Prevalence of American Heart Association defined ideal cardiovascular health metrics in Nepal: findings from a nationally representative cross-sectional study

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Background: The ever-increasing burden of non-communicable diseases (NCDs) is posing a serious health challenge for Nepal. This study examines the status of ideal cardiovascular health (ICH) and its associated determinants in Nepal using the American Heart Association (AHA) definition of ICH metrics.

Methods: The AHA has defined ICH as having five to seven of the ideal health metrics. A representative sample from the NCD risk factors STEPS survey 2013 were drawn to analyse the prevalence and sociodemographic determinants of ideal, intermediate and poor cardiovascular health in Nepal. Multivariable logistic regression was used to measure the determinants of ICH.

Results: More than half of the participants had ICH metrics (51.6%), with the 45–69 y age group having the lowest prevalence of ICH (28%) and females having better cardiovascular health compared with their male counterparts (60.6% vs 41.7%). The prevalence of low intake of fruit and vegetables, tobacco smoking and elevated blood pressure were quite high (99%, 18.8% and 31.4%, respectively). The status of ICH declined with age: individuals 15–29 y of age had 6 times (95% confidence interval [CI] 4.80–8.60) higher odds of having ICH compared with those who were 45–69 y, and it was low among urban residents (referent: rural; adjusted odds ratio 0.77 [95% CI 0.58–1.01]).

Conclusions: Nearly half of the participants had ICH, which declined with ageing. Further, urban residents had poor cardiovascular health. This highlights the need for a comprehensive population-based intervention targeting elderly and urban residents to improve overall cardiovascular health.

Keywords: Cardiovascular health, ICH health metrics, Nepal, Non-communicable diseases

Introduction

With unprecedented urbanization and population growth, the burden of non-communicable diseases (NCDs), notably cardiovascular diseases (CVDs), has emerged as a serious health challenge in Nepal. CVDs and their associated known risk factors account for 17% of disability-adjusted life years lost in Nepal. The major CVD risk factors, including high blood pressure (BP), high blood glucose, smoking, alcohol consumption, obesity and high triglycerides, along with increasing age, are well established. To combat this escalating threat of CVDs, the focus of their prevention and treatment needs to be shifted to the promotion of health and control of risk factors.

In 2010, the American Heart Association (AHA) came up with the concept of cardiovascular health on the basis of seven health factors and behaviours, including smoking, body mass index (BMI), nutritional intake, physical activity, BP, blood glucose level and total cholesterol level. Criteria were set to define the ideal levels of each health factor or behaviour. To attain ideal cardiovascular health (ICH), a person should have a simultaneous presence of four ideal health behaviours (non-smoking, normal weight, sufficient physical activity and ideal diet) and three ideal
health factors (normal cholesterol, normal BP and normal blood
glucose levels). Various studies have reported that higher ICH
metrics are associated with lower rates of cardiovascular events
compared with poor ICH metrics. The previous studies have
investigated these behaviours and risk factors separately and
failed to show how they interrelate to define cardiovascular
status in the Nepalese population. Furthermore, in a recent inves-
tigation involving a population from a semi-urban area of Nepal,
Gyawali et al. stated that the prevalence of ICH was very low in
Nepal. However, this study exhibited a limitation by not including
a cholesterol level in the definition of ICH, which could affect
the true estimate of the total ICH. The AHA’s metrics have not
been broadly studied before, and there is a need for studies
to capture the complete effect of the seven metrics selected
by the AHA on cardiovascular health in a Nepalese context.
Hence this study fills this gap by analysing the prevalence and
sociodemographic determinants of ideal, intermediate and poor
cardiovascular health in the Nepalese population using the AHA’s
cardiovascular health metrics from the STEPS Nepal 2013 survey,
a nationally representative household survey.

Materials and methods
Study design and sampling technique
We used the NCD risk factors of the STEPS survey 2013 data
for this analysis. Details of the methodology and a report of the
survey have been presented elsewhere. This survey, carried out
from January to June 2013, aimed to assess the risk factor of
NCDs in Nepal. The sample selection was done in two stages.
In the first stage, the primary sampling unit (PSU), compris-
ing 70 of 921 Ilakas (an administrative unit at the subdistrict
level) in Nepal, maintaining a proportionate distribution of Nepal’s
three ecological zones (mountains, hills and Terai/plains), were
selected using probability proportionate to population size. From
each PSU and three ecological zones, the wards (the smallest
administrative units) were selected, forming a total of 210 clus-
ters to serve as a secondary sampling unit. Twenty households
per cluster were then selected using systematic sampling. Finally,
a sample size of 4200 adults was obtained after the selection of
one participant from the eligible frame of adults (15–69 y of age)
in each selected household.

