Round 1

Dear editor,

We would like to thank both reviewers for their constructive suggestions that would help to improve our manuscript substantially. Below are our point-to-point responses to reviewers' comments:

Reviewer#1:

1

Needs fine English polishing

Response:

The revised manuscript will be polished by a professional English editing service after this revision is approved by the reviewers and editor. The corresponding author, Dr. Guoqiang Xing, have published nearly 100 research papers in peer-reviewed biomedical journals without professional English editing service.

2

Use standard abbreviations, not of its own.

Response:

The suggestion is well-taken. Therefore, we have changed "type 2 diabetes (**T2D**)" to type 2 diabetes **mellitus** (**T2DM**) throughout the text. Most of other uncommon abbreviations including BCAA/AAA, lean health (LH), metabolically healthy normal weight (MHNW), metabolically healthy overweight (MHOW), metabolic healthy obesity (MHO), metabolically unhealthy normal weight (MUNW) metabolically unhealthy overweight (MUOW), metabolic unhealth obesity (MUO), VAT, BCAA aminotransferase (BCAT), BCATm and BCKD etc. are consistent with the literature.

3

Many unreferenced statements in text.

Response:

We have added a few more references (1,21,75,91, {Collaborators, 2023 #1428}{Han, 2022 #1432;Rannan-Eliya, 2023 #1431;Rooney, 2023 #1430}) that the total of 90 references has well passed the 50-references limit recommended by the journal. We could include more references if this is a review article.

4

There are excessive abbreviations and throughout text

Response:

The long list of the excessive abbreviations have been shortened to include only those frequently used with those appeared once being deleted such as 3-hydroxyisobutyrate (3-HIB) etc. Other abbreviations such as lean health (LH), metabolically healthy normal weight (MHNW), metabolically healthy overweight (MHOW), metabolic healthy obesity (MHO), metabolically unhealthy normal weight (MUNW) metabolically unhealthy overweight (MUOW),

metabolic unhealth obesity (MUO) individuals, visceral adipose tissue (VAT) etc. are consistent with the literature and are used prudently.

5
Introduction too long.
Response: The reviewer's judgment is well-taken. We have tried to engage the audience on this rather complex topic from several aspects: 1) the impact of T2DM prediction; 2) evidence of BCAA/AAA as a T2DM predictor; 3) the unresolved problem or confounding factors of BCAA/AAA as the predictor. We also assume that the broad audience need some background knowledge whereas the experts like the reviewers are more interested in mechanisms. Furthermore, considering numerous studies including many controversial and/or conflicting studies published on this BCAA/AAA over the last 5 decades, it may be necessary to summarize and review some of the key findings.

6
Your Aim is confusing.
Response: Thanks for the feedback. The aim of the study has been clarified: This study aimed evaluate the effects of BMI and gender on BCAA/AAA in new-onset T2DM individuals differing in BMI categories.

7
limitations of study
Response: Thanks for the reminder. A limitation subhead is inserted before the limitation paragraph as below:

Limitations

This study has limitations. The lack of BMI-matched health controls makes it impossible to evaluate a potential low grade hyperaminoacidemia/BCAA/AAA elevation that may exist in normal weight T2DM patients. Only fasting sample but not post-prandial/OGTT sample or BCKA were studied that may have missed the dynamic changes in BCAA catabolism. The moderate sample size of this cross-sectional study could not discern the causality of the findings. The lack of lifestyle data limits exploration of the influences of diet, social, psychological, and physical activities on BCAA/AAA. Anti-diabetic medication taken by some T2DM patients may have compromised the results.[72-74] In addition, no genetic, race or ethnic influences can be derived from this study although gender differences and significant correlations between BCAA/AAA and anthropometric parameters were demonstrated.

Reviewer #2:
Reviewer #2: Here are my comments related with the paper by Wangh et al.
1
-In the abstract section, please add the nationality of the population and statistical methods used in the study. In the results section, the amino acids that belong to branched-chain amino acids (BCAAs) or aromatic amino acids (AAAs) should be indicated.

Response: The First People's Hospital of Yunnan Province, Kunming, China has been included to indicate the nationality of the population studied. One-way and 2-way ANOVA were conducted to determine the effects of BMI and gender. **AAA (**tyrosine, tryptophan, phenylalanine**)**, **BCAA (**isoleucine, valine**)** were specified.

2
-The acronym WHO should be defined and also contain the link to its website.
Response: World Health Organization (WHO) has been defined and linked to its website (https://www.who.int/news-room/fact-sheets/detail/diabetes) {Collaborators, 2023 #1428}.

