Characterising the relationships between physiological indicators and all-cause mortality (NHANES): a population-based cohort study

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Summary

Background Mortality risk stratification based on dichotomising a physiological indicator with a cutoff point might not adequately capture increased mortality risk and might not account for non-linear associations. We aimed to characterise the linear and non-linear relationships of 27 physiological indicators with all-cause mortality to evaluate whether the current clinical thresholds are suitable in distinguishing patients at high risk for mortality from those at low risk.

Methods For this observational cohort study of the US non-institutionalised population, we used data from adults (≥18 years) included in the 1999–2014 National Health and Nutrition Examination Survey (NHANES) linked with National Death Index mortality data collected from Jan 1, 1999, up until Dec 31, 2015. We used Cox proportional hazards regression models adjusted for age, sex, and race or ethnicity to assess associations of physiological indicators with all-cause mortality. We assessed non-linear associations by discretising the physiological indicator into nine quantiles (termed novemtiles) and by using a weighted sum of cubic polynomials (spline). We used ten-fold cross validation to select the most appropriate model using the concordance index, Nagelkerke R², and Akaike Information Criterion. We identified the level of each physiological indicator that led to a 10% increase in mortality risk to define our cutoffs used to compare with the current clinical thresholds.

Findings We included 47 266 adults of 82 091 assessed for eligibility. 25 (93%) of 27 indicators showed non-linear associations with substantial increases compared with linear models in mortality risk (1.5–2.5-times increase). Height and 60 s pulse were the only physiological indicators to show linear associations. For example, participants with an estimated glomerular filtration rate (GFR) of less than 65 mL/min per 1.73 m² or between 90–116 mL/min per 1.73 m² are at moderate (hazard ratio 1–2) mortality risk. Those with a GFR greater than 117 mL/min per 1.73 m² show substantial (hazard ratio ≥2) mortality risk. Both lower and higher values of cholesterol are associated with increased mortality risk. The current clinical thresholds do not align with our mortality-based cutoffs for fat deposition indices, 60 s pulse, triglycerides, cholesterol-related indicators, alkaline phosphatase, glycohaemoglobin, homoeostatic model assessment of insulin resistance, and GFR. For these indicators, the misalignment suggests the need to consider an additional bound when only one is provided.

Interpretation Most clinical indicators were shown to have non-linear associations with all-cause mortality. Furthermore, considering these non-linear associations can help derive reliable cutoffs to complement risk stratification and help inform clinical care delivery. Given the poor alignment with our proposed cutoffs, the current clinical thresholds might not adequately capture mortality risk.

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Introduction Identification of patients at high risk for poor health and determination of required health-care services (eg, additional diagnostic testing) often rely on the dichotomisation of physiological indicators. For example, glomerular filtration rate (GFR) of less than 90 mL/min per 1.73 m² is used to stage chronic kidney disease; however, this threshold is under fierce debate as it can vary depending on the investigated outcome (eg, all-cause mortality or cardiovascular disease). More importantly, the thresholds are sometimes chosen arbitrarily. Dichotomising a physiological indicator with a cutoff can be inadequate in discerning changes in mortality risk as it does not account for deviation from the cutoff. Thus, there is a need to evaluate whether the current clinical thresholds are appropriate in distinguishing patients at high mortality risk from those at low mortality risk.

Defining reliable risk thresholds requires understanding of whether associations between physiological indicators and mortality risk are linear or non-linear. The simplest approach is to model a physiological indicator as linear with respect to mortality. However, a linear approach is limited to only capturing risk that is...
To address these limitations, we aimed to characterise the relationships between 27 physiological indicators and all-cause mortality in a sample of adults in the USA to compare the current clinical thresholds with the risk cutoffs identified in our study. We focused on all-cause mortality as it is the ultimate health outcome and it was an outcome used to derive the current clinical thresholds for some physiological indicators such as body-mass index (BMI) and GFR.10 For each physiological indicator, we compared the prediction performance of linear and non-linear models to help select the most appropriate model for risk surveillance and management.

Evidence before this study
Mortality risk stratification based on dichotomising a physiological indicator with a cutoff can be poor. We searched PubMed for studies in any language published from database inception up to Jan 20, 2020, using the key words “mortality”, “linear”, “non-linear”, “validate”, and the name of a given physiological indicator. We found 12 753 studies characterising linear associations. We found 436 cohort studies that identified non-linear associations. 29 (7%) of these studies compared non-linear and linear associations. We found several studies (n=398) that compared the prediction performance across the different models but that have not validated their findings by defining separate training and test sets.