Study implementation
Data were collected by a team comprising a field supervisor,
medical laboratory technologist, laboratory technician and 10
enumerators having nursing, public health or a paramedic back-
ground. Two such teams with 13 researchers in each team were
assigned for data collection, which included filling out the ques-
tionnaires, carrying out physical measurements and collecting
blood samples. Maintaining a cold chain, sample processing and
the recording and reporting of biochemical measurements were
assigned to a laboratory technician. Medical laboratory technolo-
gists were responsible for assessing blood glucose levels and lipid
profiles, and for dispatching a 10% blood sample to the reference
laboratory for external quality control.

The researchers attended a week-long training conducted
by the STEPS team from the WHO headquarters (Geneva,
Switzerland) and the WHO Regional Office for South-East
Asia (New Delhi, India) prior to data collection. The training
consisted of various modules, such as the data collection
tools, interview techniques, sampling process, household and
individual selection, the use of the different kinds of templates
and forms in the survey, the use and care of personal digital
assistants (PDAs), a detailed explanation of the questionnaire
and the technique to be used for physical measurements. During
the training session, data collection teams were instructed on the
tools to be used to collect data. A pilot study was carried out to
validate the Nepali version of the WHO NCD STEPS questionnaire.

NCD risk factors were measured using three STEPS: STEP 1
involved questionnaires, STEP 2 included physical measurements
and STEP 3 was comprised of biochemical measurements. BP was
measured with a digital device (OMRON, Kyoto, Japan). Height,
weight, and waist and hip circumference were also measured.
Height was measured with a portable standard stature scale
and a portable digital weighing scale was used to measure the
participant’s weight. A constant tension tape (Seca, Hamburg,
Germany) was used to measure waist and hip circumference.

Definition of variables
ICH is defined as the simultaneous presence of ideal health
factors (untreated BP <120/<80 mmHg, untreated total chol-
esterol [TC] <200 mg/dL and untreated fasting plasma glucose
<100 mg/dL) and ideal health behaviours (never smoker, ideal
BMI, adequate physical activity and ideal diet with four of the
five components of the Dietary Approach to Stop Hypertension
diet).10 ICH was described in two ways: as the percentage of
the population with ICH (five to seven ideal metrics) and as the
mean healthy lifestyle score (range 0–7). Intermediate and poor
cardiovascular health were also assessed (three to four and zero
to two ideal metrics, respectively). ICH metrics, both biological
and behavioural, are defined individually in Box 1.

The age of the participants was categorized as 15–29, 30–
44 or 45–69 y and the level of education was categorized as
no formal education, primary, secondary or higher. The marital
status of the participants was categorized as never married,
currently married or divorced/widowed/separated. The partici-
 pant’s place of residence was classified as rural or urban and the
ecoregion was classified as mountains, hills or Terai (flat lands).
Ethnicities that were classified in the health management infor-
mation system of Nepal were further categorized as advantaged
or disadvantaged.11

Statistical analysis
The percentages and frequencies were part of the descriptive
analysis. We calculated the 95% CIs of the estimates instead
of point estimates to better reflect the variability in estimates
across the clusters. Only those participants that had complete
information on all of the cardiometabolic and behavioural
factors were included in the analysis; the rest of the data
were treated as missing and were excluded from the analysis.
Cardiovascular health metrics were compared across three ICH
metrics (ideal, intermediate and poor cardiovascular health)
according to the participant’s sociodemographic variables. The
association between ICH metrics (five to seven metrics) and
Half of the population were from the Terai belt and belonged to the study participants were primarily from rural areas (80.9%). Participants completed only primary school or less (56.4%) and 26.7% of the study population were demographic characteristics of the total participants by sex. Only in the model. A p-value adjusted and crude odds ratios were calculated simultaneously.