3.
-I recommend the next reference (PMID: 34769060) for the introduction section of this interesting manuscript.
Response: The reference (PMID: 34769060) **{Gutierrez-Cuevas, 2021 #1433}** is included in the introduction section (Line#67). Obesity, fatty liver and nonalcoholic steatohepatitis could be the common causes underlying CVD and diabetes.

4.
-With respect to the following phrase (line 68-69): "The prevalence of diabetes among Chinese adults has increased from 10.9% in 2013 to 12.4% in 2018. 2" The reference 2 does not contain information related to the phrase, please add an appropriate one.
Response: That reference has been replaced with appropriate ones: [3,4]{Wang, 2017 #1429}{Pan, 2021 #667}

5.
-There are some typos and phrases that need to be corrected. In addition, the percentage symbol is in different ways, please use only one way.
Response: We have tried to fix those typos and phrases as well as percentage symbols as we can find. It would be very helpful if the reviewer can specify the errors and flaws so that we can fix all of them. A professional English Editing certificate is also attached.

6.
-The following phrase (line 99-101): "Furthermore, T2D prevalence/incident rates of the BCAA/AAA studies …….data (~10% for diabetes and ~20% for prediabetes).5 101" The reference 5 does not contain information related to the phrase, please add an appropriate one.
Response: appropriate references are added:
Furthermore, T2D prevalence/incident rates in these BCAA/AAA studies (3-5%) were often much lower than the population-based epidemiological data (~10% for diabetes and ~20% for prediabetes). (WHO: https://www.who.int/news-room/fact-sheets/detail/diabetes, CDC: https://www.cdc.gov/diabetes/prevention/about-prediabetes.html) {Han, 2022 #1432;Rannan-Eliya, 2023 #1431;Rooney, 2023 #1430}

7.

-In the material and methods section, the acronym VAT should be defined correctly as visceral adipose tissue (VAT) on line 154: “The area of abdominal fat area (VAT).....”
Response: the acronym VAT has been corrected as visceral adipose tissue (VAT).

8.
-Because the study considers patients with obesity, the authors should consider evaluating lipid profiles such as Triglycerides, Total-cholesterol, HDL-cholesterol, and LDL-cholesterol and correlating them with amino acid levels. In addition, since abnormal BCAA catabolism in muscle may play a key role in the pathogenesis of elevated BCAA in metabolic syndrome, insulin resistance, and diabetes, the authors should evaluate the homeostatic model of insulin resistance (HOMA-IR).
Response: The constructive suggestions are well taken. We have analyzed and included the lipid profiles of Triglycerides, Total-cholesterol, HDL-cholesterol, and LDL-cholesterol and their correlations with amino acid levels in this revision. Thanks very much for the constructive suggestion of HOMA-IR as our future research goals.

9.
-In the figure 1a and 1b, please add the units for amino acids levels. What does the letter D mean? Moreover, the y-axis has different formats. What does the symbol a on bars mean?
Response: The units for amino acids (ug/L) levels are included now. D is replaced with B to indicate BMI’s effect and S is used to indicate sex/Gender’s effect.
The y-axis has adopted different scales according to the range of each amino acid for easy visualization of the differences. A uniformed scale i.e. based on the highest concentration of valine, would make it hard to visualize the BMI and gender on the low-leveled amino acids i.e. citrulline, homocysteine and asparagine. We have modified the Figure legend accordingly. The symbol a on bars means a significant difference ($P<0.05$) between the Obese group and Overweight group.

10.
-The results belonging to table 2 are described as figures 1A and 1B, and Table 1 in the results section, please make the correction.
Response: Thanks for checking and pointing out the errors. We have made the corrections.

11.
-According to the statistical analysis section, $P\leq0.05$ was used as a significant level. However, the authors use $P<0.1$ in table 2 and 3, please clarify this value.
Response: Yes, we have used $P<0.1$ as an arbitrary indicator to indicate trend level differences.

12.
-With respect to the following phrase (line 281-284): “Simultaneous hyperaminoacidemia and …….. intermediate between lean health (LH) and MUO individuals.40,41,42” The reference 41 and 42 do not contain information related to amino acids, please add the appropriate ones.

Response: The redundant references 41 and 42 have been deleted and replaced with reference 44 {Telle-Hansen, 2020 #1255}

Round 2

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RE-REVIEW REPORT OF REVISED MANUSCRIPT

Dear editor,

We would like to thank the reviewer for the careful examination to improve our manuscript further. Therefore, we have made point-to-point corrections. Proofreading and minor language polishing (color highlighted) have been made by Evan Xing, a Native speaker of American English. All corrections are highlighted in the attached file.