Added value of this study
The linear and non-linear models performed similarly in characterising the association between a given physiological indicator and mortality, but this result creates challenges in selecting the most appropriate model. We developed a visualisation tool to compare the linear and non-linear shapes of the association to help select the most appropriate model. Our analyses highlighted the unexpected non-linear associations of glomerular filtration rate and cholesterol-related indicators, which could have important clinical implications as both lower and higher values than the mid-range are associated with increased mortality risk. We used the non-linear associations to identify substantial differences between the current clinical thresholds and our proposed cutoffs based on mortality risk for several physiological indicators. Such differences suggest the need to understand why physiological risks of mortality are changing over time and the need for replication in other databases and using clinical outcomes to establish the utility of an additional bound or to reconsider the thresholds used for minimal risk.

Implications of all the available evidence
Choosing modelling approaches that do not capture the non-linear relationship with mortality can lead to non-optimal, non-precise, and non-sensitive risk stratification. Systematically characterising the non-linear relationships can provide clinical guidance on deriving reliable thresholds to differentiate patients who are at high risk and low risk for mortality and other disease endpoints. However, replication of our findings with respect to mortality and additional diverse clinical outcomes (eg, disease) requires follow-up in other databases to help provide insights on how clinical guidelines might be updated.

Methods
Study design and participants
The National Health and Nutrition Examination Survey (NHANES) is a cross-sectional study done by the US Centers for Disease Control and Prevention to characterise the health of a non-institutionalised, civilian US population. For this analysis, we used the continuous 1999–2014 NHANES data and started with a sample of 82 091 participants. We also used data linked with the National Death Index to obtain mortality information (eg, mortality status at follow-up and duration of follow-up) collected from Jan 1, 1999, up until Dec 31, 2015.22 We excluded participants younger than 18 years as their mortality status was not available for public release (n=34737). We also excluded participants who were deemed ineligible for mortality linkage if they...
had insufficient identifying data (n=75) and those who were not followed up (n=13) as their time of follow-up was recorded as 0, resulting in a sample of 47,266 participants. Exclusion criteria are described further in the appendix (p 30).

The National Center for Health Statistics research ethics review board provided ethical approval of the study. All participants provided written informed consent.

Procedures
We identified 60 biomarkers and measures that characterise physiological function. In NHANES, not all physiological indicators are measured in all study participants. As such, many participants with mortality data do not have measurements for some physiological indicators. Thus, we excluded physiological indicators that have low overlap with mortality data by excluding those with measurements in fewer than six NHANES cycles (n=10) and with a sample size of less than 10,000 participants (n=21). As we focused on continuous variables for studying linear and non-linear associations, we also excluded physiological indicators that are categorical (n=2). The final dataset for analysis consisted of 27 physiological indicators. Laboratory methods used to measure the physiological indicators are provided on the NHANES Laboratory Data website. A comparison of the observed characteristics between participants with complete and incomplete data is shown in the appendix (pp 3–8).

To characterise how the current clinical thresholds align with the cutoffs of increased mortality risk, we compiled a database of thresholds for 23 (85%) of 27 physiological indicators from information provided in US clinical and medical news, medical associations, and health institute webpages (appendix pp 9–17). Thresholds were not available for height, subcapular skinfold, triceps skinfold, and weight. As the same indicator might have several thresholds, the range was only used for those indicators with several thresholds. We used sex-specific thresholds when available.

Statistical analysis
To produce estimates that are representative of the non-institutionalised, civilian US population, we accounted for NHANES sampling designs by applying the survey weights to our statistical models. We did all analyses using R, version 3.6.0. Our analytical code is publicly available online.

Role of the funding source
The funders of the study had no role in study design, data collection, data analysis, data interpretation, or writing of the manuscript.

Results
We included 47,266 adults of 82,091 assessed for eligibility, with a mean age of 47.4 (SD 19.7) years, of which 24,486 (51.8%) were women (table 1). Table 2 shows the observed characteristics between participants with complete and incomplete data.
population characteristics for each physiological indicator, and the distributions of each indicator are shown in the appendix (p 32).