Figure 1. Of the three ICH factors, ideal blood cholesterol was the vascular health metric in the study population is shown in the distribution of ideal, intermediate and poor for each cardio-

Prevalence of ICH factors

The distribution of ICH factors and behaviours was examined by generating multivariable logistic regression with 95% CIs using Stata version 15 statistical software (StataCorp, College Station, TX, USA). Both adjusted and crude odds ratios were calculated simultaneously in the model. A p-value <0.05 was considered statistically significant. All the analyses were performed using a complex survey design and sampling weights were applied to adjust for design effects and non-response rates.

Results

A total of 4143 respondents participated in the STEP cohort survey, of whom 3238 (78.2%) completed the information on all seven ICH factors. Table 1 presents the baseline socio-demographic characteristics of the total participants by sex. Only 26.7% of the study population were ≥45 y of age. Most of the participants completed only primary school or less (56.4%) and the study participants were primarily from rural areas (80.9%). Half of the population were from the Terai belt and belonged to the advantaged caste group (50.5%).

Prevalence of ICH behaviours

The distribution of ICH behaviours overall and by sex is shown in Supplementary Figure 2. Poor consumption of fruit and vegetables was the least prevalent ICH behaviour, whereas ideal physical activity, non-smokers and ideal BMI were the most prevalent ICH behaviours. Supplementary Table 1 shows the very low distribution of consumption of adequate fruit and vegetables among both sexes (0.1% ideal metric). More females were non-smokers compared with males (87.4% vs 66.3%, respectively), with smoking prevalence of 77.2% among the overall population. Around three-quarters of both females and males had an ideal BMI (75.5% among the overall population; men vs women: 75% vs 75.9%). Ideal physical activity was highly prevalent in both females and males (94.6% among the overall population; men vs women: 95.9% vs 93.3%).

Number of ICH factors and behaviours

Table 2 shows that the ideal cardiovascular health (five to seven ideal metrics) was the most prevalent ICH metric among the participants (51.6%), while just 4.7% had poor cardiovascular health (zero to two ideal metrics). The mean ICH score was 4.43 (95% CI 4.38 to 4.48). None of the participants had all seven ICH metrics, while 16.2% of participants had six ICH metrics and 0.4% had one or no ICH metric (Supplementary Figure 3). The prevalence of ideal cardiovascular health was higher among females, people with a secondary level education, those never

Box 1. Definition of ICH metrics

Biological

1. Ideal BP is defined as BP <120/<80 mmHg and without any antihypertensive medication, intermediate is systolic BP 120–139 mmHg or diastolic BP 80–89 mmHg or treated to BP <120/<80 mmHg and poor is BP ≥140/≥90 mmHg.
2. Ideal TC is <200 mg/dL and without any cholesterol-lowering medication, intermediate is TC 200–239 mg/dL or treated to TC <200 mg/dL and poor is TC ≥240 mg/dL.
3. Ideal fasting blood glucose is <100 mg/dL and without any glucose-lowering medication, intermediate is glucose 100–125 mg/dL or treated to <100 mg/dL and poor is glucose ≥126 mg/dL.

Behavioural

1. BMI (kg/m²) is computed from weight and standing height, measured in the clinic setting. Ideal BMI is defined as 18.5–24.9 kg/m², intermediate as 25.0–29.9 kg/m² and poor as BMI ≥30 kg/m².
2. Smoking is defined as ideal if self-report of never having smoked, intermediate if a former smoker who quit smoking and Poor if current smoker.
3. Fruits and vegetables ≥4.5 servings/d is an ideal diet and <4.5 servings/d is a poor diet.
4. Physical activity ≥300 min of moderate or ≥150 min of vigorous intensity physical activity, or an equivalent combination of both, is ideal. Physical activity ≥150 min of moderate or ≥75 min of vigorous intensity physical activity, or an equivalent combination of both, is intermediate. Poor is not meeting these recommendations.

BP was measured with a digital, automated BP monitor (OMRON) with appropriate-sized cuffs. Three readings were taken and the mean of the second and third readings was used for reporting.

Fasting blood glucose and TC were estimated using semi-automated procedures (Bioanalyzer, Analytycon Biotechnologies, Lichtenfels, Germany) and commercially available kits (Analytycon). Blood glucose and TC were estimated using the glucose oxidase/peroxidase–phenol–4-amenophenazone (GOD–PAP) and cholesterol oxidase/peroxidase-4-phenol-aminoantipyrine (CHOD–PAP) methods, respectively.