SPECIFIC COMMENTS TO AUTHORS:

1

-Clinical Laboratory , correct the comma (review all)

Response: Corrections are made throughout the text as suggested, with the spaces deleted.

2

-waistline: heigh ratio (WHR), correct to height -preclinic stage, correct to preclinical

Response: "waistline: heigh ratio (WHR)" has been changed to "waistline to height ratio (WHR)"

3

-increased(~4-fold), correct space

Response: The space has been inserted.

4

-heterogenous elevation, correct to heterogeneous

Response: heterogenous has been changed to heterogeneous

5

-Please check that all writing has the same font color (e.g., when BCAA/AAA is to be used..)

Response: The inconsistent AAA/BCAA has been changed to BCAA/AAA.

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6

-Restructure the phrase and metabolic unhealth (MUO) to metabolically Unhealthy Obesity (MUO)

Response: metabolic unhealth (MUO) has been changed to metabolically Unhealthy Obesity (MUO).

7

-individuals[42], muscle[60] correct space

Response: The space has been inserted.

8

-vs and vs, this abbreviation is in different ways (italics), and it should end with a period (e.g., vs.)

Response: All "*vs*" have been changed to "vs."

9

-measured via an abdominal, correct via without italics

Response: The italics of via has been corrected without italics.

Response to JOURNAL EDITORIAL BOARD COMMENTS

JOURNAL EDITORIAL BOARD COMMENTS TO AUTHORS

The limitations and prospective use or utility in different sub types of T2DM needs to be clarified

Response: Thanks for the very important suggestion**.** Briefly speaking, more work need to be done in this area before BCAAs/AAAs can be used as a T2DM predictor. Nevertheless, we try to provide some basic facts and perspectives that may give some insights regarding current and future research directions. Unlike the gold standards for T2DM diagnosis (fasting glucose, OGTT and HbA1c), so far, no standards or reference range of BCAAs/AAAs concentrations have been established for screening at-risk individuals of subtypes of T2DM. Most research used risk ratios, odds ratios and hazard ratios of BCAAs/AAAs that compared the highest and lowest quartiles of individuals who developed obesity (but excluded non-obese individuals) that has limited value in clinical settings. Further, the influence of assay standards (LC-MS techniques *vs.* nuclear magnetic resonance spectroscopy etc.), race/ethnicity, genetics, age, sex, BMI, and subtypes of T2DM on the utility of BCAAs/AAAs still need to be validated. Due to the limited nature of this cross-section study and lack of BMI-matched healthy controls, our results could only suggest that BCAAs/AAAs is more likely a T2DM predictor in individuals with obesity(-propensity). Thus, we have modified the discussion and added the following information:

Limitations and prospective use of BCAAs/AAAs in predicting different subtypes of T2DM

While elevated BCAAs/BCKAs may induce and interact with FFA accumulation in obese T2DM candidates, T2DM development in lean/NW populations may arise from different mechanisms. Unlike the gold standard for predicting different subtypes of T2DM diagnosis (i.e. fasting glucose and HbA1c), so far, there is no established standard for T2DM prediction. Although BCAAs/AAAs is a promising predictor, its utility may be affected by a range of factors including race/ethnicity, age, sex, body weight/BMI, and subtypes of T2DM. The heterogeneous elevation of BCAAs/AAAs among new-onset T2DM patients indicates limitations and restricted utility of BCAAs/AAAs as the predictor for different subtypes of T2DM. While the greater BCAAs/AAAs elevation in obese T2DM patients would support its' prediction in individuals with obesity(-propensity) which account for a large portion of T2DM population, the lower level, or a lack of BCAAs/AAAs elevation in normal-weight and reproductive-aged females would diminish its' predicting power in these individuals.

A longitudinal 12-year follow up study of old adults (56±8 years) showed that when BMI-matched obese non-T2DM individuals (n=189/group, BMI=30) were compared, the highest quartile of individual with elevated baseline BCAAs/AAAs had a 2- to 3.5-fold higher odds of risk of developing diabetes per standard deviation (SD) increment over a 12-year follow-up period based on individual BCAAs/AAAs, or a 5- to 7-fold higher odds of developing diabetes if all BCAAs/AAAs were combined, in comparison with those individuals whose plasma amino acid levels were in the lowest quartile[1]. However, such increments in odds of risk were reduced to 1.3 and 2.0, respectively if the obese T2D candidates were compared with normal weight controls (n=400, BMI=25) randomly selected form a larger pool. Thus, how the controls are selected can lead to very different outcomes.