Figure 1 shows the concordance index and Nagelkerke $R^2$ across all the models and physiological indicators for two populations: one including all participants and the other including those participants who have measurements within the first and 99th percentiles. The Akaike Information Criterion and concordance index for these populations are shown in the appendix (pp 33–34). The concordance index ranged from 0.8 to 0.9, which is fairly high compared with the minimum concordance index at 0.5. The Nagelkerke $R^2$ ranged from 0.00 to 0.15, which is low compared with the maximum $R^2$ at 1.00. Across the fit measures, the non-linear models showed better prediction performances than the linear models. The prediction performance of the novemtile models was consistently high across all physiological indicators regardless of whether extreme measurements were excluded and was also stable as reflected by the narrower CIs compared with other models. By contrast, linear models showed low $R^2$s for inflammatory (C-reactive protein [CRP]), alkaline phosphatase, white blood cell counts), metabolic (homeostatic model assessment of insulin resistance [HOMA-IR]), and nephrological (blood urea nitrogen, creatinine) biomarkers when all participants were included. The spline model $R^2$s were also low for alkaline phosphatase and cardiovascular biomarkers involving LDL cholesterol. For alkaline phosphatase, ratio of total to HDL cholesterol, LDL cholesterol, and ratio of LDL to HDL cholesterol, exclusion of measurements outside the first and 99th percentiles resulted in improved prediction performance, particularly for the linear and spline models. The overfitting is due to the linear and spline models attempting to fit to the outliers. Appendix p 35 shows the correlations between decreased sample size and improved prediction performance. The sample sizes ranged from 14,127 to 44,288 with indicators of the cardiovascular and metabolic system having the smallest sample size, whereas indicators of body composition had the largest sample size.

A comparison of the prediction performance of models when including all participants with the prediction performance from sensitivity analyses restricting the distribution of the physiological indicators to the fifth to 95th and the tenth to 90th percentiles is shown in the appendix (pp 36–38). Appendix p 39 shows the statistical significance of all models with respect to the prediction performances. Although prediction performance and statistical significance improved when the distribution was restricted to the first and 99th percentiles, further exclusion did not lead to further improvement for the linear or spline models. By contrast, the novemtiles models showed consistent prediction performance and significance regardless of the restrictions. This result implies that studying non-linearity with quantile-based models will result in stable predictions, as outliers do not heavily influence these models when there are enough participants in each quantile. In our case, we ensured that there were at least 1,100 participants in each novemtile.

Figure 2 shows the hazard ratios for mortality risk across the distribution of BMI, systolic blood pressure, ratio of total to HDL cholesterol, CRP, HOMA-IR, and GFR for the different models. We selected these examples to highlight expected and unexpected findings, challenges in model interpretation, and relevance of the clinical thresholds. To aid visualisation, we showed the associations when measurements outside the first and 99th percentiles were excluded. Appendix p 40 shows the associations for these physiological indicators in the entire population, and appendix pp 41–45 shows the relative mortality risk for the remaining physiological indicators. As the prediction performance and mortality risks for height and 60 s pulse were similar across the models, these two measures were linearly associated with mortality. By contrast, the other 25 (93%) of 27 indicators showed non-linear associations. The sex-specific associations are shown in the appendix (pp 46–51). These figures, along with those comparing the associations with and without covariate adjustment are available online on our interactive application with an option to log-scale the axis of the physiological indicator.

For BMI, while the non-linear models showed a parabolic association with mortality in figure 2A, the linear model suggested that mortality risk is the same across the entire distribution. The BMI measurements, showing a 10% increase from minimum mortality risk, are shifted higher compared with the current clinical thresholds. After further adjusting for socioeconomic status, multimorbidity, and smoking, findings for the non-linear associations were fairly robust, although there was some attenuation in mortality risk for participants who were obese (36.1–49.9 kg/m²). However, the linear models were not robust, as shown by the transition from
null associations to negative associations for BMI and mortality risk (appendix p 52). Similar findings were observed for the other physiological indicators. Figures comparing the associations with and without confounders are available online on our interactive application. Low BMI (17·7–23·9 kg/m²) and high BMI (31·7–49·9 kg/m²) were more strongly associated with all-cause mortality in men than in women (appendix p 47). For mean systolic blood pressure, the linear model identified increased risk with elevated blood pressure (figure 2B). However, the non-linear models suggest a parabolic association between mortality and systolic blood pressure. In figure 2C, the non-linear models suggest that lower and higher ratios of total to HDL cholesterol are associated with higher mortality risk than mid-range values. By contrast, the linear model shows a positive association between the ratios and mortality. Increased mortality risk aligns with the current clinical threshold for the ratio of total to HDL cholesterol for men but is higher than the threshold for women (appendix p 46). In figure 2D, discretisation of CRP concentrations into novemtiles shows that a sigmoidal function better characterises this association compared with the linear model. Mortality risk becomes apparent for CRP at the current clinical threshold of 0·1 mg/dL. In figure 2E, all models agree that elevated HOMA-IR is associated with increased