Definition of ICH metrics

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### Table 1. Characteristics of the study population overall and by sex (n=4143)

<table>
<thead>
<tr>
<th>Background characteristics</th>
<th>Total population (n=4143)</th>
<th>Male (n=1336)</th>
<th>Female (n=2807)</th>
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<td>Weighted, %</td>
<td>Unweighted, n</td>
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<tr>
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<td>46.53</td>
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<td>30–44</td>
<td>1558</td>
<td>26.82</td>
<td>417</td>
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<tr>
<td>45–69</td>
<td>1613</td>
<td>26.65</td>
<td>630</td>
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<tr>
<td>Primary</td>
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<td>25.39</td>
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<td>18.68</td>
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<td>78.00</td>
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<td>Divorced/widowed/separated</td>
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<td>3.32</td>
<td>53</td>
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<td>80.94</td>
<td>1067</td>
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<td>Urban</td>
<td>777</td>
<td>19.06</td>
<td>269</td>
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<tr>
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<td></td>
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<tr>
<td>Hill</td>
<td>1767</td>
<td>42.82</td>
<td>570</td>
</tr>
<tr>
<td>Mountain</td>
<td>297</td>
<td>6.54</td>
<td>104</td>
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<tr>
<td>Terai</td>
<td>2077</td>
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<tr>
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<td>49.53</td>
<td>652</td>
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<tr>
<td>Advantaged</td>
<td>2106</td>
<td>50.47</td>
<td>684</td>
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</table>

**Figure 1.** Prevalence estimates for poor, intermediate and ICH metrics for each of the seven AHA cardiovascular health metrics.
Table 2. Cardiovascular health metrics by sociodemographic variables

<table>
<thead>
<tr>
<th>Categories</th>
<th>Unweighted, n</th>
<th>Ideal cardiovascular health (5–7 ideal metrics), weighted % (95% CI)</th>
<th>Intermediate cardiovascular health (3–4 ideal metrics), weighted % (95% CI)</th>
<th>Poor cardiovascular health (0–2 ideal metrics), weighted % (95% CI)</th>
</tr>
</thead>
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<tr>
<td>Age (y)</td>
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<td></td>
<td></td>
</tr>
<tr>
<td>15–29</td>
<td>716</td>
<td>70.1 (65.2 to 74.6)</td>
<td>28.9 (24.5 to 33.8)</td>
<td>1 (0.4 to 2.2)</td>
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<tr>
<td>30–44</td>
<td>1261</td>
<td>45.2 (41.2 to 49.3)</td>
<td>50 (46.1 to 53.8)</td>
<td>4.8 (3.3 to 7)</td>
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<td>45–69</td>
<td>1261</td>
<td>27.9 (24.8 to 31.1)</td>
<td>61.3 (57.8 to 64.7)</td>
<td>10.8 (8.5 to 13.7)</td>
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<td>Sex</td>
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<tr>
<td>Male</td>
<td>1019</td>
<td>41.7 (37.2 to 46.5)</td>
<td>51.5 (47 to 55.9)</td>
<td>6.8 (5.3 to 8.7)</td>
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<tr>
<td>Female</td>
<td>2219</td>
<td>60.6 (57.3 to 63.4)</td>
<td>36.6 (34 to 39.2)</td>
<td>2.8 (2.2 to 3.7)</td>
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<td>Education</td>
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<tr>
<td>No formal schooling</td>
<td>1372</td>
<td>48.5 (44.4 to 52.6)</td>
<td>46.7 (42.9 to 50.6)</td>
<td>4.8 (3.6 to 6.4)</td>
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<tr>
<td>Primary</td>
<td>814</td>
<td>47.2 (42 to 52.4)</td>
<td>45.4 (40.4 to 50.4)</td>
<td>7.5 (5.3 to 10.4)</td>
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<tr>
<td>Secondary</td>
<td>900</td>
<td>57.1 (52 to 62.1)</td>
<td>39.6 (35 to 44.5)</td>
<td>3.3 (2.3 to 4.6)</td>
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<tr>
<td>Higher</td>
<td>152</td>
<td>49.7 (40.3 to 59.2)</td>
<td>47.4 (38.3–56.6)</td>
<td>2.9 (1.2 to 6.9)</td>
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<tr>
<td>Marital status</td>
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<tr>
<td>Never married</td>
<td>239</td>
<td>67.8 (60.3 to 74.5)</td>
<td>30.9 (24.3 to 38.4)</td>
<td>1.3 (0.5 to 3.1)</td>
</tr>
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<td>Currently married</td>
<td>2831</td>
<td>48.9 (45.7 to 52.2)</td>
<td>45.8 (42.8 to 48.8)</td>
<td>5.3 (4.2 to 6.6)</td>
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<tr>
<td>Divorced/widowed/separated</td>
<td>168</td>
<td>34 (24.7 to 44.7)</td>
<td>56.6 (46.2 to 66.4)</td>
<td>9.4 (5.5 to 15.7)</td>
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<tr>
<td>Residence</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Rural</td>
<td>2598</td>
<td>53.1 (49.6 to 56.6)</td>
<td>42.4 (39.1 to 45.7)</td>
<td>4.5 (3.5 to 5.8)</td>
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<tr>
<td>Urban</td>
<td>640</td>
<td>45.4 (38.2 to 52.8)</td>
<td>48.9 (41.9 to 55.9)</td>
<td>5.7 (4.2 to 7.8)</td>
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<td>Ecoregion</td>
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<td></td>
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<tr>
<td>Hill</td>
<td>1442</td>
<td>49.9 (44.8 to 54.9)</td>
<td>45.7 (41 to 50.4)</td>
<td>4.5 (3.4 to 6)</td>
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<tr>
<td>Mountain</td>
<td>238</td>
<td>59.4 (46.2 to 71.4)</td>
<td>35.3 (23.9 to 48.7)</td>
<td>5.3 (1.8 to 14.3)</td>
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<tr>
<td>Terai</td>
<td>1558</td>
<td>52.2 (47.9 to 56.4)</td>
<td>43 (39 to 47)</td>
<td>4.9 (3.6 to 6.5)</td>
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<td>Ethnicity</td>
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<tr>
<td>Disadvantaged</td>
<td>1527</td>
<td>54.2 (50.2 to 58.2)</td>
<td>41.7 (37.9 to 45.6)</td>
<td>4.1 (3 to 5.6)</td>
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<tr>
<td>Advantaged</td>
<td>1711</td>
<td>49.2 (45.3 to 53.1)</td>
<td>45.5 (41.8 to 49.2)</td>
<td>5.3 (4.2 to 6.7)</td>
</tr>
<tr>
<td>Total</td>
<td>3238</td>
<td>51.6 (48.4 to 54.8)</td>
<td>43.7 (40.7 to 46.7)</td>
<td>4.7 (3.8 to 5.8)</td>
</tr>
</tbody>
</table>

