical utility of a fingerstick technology to identify individuals with abnormal blood lipids and high-sensitivity C-reactive protein levels. Am J Health Promot 2009;**23**(4):279-82.

32. West SG, Finch JF, Curran PJ. Structural equation models with nonnormal variables: Problems and remedies. In: Hoyle RH, (ed). *Structural equation modeling. Concepts, issues, and applications*. Thousand Oakes: Sage; 1995, 56-75.

33. Norman K, Stobäus N, Cristina Gonzalez M, Schulzke J-D, Pirlich M. Hand grip strength: Outcome predictor and marker of nutritional status. Clin Nutr 2011;**30**:135-142.

34. Kim Y, Wijndaele K, Lee D-C, Sharp SJ, Wareham N, Brage S. Independent and joint associations of grip strength and adiposity with all-cause and cardiovascular disease mortality in 403'199 adults: The UK Biobank study. Am J Clin Nutr 2017;**106**:773-782.

35. Kim HR, Kwon O. Higher diet quality is associated with lower odds of low hand grip strength in the Korean elderly population. Nutrients 2019;**11**:1487.

36. Thorpe RJJ, Simonsick E, Zonderman A, Evans MK. Association between race, poverty status and grip strength in middle to old age adults. Ethn Dis 2016;**26**:493-500.

37. Magnussen CG, Schmidt MD, Dwyer T, Venn A. Muscular fitness and clustered cardiovascular disease risk in Australian youth. Eur J Appl Physiol 1012;**112**:3167-3171.

38. Stavnsbo M, Resaland GK, Anderssen SA, Steene-Johannessen J, Domazet SL, Skrede T, Sardinha LB, Kriemler S, Ekelund U, Andersen LB, Aadland E. Reference values for cardiometabolic risk scores in children and adolescents: Suggesting a common standard. Atherosclerosis 2018;**278**:299-306.

39. Ashdown-Franks G, Stubbs B, Koyanagi A, Schuch FB, Firth J, Veronese N, Vancampfort D. Handgrip strength and depression among 34,129 adults aged 50 years and older in six low- and middle-income countries. J Affect Disord 2019;**243**:448-454.

40. Gasior JS, Pawlowski M, Williams CA, Dabrowski MJ, Rameckers EA. Assessment of maximal isometric hand grip strength in school-aged children. Open Med 2018;**13**:22-28.

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**FIGURE LEGENDS**

Figure 1. Participant flow chart

Figure 2. Differences in muscle mass between HGS quartiles, separately for girls and boys and for

each study site

Table 1. Sample characteristics and descriptive statistics, separately for each study site

| Categorical variables | Côte d'Ivoire (n=467) | | South Africa (n=864) | | Tanzania (n=695) | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | n | % | n | % | n | % | χ² | φ | | |
| Sex | | | | | | | | | | |
| Male | 210 | 45.0 | 429 | 49.7 | 334 | 48.1 | 2.67 | 0.036 | | |
| Female | 257 | 55.0 | 435 | 50.3 | 361 | 51.9 | | | | |
| | | | | | | | Uncontrolled | | Controlled for sex, height and weight | |
| Metric variables | M | SD | M | SD | M | SD | F | η² | F | η² |
| Age (in years) | 7.86[ab] | 1.69 | 8.24[ac] | 1.42 | 9.34[bc] | 1.74 | 113.15*** | 0.118 | 60.12*** | 0.067 |
| Height (in cm) | 123.53[a] | 9.44 | 124.01[b] | 8.95 | 128.67[ab] | 10.28 | 49.83*** | 0.056 | 20.21***[2] | 0.020 |
| Weight (in kg) | 23.09[ab] | 4.43 | 24.54[ac] | 5.49 | 26.77[bc] | 5.86 | 50.07*** | 0.056 | 29.91***[3] | 0.029 |
| BMI (in kg/m²) | 15.00[ab] | 1.29 | 15.79[a] | 2.04 | 16.01[b] | 1.90 | 24.89*** | 0.029 | 32.79***[4] | 0.042 |
| Grip strength | | | | | | | | | | |
| Absolute grip strength (in kg) | 8.47[ab] | 2.63 | 11.23[ac] | 4.38 | 12.43[bc] | 4.40 | 82.74*** | 0.089 | 56.19*** | 0.063 |
| Body weight adjusted grip strength[1] | 0.36[ab] | 0.09 | 0.46[a] | 0.17 | 0.46[b] | 0.11 | 52.92*** | 0.059 | 48.13*** | 0.065 |
| Body composition | | | | | | | | | | |
| Body fat (in %) | 19.50[ab] | 2.96 | 22.13[ac] | 4.37 | 20.23[bc] | 3.95 | 68.94*** | 0.076 | 113.16*** | 0.118 |
| Muscle mass (in %) | 76.01[ab] | 2.73 | 73.50[ac] | 4.04 | 75.38[bc] | 3.68 | 74.52*** | 0.081 | 115.17*** | 0.120 |
| Fat-free mass (in %) | 80.51[ab] | 2.95 | 77.88[ac] | 4.38 | 79.78[bc] | 3.96 | 68.94*** | 0.075 | 112.50*** | 0.118 |
| Cardiovascular risk factors | | | | | | | | | | |
| Systolic blood pressure (in mm Hg) | 99.47[ab] | 10.44 | 101.36[ac] | 11.10 | 104.66[bc] | 11.07 | 20.35*** | 0.024 | 6.49** | 0.008 |
| Diastolic blood pressure (in mm Hg) | 64.95[ab] | 9.46 | 62.88[ac] | 8.50 | 67.34[bc] | 8.32 | 38.44*** | 0.044 | 32.66*** | 0.037 |
| Total cholesterol (in mmol/L) | 3.57[a] | 0.67 | 3.63[b] | 0.60 | 3.78[ab] | 0.67 | 14.43*** | 0.017 | 11.16*** | 0.013 |
| HDL cholesterol (in mmol/L) | 1.99[ab] | 0.52 | 2.06[ac] | 0.50 | 2.15[bc] | 0.53 | 35.98*** | 0.041 | 29.32*** | 0.034 |
| LDL cholesterol (in mmol/L) | 1.05[ab] | 0.36 | 1.24[ac] | 0.29 | 1.28[bc] | 0.41 | 12.86*** | 0.015 | 13.05*** | 0.015 |
| Triglycerides (in mmol/L) | 1.16[ab] | 0.35 | 0.74[a] | 0.22 | 0.77[b] | 0.33 | 241.99*** | 0.223 | 252.92*** | 0.231 |
| Glycated hemoglobin (HbA1c, in %) | 4.94[ab] | 0.38 | 5.42[ac] | 0.25 | 5.22[bc] | 1.73 | 266.29*** | 0.240 | 266.02*** | 0.240 |

Notes. [1]Body weight adjusted grip strength is calculated with the following formula: Absolute grip strength / body weight. [2]ANCOVA controlled for sex and weight. [3]ANCOVA controlled for sex and height. [4]ANCOVA controlled for sex. Mean scores with the same superscript letters are significantly different (p<0.05), based on Bonferroni post-hoc tests. Significant results are marked in bold font. *p<0.05. **p<0.01. ***p<0.001.

Table 2. Association between weight-adjusted HGS, body composition and cardiometabolic risk markers, separately for each study site

| | Côte d'Ivoire (n=467) | | | South Africa (n=864) | | | Tanzania (n=695) | | |
|---|---|---|---|---|---|---|---|---|---|
| | Model 1: Uncontrolled | | | | | | | | |
| | B (SE) | 95% CI | p | B | 95% CI | p | B | 95% CI | p |
| Body composition | | | | | | | | | |
| Body fat | **-12.65 (1.48)** | **-15.55 to -9.74** | **<0.001** | **-7.69 (0.85)** | **-9.36 to -6.03** | **<0.001** | **-12.49 (1.19)** | **-14.83 to -10.16** | **<0.001** |
| Muscle mass | **11.66 (1.36)** | **8.99 to 14.34** | **<0.001** | **7.03 (0.79)** | **5.48 to 8.59** | **<0.001** | **11.70 (1.11)** | **9.53 to 13.87** | **<0.001** |
| Fat free mass | **12.64 (1.47)** | **9.75 to 15.53** | **<0.001** | **7.69 (0.85)** | **6.02 to 9.36** | **<0.001** | **12.47 (1.19)** | **10.14 to 14.81** | **<0.001** |
| Cardiovascular risk markers | | | | | | | | | |
| Systolic blood pressure | **13.98 (5.91)** | **2.37 to 25.59** | **<0.001** | 1.36 (2.15) | -2.87 to 5.58 | 0.529 | **10.05 (3.83)** | **2.54 to 17.56** | **0.009** |
| Diastolic blood pressure | 4.07 (5.09) | -5.93 to 14.06 | 0.425 | -1.25 (1.69) | -4.47 to 2.17 | 0.496 | 0.84 (2.79) | -4.63 to 6.32 | 0.762 |
| Total cholesterol | 0.66 (0.37) | -0.07 to 1.38 | 0.078 | 0.20 (0.12) | -0.04 to 0.44 | 0.098 | -0.27 (0.20) | -0.66 to 0.11 | 0.162 |
| HDL cholesterol | 0.33 (0.18) | -0.04 to 0.69 | 0.076 | 0.10 (0.06) | -0.01 to 0.22 | 0.075 | 0.19 (0.11) | -0.02 to 0.40 | 0.082 |
| LDL cholesterol | 0.48 (0.28) | -0.07 to 1.03 | 0.088 | 0.13 (0.10) | -0.07 to 0.34 | 0.189 | **-0.51 (0.17)** | **-0.85 to -0.18** | **0.003** |
| Triglycerides | -0.32 (0.20) | -0.71 to 0.08 | 0.113 | -0.07 (0.04) | -0.16 to 0.01 | 0.091 | 0.12 (0.09) | -0.05 to 0.29 | 0.169 |
| Glycated hemoglobin (HbA1c) | -0.16 (0.21) | -0.56 to 0.24 | 0.433 | 0.06 (0.05) | -0.04 to 0.16 | 0.229 | -0.04 (0.10) | -0.24 to 0.17 | 0.712 |
| | Model 2: Controlled for sex and height | | | | | | | | |
| | B | 95% CI | p | B | 95% CI | p | B | 95% CI | p |
| Body composition | | | | | | | | | |
| Body fat | **-8.30 (1.45)** | **-11.16 to -5.45** | **<0.001** | **-5.93 (0.77)** | **-7.45 to -4.42** | **<0.001** | **-7.02 (1.34)** | **-9.66 to -4.39** | **<0.001** |
| Muscle mass | **7.68 (1.39)** | **4.95 to 10.41** | **<0.001** | **5.53 (0.73)** | **4.10 to 6.96** | **<0.001** | **6.55 (1.26)** | **4.08 to 9.03** | **<0.001** |
| Fat free mass | **8.29 (1.45)** | **5.43 to 11.15** | **<0.001** | **5.93 (0.77)** | **4.41 to 7.45** | **<0.001** | **7.02 (1.34)** | **4.38 to 9.65** | **<0.001** |
| Cardiovascular risk markers | | | | | | | | | |
| Systolic blood pressure | 1.94 (5.97) | -9.80 to 13.68 | 0.746 | -0.65 (2.02) | -4.62 to 3.32 | 0.747 | 4.88 (1.17) | -3.28 to 13.04 | 0.241 |
| Diastolic blood pressure | -0.31 (5.41) | -10.95 to 10.32 | 0.954 | -1.06 (1.67) | -4.34 to 2.23 | 0.523 | 0.06 (3.04) | -5.91 to 6.03 | 0.984 |
| Total cholesterol | **0.83 (0.39)** | **0.06 to 1.59** | **0.035** | 0.21 (0.12) | -0.04 to 0.45 | 0.094 | -0.15 (0.23) | -0.59 to 0.30 | 0.519 |
| HDL cholesterol | 0.27 (0.20) | -0.13 to 0.67 | 0.178 | 0.06 (0.06) | -0.06 to 0.17 | 0.350 | 0.19 (0.12) | -0.05 to 0.43 | 0.125 |
| LDL cholesterol | **0.07 (0.30)** | **0.15 to 1.32** | **0.014** | 0.19 (0.11) | -0.02 to 0.39 | 0.079 | -0.35 (0.20) | -0.73 to 0.04 | 0.079 |
| Triglycerides | -0.39 (0.22) | -0.81 to 0.04 | 0.075 | -0.07 (0.04) | -0.16 to 0.02 | 0.119 | 0.04 (0.10) | -0.16 to 0.23 | 0.699 |
| Glycated hemoglobin (HbA1c) | -0.23 (0.22) | -0.66 to 0.20 | 0.297 | 0.04 (0.05) | -0.06 to 0.14 | 0.463 | -0.05 (0.11) | -0.26 to 0.17 | 0.669 |

*Notes.* Significant results are marked in bold font.