| Table 2: Distribution statistics of the 27 physiological indicators |
|---------------------------------------------------------------|
| **Demographics**                                           |
| Duration of follow-up, months                               | 47266 | 10 | 110 | 190 | 260 | 920 (910) | 973 (54.4) | 170 | 189 | 199 | 2010 |
| Age, years                                                 | 47266 | 180 | 180 | 190 | 210 | 460 (340) | 474 (197) | 76 | 80 | 85 | 850 |
| **Body composition**                                       |
| Body-mass index, kg/m²                                      | 44047 | 120 | 177 | 198 | 212 | 274 (80) | 285 (6.7) | 371 | 409 | 499 | 130.2 |
| Standing height, cm                                         | 44288 | 123.3 | 145.7 | 153.1 | 154.3 | 166.9 (14.7) | 167.3 (10.2) | 180.8 | 184.4 | 190.9 | 204.5 |
| Subscapular skinfold, mm                                   | 26374 | 2.4 | 6.5 | 8.4 | 9.9 | 19.4 (12.0) | 20.1 (8.0) | 31.3 | 34.5 | 38.5 | 44.0 |
| Triceps skinfold, mm                                       | 29824 | 2.4 | 4.9 | 7.0 | 8.5 | 17.8 (13.0) | 18.9 (8.5) | 31.2 | 34.2 | 38.4 | 45.0 |
| Waist circumference, cm                                     | 42588 | 55.5 | 67.5 | 73.4 | 77.4 | 96.2 (21.4) | 97.3 (16.1) | 118.2 | 126.0 | 142.2 | 179.0 |
| Weight, kg                                                 | 44233 | 25.6 | 45.1 | 52.2 | 56.7 | 77.1 (25.7) | 80.0 (20.9) | 106.8 | 118.0 | 145.4 | 371.0 |
| Relative fat mass index                                     | 42425 | 9.0 | 15.5 | 20.2 | 23.5 | 34.4 (13.7) | 34.9 (8.8) | 46.6 | 48.7 | 51.9 | 58.4 |
| **Cardiovascular system**                                  |
| Heart rate, min                                            | 40417 | 32.0 | 48.0 | 54.0 | 58.0 | 72.0 (16.0) | 72.8 (12.5) | 90.0 | 96.0 | 106.0 | 224.0 |
| Systolic blood pressure, mm Hg                             | 42647 | 3.3 | 39.3 | 50.0 | 54.7 | 70.0 (15.3) | 69.9 (12.2) | 84.7 | 89.7 | 100.0 | 132.0 |
| Direct HDL cholesterol, mg/dL                              | 42881 | 64.7 | 91.3 | 98.7 | 102.7 | 120.0 (22.7) | 123.7 (19.5) | 149.3 | 161.3 | 186.0 | 270.0 |
| LDL cholesterol, mg/dL                                     | 19696 | 9.0 | 45.0 | 62.0 | 72.0 | 112.0 (47.0) | 114.8 (35.9) | 161.0 | 177.0 | 213.0 | 629.0 |
| Ratio of LDL to HDL cholesterol                            | 19696 | 0.10 | 0.7 | 1.0 | 1.2 | 2.1 (1.3) | 2.3 (1.0) | 3.6 | 4.1 | 5.2 | 31.1 |