married and those living in rural and mountain regions. Similarly, disadvantaged ethnicity had a higher percentage of ICH metrics (Figure 2).

The associations between the sociodemographic variables and ICH using multivariate analyses are presented in Supplementary Table 2. After adjusting for sex, marital status, residence, education and ethnicity, the 15–29 y age group (adjusted OR [aOR] 6.42 [95% CI 4.80 to 8.60], p<0.001) and the 30–44 y age group (aOR 2.19 [95% CI 1.78 to 2.69], p<0.001) were more likely to have ICH compared with the 45–69 y age group. Adjusted analysis suggests that female (aOR 2.28 [95% CI 1.82 to 2.85], p<0.001) and mountain residents (aOR 1.51 [95% CI 1.01 to 2.25], p=0.044) had higher odds of having ICH than male and hill residents, respectively.

**Discussion**

This is the first study from Nepal to explore the differences in ICH metrics according to age group, sex, marital status, education status, residence and ethnicity. None of the participants had all seven ICH metrics, 16.2% participants had six ICH metrics and 0.4% had one or no ICH metric. More than half of the participants (51.6%) were found to have five to seven ideal metrics, which was higher compared with similar studies conducted in Nepal and Peru. This rate is considerably greater than that of a community setting for the general population of Nepal, where the study reported an ICH rate of 14.3% and only 0.8% of adults (≥25 y of age) had all seven ICH metrics. Similarly, in the Peruvian study that included 3058 participants, none of the participants met all seven components of the AHA’s definition of ICH and only 12.7% of participants had five to seven components of ICH. Caution should be observed when comparing the results of this study with those from other studies, as the numbers and measurements are different. Indeed, the NCD risk factors STEPS survey 2013 included a relatively younger age group, explaining the higher prevalence of ICH found in our study.