Table 3. Differences between HGS quartiles in body composition and cardiometabolic risk markers, separately for each study site

| | Côte d'Ivoire (*n*=467) | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | Quartile 1 (*n*=121) | | Quartile 2 (*n*=112) | | Quartile 3 (*n*=121) | | Quartile 4 (*n*=113) | | ANCOVA | |
| | *M* | *SD* | *M* | *SD* | *M* | *SD* | *M* | *SD* | *F* | $\eta^2$ |
| BF | **21.14**[abc] | **2.86** | **19.75**[ade] | **2.85** | **18.63**[bdf] | **2.47** | **18.42**[cef] | **2.82** | **13.33*** | **0.080** |
| MM | **74.49**[abc] | **2.65** | **75.79**[ade] | **2.65** | **76.82**[bd] | **2.24** | **76.99**[ce] | **2.62** | **12.88*** | **0.077** |
| FFM | **78.88**[abc] | **2.85** | **80.24**[ade] | **2.84** | **81.36**[bd] | **2.47** | **81.61**[ce] | **2.83** | **13.43*** | **0.080** |
| SBP | 97.89 | 11.81 | 100.87 | 10.20 | 99.77 | 9.64 | 99.44 | 9.82 | 0.25 | 0.002 |
| DBP | 64.90 | 9.64 | 65.36 | 9.95 | 64.83 | 9.11 | 64.72 | 9.26 | 0.37 | 0.002 |
| TC | 3.59 | 0.72 | 3.47 | 0.61 | 3.56 | 0.69 | 3.64 | 0.67 | 1.32 | 0.009 |
| HDL-C | 1.04 | 0.35 | 1.01 | 0.37 | 1.06 | 0.37 | 1.10 | 0.37 | 1.34 | 0.009 |
| LDL-C | 2.00 | 0.55 | 1.93 | 0.48 | 1.98 | 0.53 | 2.04 | 0.50 | 0.89 | 0.006 |
| TRIG | 1.21 | 0.37 | 1.18 | 0.35 | 1.17 | 0.37 | 1.10 | 0.32 | 1.89 | 0.012 |
| HbA1c | 4.97 | 0.38 | 4.98 | 0.37 | 4.90 | 0.37 | 4.92 | 0.40 | 1.41 | 0.009 |
| | South Africa (*n*=864) | | | | | | | | | |
| | Quartile 1 (*n*=213) | | Quartile 2 (*n*=218) | | Quartile 3 (*n*=216) | | Quartile 4 (*n*=217) | | ANCOVA | |
| | *M* | *SD* | *M* | *SD* | *M* | *SD* | *M* | *SD* | *F* | $\eta^2$ |
| BF | **24.13**[ab] | **4.88** | **23.13**[cd] | **4.47** | **21.35**[ace] | **3.72** | **19.93**[bde] | **2.96** | **31.69*** | **0.100** |
| MM | **71.64**[ab] | **4.50** | **72.58**[cd] | **4.14** | **74.24**[ace] | **3.44** | **75.52**[bde] | **2.73** | **31.58*** | **0.099** |
| FFM | **75.88**[ab] | **4.89** | **76.88**[cd] | **4.48** | **78.66**[ace] | **3.73** | **80.08**[bde] | **2.97** | **31.58*** | **0.099** |
| SBP | 99.79 | 11.99 | 102.06 | 10.37 | 101.57 | 11.46 | 101.98 | 10.43 | 0.60 | 0.002 |
| DBP | 62.32 | 8.79 | 63.63 | 8.64 | 62.64 | 8.15 | 62.93 | 8.40 | 0.90 | 0.003 |
| TC | 3.61 | 0.63 | 3.57 | 0.61 | 3.64 | 0.53 | 3.70 | 0.61 | 2.01 | 0.007 |
| HDL-C | 1.22 | 0.29 | 1.21 | 0.28 | 1.24 | 0.31 | 1.27 | 0.29 | 1.20 | 0.004 |
| LDL-C | 2.03 | 0.52 | 2.02 | 0.51 | 2.07 | 0.47 | 2.10 | 0.51 | 1.81 | 0.006 |
| **TRIG** | **0.78**[a] | **0.25** | **0.75** | **0.22** | **0.70**[a] | **0.20** | **0.73** | **0.22** | **4.58** | **0.016** |
| HbA1c | 5.49 | 0.25 | 5.43 | 0.26 | 5.44 | 0.24 | 0.42 | 0.26 | 0.06 | 0.002 |
| | Tanzania (*n*=695) | | | | | | | | | |
| | Quartile 1 | | Quartile 2 | | Quartile 3 | | Quartile 4 | | ANCOVA | |
| | *M* | *SD* | *M* | *SD* | *M* | *SD* | *M* | *SD* | *F* | $\eta^2$ |
| BF | **21.91**[ab] | **4.15** | **21.01**[cd] | **3.57** | **19.69**[ace] | **3.57** | **18.29**[bde] | **3.55** | **9.49*** | **0.040** |
| MM | **73.78**[ab] | **3.86** | **74.67**[cd] | **3.32** | **75.91**[ace] | **3.28** | **77.16**[bde] | **3.31** | **9.40*** | **0.039** |
| FFM | **78.09**[ab] | **4.16** | **79.01**[cd] | **3.56** | **80.30**[ace] | **3.57** | **81.71**[bde] | **3.56** | **9.34*** | **0.039** |
| SBP | 103.36 | 10.38 | 104.28 | 10.38 | 104.85 | 11.75 | 106.16 | 11.59 | 0.44 | 0.002 |
| DBP | 67.20 | 8.05 | 67.07 | 8.49 | 67.61 | 8.49 | 67.47 | 7.84 | 0.09 | 0.000 |
| TC | 3.75 | 0.65 | 3.86 | 0.64 | 3.77 | 0.64 | 3.71 | 0.56 | 1.44 | 0.006 |
| HDL-C | 1.24 | 0.32 | 1.30 | 0.37 | 1.28 | 0.34 | 1.30 | 0.35 | 1.19 | 0.005 |
| LDL-C | 2.17 | 0.55 | 2.22 | 0.50 | 2.13 | 0.57 | 2.07 | 0.49 | 0.86 | 0.004 |
| TRIG | 0.72 | 0.23 | 0.79 | 0.31 | 0.79 | 0.36 | 0.76 | 0.31 | 1.02 | 0.004 |
| HbA1c | 5.17 | 0.31 | 5.15 | 0.32 | 5.14 | 0.32 | 5.16 | 0.33 | 0.20 | 0.001 |

*Notes.* BF = Body fat (in %). MM = Muscle mass (in %). FFM = Fat-free mass (in %). SBP = Systolic blood pressure (in mm Hg). DBP = Diastolic blood pressure (in mm Hg). TC = Total cholesterol (in mmol/L). HDL-C = HDL cholesterol (in mmol/L). LDL-C (in mmol/L). TRIG = Triglycerides (in mmol/L). HbA1c = Glycated hemoglobin (in %). ANCOVAs controlled for sex and height. Mean scores with the same superscript letters are significantly different (*p*<0.05), based on Bonferroni post-hoc tests. Significant results are marked in bold font.
*\*p*<0.05. \*\**p*<0.01. \*\*\**p*<0.001.

| Côte d'Ivoire | South Africa | Tanzania |
| --- | --- | --- |

**Côte d'Ivoire**

Number of schools: *N*=4
Number of classes: *N*=32
Total number of children in classes: *N*=1428

Number of children without parental consent: *N*=35

Number of children with parental consent: *N*=1393

Number of children excluded because of:
- Informed consent withdrawn or lost to data assessment due to relocation: *N*=78
- No grip strength data: *N*=349
- At least one cardiovascular risk marker missing: *N*=497
- Child oder than 12 years: *N*=1
- No information about child's sex: *N*=1

Number of children included in analyses: *N*=467 (32.7% of initial sample)

**South Africa**

Number of schools: *N*=4
Number of classes: *N*=32
Total number of children in classes: *N*=1413

Number of children without parental consent: *N*=45

Number of children with parental consent: *N*=1368

Number of children excluded because of:
- Informed consent withdawn: *N*=3
- Child did not pass grade: *N*=4
- Child left school: *N*=51
- No grip strength data: *N*=349
- At least one cardiovascular risk marker missing: *N*=497
- Child oder than 12 years: *N*=1
- No information about child's sex: *N*=1

Number of children included in analyses: *N*=864 (61.1% of initial sample)

**Tanzania**

Number of schools: *N*=4
Number of classes: *N*=16
Total number of children in classes: *N*=1209

Number of children without parental consent: *N*=81

Number of children with parental consent: *N*=1128

Number of children excluded because of:
- Informed consent withdrawn or lost to data assessment due to relocation: *N*=32
- No grip strength data: *N*=113
- At least one cardiovascular risk marker missing: *N*=229
- Child oder than 12 years: *N*=30
- No information about child's sex: *N*=9

Number of children included in analyses: *N*=695 (57.5% of initial sample)

Figure 1. Participant flow chart

185x185mm (96 x 96 DPI)

Figure 2. Differences in muscle mass between HGS quartiles, separately for girls and boys and for each study site

190x190mm (96 x 96 DPI)

Trials

# Effects of school-based physical activity and multi-micronutrient supplementation intervention on growth, health and well-being of schoolchildren in three African countries: the *KaziAfya* cluster randomised controlled trial protocol with a 2 × 2 factorial design

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## Abstract

**Background:** In low- and middle-income countries, infectious diseases remain a key public health issue. Additionally, non-communicable diseases are a rapidly growing public health problem that impose a considerable burden on population health. One way to address this dual disease burden, is to incorporate (lifestyle) health promotion measures within the education sector. In the planned study, we will (i) assess and compare physical activity, physical fitness, micronutrient status, body composition, infections with soil-transmitted helminths, *Schistosoma mansoni*, malaria, inflammatory and cardiovascular health risk markers, cognitive function, health-related quality of life, and sleep in schoolchildren in Côte d'Ivoire, South Africa and Tanzania. We will (ii) determine the bi- and multivariate associations between these variables and (iii) examine the effects of a school-based health intervention that consists of physical activity, multi-micronutrient supplementation, or both.

**Methods:** Assuming that no interaction occurs between the two interventions (physical activity and multi-micronutrient supplementation), the study is designed as a cluster-randomised, placebo-controlled trial with a 2 × 2 factorial design. Data will be obtained at three time points: at baseline and at 9 months and 21 months after the baseline assessment. In each country, 1320 primary schoolchildren from grades 1–4 will be recruited. In each school, classes will be randomly assigned to one of four interventions: (i) physical activity; (ii) multi-micronutrient supplementation; (iii) physical activity plus multi-micronutrient supplementation; and (iv) no intervention, which will serve as the control. A placebo product will be given to all children who do not receive multi-micronutrient supplementation. After obtaining written informed consent from the parents/guardians, the children will be

(Continued on next page)

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subjected to anthropometric, clinical, parasitological and physiological assessments. Additionally, fitness tests will be performed, and children will be invited to wear an accelerometer device for 7 days to objectively assess their physical activity. Children infected with *S. mansoni* and soil-transmitted helminths will receive deworming drugs according to national policies. Health and nutrition education will be provided to the whole study population independently of the study arm allocation.

**Discussion:** The study builds on the experience and lessons of a previous study conducted in South Africa. It involves three African countries with different social-ecological contexts to investigate whether results are generalisable across the continent.

**Trial registration:** The study was registered on August 9, 2018, with ISRCTN. https://doi.org/10.1186/ISRCTN29534081.

**Keywords:** Children, Côte d'Ivoire, Dual disease burden, Health, Multi-micronutrient supplementation, Placebo, Physical activity, South Africa, Tanzania, Well-being

## Background

Ensuring healthy lives and promoting well-being among children is a complex and challenging endeavour. Indeed, children's health depends on cultural, environmental, genetic and socioeconomic factors as well as current living conditions and social and community networks [1]. In low- and middle-income countries (LMICs), infectious diseases remain a key public health issue, negatively impacting children's physical and cognitive development [2]. For example, more than a billion people are infected with parasitic worms (helminths) [3]. Helminth infections can cause abdominal pain, anaemia and (bloody) diarrhoea and might impair cognitive and physical development [4], resulting in reduced fitness and work productivity [5]. Moreover, helminth infections can have a negative impact on a child's nutritional status [6]. A deprived socioeconomic status (SES) of parents can put children at risk of malnutrition and growth retardation. Malnutrition has been found to be associated with stunting and poor cognitive development, thereby resulting in low IQ, cognitive delays and problems with motor development. This, in turn, can cause problems with a child's ability to concentrate, process information and focus on school work [7]. Children from low SES families are also less likely to have access to health care or health insurance and are more prone to be absent from school, which may have negative consequences on their academic performance. These deficiencies can prevent school-aged children from realising their full potential and perpetuate a vicious cycle of poverty and poor health.

Non-communicable diseases (NCDs) are a rapidly growing public health problem that impose a considerable burden on population health [8]. New research revealed that African populations have moved towards a disease profile similar to Western countries, with increasing proportions of deaths attributed to chronic, lifestyle-related diseases [9] and overweight, replacing undernutrition as a risk factor [2, 10]. Consequently, children are at an increased risk of compromised health due to a dual burden of diseases, which may hamper their development and well-being [8, 11]. The drivers of this dual burden may relate to the shift in dietary consumption patterns and energy expenditure as these countries pass through rapid nutritional and epidemiological changes. This trend might also result from the effect that underlying micronutrient deficiencies have on childhood stunting and changes in body composition, leading to greater adiposity and possibly contributing to long-term risks of obesity [12–15]. This dual burden constitutes a challenge for health systems in Africa and elsewhere. Although many children are still affected by infectious diseases, at a young age, they may already have developed risk factors predisposing them to NCDs in early adulthood [16, 17].

Given that (i) childhood physical inactivity is an independent risk factor for NCDs, which can lead to poor health outcomes in later life [18–20] and that (ii) micronutrient status influences health and body composition and subsequently the development of obesity and obesity-related conditions [21], one plausible strategy is to focus on the promotion of physical activity and multi-micronutrient supplementation through school-based health promotion programmes. School-based physical activity interventions are worthwhile because a considerable amount of children's daily physical activity is acquired during school hours [22]. Moreover, school-based physical activity programmes are generally effective in increasing physical activity and physical fitness in children and adolescents aged 6–18 years [23]. For instance, a randomised controlled trial with Swiss primary schoolchildren (first and fifth graders) showed that physical activity and fitness could be significantly improved with a 1-year, school-based, physical activity intervention, whereas adiposity could be decreased [24].

An attempt to increase health literacy in South African children from disadvantaged schools was undertaken in

the 'Disease, Activity and Schoolchildren's Health' (DASH) project [3]. The study primarily focused on the development of healthy school environments by implementing a series of clearly defined and standardised intramural measures. The developed intervention toolkit was pilot-tested among fourth graders in disadvantaged primary schools in Port Elizabeth, South Africa. The preliminary findings suggest that (i) the prevalence of parasitic worm infection was high in several schools [25]; (ii) children infected with soil-transmitted helminths had lower maximal oxygen uptake compared to their non-infected peers [26]; (iii) helminth infections and low physical fitness were significant predictors of low selective attention and poor academic achievement [27]; (iv) increased levels of physical activity were associated with a higher health-related quality of life (HRQoL) [28]; (v) increased physical activity levels were associated with lower risks of obesity and hypertension, but increased risk for soil-transmitted helminth infections [29]; and (vi) the DASH physical activity component resulted in small but significant decreases in the body mass index (BMI) of the children [30] and had a positive impact on academic performance within one year [31]. The intervention teaching material used in the DASH study was further elaborated and adapted and then pilot-tested among schoolchildren in grades 4–7 from two disadvantaged primary schools in Port Elizabeth. After the pilot-testing, final adaptations were made according to the feedback of school teachers, teacher-coaches and students.