| Ratio of total to HDL cholesterol                          | 42144 | 1.3 | 1.9 | 2.3 | 2.5 | 3.7 (1.7) | 4.0 (1.4) | 5.8 | 6.6 | 8.4 | 37.1 |
| Total cholesterol, mg/dL                                   | 42147 | 59.0 | 122.0 | 133.0 | 144.0 | 192.0 (56.0) | 195.4 (43.0) | 250.0 | 270.0 | 313.0 | 813.0 |
| Triglycerides, mg/dL                                       | 42018 | 9.0 | 34.0 | 46.0 | 50.0 | 116.0 (102.0) | 1483 (1314) | 269.0 | 247.0 | 389.0 | 6057.0 |
| **Immune system**                                          |
| C-reactive protein, mg/dL                                  | 31478 | 0.01 | 0.01 | 0.02 | 0.04 | 0.21 (0.41) | 0.45 (0.88) | 1.06 | 1.63 | 3.77 | 29.6 |
| White blood cell count, 1000 cells per µL                  | 42703 | 1.5 | 3.5 | 4.3 | 4.8 | 6.9 (2.7) | 7.3 (2.4) | 10.0 | 11.2 | 13.8 | 100.0 |
| **Metabolic system**                                       |
| Alkaline phosphatase, units per L                          | 36837 | 7.0 | 32.0 | 41.0 | 46.0 | 68.0 (28.0) | 71.9 (278) | 101.0 | 115.0 | 132.0 | 1378.0 |
| Glucose, plasma, mg/dL                                     | 20757 | 36.0 | 72.6 | 81.0 | 84.7 | 98.0 (16.9) | 105.9 (35.3) | 128.0 | 159.0 | 281.0 | 686.2 |
| Glycohaemoglobin, %                                        | 42667 | 2.0 | 4.5 | 4.8 | 4.9 | 5.4 (0.7) | 5.6 (1.0) | 6.4 | 7.4 | 10.7 | 18.8 |
| Homoeostatic model assessment of insulin resistance       | 17659 | 0.05 | 0.40 | 0.74 | 0.98 | 2.43 (2.74) | 3.81 (5.64) | 7.24 | 10.31 | 24.72 | 204.47 |
| Ratio of insulin to glucose, μU/mL:mg/dL                   | 17659 | 0.00 | 0.02 | 0.03 | 0.04 | 0.10 (0.09) | 0.13 (0.13) | 0.24 | 0.31 | 0.56 | 5.06 |
| **Nephrology**                                             |
| Albumin, g/dL                                              | 42041 | 1.2 | 3.1 | 3.6 | 3.8 | 4.3 (0.5) | 4.3 (0.4) | 4.7 | 4.8 | 5.0 | 5.7 |
| Blood urea nitrogen, mg/dL                                 | 42038 | 1.0 | 4.0 | 6.0 | 7.0 | 12.0 (6.0) | 13.2 (6.0) | 20.0 | 23.0 | 35.0 | 122.0 |
| Creatinine, mg/dL                                          | 36831 | 0.2 | 0.4 | 0.6 | 0.8 | 0.8 (0.3) | 0.9 (0.5) | 1.2 | 1.3 | 2.0 | 17.8 |
| Estimated glomerular filtration rate, mL/min per 1.73 m²  | 36831 | 1.9 | 30.5 | 51.9 | 62.7 | 98.6 (34.6) | 97.2 (25.9) | 128.5 | 136.8 | 151.5 | 207.6 |