Similarly, a meta-analysis show that MUO, MUOW and MUNW individuals would show similar 4-fold risk increase of developing T2DM if each of them is compared with healthy counterparts of their corresponding BMI category (*vs.* MHO, MHOW, and MHNW, respectively), but MUO and MUOW would have 2-3-fold higher risks than the MU-NW group if each of them

is compared with MH-NW [45]. Thus, BCAAs/AAAs elevation would be greater in obese T2DM candidates than in NW T2DM candidates if all were compared with normal weight controls, but similar BCAAs/AAAs elevations across different BMI categories if obese, OW and NW T2DM candidates were compared with same BMI categories controls, respectively.

T2DM is a highly heterogeneous disease that include latent autoimmune diabetes in adults (LADA, defined by the presence of glutamic acid decarboxylase antibodies (GADA), maturity onset diabetes of the young (MODY, defined by gene mutations that disrupt insulin production) and neonatal diabetes, in addition to insulin resistant and BMI-related subgroups.

A recent data-driven cluster analysis of 14755 European T2DM patients using six variables (GADA, age at diagnosis, BMI, HbA1c, β-cell function and insulin resistance) resulted in 5 well-separated novel subgroups of adult-onset diabetes with distinct outcomes: a cluster of more severe insulin resistant individuals associated with higher risk of diabetic kidney disease; insulin deficiency cluster associated with highest risk of retinopathy; relatively young insulin deficient individuals with poor glycemic control (high HbA1c) and; a larger group of elderly patients with benign disease course [2]. That finding has been confirmed and extended by another cluster analysis of 2316 Chinese T2D patients and 685 US T2D patients using five variables (age at diagnosis, BMI, HbA1c/glucose, β-cell function and insulin resistance) that resulted in 4 clusters: half of the patients were elders with milder metabolic derangements; 25% of the patients had the highest BMI values but average blood glucose, β-cell function and insulin resistance; 14% of the patients had severe insulin deficiency and highest blood glucose; 8% of the patients were elders with severe insulin resistance and β-cell function [3]. Similar results of cluster analysis were reported in 55777 individuals with prediabetes [4]. However, none of these studies included BCAAs/AAAs as the study variable. Thus, the contributions of BCAAs/AAAs to different clusters or subtypes of T2DM remain unknown.

Nevertheless, BCAAs/AAAs elevation, if standardized based on different age and BMI sub-groups, could be useful in screening future prediabetes and obesity-related diabetes in infants and adolescents as blood BCAAs/AAAs were found significantly correlated with BMI standard deviation score (BMI-SDS), fasting glucose, HbA1c, triglycerides, cystatin C and creatinine in 2191 healthy participants aged 3 months to 18 years[5]. A 7.5-year longitudinal study of 396 nondiabetic Finnish girls showed that serum BCAA profile in childhood (11.2 ± .8 years at baseline) were associated with insulin resistance during pubertal development (significant both before and after menarche) independent of adiposity, and it predicted triglyceride level in adulthood[6, 7]. Blood BCAA/AAA were also found significantly elevated in overweight and obese prepubertal children than in healthy controls[8].

As the global prevalence of prediabetes in children and adults have reached alarming levels [9] that has an annualized diabetes conversion rate of 5%–10% [10], and 500 million adults in China (50% of China's adult population) and 98 million adult Americans (38.0% of the US adult population) have prediabetes (https://www.cdc.gov/diabetes/data/statistics-report/index.html#anchor_23827, the world T2DM population could reach unbearable level. In the US, the total cost of diagnosed diabetes in 2022 was $413 billion, including $307 billion in direct medical costs and $106 billion in indirect costs (https://www.cdc.gov/diabetes/health-equity/diabetes-by-the-numbers.html). Because preventive lifestyle modification can reduce the risk of diabetes by up to 70% [10], identification of "at-risk" individuals 10-20 years prior to T2DM onset based on BCAAs/AAAs elevation would leave plenty time for the at-risk individuals to learn and adapt to lifestyle modifications.

While BCAAs/AAAs elevation alone may not predict all subtypes of T2DM, its combined use with other anthropogenic, metabolic, and genetic biomarkers such as visceral adiposity index, muscle mass index, fasting glucose, GADA (associated with LADA), genetic polymorphisms (associated with MODY), and metabolic parameters associated with insulin deficiency, diabetic kidney disease and retinopathy should be evaluated in future studies.

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