The present study will build on and expand DASH by implementing this school-based health promotion programme in two other African countries and by providing multi-micronutrient supplementation. Placing an additional emphasis on multi-micronutrient supplementation is justified on multiple grounds. First, a recent meta-analysis concluded that helminth infections and micronutrient deficiencies are highly prevalent in LMICs. Second, a strong relationship exists between helminth infections and serum retinol in school-aged children. Third, micronutrient-supplementation randomised controlled trials (RCTs) showed a modest, but significant protective effect on helminth infection and reinfection rates [32]. Fourth, periodic deworming has not translated into the expected health gains according to recent systematic reviews and meta-analyses [33, 34]. One reason for this may be the concurrent problem of malnutrition and underlying micronutrient deficiencies, which makes it difficult for children to compensate for delays in growth and development that resulted from infectious diseases. Thus, multi-micronutrient supplementation might make deworming more effective in terms of a positive health impact. Fifth, as shown in previous studies [35, 36], multi-micronutrient supplementation might

result in a decrease in fat mass and increased lean body mass.

## Specific aims of the study

There are three interrelated specific aims that will be addressed in the planned study:

- To assess and compare physical activity, physical fitness, micronutrient status, body composition, infections with soil-transmitted helminths, *S. mansoni*, malaria, inflammatory and cardiovascular health risk markers, cognitive function, HRQoL, and sleep in schoolchildren in Côte d'Ivoire, South Africa and Tanzania.
- To determine the bi- and multivariate associations that exist among physical activity, physical fitness, micronutrient status, body composition, infections with soil-transmitted helminths, *S. mansoni*, malaria, inflammatory and cardiovascular health risk markers, cognitive function, HRQoL, and sleep in schoolchildren in the three study countries.
- To examine the effects of a school-based health intervention (physical activity, multi-micronutrient supplementation or both) on physical activity, physical fitness, micronutrient status, body composition, infections with soil-transmitted helminths, *S. mansoni*, malaria, inflammatory and cardiovascular health risk markers, cognitive function, HRQoL, and sleep in schoolchildren in the three study countries.

Additionally, secondary aims are to determine how the intervention is perceived by the school principals, teachers, parents and children, and whether or not the teachers can be empowered to implement the teaching material successfully by themselves. The study also has the potential for highlighting how micronutrient deficiencies may contribute to the observed increases in obesity prevalence, which is on the rise in some African countries.

Specific hypotheses for each of the outcome variables were formulated, hereby taking into account existing evidence from previous studies, mostly carried out with children living in Western societies. These hypotheses are described in detail in the supplementary material (see Additional file 1).

## Methods/Design
### Study design
We assume that no interaction occurs between the two interventions (physical activity and multi-micronutrient supplementation). The study is designed as a cluster randomised, placebo-controlled trial [37] using a 2 × 2 factorial design to assess the effect of physical activity and multi-micronutrient supplementation on children's

growth, health and well-being (Fig. 1). Data will be assessed at three time points (baseline and 9 months and 21 months after the baseline assessment). At baseline, children from grades 1–4 (most of them aged 6–10 years) will be recruited. The intervention will span two school years. The project officially started in January 2018. After 9 months of preparation and obtaining ethical approval from the relevant authorities, interventions have been launched in 2019. The project will last until December 2021 (see SPIRIT Flow Chart in Additional file 2).

The study will be carried out in primary schools in the area of Taabo in south-central Côte d'Ivoire, in Port Elizabeth in the Eastern Cape Province of South Africa, and in Ifakara in the Kilombero district of Tanzania. To obtain an adequate sample size for overweight/obese children, the intervention will not take place in the most remote areas but rather in villages that are located in rural (Côte d'Ivoire and Tanzania) or peri-urban (South Africa) settings in the three study countries.

In each country, we aim to recruit 1320 children from public school classes. Classes will be randomly assigned to one of four interventions (for more details, see the next section). To ensure allocation concealment, the four treatment arms will be determined by a computer-generated code after the baseline assessment. The four intervention arms are (i) physical activity; (ii) multi-micronutrient supplementation; (iii) physical activity plus multi-micronutrient supplementation; and (iv) no specific interventions, which will serve as the control. Based on the four intervention arms, the main and inter-action effects of the two intervention components (physical activity and multi-micronutrient supplementation) will be examined.

We will provide a placebo product to all children who do not receive multi-micronutrient supplementation. For physical activity, controlling with a placebo is not feasible. Thus, classes not involved in the physical activity intervention will follow their routine lesson plans so that, during the intervention phase, all students will have similar amounts of contact with schoolmates and teachers. To minimise subjective biases, teachers and local study personnel will be blinded with regard to the multi-micronutrient supplementation or placebo tablets.

In case of missing values (e.g. when children drop out during the intervention), all analyses will be performed



**Fig. 1** Overview of the planned study design (*Note*. MMNS = multi-micronutrient supplementation; PA = physical activity; STH = soil-transmitted helminth; T2 = 9-month follow-up; T3 = 21-month follow-up). All children receive health and nutritional education during the 2-year study period 4

with and without intention-to-treat [38]. After a thorough dropout analysis, a decision will be made on the most suitable method to use to analyse the intention-to-treat effects (e.g. imputation of missing values) [39].

### Participants and procedures

All children will be recruited in the schools involved in the project. School authorities will be contacted first. Contact with schools is made through the school principals. School principals will be informed about the objectives, procedures and potential risks and benefits of the study. Based on this information, the principals can state their interest in being part of the project.

To achieve at least small effects (f = 0.10) in the primary outcomes (physical activity and micronutrient deficiency) and to take into account the children's weight status (underweight, normal weight, or overweight/obese), power calculations indicate that a total sample of 1096 children is needed per study site (calculations based on G*power 3.1: alpha error probability = 0.05, power = 0.80, number of groups = 12 (2x2x3: physical activity intervention: yes/no, multi-micronutrient supplementation: yes/no; weight status: underweight, normal weight, or overweight), number of measurements = 3). Assuming a yearly dropout-rate of 10%, the targeted sample size is 1320 children per country at baseline. Thus, in each country, approximately 330 students will be assigned to one of the four intervention arms.

After having identified suitable schools, we will proceed in two steps to allocate classes to the four intervention arms. First, we develop a stratification table to ensure that each intervention arm will be represented in every grade across all schools. We then randomly assign the schools to the different strata, as shown in Fig. 2. If a school has several classes per grade, the classes will be randomly selected. Given that our intervention takes place in a school setting, is implemented during official class hours, is integrated in the current curriculum, and is provided by the class teachers, we have no choice other than to randomise the participants in clusters

(classes). A division of classes (in order to allow individual randomisation) would affect the everyday school life in a negative way and would be considered interruptive by the teacher staff. As a consequence, our intervention would not be acceptable to the school principals and education authorities.

Before baseline data assessment, written informed consent will be sought from the parents/guardians of the children. In line with the Ottawa Statement [40], despite cluster randomisation, we will ask each research participant and his/her parents guardians for informed consent before the children and their parents guardians know to which cluster they will be allocated. Research assistants will explain the purpose and procedures of the study, the expected duration, potential risks and benefits and any discomfort it may entail for the children. The parents/guardians will be provided with a participant information sheet and a consent form describing the study and providing sufficient information to make an informed decision about whether or not to participate. For illiterate parents, the information sheet will be read aloud and, if necessary, an oral translation of the information sheet into any of the local languages will be provided. Parents/guardians will be informed that participation is voluntary, data will be handled confidentially, and withdrawal from the study can occur anytime without further obligation, and that withdrawal of consent will not have any negative consequences except forgoing the potential benefits of the allocated intervention. After having obtained written informed consent, the parents/guardians will be asked a few specific questions regarding the families' SES, and the dietary intake information, sleep and physical activity behaviour of the children. Additionally, oral assent will be sought from children before the start of the study.

### Criteria for inclusion/exclusion

School eligibility criteria include (i) public schools from disadvantaged areas; (ii) facilities for the implementation of physical education lessons (free space: lawn, sand or

|  | School 1 | School 2 | School 3 | School 4 |
|---|---|---|---|---|
| **Grade 1** | PA | Control | PA + MMNS | MMNS |
| **Grade 2** | MMNS | PA | Control | PA + MMNS |
| **Grade 3** | PA + MMNS | MMNS | PA | Control |
| **Grade 4** | Control | PA + MMNS | MMNS | PA |

**Fig. 2** Assignment of classes to conditions via stratification to ensure that each intervention arm is represented at each grade across all schools (*Note.* PA = physical activity; MMNS = multi-micronutrient supplementation)

concrete ground); and (iii) not engaged in any other research project or clinical trial or located in areas where governmental nutrition interventions take place.

To be included in the data assessment, children must accomplish the following inclusion criteria: (i) attend grades 1–4 at baseline; (ii) aged 6–12 years at baseline; (iii) have written informed consent from their parents/guardians; (iv) not participating in other research projects or clinical trials; (v) not participating in any food/nutritional programme; and (vi) not suffering from clinical conditions that prevent participation in physical activity, as determined by qualified medical personnel.

Children will be excluded from data analyses if (i) they have a congenital or acquired alteration of the gastro-intestinal tract, which could impair absorption of the multi-micronutrient supplements; (ii) they participated in food/nutritional programmes in the past 6 months, and hence, received regular vitamin and mineral supplements; (iii) their parents/guardians did not provide written informed consent; and (iv) the children denied oral assent.

Since some parts of the intervention are integrated in the compulsory school curriculum, children will be automatically exposed to physical activity and health/nutrition education intervention. In fact, physical education is foreseen in the curriculum in all three study countries, not as a separate subject but as part of other (broader) subjects. For instance, in South Africa, physical education is part of the 'life orientation' subject. Nevertheless, physical education is not implemented as it should be in any of the involved countries. Typically, this slot is used to increase learning time for academic subjects. Therefore, our intervention aims to ensure that the time allocated to physical education in the curriculum is used appropriately and filled with meaningful content.

In the event that qualified medical personnel identify children during the baseline data assessment as having a clinical condition that prevents their participation in physical activity, we will contact the school principal and suggest that these children be removed from the physical activity intervention. Moreover, as multi-micronutrient supplementation and deworming are additional components, children and their parents/guardians who did not provide informed consent can decide whether or not the child should participate in the multi-micronutrient supplementation and/or deworming. Finally, all children have the possibility to withdraw from the study anytime without consequences from these two additional components.

### Intervention and control conditions

Participants allocated to the physical activity intervention will receive the so-called *KaziKidz* physical activity component (see: www.kazibantu.org). Of note, regular physical activity opportunities are incorporated into the main school curriculum, including daily in-class activity breaks as well as one weekly 40-min playful physical education lesson and one 40-min moving-to-music lesson. These measures are designed towards improving children's physical activity levels and positively affecting their school satisfaction and psychosocial well-being. The intervention materials presented above were pilot-tested in 2015 and 2016 in the DASH study with fourth grade children from disadvantaged schools in Port Elizabeth, South Africa. Qualitative data revealed that the physical activity materials were well received at the pilot schools.

Participants allocated to the multi-micronutrient supplementation condition will receive a daily chewable tablet containing vitamins and trace elements. The multi-micronutrient supplement is provided free of charge by DSM Nutritional Products Ltd. (Basel, Switzerland; see: www.dsm.com). The exact composition of the multi-micronutrient supplement is summarised in Table 1. During school days, the supplement is taken at schools under the direct supervision of a teacher. To avoid the risk that supplements are exchanged between students or given to other family members during weekends or public holidays, no supplements will be provided on non-school days.

Participants allocated to the physical activity plus multi-micronutrient supplementation condition will receive both intervention measures, as described above. Children who do not receive multi-micronutrient supplementation (physical activity only and control conditions) will receive a placebo product. Thus, during school days, they will receive a daily chewing tablet, similar in taste and appearance to the multi-micronutrient supplementation and administered to the children from identical packages. The tablet will not contain any macronutrients or micronutrients, except sugar, citric acid, water and artificial flavour (orange) to mask the taste and to ensure similar appearance.

### Complementary interventions for all participating children

Independently of the study arm allocation, children diagnosed with helminth infections will receive deworming medication after each data assessment. The treatment strategy chosen for each school will follow national and international guidelines, including recommendations by the World Health Organization (WHO) [41]. In brief, in schools where the infection prevalence of soil-transmitted helminths is below 20%, infected children will be treated individually; in schools where the infection prevalence of soil-transmitted helminths is between 20% and 50%, all children will be treated once a year; and, finally, in schools were the prevalence of soil-

**Table 1** Composition of the multi-micronutrient supplement

| No. | Nutrient | Average per 1 tablet |
| --- | --- | --- |
| 1 | β-carotene (as BetTab 20%S) | 3.6 mg |
| 2 | Vitamin D | 400 IU/10 mcg |
| 3 | Vitamin E | 9 mg TE |
| 4 | Vitamin K | 30 mcg |
| 5 | Vitamin C | 60 mg |
| 6 | Vitamin B1 Thiamine | 1.1 mg |
| 7 | Vitamin B2 Riboflavin | 1.3 mg |
| 8 | Vitamin B6 Pyriodoxine | 0.5 mg |
| 9 | Vitamin B12 | 1.2 mcg |
| 10 | Folic acid | 200 mcg |
| 11 | Niacinamide | 8 mg |
| 12 | Iron (added as Fe-EDTA) | 8 mg |
| 13 | Zinc (added as zinc oxide) | 5 mg |
| 14 | Selenium (added as sodium selenite anhydrous) | 20 mcg |
| 15 | Iodine (added as potassium iodate) | 100 mcg |

*Notes.* Ingredients 1–15 are nutrients and will be produced with an overage to ensure required amounts during the shelf life
Inactive ingredients: sugar, citric acid, sorbitol, non-nutritive sweetener, flavour
Placebo tablets do not contain nutrients, but colorants will be added
Dosage and directions: one tablet daily during the first school lesson
The tablets will be stored in a climate-controlled storage room at the respective research institution and will be provided to school on a fortnightly basis. At school, the tablets will be stored in locked cupboards in the teachers' room

transmitted helminth infection is 50% and above, mass treatment will be carried out twice a year. We will either administer a single 400-mg oral dose of albendazole or a single 500-mg oral dose of mebendazole. Additionally, children infected with *S. mansoni* will receive praziquantel (single 40 mg/kg oral dose).

All children will additionally benefit from health and nutritional education lessons. The developed *KaziKidz* teaching material includes a series of classroom-based health education lessons to increase awareness of helminth infections, how to prevent helminth infections (e.g. hygiene behaviour, adequate sanitation habits and the importance of consuming clean water and healthy food). Likewise, nutritional education lessons aim at highlighting the importance of healthy nutrition.