For the interactive app see https://chiragjp.shinyapps.io/nhanes_mortality_associations/
Figure 1: Concordance index and Nagelkerke $R^2$ for the associations of all physiological indicators with all-cause mortality

The prediction performances are displayed for two populations: one including all participants and the other including those participants who have measurements within the first and 99th percentiles for a given physiological indicator. Sample sizes for each physiological indicator are provided to indicate the number of participants who had data for mortality, age, sex, race or ethnicity, and the given indicator. Results were adjusted for age, sex, and race or ethnicity.
Figure 2: HRs relative to physiological indicators across all models
Relative mortality risk for body-mass index (A), mean systolic blood pressure (B), ratio of total to HDL cholesterol (C), C-reactive protein (D), homoeostatic model assessment of insulin resistance (E), and glomerular filtration rate (F). Participants with measurements between the first and 99th percentiles of a physiological indicator are included. Relative risks for mortality from the novemtiles model are represented by the boxes with the width representing the range of a novemtile and the height representing the 95% CI of the HR. The mean HR for each novemtile is represented by a digit. The hazard compares participants in a novemtile to those in the reference group at the novemtile shown without a box. The purple dot represents the reference point and the measurement of a physiological indicator shown to have the lowest HR for the linear and spline models. The red and blue lines represent the relative mortality risk with respect to reference point for the linear (red) and spline models (blue). The black dot represents the median of a physiological indicator. The dashed green line represents when the HR is 10% higher than the minimum HR—ie, where the HR is 1.1. The blue diamonds indicate the concentration at which the HR shows a 10% increase from the minimum HR. The light purple lines and rectangles represent the values of the clinical thresholds with the width of the rectangles representing the ranges of the threshold. The values to the left of the purple box indicate early stage of risk and the values to the right indicate substantial risk except for F where the direction is reversed. The set of tick marks along the base of the plot represent the distribution of a physiological indicator with increased opacity implying increased number of participants. The y-axis is log10-scaled. Results were adjusted for age, sex, and race and ethnicity. HR=hazard ratio.
mortality risk but disagree with the mortality risks at lower HOMA-IR, with the spline suggesting increased risk, novemtiles suggesting no effect, and linear models suggesting lower risk. In figure 2F, for GFR, the linear model suggests that participants with values less than the clinical threshold of 90 mL/min per 1·73 m² are at higher risk of mortality. However, the non-linear models suggest that participants with a GFR of less than...
65 mL/min per 1·73 m² or between 90–116 mL/min per 1·73 m² are at moderate risk. Risk becomes substantial for participants with a GFR greater than 117 mL/min per 1·73 m².

Table 3 shows a comparison of the current clinical thresholds for the physiological indicators with measurements indicative of a 10% increase from the minimum mortality risk to help identify discrepancies between the current clinical thresholds and those derived from mortality risk. The difference between the clinical thresholds and those derived from mortality risk is minimal for CRP, creatinine, the upper bound of glycohaemoglobin, and blood urea nitrogen, suggesting that the physiological state is equivalent to the risk for mortality. By contrast, such differences are substantial for BMI, waist circumference, 60 s pulse, triglycerides, cholesterol-related indicators, alkaline phosphatase, HOMA-IR, and GFR.

Discussion

We present a data-driven approach to identify the models that most appropriately describe the association between physiological indicators and all-cause mortality in a sample representative of the US population. We analytically tested the prediction capability and robustness of linear and non-linear models while penalising models that are more prone to overfitting. Prediction performance can be similar across the linear and non-linear models (eg, for BMI), which creates challenges in selecting the most appropriate model. Thus, we developed a visualisation tool to compare the shape of the associations across the different models to help select which model is most informative for characterising physiological risk of death. Lastly, we determined whether established clinical thresholds used for medical decision-making align with values identified at which mortality risk increases over baseline mortality risk. Our findings showed that associations of most physiological indicators with mortality were non-linear and that the novemtile models showed the most consistent prediction performance regardless of the exclusion of outliers.

Obesity in the USA is a major health problem as 71% of Americans are considered overweight or obese; at the same time, the prevalence of underweight is also high (10–15% of Americans). Participants with a lower BMI have an increased mortality risk than those with a higher BMI, which could be attributed to efficacy of public health interventions or improvements in health care for obesity-related conditions. In addition, the associations with mortality were shown in our study to have shifted, with the minimum risk found within 24–30 kg/m² instead of at 24 kg/m², which is consistent with other studies. This shift further emphasised how our proposed thresholds, defined as measurements showing a 10% increase from minimum mortality risk, are shifted upward from the current clinical thresholds. This shift might be due to secular changes in the population such as variations in dietary habits, food availability, and national prosperity. The thresholds calibrated to all-cause mortality in 1995 are still being used, even though mounting evidence indicates that the associations between BMI and mortality have changed since then. In addition, the associations of all-cause mortality with lower and higher BMI were weaker in women than in men, which might suggest residual confounding due to sex differences or the survival advantage of adipose distribution in women. These findings prompt the need for future research to assess the influence of caloric intake and adipose tissue mass on mortality risk.