ICH behaviours were found to be more frequent compared with ICH factors in our study, with more than two-thirds of the participants having ideal physical activity, ideal BMI and non-smoking status. The explanation for this difference could again be attributable to the inclusion of younger age groups in the STEPS
The high prevalence of ideal physical activity reported in the current study might be because, unlike in western countries, mechanization of jobs has not been possible in many sectors in Nepal. Nepalese people still engage in labour-intensive jobs and in very little leisure physical activity. High levels of occupational physical activity and low levels of leisure physical activity is not ideal for good cardiometabolic health. Thus Nepalese people should aim for a more balanced distribution of occupational and leisure-time physical activity. Food and vegetable intake according to WHO guidelines was found to be very low, which has also been reported in a community-based study. Regarding the smoking metric, smoking was higher among males compared with females (27.7% vs 10.3%, respectively). According to the Nepal Demographic and Health Survey 2016, the prevalence of cigarette smoking has decreased among both men and women, which could have improved the overall ICH score in the current scenario. Overweight and obesity are closely linked with ICH metrics, and more females were overweight or obese compared with males (25% vs 21%) in our study. Similar findings were reported in another large survey, the Nepal Demographic Health Survey 2016. Poor BP or hypertension was reported among 28% of participants, which is similar to the prevalence of hypertension in Nepal (33.8%) reported in a systematic review by Neupane et al. The ICH score could be a good metric to evaluate the overall cardiovascular health of Nepalese people. It also allows for comparisons of cardiovascular health across nations. Estimating cardiovascular health is an important tool in predicting the morbidity and mortality of several cardiovascular diseases. The importance of maintaining a high prevalence of ICH in populations has been demonstrated in recent studies, which showed a lower risk of CVDs in groups with high numbers at ideal levels. In a cohort with 15 792 participants 45–64 y of age, the group presenting an increasing number of ideal factors showed reductions in the risk of incidence of cardiovascular events, ranging from 89 to 55%, when compared with the group that did not have any ideal factors. Hence efforts should be directed towards improving the overall ICH mean population score by moving from poor to intermediate and from intermediate to ideal in any metric.

Interventions directed towards decreasing obesity, increasing physical activity, encouraging consumption of more fruit and vegetables and strictly implementing an anti-tobacco law in Nepal could significantly improve the ICH. At the same time, there is an immediate need for new policies to address the production and consumption of foods rich in trans fat and sugar-sweetened beverages.

Our study has several strengths. First, this is a population-based study that is a representative random sample of the general population of Nepal, thus the results can be generalized to the target population (individuals ≥15 y of age in Nepal). Our study is less prone to measurement error compared with other cross-sectional studies conducted in Nepal because of the use of validated and standardized STEPS tools and robust methodology.

![Figure 2. ICH metrics by age group. To the right are six ideal metrics (except fruit and vegetable intake, which was removed because nearly everyone had poor intake). Overall, the ICH was highest among individuals 26–35 y of age and decreased afterwards.](https://academic.oup.com/inthealth/advance-article-abstract/doi/10.1093/inthealth/ihz088/5613945)
However, this study has some potential limitations. The current study was cross-sectional, which limited the ability to examine causal relationships, for which longitudinal studies are needed. The self-reported physical activity measures, dietary measures and smoking measures are subject to bias that could have increased the possibility of exposure misclassification. Although a total of 4143 individuals participated in the survey, only 78.16% provided information on all seven ICH factors, which prevents us from running the analysis for all the participants. Those cases who had complete information on all seven ICH factors were included in the analysis.

Conclusions
This study shows a high prevalence of ICH in Nepal that declined with ageing. Our findings underscore the importance of promoting cardiovascular health among older and urban populations. Health education on modifiable risk factors such as physical activity, smoking and the consumption of fruits and vegetables, and screening of vascular risk factors such as BP, blood glucose and cholesterol, should be priorities.

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Ethical approval: Ethical approval for the STEPs survey 2013 was provided by the Nepal Health Research Council (Ramshah Path, Kathmandu, Nepal). Written informed consent was obtained from all participants before data collection. For those <18 y of age, consent from a legal guardian was obtained. Laboratory waste was disposed as per protocol and all the biological samples were discarded after biochemical measurements.

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