### Education and training of the teachers

The initial experiences in the DASH study showed that teachers needed considerable support for the implementation of the physical education lessons. In the planned study, during the initial phase, the intervention will be carried out in close collaboration among the teacher-coach, teachers, and school staff to allow sufficient time for capacity building and to empower schools to incorporate the programme in their given structures and to implement the programme with their own resources. Hence, teachers will be assisted by a teacher-coach during the first year of the intervention, whereas during the second year, the physical activity intervention will be

carried out by the teachers themselves without external assistance.

### Monitoring of compliance with intervention implementation

One of the specific aims of the study is to obtain new insights regarding the possibility to empower schools to implement sustainable health-promotion measures, including an appraisal of the implementation quality of health-promotion measures, if carried out under the direction of the schools themselves. Using both quantitative and qualitative research methods, such as the systematic observation of lessons; (semi-)structured interviews with school principals and parents; and focus group discussions (FGDs) with teachers, teaching staff members, and children, the study will shed light on the quality of the implementation and sustainability of the proposed health promotion measures, as well as factors affecting the quality of the implementation and the sustainability of the programme. Thereof, important insights will result with regard to the training of the teachers and school staff, as well as the support needed by the schools to incorporate health-promotion measures in the existing structures.

### Data collection and measures

Data collection will take place in the schools. Data assessment procedures will be based on a series of standardised, validated and quality-controlled tools. The same

methods will be employed at each measuring occasion (except for the parent questionnaires, which will only take place during the baseline assessment). The collected data will be double-entered and validated using EpiData (version 3.1) and merged into a single database.

The following parameters are defined as equivalent primary outcomes: (i) physical activity (7-day accelerometry), and (ii) multi-micronutrient status (vitamin A, vitamin D, transferrin and zinc). In our study, physical activity is assessed via self-reports and 7-day accelerometry. Although both methods have advantages and disadvantages [42, 43], many researchers consider accelerometer-derived data as the most appropriate way to validly assess physical activity [44]. This particularly applies to children, where the assessment of self-reported physical activity is complicated by the fact that some cognitive functions are not yet fully developed. Multi-micronutrient status, per definition, is a multi-dimensional construct. Given that our supplement contains several (nutritional) ingredients, one can expect that the product will be effective in terms of multiple outcomes. As highlighted by Bailey et al. [45], iron, iodine, folate, vitamin A and zinc deficiencies are the most widespread multi-micronutrient deficiencies and are common contributors towards poor growth, intellectual impairment, and increased risk of morbidity. Making a decision about whether one nutritional indicator is more important than another is arbitrary and cannot be convincingly substantiated. Therefore, we consider vitamin A, vitamin D, transferrin and zinc as equivalent primary outcomes.

Table 2 provides an overview of all types of data to be collected and the specific parameters that will be assessed either as primary outcomes, secondary outcomes, moderators or control variables.

### Clinical examination

A research assistant will assess detailed disease history in a face-to-face interview. Features of disease history will focus on fevers, abdominal pain, change in bowel movements, diabetes, and psychosomatic symptoms. Additionally, a qualified nurse will conduct an abdominal examination.

For the detection of hypertension, the blood pressure of each child will be taken three times after the child has been resting for approximately 5 min, with a 1-min rest in between the assessments. An Omron M3® digital blood pressure monitor (Omron Healthcare Europe; Hoofddorp, The Netherlands) will be used. A cuff size appropriate to the arm circumference of the child will be chosen.

### Blood testing

Capillary blood will be collected for haematological analyses. The child's finger will be pricked once (or if

**Table 2** Overview of parameters assessed in the planned study

Clinical examinations
    Disease history of children and parents/guardians
    Subjective health complaints (15 items)
    Blood pressure (SBP, DBP)
Blood testing
    Haemoglobin concentration (Hb)
    Blood lipids (TC, HDL-C, LDL-C, TG, Non-HDL, C-HDL ratio)
    Blood glucose (HbA1c)
    Micronutrient status (vitamin A, vitamin D, zinc, transferrin)
    Cytokines (IL-6)
    Leptin
Anthropometric measurements
    Body weight and height
    Body composition (body fat)
    Waist-to-hip ratio
    Body mass index
Parasitological examinations
    Soil-transmitted helminths (*Ascaris lumbricoides*, hookworm, *Trichuris trichiura*)
    *Schistosoma mansoni*
Cognitive function and academic performance
    Flanker task (executive function)
    School grades
Student survey
    Socio-demographic background (sex, ethnicity, home language)
    Self-reported physical activity
    Health-related quality of life (KIDSCREEN-10)
    Perceived stress (1 item)
    School satisfaction (1 item)
    Perceived academic competence (1 item)
    Sleep (6 items)
Objective assessment of physical activity
    7-day actigraphy
Fitness testing
    20-m shuttle run test (cardiorespiratory fitness)
    Grip strength test (upper body strength)
Parental survey
    Family socioeconomic status
    Dietary intake information (food frequency questionnaire)
    Food insecurity
    Sleep
Other variables
    Country, school, grade, class, distance/traveling to/from school

necessary twice) to collect approximately 10 blood drops. Haemoglobin (Hb), blood lipid and blood glucose analyses will be performed on the spot (further details are provided below), with rapid finger prick malaria tests being done in Côte d'Ivoire and Tanzania. All tests will be performed with the same measurement devices and are carried out by trained research assistants. Testing cassettes will be disposed immediately after completion of the analyses.

**Haemoglobin** For the detection of anaemia, Hb concentration will be measured once with a HemoCue® Hb 301 system according to the manufacturer's instructions (HemoCue AB; Ängelholm, Sweden).

**Blood glucose** For the measurement of glycated haemoglobin (HbA1c) level, a point-of-care (POC) instrument employing the Afinion test (Alere Technologies, Abbott; Wädenswil, Switzerland) will be used. Notably, the HbA1c level reflects the average plasma glucose concentration levels over the previous 8–12 weeks before measurement with no prior fasting required.

**Blood lipids** For the assessment of blood lipid profiles (total cholesterol (TC), low-density-lipoprotein cholesterol (LDL-C), high-density-lipoprotein cholesterol (HDL-C) and triglycerides (TG), capillary samples for blood lipid will be analysed by the Afinion test (Alere Technologies, Abbott; Wädenswil, Switzerland). One drop of blood will be taken up by the test strip and read by the machine. Children will be instructed to fast during the 3 h prior to the data assessment.

**Micronutrient status, inflammatory cytokines and leptin** The finger prick technique will also be used to prepare dried blood spots to assess children's micronutrient status/deficiencies (concentrations of vitamin A, vitamin D, zinc and transferrin) and to determine the inflammatory cytokine (IL-6) and leptin concentrations. Blood drops will be collected on a filter paper for further examination in a specialised laboratory. Dried blood spot samples on the filter paper will be shipped to the Global Clinical and Viral Laboratory (Durban, South Africa). The laboratory was involved in the WHO SAGE study [46] and is a partner institution of the Global Health Biomarker Lab at the University of Oregon, USA.

#### Anthropometric measurements

**Body weight, body height and body composition** Body composition will be assessed via bioelectrical impedance analysis (BIA) with a wireless body composition monitor (Tanita MC-580, Tanita Corp.; Tokyo, Japan). The participants will be asked to fast for 3 h before the data

assessment, to void their bladder immediately before the assessment, and to wear only light sport clothing (≤1 kg). Participants will be asked to stand barefoot on the metal plates of the machine, while being guided by the research assistant to ensure optimal contact according to the device manufacturer's instructions. The MC-580 is also able to assess body weight, which will be measured to the nearest 0.1 kg. With shoes off, each child will stand against a stadiometer with the back erect and shoulders relaxed. Body height will be taken to the nearest 0.1 cm. Sex-specific height or length-for-age and weight-for-age $z$ scores will be computed from the CDC/WHO growth reference data [47].

#### Parasitological examinations

For parasitological examinations, a researcher will visit the schools and distribute pre-labelled plastic containers to each class for the children to take home and use to collect a stool sample. These plastic containers will be returned to the research assistant in the morning of the following day. Further visits may be required to catch up with absent children. To reduce discomfort, paper bags are provided along with the containers. All stool samples will be processed on the day of collection at the study site.

Parasitic infections to be detected with the Kato-Katz technique [48] include the three main soil-transmitted helminths (*Ascaris lumbricoides*, hookworm and *Trichuris trichiura*), and *Schistosoma mansoni*. In brief, stool samples (at least 10–15 g) will first be visually examined for the presence of blood, mucus and diarrhoea. Second, duplicate 41.7 mg Kato-Katz thick smears will be prepared from each stool sample [48]. For quality control, a random sample of 10% of the Kato-Katz slides will be re-examined by a senior technician. In case of discordant results, the slides will be read a third time, and the results will be discussed among the technicians until agreement has been reached [49, 50]. Parasitological status will be established in terms of prevalence and intensity of infection with individual helminth species, and the extent of multiparasitism will be determined.

#### Cognitive function and academic performance

**Executive function** Inhibitory control is a core component of executive function and will be assessed with a computer-based version of the Flanker task [51], a standardised psychological test [52] suitable for repeated measures [53]. The task requires participants to respond to the direction of a centrally presented target stimulus, while flanking stimuli are facing in the same (congruent trials) or opposite direction (incongruent trials). Performance is assessed by calculating the mean reaction

time for correct responses as well as mean accuracy separately for different trial types. Congruent trials are a measure of basic processing speed and attention, whereas incongruent trials assess selective attention and inhibitory control.

**Academic performance** In cooperation with the schools, the end of year marks will be obtained from the following subjects: school/home language, first additional language, mathematics and life orientation. The sum-score of the four subjects will be used to estimate a child's overall academic achievement.

### Student survey

**Self-reported physical activity** Two self-report instruments will be used to assess physical activity in the present study [42, 54]. The first instrument is a single-item tool taken from the HBSC survey. The exact wording of this item is as follows: 'Physical activity is any activity that increases your heart rate and makes you get out of breath some of the time. Over the past 7 days, on how many days did you engage in such activity?' Answering options range from 1 to 7 days [55]. A similar item has been used in previous research enrolling children [56–58], including the DASH study in South Africa [28].

The second instrument is the Physical Activity Questionnaire for Children (PAQ-C), a 9-item instrument specifically designed for school-aged children [59]. Due to the limited age of our sample and time constraints, only five items will be included. The PAQ-C consists of a 7-day recall that provides a summary physical activity score derived from several items, which are each scored on a 5-point Likert-scale (from 1 to 5). Items included in the present study refer to physical activity accumulated during physical education, recess, after school, in the evening and on weekends. Previous research has shown that the PAQ-C has acceptable reliability and convergent validity [60–62]. Furthermore, cut-off values have been established which are suitable to distinguish between those children who accomplish the recommended levels of physical activity (≥60 min of moderate-to-vigorous physical activity (MVPA) per day) and children who do not meet these criteria [63].

**Health-related quality of life** The KIDSCREEN-10 will be used to assess children's HRQoL. The KIDSCREEN-10 proved to be a valid instrument to assess the psycho-social health of children aged 8–18 years in different countries [64]. The construct validity of the KIDSC-REEN instrument has been documented in an African context [65]. Moreover, we have used the KIDSCREEN in the DASH study, where we found satisfactory

psychometric properties of this instrument [28]. The KIDSCREEN-10 consists of 10 items, which can be used to build an overall HRQoL index. Following recommended procedures, item scores first will be summed up to obtain raw scores and then will be transformed into Rasch person parameter estimates using the available SPSS software version syntax for each dimension [66]. These steps will result in T-values with a scale mean of 50 and a standard deviation (SD) of 10. Higher mean scores generally reflect higher HRQoL. To be classified as 'normal', the threshold chosen by the KIDSCREEN developers was the mean, plus or minus half a SD. The KIDSCREEN scores can be compared with the norm scores of an international survey sample of 5754 European children, stratified by sex.

**Stress, school satisfaction and perceived academic competence** Perceived school-related stress, satisfaction with school and perceived academic competence will be assessed with three items from the HBSC survey. The stress measure has been used previously to show evidence for the stress-buffering effects of physical activity in European youngsters [58]. To assess school-related stress, students are asked how pressured they feel by the schoolwork they must pursue. Possible answers are as follows: not at all, a little, some, and a lot. To measure school satisfaction, students respond to the question of how they feel about school at present. Possible answers are as follows: 'I like it a lot', 'I like it a bit', 'I don't like it very much', and 'I don't like it at all'. Finally, perceived academic performance is assessed with the following question: 'In your opinion, what does your class teacher(s) think about your school performance compared to your classmates?' Possible answers are as follows: 'much better than classmates', 'better than classmates', 'similar/same as classmates', and 'worse than classmates'.

**Sleep** To assess sleep quality, questions from the Pittsburgh Sleep Quality Index (PSQI) [67] will be adapted. To screen for sleep disturbances, the three items of the Insomnia Severity Index [68] will ask about difficulty falling asleep, staying asleep and waking up too early in the morning. Evidence for the reliability and validity of this measure has been provided previously [69]. To assess further information about sleep quality and daytime functioning, children will be invited to rate their overall sleep quality and to report how restored they feel in the morning, how tired they feel during the day and how exhausted they feel in the evening. Children will also provide basic information about their sleep environment (e.g. room, type of bed, and people sleeping in the same room/bed).

*Actigraphy*

Objective physical activity will be assessed with an accelerometer device (Actigraph wGT3x-BT; Shalimar, FL, USA). The devices will be worn around the hip for 7 consecutive days to assess a full week, with a sampling epoch of 15 s [70]. Time per day spent in moderate physical activity (MPA; ≥3 metabolic equivalents of task (MET)) and vigorous physical activity (VPA; ≥6 MET) will be determined based on the raw accelerometry counts and the ActiLife® computer software (Actigraph; Shalimar, FL, USA), with cut-off values derived from Freedson et al. [71]. Of note, the ActiGraph accelerometers have been validated for children [72, 73].

*Fitness testing*

**Cardiorespiratory fitness** The children's cardiorespiratory fitness will be measured with the 20-m shuttle run test [74], which is part of the Eurofit fitness testing battery [75]. Before the start of the test, all children will be told to indicate any body discomfort and anyone who feels unwell or uncomfortable will not take part in the test. The pre-recorded sound signals will be played to the children, and they will be able to do a trial run of 2 intervals (40 m) under the supervision of a research staff member. Once children are familiar with the test procedures, they will be asked to run back and forth on the 20-m flat course (marked with colour-coded cones) in groups of 10–15 children, following the pace of the sound signals. Starting with a running speed of 8.5 km/h, the frequency of the signal increases every minute by 0.5 km/h. When a child fails to follow the pace in two consecutive intervals, the stage and the distance completed fully will be recorded. The age of the participating child and the speed at which the child stopped running will be converted into $VO_2$ max estimates.