Our results support low GFR (hypofiltration) and surprisingly elevated GFR (hyperfiltration) as potentially reflecting renal injury. As the curation criteria of the Chronic Kidney Disease Prognosis Consortium restricted the distribution of GFR to a maximum of 120 mL/min per 1·73 m², hyperfiltration was not associated with all-cause mortality, but hypofiltration showed a significant and substantial increase in mortality risk. Thus, a lower but not upper bound was established for the threshold for GFR. As the maximum GFR was 151·5 mL/min per 1·73 m² for NHANES participants within the first and 99th percentiles, we found that mortality risk for participants with hyperfiltration is twice as high as those with hypofiltration in the novemtile model. Mortality risk for participants with hyperfiltration was just as high when we included all participants with GFR estimates and the maximum GFR was 207·6 mL/min per 1·73 m². These results and findings from existing literature indicate associations between hyperfiltration and mortality risk, diabetes risk, and early kidney disease. A plausible explanation for these associations might be related to how elevated GFR can overwhelm and subsequently damage the capacity of the renal tubules to reabsorb fluids and minerals from the urine. Damaged or destroyed tubules can lead to a common type of kidney injury known as acute tubular necrosis, which has been implicated in kidney failure. Such damage is highly associated with mortality.

In addition, these results are especially pertinent to Native Americans and non-Hispanic Black Americans, who have higher mean GFR levels. Overall, these findings emphasise the need for future investigations to study the associations of GFR with other health endpoints to verify the relevance of lower and upper thresholds.

We found non-linear associations for cholesterol-related biomarkers. The non-linear models supported the growing literature on the associations between higher HDL concentrations and increased mortality risk. The current notion of LDL being the unhealthy cholesterol implies that higher LDL concentrations are associated with increased risk. However, our non-linear results show a stronger association between mortality and lower LDL concentrations compared with higher concentrations. It is unclear if these associations are due to pre-existing disease leading to low cholesterol concentrations, adverse side-effects from lipid-lowering medications, or low LDL.
concentrations being a causal factor of mortality. There is a need to study the role of low cholesterol levels on mortality beyond cardiovascular disease to help establish the need for lower and upper thresholds.

Several mortality risk scores that dichotomised physiological indicators use a cutoff, such as a clinical threshold or a percentile. For physiological indicators that have parabolic associations with mortality, using only one cutoff to dichotomise the indicator will result in mischaracterising mortality risk, often in the opposite direction away from increased mortality risk. Mortality risk, at times, increases substantially when biomarker concentrations deviate from the cutoff point but is not accounted for in these risk scores. Therefore, our findings prompt the need for future studies to investigate whether accounting for the non-linear associations helps to discern risk for death.

We observed that for some indicators, the current clinical thresholds and our thresholds derived from mortality risk differ substantially. For example, our proposed thresholds for BMI and relative fat mass index are shifted upward from the current clinical thresholds. Such results suggest that understanding why physiological risks of mortality are changing over time is a prerequisite for determining how to recalibrate the thresholds to improve risk stratification. In addition, differences between our proposed versus current thresholds show the need to consider an additional bound when only one bound is clinically recommended. This requirement is especially important for waist circumference, 60 s pulse, triglycerides, cholesterol related indicators, alkaline phosphatase, glycohaemoglobin, HOMA-IR, and GFR. Additionally, differences between our proposed versus current thresholds show disagreement on defining the range of minimal risk. For example, estimated GFR between 60–90 mL/min per 1·73 m² is clinically defined as indicating mild decline in kidney function, but our results show that this range is associated with minimal risk. However, replication of our findings with respect to mortality requires follow-up in other databases to establish the utility of an additional bound or to reconsider the bounds used for minimal risk. Furthermore, future studies could investigate the associations of the physiological indicators with other clinical outcomes (eg, disease), which are more clearly linked to clinical care, to help elucidate mismatches between the current clinical thresholds and those derived from disease risk.

Characterising the shape of the associations between physiological indicators and risk for death is essential to prevent false negative findings when using the wrong model (eg, a linear one). Modelling the shape of the association with quantiles and splines enables us to observe trends in mortality risk across the distribution of a physiological indicator. As the spline model enabled us to observe a smooth dose-response curve, it was particularly useful for defining cutoffs based on mortality risk to compare with the clinical thresholds. Although the linear model was limited in characterising the non-monotonic associations, it served as a comparison for the non-linear models, revealing that height and 60 s pulse have linear associations with mortality. A systematic approach to characterising the shape of the associations is integral to identifying patients at high risk for mortality so as to inform clinical care delivery. Although we did not evaluate how non-linear associations with all-cause mortality can be directly used in clinical care delivery, other studies have used associations observed in an institutionalised population to provide evidence-based guidance for delivering clinical care to patients at high risk.