**Upper body strength** Upper body strength will be determined with the grip strength test, with both right and left hands. The Saehan hydraulic hand dynamometer (MSD Europe BVBA; Tisselt, Belgium) will be used for this test. Before the start of the test, the hand span (distance from the tip of the thumb to the tip of the little finger) of the child's dominant hand will be measured (to the nearest 0.5 cm), and the grip span on the dynamometer will be adjusted accordingly [76, 77]. The outdoor staff will explain to the child how to hold the dynamometer correctly. The child, while sitting in an upright position, should grip the dynamometer with the arm fully extended. During this time, no other parts of the body should touch the dynamometer, and the arm being tested may not be squeezed against the body. Each child will then have three tries, or six tries in total (with a 30-s rest in between) to grip the dynamometer as hard

as possible with alternating hands. The maximum reading, measured to the nearest 1 kg, will be recorded.

*Parental survey*

**Dietary intake information** Information on dietary intake will be obtained from the parents/guardians to determine the adequacy of child's intake of macro- and micronutrients. Dietary intake of children will be assessed using a culturally sensitive food frequency questionnaire (FFQ) for each country [78, 79]. The FFQ will be administered to the child's carer at baseline.

**Food insecurity** USAID (www.usaid.gov) defines food security as a situation in which all people at all times have physical and economic access to sufficient food to meet their dietary needs for a productive and healthy life. For the purpose of this study, food insecurity will be measured with a questionnaire based on the Household Food Insecurity Access Scale [80], which has been validated in industrialized countries and LMICs. For example, the study by Knueppel et al. [81] showed satisfactory validity and reliability among poor households in rural Tanzania.

**Sleep** To gather information about children's sleep duration, parents/guardians will report the time at which their child goes to bed and wakes up in the morning on school nights and school days.

**Socioeconomic status** To estimate the SES, parents/guardians will be asked to answer nine items, covering household-level living standards, such as infrastructure and housing characteristics (house type, number of bedrooms, type of toilet and access to indoor water, indoor toilet/bathroom and electricity) and questions related to the ownership of three durable assets (presence of a working refrigerator, washing machine and car). The dichotomized items (0 = poor quality, not available; 1 = high quality, available) will be summed up to build an overall SES index, with higher scores reflecting higher SES. The validity of similar measures was established in previous research [82].

**Distance from home to school** Distance from home to school and mode of traveling to/from school will be taken into consideration as a confounding variable. Therefore, we will ask the parents/guardians about their living place (township, village and area). Using Google maps, we will determine the distance between the residence and the school location.

## Statistical analyses

To assess the effects of the intervention, changes in outcome variables over the three time points will be analysed using repeated measures analyses of variances (ANCOVAs), with three between-subject factor groups (physical activity intervention: yes/no; multi-micronutrient intervention: yes/no; and weight status: underweight, normal weight, or overweight/obese) and a within-subject factor time (baseline, 9 months, and 21 months), after controlling for relevant confounders. To take into account the non-independence of the children within a class, we will examine between-cluster differences in potentially relevant confounders, which might include characteristics of the children (e.g. sex, socioeconomic status, and ethnicity), class (e.g. class size) and the intervention (e.g. compliance with intervention). If we find systematic and substantial differences between the clusters for these variables, the factors will be controlled as covariates. Moreover, we will screen the data for univariate and multivariate outliers before performing the main analyses in order to exclude students with unrealistically high or low values. In case of missing values (e.g. when participants drop out), all analyses will be performed with and without an intention-to-treat [38]. After a thorough dropout analysis, a decision will be reached regarding the best suited method to analyse intention-to-treat effects (e.g. imputation of missing values) [39]. If significant group or time interactions are present, Bonferroni-adjusted post-hoc tests will be performed to identify individual differences. Statistical significance level will be defined at an alpha level of 0.05. Effect size will be calculated according to the recommendations of Cohen [83], with $0.49 \geq d \geq 0.20$ indicating small (negligible practical importance), $0.79 \geq d \geq 0.50$ indicating medium (moderate practical importance) and $d \geq 0.80$ indicating large effects (crucial practical importance). Additionally, bivariate and multivariate relationships between study variables will be tested using correlational analyses, regression analyses or structural equation modelling.

## Ethical considerations

### Ethical approval and trial registration

The study will be carried out in accordance with the protocol and with principles in the current version of the Declaration of Helsinki and the guidelines of Good Clinical Practice (GCP) issued by the International Conference of Harmonisation (ICH).

The study can only begin once approval from the required authorities has been received. Any additional requirements imposed by the authorities shall be implemented. Ethical approval has been obtained from the 'Ethikkommission Nordwest- und Zentralschweiz' in Switzerland (EKNZ; reference number: Req-2018-00608). The intervention study has been registered in the ISRCTN registry (http://www.isrctn.com/ISRCTN29534081).

The responsible investigator at each site ensures that ethical approval from an appropriately constituted competent ethics committee (CEC) is sought for the clinical study. The study protocol has been approved in Côte d'Ivoire by the Institutional Review Board (IRB) of the Centre Suisse de Recherches Scientifiques en Côte d'Ivoire (CSRS; Abidjan, Côte d'Ivoire) and the Comité National d'Ethique et de la Recherche (CNER), reference number: 100–18/MSHP/CVESVS-km. Approval has also been obtained in South Africa from the research ethics committee of the Nelson Mandela University in Port Elizabeth (reference number: H18-HEA-HMS-006) and the Department of Education of the Eastern Cape Province. Moreover, the study protocol has been approved by the responsible ethics committee in Tanzania (Ifakara Health Institute (IHI–IRB), the National Institute for Medical Research (NIMR) and the Tanzania Food and Drugs Authority (TFDA).

The principal investigator (MG) and local project leaders (BB, CW, and HM) are allowed to amend the protocol or to provide suggestions for a protocol amendment. Should amendments to the approved protocol be required during the project, these would only be implemented after receiving the approval of the CEC.

### Right to privacy and confidentiality

The principal investigator and local project leaders affirm and uphold the principle of the participants' rights to privacy and that they will comply with applicable privacy laws. Anonymity of the participants will be guaranteed when presenting the data at national or international conferences or publishing key findings in the peer-reviewed literature. Individual participant medical information obtained as a result of this study will be considered confidential, and disclosure to third parties is prohibited. Participant confidentiality will be further ensured by utilizing participant identification code numbers to correspond to treatment data in the computer files. For data verification purposes, authorised representatives of the EKNZ and the respective Human Ethics Committee at site may require direct access to parts of the clinical records relevant to the study, including participants' medical histories.

### Referral to local clinics

Children who suffer from severe medical conditions and/or malnourishment (as diagnosed by a nurse, following national guidelines) will be referred to local clinics. If children are infected with malaria at the time of the data assessment, immediate treatment will be offered to the children.

### Incentive for schools

As an incentive for schools, schools will be equipped with basic sports equipment and a music centre that allows for the implementation of moving-to-music lessons. Moreover, in South Africa, physical activity-friendly school environments will be developed through the implementation of simple painted games to encourage the free play of children.

### Data processing and archiving

Data will be double-entered, checked, and merged into a single SPSS file. Survey data obtained from the paper and pencil questionnaire will be scanned and entered automatically (using EvaSys software). Data analysis will be performed with established software packages (e.g. SPSS, STATA and Mplus). SPSS syntax files will be documented to assure transparency of the conducted data analysis and to assure GCP.

Data will be saved electronically. Backup files will be stored regularly on the external cloud *Switchdrive@Universität Basel*. The personal data of the participants will be encrypted, and all the data obtained (e.g. handwritten paper questionnaires and the case report forms (CRFs)) will be used exclusively for scientific research. The local study leader will keep records in locked cupboards, and after 10 years, these records will be destroyed. Completed paper sheets will be locked and stored at the respective home institution. Electronic data files will be archived on the database *Switchdrive@Universität Basel*. Only authorised investigators will have access to data files. However, in line with the guidelines and open access policies of nationally and internationally recognised foundations and institutions, the published data from our project will be made publicly available (as Additional file 1).

### Storage of biological material

Dried blood spots will be collected on filter paper and stored in a freezer at the home institution. Due to the complexity of assaying cytokines in dried blood spot samples, all samples will be shipped to the Global Clinical and Viral Laboratory in Durban, South Africa. Thus, biological samples from Côte d'Ivoire and Tanzania will leave the country conditional to material transfer agreements. Dried blood spot analyses will be carried out soon thereafter at the laboratory. Samples will be destroyed upon completion of all required assays.

### Safety

Research assistants shall report all serious adverse reactions and adverse events (AEs) that might occur after deworming, finger pricking or multi-micronutrient supplementation immediately to the local study leader and principal investigator of the clinical trial. More

specifically, during the entire duration of the study, all AEs and all serious adverse events (SAEs) will be collected, fully investigated and documented in source documents and CRFs. The study duration encompasses the time from when the first participant signs the informed consent until the last protocol-specific procedure has been completed, including a safety follow-up period. In case of any SAE, the participant's well-being will be followed up until he or she shows normal laboratory values or vital signs below alert. Project leaders at the site are obliged to document and report the process, independently of the participant's termination of study. Each research institution will ensure that the required insurance coverage is in place for the trial under applicable laws. In case of AEs or SAEs, unblinding of the participant is permissible.

### Data monitoring and publication of data

The trial steering committee (composed of the sponsor-investigator (MG), the director of the Swiss Tropical and Public Health Institute (JU), and the principal investigators of the three study countries (BB, CW, HM)) will coordinate data monitoring, interim analysis, and dissemination of the study results through presentations at national and international conferences and publications in primarily open-access peer-reviewed journals. The trial steering committee will decide which researchers (beyond those listed as co-authors in the current study protocol) will have access to the final trial dataset. In agreement with the other members of the trial steering committee, the sponsor–investigator has the right to terminate the study prematurely according to certain circumstances, including ethical concerns, insufficient participant recruitment, and safety issues. Additional reasons for study termination include alterations in accepted clinical practice that render the continuation of the trial unwise and evidence of benefit or harm of the experimental intervention. On request of the local ethical review boards, an independent data monitoring committee will be appointed. The data monitoring board will have access to the unblinded interim data and can make recommendations to the trial steering committee. Trial auditing is carried out at the end of each year, whereas annual financial reports will be audited by an external auditor in each study country.

At the end of the study, the results will be communicated to the respective health and education authorities in the study countries and to the involved schools. All intervention materials will be made available to the respective control groups, so that the whole community can benefit from this project. Workshops will be offered to all teachers at the involved schools to assist with the

implementation of the school-based health promotion programme.

### Schedule and milestones
Recruitment of participants started in Côte d'Ivoire on October 18, 2018. Follow-up assessments will be complete in March 2021. The project will end in December 2021. An approximate schedule is presented in Table 3.

### Discussion
This investigation has scientific relevance because it is among the first to explore the associations between objectively assessed physical activity/fitness, micronutrient deficiencies, body composition, infectious diseases, inflammatory and cardiovascular health risk markers and sleep indicators in schoolchildren in three African settings. Moreover, our study will contribute to a deeper understanding of the effects of a school-based intervention, combining physical activity and multi-micronutrient supplementation. The study will provide new insights in how schools can be empowered to implement school-based health promotion measures. Finally, as happens in many African countries, physical education and sport activities are not implemented as foreseen because academic subjects are considered more important, and this project could help to maintain or even increase the time allowed for physical activities in the schools. Time allocation is crucial along with empowerment of the actors.

Additionally, the study will provide important information about factors that may negatively affect the quality of the implementation of the intervention. Our study will also highlight the opportunities and challenges associated with capacity building in disadvantaged schools, to empower them to carry out health promotion measures with their own limited resources. Using accelerometer-based physical

activity data will allow a more valid estimate of the level of physical activity of African schoolchildren inside and outside the school and will provide new insights into how a physical activity intervention impacts schoolchildren's physical activity behaviour.

In addition, our research will provide a comprehensive update on the status of micronutrient deficiencies, inflammatory and cardiovascular health risk markers and communicable diseases in the selected communities in Port Elizabeth (South Africa), Ifakara (Tanzania), and Taabo (Côte d'Ivoire) that are at different stages of an epidemiological and nutrition transition. By linking these factors with cognitive and academic performance, children's subjective health perceptions, and their psychosocial well-being, this wealth of information will reveal the true health consequences associated with the potential dual burden of diseases and will provide guidance for further health interventions to be implemented among school-aged children in these settings and elsewhere.

Thus far, only a few trials have investigated the potential of multi-micronutrient supplementations in African children. While physical activity was offered as part of our initial DASH study, the data assessment was limited to urban townships in South Africa. Hence, the current study will provide additional relevant information on whether or not the findings can be generalized to rural or peri-urban areas and to other African countries.

### Trial status
The study protocol corresponds to the first version of the protocol, as submitted to the EKNZ on July 19, 2018. The recruitment will start on October 18, 2018, in Côte d'Ivoire, in January 2019 in South Africa and in July 2019 in Tanzania. Data assessment should be complete in June 2020 in Côte d'Ivoire,

**Table 3** Planned schedule and milestones

| Due date | Milestone title |
| --- | --- |
| April 30, 2018 | Establishing contacts with authorities and schools |
| June 30, 2018 | Development of intervention materials |
| December 31, 2018 | Completion of initial teacher training in all countries |
| March 31, 2019 | Completion of baseline (T1) data assessment in all countries |
| November 30, 2019 | Completion of T2 follow-up data assessment in all countries |
| November 30, 2019 | Completion of first year of intervention in all countries |
| November 30, 2020 | Completion of T3 follow-up data assessment in all countries |
| March 31, 2021 | Completion of second year of intervention in all countries |
| November 30, 2021 | Completion of presentation of results to local authorities |
| December 31, 2021 | Final report |

in December 2020 in South Africa, and in March 2021 in Tanzania. Ethical approval has been obtained from the relevant review boards in Switzerland, Côte d'Ivoire, South Africa and Tanzania.

## Supplementary information

**Supplementary information** accompanies this paper at https://doi.org/10.1186/s13063-019-3883-5.

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**Additional file 1:** Hypotheses associated with each of the study's outcomes.

**Additional file 2:** Spirit flow chart.

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## Abbreviations

AE: adverse event; ANCOVA: analysis of covariance; BIA: bioelectrical impedance analysis; BMI: body mass index; CDC: Centers for Disease Control and Prevention; CEC: competent ethics committee; CNER: Comité National d'Ethique et de la Recherche in Côte d'Ivoire; CSRS: Centre Suisse de Recherches Scientifiques en Côte d'Ivoire; DASH: Disease, Activity and Schoolchildren's Health project; EKNZ: Ethikkommission Nordwest- und Zentralschweiz; FFQ: food frequency questionnaire; FGD: focus group discussion; GCP: good clinical practice; Hb: haemoglobin; HbA1c: glycated haemoglobin; HDL-C: high-density lipoprotein; HRQoL: health-related quality of life; ICH: International Conference of Harmonisation; IHI-IRB: Institutional Review Board of the Ifakara Health Institute; IL-6: human interleukin 6; ISRCTN: International Standard Randomized Controlled Trial Number; KIDSCREEN-10: 10-item instrument to assess health-related quality of life among children; LDL-C: low-density lipoprotein; LMICs: low- and middle-income countries; NCDs: non-communicable diseases; PAQ-C: physical activity questionnaire for children; POC: point-of-care; PSQI: Pittsburgh sleep quality index; RCT: randomized controlled trial; SAE: serious adverse event; SD: standard deviation; SES: socioeconomic status; SPSS: Statistical Package for the Social Sciences; STATA: statistics and data software; TC: total cholesterol; TFDA: Tanzania Food and Drugs Authority; TG: triglycerides; USAID: US Agency for International Development; WHO: World Health Organization

## Authors' contributions

MG, SAA, JB, BB, JTC, DD, RdR, LF, SG, GJM, CL, KZL, SL, HM, IM, SN, FO, NPH, UP, PS, SGT, CW and JU were involved in the design of the study. MG wrote the manuscript draft. SAA, JB, BB, JTC, DD, RdR, LF, SG, GJM, CL, KZL, SL, HM, IM, SN, FO, NPH, UP, PS, SGT, CW and JU read and critically commented on the draft. All authors read and approved the final manuscript.

## Availability of data and materials

All data analysed during this study will be included in the published articles and their supplementary information files. The *KaziKidz* intervention material will be made publicly available on www.kazibantu.org.

## Ethics approval and consent to participate

Data assessment can only begin in a study country once approval from the required authorities has been received. Ethical approval has been obtained from the 'Ethikkommission Nordwest- und Zentralschweiz' in Switzerland (EKNZ, reference number: Req-2018-00608:). The intervention study has been registered in the ISRCTN registry (http://www.isrctn.com/ISRCTN29534081). The study protocol has been approved in Côte d'Ivoire by the Institutional Review Board of the Centre Suisse de Recherches Scientifiques en Côte d'Ivoire (CSRS; Abidjan, Côte d'Ivoire) and the Comité National d'Ethique et de la Recherche (CNER) (reference number: 100–18/MSHP/CVESVS-km). Approval has also been obtained in South Africa from the research ethics committee of the Nelson Mandela University in Port Elizabeth (reference number: H18-HEA-HMS-006) and the Department of Education of the Eastern Cape Province. Moreover, the study protocol has been approved by the responsible ethics committee in Tanzania (Ifakara Health Institute–Institutional Review Board (IHI-IRB), National Institute for Medical Research (NIMR)) and the Tanzania Food and Drugs Authority (TFDA). Oral assent will be sought from children, and written informed consent will be sought from the parents/guardians before the start of the data assessment.

## Consent for publication

Not applicable. Our manuscripts will not contain the personal data of any individual.

## Competing interests

The authors declare that they have no competing interests.

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## References

1.  Dahlgren G, Whitehead M. Policies and strategies to promote social equity in health. Stockholm: Institute for Future Studies; 1991.
2.  Murray CJL, Vos T, Lozano R, Naghavi M, Flagman AD, Michaud C, et al. Disability-adjusted life years (DALYs) for 291 diseases and injuries in 21 regions, 1990-2010: a systematic analysis for the Global Burden of Disease Study 2010. Lancet. 2012;380:2197–223.
3.  Yap P, Müller I, Walter C, Seelig H, Gerber M, Steinmann P, et al. Disease, Activity and Schoolchildren's Health (DASH) in Port Elizabeth, South Africa: a study protocol. BMC Public Health. 2015;15:1285. https://doi.org/10.1186/s12889-015-2636-y.
4.  Utzinger J, Becker SL, Knopp S, Blum J, Neumayr AL, Keiser J, et al. Neglected tropical diseases: diagnosis, clinical management, treatment and control. Swiss Med Wkly. 2012;142:w13727.
5.  Yap P, Du ZW CR, Zhang LP, Wu FW, Wang J, Wang XZ, Zhou H, Zhou XN, Utzinger J, Steinmann P. Soil-transmitted helminth infections and physical fitness in school-aged Bulang children in southwest China: results from a cross-sectional survey. Parasit Vectors. 2012;5:50. https://doi.org/10.1186/756-3305-5-50.
6.  Hürlimann E, Houngbedji CA, N'Dri PB, Bänninger D, Coulibaly JT, Yap P, et al. Effect of deworming on school-aged children's physical fitness, cognition and clinical parameters in a malaria-helminth co-endemic area of Côte d'Ivoire. BMC Infect Dis. 2014;14:411. https://doi.org/10.1186/471-2334-14-411.

7. Bloom B, Dey AN, Freeman G. Summary health statistics for U.S. children: National Health Interview Survey, 2005. Vit Health Stat. 2006;231:1–84.

8. Marshall SJ. Developing countries face double burden of disease. Bull World Health Organ. 2004;82:556.

9. Steyn K, Damasceno A. Lifestyle and related risk factors for chronic diseases. In: Jamison DT, Feachem RG, Makgoba MW, Bos ER, Baingana FK, Hofman KJ, et al., editors. Disease and mortality in sub-Saharan Africa. Washington: World Bank; 2006.

10. Lim SS, Vos T, Flaxman AD, Danaei G, Shibuya K, Adair-Rohani H, et al. A comparative risk assessment of burden of disease and injury attributable to 67 risk factors and risk factor clusters in 21 regions, 1990-2010: a systematic analysis for the Global Burden of Disease Study 2010. Lancet. 2012;380: 2224–60.

11. Santosa A, Wall S, Fottrell E, Hogberg U, Byass P. The development and experience of epidemiological transition theory over four decades: a systematic review. Glob Health Act. 2014;7:23574. https://doi.org/10.3402/gha.v7.23574.

12. Popkin BM, Richards MK, Montiero CA. Stunting is associated with overweight in children of four nations that are undergoing the nutrition transition. J Nutr. 1996;126:3009–16.

13. Brown KH, Peerson JM, Rivera J, Allen LH. Effect of supplemental zinc on the growth and serum zinc concentrations of prepubertal children: a meta-analysis of randomized controlled trials. Am J Clin Nutr. 2002;75:1062–71.

14. Gilbert-Diamond D, Baylin A, Mora-Plazas M, Marin C, Arsenault JE, Hughes MD. Vitamin D deficiency and anthropometric indicators of adiposity in school-age children: a prospective study. Am J Clin Nutr. 2010;92:1446–51.

15. Gunanti IR, Marks GC, Mamun AA, Long KZ. Low serum concentrations of carotenoids and vitamin E are associated with high adiposity in Mexican-American children. J Nutr. 2014;144:489–95.

16. Herman KM, Craig CL, Gauvin L, Katzmarzyk PT. Tracking of obesity and physical activity from childhood to adulthood: the Physical Activity Longitudinal Study. Int J Pediatr Obes. 2009;4:281–8.

17. The NCD Alliance. A focus on children and non-communicable diseases (NCDs). 2016. https://ncdalliance.org/sites/default/files/rfiles/20110627_A_Focus_on_Children_&_NCDs_FINAL_2.pdf. Accessed 14 May 2016.

18. Dwyer T, Magnussen CG, Schmidt MD, Ukoumunne OC, Ponsonby AL, Raitakari OT, et al. Decline in physical fitness from childhood to adulthood associated with increased obesity and insulin resistance in adults. Diabetes Care. 2009;32:683–7.

19. Gerber M, Börjesson M, Ljung T, Lindwall M, Jonsdottir I. Fitness moderates the relationship between stress and cardiovascular risk factors. Med Sci Sports Exerc. 2016;48:2075–81.

20. Walter CM, Du Randt R, Venter DJL. The physical activity and health status of two generations of black South African professional women. Health SA Gesondheid. 2011;16:538.

21. Garcia OP, Long KZ, Rosado JL. Impact of micronutrient deficiencies on obesity. Nutr Rev. 2009;67:559–72.

22. Meyer U, Roth R, Zahner L, Gerber M, Puder J, Hebestreit H, et al. Contribution of physical education to overall physical activity. Scand J Med Sci Sports. 2013;23:600–6.

23. Dobbins M, Husson H, DeCorby K, LaRocca RL. School-based physical activity programs for promoting physical activity and fitness in children and adolescents aged 6 to 18. Cochrane Database Syst Rev. 2013;2. https://doi.org/10.1002/14651858.CD007651.pub2.

24. Kriemler S, Zahner L, Schindler C, Meyer U, Hartmann T, Hebestreit H, et al. Effect of school based physical activity programme (KISS) on fitness and adiposity in primary schoolchildren: cluster randomised controlled trial. BMJ. 2010;340:c785.

25. Müller I, Gall S, Beyleveld L, Gerber M, Pühse U, du Randt R, et al. Shrinking risk profiles after deworming of children in Port Elizabeth, South Africa, with special reference to *Ascaris lumbricoides* and *Trichuris trichiura*. Geospat Health. 2017;12:601. https://doi.org/10.4081/gh.2017.601.

26. Müller I, Yap P, Steinmann P, Damons BP, Schindler C, Seelig H, et al. Intestinal parasites, growth and physical fitness of schoolchildren in poor neighbourhoods in Port Elizabeth, South Africa: a cross-sectional survey. Parasit Vectors. 2016;9:488. https://doi.org/10.1186/s13071-016-1761-5 .

27. Gall S, Müller I, Walter C, Seelig H, Steenkamp L, Pühse U, et al. Associations between selective attention and soil-transmitted helminth infections, socioeconomic status and physical fitness in disadvantaged children in Port Elizabeth, South Africa: an observational study. PLoS Negl Trop Dis. 2017;11: e0005573. https://doi.org/10.1371/journal.pntd.0005573.

28. Salvini M, Gall S, Müller I, Walter C, Du Randt R, Steinmann P, et al. Physical activity and health-related quality of life among schoolchildren from disadvantaged neighbourhoods in Port Elizabeth, South Africa. Qual Life Res. 2018;27:205–16.

29. Gerber M, Müller I, Walter C, Du Randt R, Adams L, Gall S, et al. Physical activity and dual disease burden among South African primary schoolchildren from disadvantaged neighbourhoods. Prev Med. 2018;112: 104–10.

30. Müller I, Schindler C, Adams L, Endes K, Gall S, Gerber M, et al. Effect of a multidimensional fitness education and hygiene intervention programme on physical fitness and body-mass-index in disadvantaged primary schoolchildren (DASH) in Port Elizabeth, South Africa: a longitudinal study. Int J Environ Res Public Health. 2019;16. https://doi.org/10.3390/ijerph16020232.

31. Gall S, Adams L, Joubert N, Ludyga S, Müller I, Nqweniso S, et al. Effect of a 20-week physical activity intervention on selective attention and academic performance in children living in disadvantaged neighborhoods in Port Elizabeth, South Africa. PLoS One. 2018;13:e0206908. https://doi.org/10.1371/journal.pone.0206908.

32. de Gier B, Campos Ponce M, van de Bor M, Doak CM, Polman K. Helminth infections and micronutrients in school-age children: a systematic review and meta-analysis. Am J Clin Nutr. 2014;99:1499–509.

33. Taylor-Robinson DC, Maayan N, Soares-Weiser K, Donegan S, Garner P. Deworming drugs for soil-transmitted intestinal worms in children: effects on nutritional indicators, haemoglobin, and school performance. Cochrane Database Syst Rev. 2015;7. https://doi.org/10.1002/14651858.CD000371.pub6

34. Welch VA, Ghogomu E, Hossain A, Awasthi S, Bhutta ZA, Cumberbatch C, et al. Mass deworming to improve developmental health and wellbeing of children in low-income and middle-income countries: a systematic review and network meta-analysis. Lancet Glob Health. 2017;5:40–50.

35. Li Y, Wang C, Zhu K, Feng RN, Sun CH. Effects of multivitamin and mineral supplementation on adiposity, energy expenditure and lipid profiles in obese Chinese women. Int J Obes. 2010;34:1070–7.

36. Arsenault JE, López de Romaña D, Penny ME, Van Loan MD, Brown KH. Additional zinc delivered in a liquid supplement, but not in a fortified porridge, increased fat-free mass accrual among young Peruvian children with mild-to-moderate stunting. J Nutr. 2008;138:108–14.

37. Campbell MK, Piaggio G, Elbourne DR, Altman DG. Consort 2010 statement: extension to cluster randomised trials. BMJ. 2012;345:e5661. https://doi.org/10.1136/bmj.e5661.

38. Gupta SK. Intention-to-treat concept: a review. Perspect Clin Res. 2011;2: 109–12.

39. White IR, Horton NJ, Carpenter J, Pocock SJ. Strategy for intention to treat analysis in randomised trials with missing outcome data. BMJ. 2011;342. https://doi.org/10.1136/bmj.d40.

40. Taljaard M, Weijer C, Grimshaw JM, Eccles MP. The Ottawa Statement on the ethical design and conduct of cluster randomised trials: précis for researchers and research ethics committees. BMJ. 2013;346. https://doi.org/10.1136/bmj.f2838.

41. WHO. Preventive chemotherapy in human helminthiasis: coordinated use of anthelminthic drugs in control interventions: a manual for health professionals and programme managers. Geneva: World Health Organization; 2006.

42. Biddle SJH, Gorely T, Pearson N, Bull FC. An assessment of self-reported physical activity instruments in young people for population surveillance: Project ALPHA. Int J Behav Nutr Phys Act. 2011;8:1. https://doi.org/10.1186/479-5868-8-1.

43. Hidding LM, Chinapaw MJM, van Poppel MNM, Mokkink LB, Altenburg TM. An updated systematic review of childhood physical activity questionnaires. Sports Med. 2018;48:2797–842.

44. Ekelund U, Sjöström M, Yngve A, Poortvliet E, Nilsson A, Froberg K, et al. Physical activity assessed by activity monitor and doubly labeled water in children. Med Sci Sports Exerc. 2001;33:275–81.