This study has several limitations. First, we focused on mortality; however, the clinical use cases for most of these clinical physiological indicators are clinical outcomes such as disease (eg, cardiovascular disease, diabetes, or kidney disease). We cannot conclude that the same non-linear relationships hold for disease risk. Relatedly, chronic disease can lead to death, and we have not considered the role of multimorbidity in the trajectory between indicators and death. We recommend that future investigations consider outcomes that mediate the association between physiological indicators and mortality using longitudinal datasets with follow-up data available between measurements, disease incidence, and mortality. Second, we examined individuals who were, for the most part, asymptomatic and non-institutionalised. Broad testing of individuals who are asymptomatic might lead to incidental findings (ie, findings unrelated to the variables assessed in the study). Future analyses could apply our data-driven approach to study how the associations between these indicators and mortality risk and the prediction performance of these models might change in a hospitalised population. Third, we did not consider changes in physiological measurements over the follow-up period. Fourth, we did not evaluate how other demographic or lifestyle factors might influence the associations between a physiological indicator and mortality. Future analyses could build upon our baseline model to quantify the type of role (eg, mediating, moderating, or confounding) that such factors have in these associations. Moreover, future directions could also examine how the prediction performance changes when including other lifestyle factors, as such analyses might lead to increased prediction performance and thus increase the reliability of the new proposed thresholds for the physiological indicators. However, the effect of bias becomes increasingly difficult to untangle with more complex models, therefore requiring other machine learning methods to account for complex interactions among the covariates. Fifth, we observed that physiological indicators with smaller sample sizes showed better prediction performance; thus we caution against using our results to infer the relative importance of the indicators on mortality. Sixth, we did not characterise the independent effects of the indicators on mortality by including all physiological indicators in...
the same multivariable model. Therefore, future studies could explore other machine learning methods to account for the complex interactions and multicollinearity (i.e., whereby one predictor variable in a multiple regression model can be linearly predicted from the others) among the physiological indicators. Accounting for such interactions and multicollinearity could change the shape of the associations between the physiological indicators and mortality and thus, in turn, change the cutoff point at which the clinical thresholds should be defined. Seventh, although we considered sex-specific thresholds, we did not consider age-specific thresholds to prevent our study from becoming overly complex. However, future work could apply our methods to age-stratified analyses to compare age-specified clinical thresholds with those derived from mortality or disease risk, or both. In addition, as our study population is from the USA, we used US-based clinical thresholds, which are based on international guidelines for some indicators (e.g., BMI and GFR). However, future investigations could incorporate clinical thresholds defined by WHO or other international guidelines to expand our methods to study participants from other countries. Finally, we were not able to confidently characterise the mortality risk for participants with extreme values due to a smaller sample size. Thus, there is a need for samples including more participants with extreme measurements.

The shape of the associations between physiological indicators and mortality has not been systematically nor precisely documented in the context of assessing how the current clinical thresholds perform in differentiating patients with high and low mortality risk. We extended a data-driven framework to establish the appropriate model to best characterise the associations with all-cause mortality for various physiological indicators. We established that most of the studied physiological factors have a non-linear association with mortality in a non-institutionalised adult population, identified unexpected directionality in the associations between estimated GFR and cholesterol-related biomarkers and increased risk of mortality, and observed substantial differences between the current clinical thresholds and those derived from mortality risk for several indicators, with differences for some indicators suggesting the need for both lower and upper bounds. Although we systematically characterised the shape of physiological risk with respect to mortality, triangulation with other clinical outcomes is required for updates to clinical guidance.

Contributors
VKN was responsible for conceptualisation, data curation, formal analysis, validation, visualisation, writing of the original draft, review, and editing. MKC was responsible for conceptualisation, funding acquisition, resources, and writing of the original draft, review, and editing. CJP was responsible for conceptualisation, funding acquisition, resources, and writing of the original draft, review, and editing. All authors had full access to all the data in the study and had final responsibility for the decision to submit for publication.

Declaration of interests
At the time of the writing of the manuscript, CJP was a consultant and shareholder of TyHealth. All other authors declare no competing interests.

Data sharing
Data for the study was collected by the Centers of Disease Control and Prevention and are publicly available online. Data include individual participant data with corresponding demographics, mortality status, time to death, and measurements for physiological indicators. A data dictionary defining each field in the dataset is available on the website. Our curated data along with related documents such as Excel documents and R code for the statistical analysis will be available online with publication. Data will be available to the requester via email to the corresponding author after approval of the request.

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