45. Bailey RL, West KP, Black RE. The epidemiology of global micronutrient deficiencies. Ann Nutr Metab. 2015;66:22–33.

46. McDade TW, Williams S, Snodgrass JJ. What a drop can do: dried blood spots as a minimally invasive method for integrating biomarkers into population-based research. Demography. 2007;44:899–925.

47. Ogden CL, Flegal KM, Carroll MD, Johnson CL. Prevalence and trends in overweight among US children and adolescents, 1999-2000. JAMA. 2002; 288:1728–32.

48. Katz N, Chaves A, Pellegrino J. A simple device for quantitative stool thick-smear technique in schistosomiasis mansoni. Rev Inst Med Trop Sao Paulo. 1972;14:397–400.

49. Sherkhonov T, Yap P, Mammadov S, Sayfuddin K, Martinez P, Amoss WP, et al. National intestinal helminth survey among schoolchildren in Tajikistan: prevalences, risk factors and perceptions. Acta Trop. 2013;126:93–8.

50. Knopp S, Mgeni AF, Khamis IS, Steinmann P, Stothard JR, Rollinson D, et al. Diagnosis of soil-transmitted helminths in the era of preventive chemotherapy: effect of multiple stool sampling and use of different diagnostic techniques. PLoS Negl Trop Dis. 2008;2:e331. https://doi.org/10.1371/journal.pntd.0000331..

51. Eriksen BA, Eriksen CW. Effects of noise letters upon the identification of a target letter in a nonsearch task. Percept Psychophys. 1974;16:143–9.

52. Diamond A. Executive functions. Ann Rev Psychol. 2013;64:135–68.

53. Wöstmann NM, Aichert DS, Costa A, Rubia K, Möller H-J, Ettinger U. Reliability and plasticity of response inhibition and interference control. Brain Cogn. 2013;81:82–94.

54. Bauman A, Chau J, van der Ploeg H, Hardy L. Physical activity measures for children and adolescents – recommendations on population surveillance: a rapid review. Sydney: Sax Institute; 2010.

55. Aït-Khaled N, Pearce N, Anderson HR, Ellwood P, Montefort S, Shah J, et al. Prevalence of symptoms of asthma, rhinitis and eczema in 13- to 14-year-old children in Africa: the ISAAC Phase III. Allergy. 2009;62:247–58.

56. Kjonniksen L, Torsheim T, Wold B. Tracking of leisure-time physical activity during adolescence and young adulthood: a 10-year longitudinal study. Int J Behav Nutr Phys Act. 2008;5:69.

57. Samdal O, Tynjala J, Roberts C, Sallis JF, Villberg J, Wold B. Trends in vigorous physical activity and TV watching of adolescents from 1986 to 2002 in seven European Countries. Eur J Pub Health. 2007;17:242–8.

58. Haugland S, Wold B, Torsheim T. Relieving the pressure? The role of physical activity in the relationship between school-related stress and adolescent health complaints. Res Quart Exerc Sport. 2003;74:127–35.

59. Crocker PRE, Bailey DA, Faulkner RA, Kowalski KC, McGrath R. Measuring general levels of physical activity: preliminary evidence for the physical activity questionnaire for older children. Med Sci Sports Exerc. 1997;29:1344–9.

60. Bervoets L, Van Noten C, Van Roosbroeck S, Hansen D, Van Hoorenbeeck K, Verheyen E, et al. Reliability and validity of the Dutch Physical Activity Questionnaires for Children (PAQ-C) and Adolescents (PAQ-A). Arch Public Health. 2014;72:47. https://doi.org/10.1186/2049-3258-72-47.

61. Kowalski KC, Crocker PRE, Faulkner RA. Validation of the physical activity questionnaire for older children. Ped Exerc Sci. 1997;9:174–86.

62. Wang JJ, Baranowski T, Lau PW, Chen TA, Pitkethly AJ. Validation of the Physical Activity Questionnaire for Older Children (PAQ-C) among Chinese children. Biomed Environ Sci. 2016;29:177–86.

63. Benitez-Porres J, Alvero-Cruz JR, Sardinha LB, Lopez-Fernandez I, Carnero EA. Cut-off values for classifying active children and adolescents using the Physical Activity Questionnaire: PAQ-C and PAQ-A. Nutr Hosp. 2016;33:1036–44.

64. Ravens-Sieberer U, Gosch A, Rajmil L, Erhart M, Bruil J, Power M, et al. The KIDSCREEN-52 quality of life measure for children and adolescents: psychometric results from a cross-cultural survey in 13 European countries. Value Health. 2008;11:645–58.

65. Taliep N, Florence M. Evaluating the construct validity of the KIDSCREEN-52 Quality of Life questionnaire within a South African context. S Afr J Psychol. 2012;42:255–69.

66. The Kidscreen Group E. The KIDSCREEN questionnaires: quality of life questionnaires for children and adolescents; 2006. p. 231.

67. Buysse DJ, Reynolds CF, Monk TH, Berman SR, Kupfer DJ. The Pittsburgh Sleep Quality Index: new instrument for psychiatric practice and research. Psychiatry Rev. 1989;28:193–213.

68. Morin CM, Belleville G, Belanger L, Ivers H. The Insomnia Severity Index: psychometric indicators to detect insomnia cases and evaluate treatment response. Sleep. 2011;34:601–8.

69. Gerber M, Lang C, Lemola S, Colledge F, Kalak N, Holsboer-Trachsler E, et al. Validation of the German version of the Insomnia Severity Index in adolescents, young adults and adult workers: results from three cross-sectional studies. BMC Psychiatry. 2016;16:174. https://doi.org/10.1186/s12888-016-0876-8.

70. Rowlands AV. Accelerometer assessment of physical activity in children: an update. Pediatr Exerc Sci. 2007;19:252–66.

71. Freedson PS, Melanson E, Sirard J. Calibration of the computer science and applications, Inc accelerometer. Med Sci Sports Exerc. 1998;30:777–81.

72. Crouter SE, Horton M, Bassett DR. Validity of ActiGraph child-specific equations during various physical activities. Med Sci Sports Exerc. 2013;45:1403–9.

73. Hänggi JM, Phillips LR, Rowlands AV. Validation of the GT3X ActiGraph in children and comparison with the GT1M ActiGraph. J Sci Med Sport. 2013;16:40–4.

74. Leger LA, Mercier D, Gadoury C, Lambert J. The multistage 20 metre shuttle run test for aerobic fitness. J Sports Sci. 1988;6:93–101.

75. Adam C, Klissouras V, Ravazzolo M, Renson R, Tuxworth W. Eurofit: European test of physical fitness. Rome: Council of European Committee for Development of Sport; 1988.

76. Espana-Romero V, Artero EG, Santaliestra-Pasias AM, Gutierrez A, Castillo MJ, Ruiz JR. Hand span influences optimal grip span in boys and girls aged 6 to 12 years. J Hand Surg Am. 2008;33:378–84.

77. Ruiz JR, Espana-Romero V, Ortega FB, Sjöström M, Castillo MJ, Gutierrez A. Hand span influences optimal grip span in male and female teenagers. J Hand Surg Am. 2008;31:1367–72.

78. Wentzel-Viljoen E, Laubscher R, Kruger A. Using different approaches to assess the reproducibility of a culturally sensitive quantified food frequency questionnaire. S Afr J Clin Nutr. 2011;24:143–8.

79. Zack RM, Irema K, Kazonda P, Leyna GH. Validity of a FFQ to measure nutrient and food intake in Tanzania. Public Health Nutr. 2018;21:2211–20.

80. Salvador Castell G, Perez Rodrigo C, Ngo de la Cruz J, Aranceta Bartrina J. Household food insecurity access scale (HFIAS). Nutr Hosp. 2015;31:272–8.

81. Knueppel D, Demment M, Kaiser L. Validation of the household food insecurity access scale in rural Tanzania. Public Heath Nutr. 2010;13:360–7.

82. Filmer D, Pritchett LH. Estimating wealth effects without expenditure data or tears: An application to educational enrollments in states of India. Demography. 2001;38:115–32.

83. Cohen J. Statistical power analysis for the behavioral sciences. Mahwah: Erlbaum; 1988.

## Publisher's Note

**Supplementary Online Material**

**Is grip strength linked to body composition and cardiovascular risk markers in primary schoolchildren? Cross-sectional data from three African countries**

Per request of one external reviewer, we have controlled our analyses for some further covariates: physical activity, cardiorespiratory fitness (VO$_2$max), household dietary diversity, and socioeconomic status. In the following sections, we provide a short description of these measures. Afterwards, we provide the results of the regression analyses and ANCOVAs, in which these factors are considered as additional covariates.

**Moderate-to-vigorous physical activity**

We used light triaxial accelerometers (ActiGraph® wGT3X-BT; Pensacola, United States of America) objectively assess children's physical activity during one regular school week. These monitors proved to be a reliable measure to assess PA levels in prior research.[1] The children wore the accelerometer on 7 consecutive days around the hip, except for activities in water. A sampling rate of 30 Hz was chosen. We then analysed the raw files with the ActiLife software (ActiGraph® version 6.13.2). Days on which children wore the monitor for at least 8 hours were considered valid.[2] Non-wear time was estimated with default settings of the Troiano et al. algorithm.[3] We included only children with valid data on ≥4 weekdays and ≥1 weekend day.[4] The applied intensity cut-points were specifically defined for children to calculate an overall index for moderate-to-vigorous physical activity.[5]

**Cardiorespiratory fitness**

We applied the 20m shuttle run test to assess cardiorespiratory fitness (CRF).[6] The test started with a pace of 8.5 km/h. Following sound signals, the speed was then steadily increased by 0.5 km/h. The test was finished when children could no longer follow the speed of the sound signal twice in a row. VO$_2$max was then predicted based on the total number of fully completed 20m laps, following the equation by Leger et al.[6] Evidence of the reliability and validity of the 20m shuttle run to assess CRF among children has been reported previously.[7]

### Dietary diversity

In order to collect information about dietary diversity, we asked parents to complete a food frequency questionnaire (FFQ) to collect information on the consumption of various food items by the children within the past 24 hours. The FFQ was modified according to main local food item consumption additionally to the recommended food items of the Food and Agriculture Organization of the United Nations (FAO).[8, 9] Food items represented the following food groups: (1) cereals (e.g., maize, rice), (2) white roots and tubers (e.g., potatoes), (3) vitamin A rich vegetables (e.g., carrot, pumpkin), (4) green, leafy vegetables (e.g., spinach, sweet potato, leaves), (5) vegetables (e.g., tomato, pepper), (6) vitamin A rich fruits (e.g., mango, peach), (7) fruits (e.g., wild fruits, melon), (8) organ meat (e.g., liver, kidney), (9) flesh meat (e.g., beef, pork), (10) eggs, (11) fish (e.g., dried fish, tuna); (12) legumes, nuts and their products (e.g., beans, peas), (13) milk, milk products (e,g., cheese, cow milk) (14) oil (e.g., oil, butter); (15) sweets (e.g., honey, chocolate); (16) sweet beverages (e.g., sweetened juice drinks, sweetened soda). In the present analyses, we used to the woman dietary diversity score (WDDS) as a measure of dietary diversity. The WDDS reflects the individual probability of nutritional qualitative food sources in the diet. The WDDS score (0-9) included the following aggregated food groups (with 0 reflecting no consumption of any food group and 9 reflecting consumption of all food groups with at least one food item per group): (1) starchy samples (cereals and white roots and tubers), (2) vitamin A rich fruits and vegetables, (3) dark green leafy vegetables, (4) other fruits and vegetables, (5) organ meat, (6) meat and fish, (7) eggs, (8) legumes (legumes, nuts and their products), (9) milk (milk and milk products).[10, 11]

### Family socioeconomic status

We assessed family socioeconomic with a questionnaire administered to the caregivers, including nine items which served as proxy variables (e.g., living standards, housing characteristics, water and sanitation condition, possession of durable assets). Each of the 9 items was a dichotomized variable with 0 = not present and 1 = present. An SES score of 9 indicates a high SES. The items were summed up and averaged to build an overall SES score ranging 0-9. In case of one missing item, a family's mean SES score was entered into the dataset. Similar items for SES calculation were previously tested.[12]

Table S1. Association between weight-adjusted HGS, body composition and cardiometabolic risk markers, separately for each study site

| | Côte d'Ivoire (n=126)[a] | | | South Africa (n=626)[a] | | | Tanzania (n=494)[a] | | |
|---|---|---|---|---|---|---|---|---|---|
| | Model 3: Controlled for sex, height, moderate-to-vigorous physical activity, accelerometer wear-time, cardiorespiratory fitness, dietary diversity, and socioeconomic status | | | | | | | | |
| | B (SE) | 95% CI | p | B | 95% CI | p | B | 95% CI | p |
| Body composition | | | | | | | | | |
| Body fat | -12.53 (2.43) | -17.33 to -7.72 | <0.001 | -5.60 (0.95) | -7.46 to -3.74 | <0.001 | -5.68 (1.54) | -8.71 to -2.66 | <0.001 |
| Muscle mass | 11.85 (2.33) | 7.24 to 16.46 | <0.001 | 5.28 (0.89) | 3.53 to 7.03 | <0.001 | 5.28 (1.44) | 2.44 to 8.12 | <0.001 |
| Fat free mass | 12.55 (2.44) | 7.73 to 17.36 | <0.001 | 5.62 (0.95) | 3.75 to 7.49 | <0.001 | 5.63 (1.54) | 2.61 to 8.66 | <0.001 |
| Cardiovascular risk markers | | | | | | | | | |
| Systolic blood pressure | -3.45 (9.24) | -21.71 to 14.80 | 0.709 | -0.10 (2.53) | -5.06 to 4.87 | 0.970 | 14.58 (6.61) | 1.58 to 27.58 | 0.028 |
| Diastolic blood pressure | 2.52 (8.67) | -14.61 to 19.65 | 0.771 | 0.51 (2.09) | -3.60 to 4.61 | 0.809 | 2.34 (3.81) | -5.12 to 9.86 | 0.535 |
| Total cholesterol | 1.17 (0.61) | -0.03 to 2.38 | 0.057 | 0.20 (0.15) | -0.10 to 0.50 | 0.193 | 0.13 (0.32) | -0.49 to 0.75 | 0.677 |
| HDL cholesterol | 0.39 (0.29) | -0.18 to 0.96 | 0.178 | -0.01 (0.08) | -0.17 to 0.14 | 0.888 | 0.25 (0.15) | -0.06 to 0.55 | 0.113 |
| LDL cholesterol | 1.02 (0.54) | -0.05 to 2.09 | 0.063 | 0.22 (0.13) | -0.03 to 0.47 | 0.087 | -0.42 (0.25) | -0.91 to 0.08 | 0.100 |
| Triglycerides | -0.52 (0.34) | -1.19 to 0.14 | 0.123 | -0.01 (0.05) | -0.12 to 0.09 | 0.814 | 0.11 (0.12) | -0.13 to 0.35 | 0.371 |
| Glycated hemoglobin (HbA1c) | -0.15 (0.37) | -0.88 to 0.58 | 0.679 | 0.08 (0.07) | -0.06 to 0.21 | 0.258 | 0.06 (0.13) | -0.20 to 0.31 | 0.676 |

Notes. [a]Lower sample sizes compared to results reported in the main manuscript are due to further missing data in additionally considered covariates (physical activity, cardiorespiratory fitness, dietary diversity, and socioeconomic status). Significant results are marked in bold font.

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Table 2. Differences between HGS quartiles in body composition and cardiometabolic risk markers, separately for each study site

| | Côte d'Ivoire (n=126)[f] | | | | | | | | | |
| | Quartile 1 (n=45) | | Quartile 2 (n=42) | | Quartile 3 (n=40) | | Quartile 4 (n=35) | | ANCOVA | |
| | M | SD | M | SD | M | SD | M | SD | F | η² |
| BF | 21.26[abc] | 3.05 | 19.81[ade] | 3.09 | 18.86[bd] | 2.49 | 17.65[ce] | 3.12 | 8.51*** | 0.145 |
| MM | 74.35[abc] | 2.77 | 75.82[ade] | 2.90 | 76.53[bd] | 2.24 | 77.66[ce] | 2.93 | 8.36*** | 0.142 |
| FFM | 78.74[abc] | 3.04 | 80.19[ade] | 3.07 | 81.11[bd] | 2.47 | 82.39[ce] | 3.09 | 8.80*** | 0.149 |
| SBP | 99.09 | 10.01 | 101.77 | 9.62 | 100.50 | 8.55 | 99.62 | 9.53 | 0.29 | 0.006 |
| DBP | 67.19 | 9.32 | 67.84 | 9.94 | 67.22 | 8.86 | 67.47 | 8.11 | 0.17 | 0.003 |
| TC | 3.67 | 0.75 | 3.63 | 0.59 | 3.65 | 0.71 | 3.71 | 0.66 | 0.33 | 0.006 |
| HDL-C | 1.07 | 0.32 | 1.12 | 0.38 | 1.18 | 0.38 | 1.16 | 0.32 | 0.62 | 0.012 |
| LDL-C | 2.06 | 0.67 | 2.00 | 0.46 | 1.97 | 0.57 | 2.07 | 0.56 | 0.67 | 0.010 |
| TRIG | 1.18 | 0.34 | 1.14 | 0.28 | 1.09 | 0.34 | 1.07 | 0.34 | 0.40 | 0.019 |
| HbA1c | 5.02 | 0.36 | 5.07 | 0.41 | 4.98 | 0.39 | 0.94 | 0.41 | 0.60 | 0.012 |
| | South Africa (n=626)[f] | | | | | | | | | |
| | Quartile 1 (n=139) | | Quartile 2 (n=158) | | Quartile 3 (n=167) | | Quartile 4 (n=162) | | ANCOVA | |
| | M | SD | M | SD | M | SD | M | SD | F | η² |
| BF | 24.58[ab] | 5.03 | 23.01[cd] | 4.59 | 21.33[ace] | 3.34 | 19.99[bde] | 2.99 | 20.99*** | 0.093 |
| MM | 71.21[ab] | 4.63 | 72.68[cd] | 4.24 | 74.28[ace] | 3.09 | 75.48[bde] | 2.76 | 21.20*** | 0.094 |
| FFM | 75.42[ab] | 5.04 | 76.99[cd] | 4.59 | 78.69[ace] | 3.35 | 80.01[bde] | 3.00 | 21.03*** | 0.093 |
| SBP | 100.11 | 12.08 | 11.85 | 10.47 | 102.11 | 11.07 | 102.15 | 10.70 | 0.18 | 0.001 |
| DBP | 62.75 | 8.33 | 63.59 | 8.85 | 63.11 | 8.38 | 63.20 | 8.75 | 0.32 | 0.002 |
| TC | 3.62 | 0.64 | 3.55 | 0.61 | 3.62 | 0.52 | 3.69 | 0.62 | 1.66 | 0.008 |
| HDL-C | 1.24 | 0.29 | 1.21 | 0.28 | 1.24 | 0.31 | 1.26 | 0.32 | 1.68 | 0.008 |
| LDL-C | 2.04 | 0.53 | 2.00 | 0.50 | 2.06 | 0.48 | 2.10 | 0.52 | 0.57 | 0.003 |
| TRIG | 0.76 | 0.24 | 0.74 | 0.22 | 0.70 | 0.19 | 0.74 | 0.22 | 2.29 | 0.011 |
| HbA1c | 5.38 | 0.25 | 5.43 | 0.26 | 5.44 | 0.24 | 5.43 | 0.25 | 0.48 | 0.002 |
| | Tanzania (n=494)[f] | | | | | | | | | |
| | Quartile 1 (n=132) | | Quartile 2 (n=122) | | Quartile 3 (n=122) | | Quartile 4 (n=118) | | ANCOVA | |
| | M | SD | M | SD | M | SD | M | SD | F | η² |
| BF | 21.91[ab] | 4.16 | 21.01[cd] | 3.57 | 19.69[ace] | 3.57 | 18.29[bde] | 3.55 | 9.49*** | 0.040 |
| MM | 73.78[ab] | 3.86 | 74.67[cd] | 3.32 | 75.91[ace] | 4.28 | 77.16[bde] | 3.32 | 9.30*** | 0.039 |
| FFM | 78.09[ab] | 4.16 | 79.01[cd] | 3.56 | 80.30[ace] | 3.57 | 81.71[bde] | 3.57 | 9.34*** | 0.039 |
| SBP | 103.36 | 10.38 | 104.28 | 10.38 | 104.85 | 11.75 | 107.51 | 17.78 | 1.33 | 0.006 |
| DBP | 67.20 | 8.05 | 67.07 | 8.49 | 67.61 | 8.91 | 67.47 | 7.84 | 0.09 | 0.000 |
| TC | 3.75 | 0.65 | 3.86 | 0.64 | 3.81 | 0.79 | 3.72 | 0.56 | 1.35 | 0.006 |
| HDL-C | 1.24 | 0.32 | 1.33 | 0.56 | 1.28 | 0.34 | 1.30 | 0.35 | 1.19 | 0.005 |
| LDL-C | 2.17 | 0.55 | 2.22 | 0.50 | 2.14 | 0.57 | 2.07 | 0.49 | 1.22 | 0.005 |
| TRIG | 0.72 | 0.23 | 0.79 | 0.31 | 0.81 | 0.43 | 0.76 | 0.31 | 1.55 | 0.007 |
| HbA1c | 5.43 | 3.40 | 5.15 | 0.32 | 5.14 | 0.32 | 5.16 | 0.33 | 1.03 | 0.004 |

*Notes.* BF = Body fat (in %). MM = Muscle mass (in %). FFM = Fat-free mass (in %). SBP = Systolic blood pressure (in mm Hg). DBP = Diastolic blood pressure (in mm Hg). TC = Total cholesterol (in mmol/L). HDL-C = HDL cholesterol (in mmol/L). LDL-C (in mmol/L). TRIG = Triglycerides (in mmol/L). HbA1c = Glycated hemoglobin (in %). ANCOVAs controlled for sex and height. Mean scores with the same superscript letters[(a-e)] are significantly different ($p < 0.05$), based on Bonferroni post-hoc tests. [f]Lower sample sizes compared to results reported in the main manuscript are due to further missing data in additionally considered covariates (physical activity, cardiorespiratory fitness, dietary diversity, and socioeconomic status). Significant results are marked in bold font.
*$p < 0.05$. **$p < 0.01$. ***$p < 0.001$.

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**References**

1.  Hills AP, Mokhtar N, Byrne NM. Assessment of physical activity and energy expenditure: An overview of objective measures. Frontiers in Nutrition 2014;**15**:doi:10.3389/fnut.2014.00005.
2.  Aadland E, Andersen LB, Skrede T, Ekelund U, Anderssen SA, Resaland GK. Reproducibility of objectively measured physical activity and sedentary time over two seasons in children; Comparing a day-by-day and a week-by-week approach. PLoS One 2017;**12**:e0189304.
3.  Troiano RP, Berrigan D, Dodd KW, Mâsse LC, Tilert T, McDowell M. Physical activity in the United States measured by accelerometer. Medicine and Science in Sports and Exercise 2008;**40**:181-188.
4.  Clemente FM, Nikolaidis PT, Martins FM, Mendes RS. Physical activity patterns in university students: Do they follow the public health guidelines? PLoS One 2016;**11**:doi:10.1371/journal.pone.0152516.
5.  Evenson KR, Catellier DJ, Gill K, Ondrak KS, McMurray RG. Calibration of two objective measures of physical activity for children. Journal of Sports Science 2008;**26**:1557-1565.
6.  Léger LA, Mercier D, Gadoury C, Lambert J. The multistage 20 meter shuttle run test for aerobic fitness. Journal of Sports Sciences 1988;**6**:93-101.
7.  Lang JJ, Wolfe Philips E, Orpana HM, Tremblay MS, Ross R, Ortega FB, Silva DAS, Tomkinson GR. Field-based measurement of cardiorespiratory fitness to evaluate physical activity. Bulletin of the World Health Organization 2018;**96**:794-796.
8.  Rose D, Bourne L, Bradshaw D. Food and nutrient availability in South African households. In. Cape Town, South Africa: MRC South Africa; 2002.
9.  FAO. Dietary Assessment: A resource guide to method selection and application in low resource settings. Rome. 2018.
10. FAO. *Guidelines for measuring household and individual dietary diversity*. Rome, Italy: FAO; 2010.
11. Swindale A, Bilinsky P. *Household dietary diversity score (HDDS) for measurement of household food access: indicator guide (v.2)*. Washington, D.C., USA: FANTA; 2006.
12. Filmer D, Pritchett LH. Estimating wealth effects without expenditure data--or tears: an application to educational enrollments in states of India. Demography 2001;**38**(1):115-32.

STROBE Statement—checklist of items that should be included in reports of observational studies

| | Item No | Recommendation |
|---|---|---|
| **Title and abstract** | 1 | (*a*) Indicate the study's design with a commonly used term in the title or the abstract (p. 1) |
| | | (*b*) Provide in the abstract an informative and balanced summary of what was done and what was found (p. 2) |
| **Introduction** | | |
| Background/rationale | 2 | Explain the scientific background and rationale for the investigation being reported (p. 4) |
| Objectives | 3 | State specific objectives, including any prespecified hypotheses (p. 5/6) |
| **Methods** | | |
| Study design | 4 | Present key elements of study design early in the paper (p. 6) |
| Setting | 5 | Describe the setting, locations, and relevant dates, including periods of recruitment, exposure, follow-up, and data collection (p. 6) |
| Participants | 6 | *Cross-sectional study*—Give the eligibility criteria, and the sources and methods of selection of participants (p. 6/7) |
| Variables | 7 | Clearly define all outcomes, exposures, predictors, potential confounders, and effect modifiers. Give diagnostic criteria, if applicable (p. 7/8) |
| Data sources/ measurement | 8* | For each variable of interest, give sources of data and details of methods of assessment (measurement). Describe comparability of assessment methods if there is more than one group (p. 7/8) |
| Bias | 9 | Describe any efforts to address potential sources of bias (p. 8) |
| Study size | 10 | Explain how the study size was arrived at (p. 6) |
| Quantitative variables | 11 | Explain how quantitative variables were handled in the analyses. If applicable, describe which groupings were chosen and why (p. 8) |
| Statistical methods | 12 | (*a*) Describe all statistical methods, including those used to control for confounding (p. 8/8) |
| | | (*b*) Describe any methods used to examine subgroups and interactions (p. 8) |
| | | (*c*) Explain how missing data were addressed (not applicable, all children had complete data, p. 8) |
| | | (*d*) *Cross-sectional study*—If applicable, describe analytical methods taking account of sampling strategy (p. 8) |
| | | (*e*) Describe any sensitivity analyses (not applicable) |

**Results**

| | | |
|---|---|---|
| Participants | 13* | (a) Report numbers of individuals at each stage of study—eg numbers potentially eligible, examined for eligibility, confirmed eligible, included in the study, completing follow-up, and analysed (p. 8) |
| | | (b) Give reasons for non-participation at each stage (not applicable) |
| | | (c) Consider use of a flow diagram (not applicable) |
| Descriptive data | 14* | (a) Give characteristics of study participants (eg demographic, clinical, social) and information on exposures and potential confounders (p. 9, Table 1) |
| | | (b) Indicate number of participants with missing data for each variable of interest (not applicable) |
| Outcome data | 15* | *Cross-sectional study*—Report numbers of outcome events or summary measures (p. 9, Table 2) |
| Main results | 16 | (*a*) Give unadjusted estimates and, if applicable, confounder-adjusted estimates and their precision (eg, 95% confidence interval). Make clear which confounders were adjusted for and why they were included (p. 10, Table 3, Figure 2) |
| | | (*b*) Report category boundaries when continuous variables were categorized (Table 3) |
| | | (*c*) If relevant, consider translating estimates of relative risk into absolute risk for a meaningful time period (not applicable) |
| Other analyses | 17 | Report other analyses done—eg analyses of subgroups and interactions, and sensitivity analyses (not applicable) |

**Discussion**

| | | |
|---|---|---|
| Key results | 18 | Summarise key results with reference to study objectives (p. 11) |
| Limitations | 19 | Discuss limitations of the study, taking into account sources of potential bias or imprecision. Discuss both direction and magnitude of any potential bias (p. 12) |
| Interpretation | 20 | Give a cautious overall interpretation of results considering objectives, limitations, multiplicity of analyses, results from similar studies, and other relevant evidence (p. 10-12) |
| Generalisability | 21 | Discuss the generalisability (external validity) of the study results (p. 10, p. 12) |

**Other information**

| | | |
|---|---|---|
| Funding | 22 | Give the source of funding and the role of the funders for the present study and, if applicable, for the original study on which the present article is based (p. 13) |