Supplementary Appendix

Alcohol consumption and metabolic syndrome: Clinical and epidemiological impact

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Metabolic syndrome (MetS)

Although, there have been several iterations of the insulin resistance syndrome since the description by Reaven in 1988 (1); in 2001, the first pragmatic criteria were published for identifying MetS using five simple, easily measured criteria. This recommendation focussed on identifying central obesity, dysglycaemia, dyslipidaemia and hypertension to diagnose MetS. Thresholds of each of: waist circumference, plasma glucose concentration, HDL-C and fasting triglyceride concentrations, and blood pressure, were identified and used to classify whether a patient had MetS. From all of these five risk factors, when three or more features were present, that identified whether a patient had MetS (or not). Since 2001, there has been considerable debate as to the numbers of features required, the thresholds of each individual risk factor, whether central obesity should be an obligatory risk factor, and whether ethnic-specific thresholds for central obesity, were required to diagnose MetS. This debate resulted in further modification of the diagnostic criteria, and between 2001 and 2009 there have been several iterations of the diagnostic criteria that are summarised in Supplementary table 1. The variations in these different classifications; the fact that central obesity, may or may not, be an obligatory factor; and the fact that some subjects may have different combination of the individual five risk factors (and yet still have MetS), predisposes to there being potential heterogeneity between individuals with MetS.

With establishing the ‘harmonised criteria’ for MetS in 2009, that was produced by members of the International Diabetes Federation (IDF), the National Heart, Lung, and Blood Institute (NHLBI), the World Heart Federation, the International Atherosclerosis Society, and the American Heart Association (AHA); a consensus statement was produced, in an attempt to further define and classify MetS, and also take account of ethnic differences in thresholds of waist circumference for defining central obesity (2-5). Although it should be noted that both the European Association for the Study of Diabetes (EASD), and the American Diabetes Association (ADA), did not sign up to, nor contribute to the consensus statement (partly due to controversy over the value of identifying the metabolic syndrome, and the polarization of views between different professional groups); this 2009 pragmatic definition and classification has stood the test of time over the last 13 years; and been widely

accepted as pragmatic criteria for identifying MetS. The 2009 criteria are also useful since widespread adoption of these diagnostic criteria is now helping to establish whether there have been, and continue to be, secular changes in global prevalence of MetS in the 21st century. That said, because the criteria (for defining MetS) changed between 2001 and 2009, and different studies have used different criteria, it is difficult to know for certain whether global prevalence of MetS has changed much in the last 20 years.

Systems biology assessment

To gain insight into the relationship between alcohol consumption and MetS components, we searched the literature with the web-based application, Pubtator (6) The search to obtain article PubMed IDs (PMID) was based on the query: ([alcohol OR ethanol AND consumption OR drinking] OR drinkers), for alcohol consumption, combined separately with queries for diabetes mellitus: (diabetes OR ogtt or oral glucose tolerance test OR t2dm OR glucose intolerance OR hba1c OR hyperglycemia), dyslipidemias (dyslipidemia OR hypertriglyceridemia OR hypercholesterolemia OR serum cholesterol OR serum triglycerides), hypertension (arterial hypertension OR ([systolic OR diastolic] AND pressure OR heart rate OR hypertensive), obesity (central obesity OR obese OR ([abdominal OR visceral] AND [adiposity OR fat]) OR BMI OR waist), and fatty liver (liver fat OR fatty liver OR liver steatosis OR steatohepatitis OR liver fibrosis OR MAFLD OR AFLD). Cancer studies and reviews were avoided. Each PMID list (including 988, 462, 1147, 1441, and 769 abstracts for diabetes, dyslipidemia, hypertension, obesity, and fatty liver, respectively) was fed into the Genie data miner application (7) to obtain the list of disease-associated genes (n=569, 716, 725, 465, and 872, for diabetes, dyslipidemia, hypertension, obesity, and fatty liver, respectively).

The ClueGO network that was created with kappa statistics indicated the relationships between the terms, based on the similarity of their associated genes. To narrow the analysis, we used a stringent criterion (Bonferroni adjusted *p*-value = 1.0 e-7, with at least five genes per pathway displayed) for the statistical test used for enrichment/depletion (two-sided hypergeometric test).

We identified enriched terms for the category "Reactome pathways". Interactions are visualized with ClueGO v2.5.8, a Cytoscape plug-in that displays the non-redundant biological terms for large clusters of genes in a functionally grouped network. The ClueGO network was created with kappa statistics and reflected the relationships between the terms based on the similarity of their associated genes/proteins. The network was constructed by filtering terms for Homo Sapiens [9606], and it showed genes in the Ontology, REACTOME-pathways_13.05.2021. We applied the statistical test, Enrichment/Depletion (a two-sided hypergeometric test), with a *p*-value cutoff of 1E-7, based on the Bonferroni correction method.

References

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Supplementary Table 1. Differences in criteria and classification used to define Metabolic syndrome according to the National Cholesterol Education Programme Adult Treatment Panel (NCEP ATP) 2001 & 2005, International Diabetes Federation (IDF) 2005, and National Heart, Lung, and Blood Institute World Heart Federation, International Atherosclerosis Society, and American Heart Association 2009 consensus criteria.

	Commonly used pragmatic definitions for Metabolic syndrome since 2001		
	NCEP 2001 (3) & NCEP 2005 criteria (4)	**IDF criteria 2005 (5)**	**Consensus statement 2009 criteria (2)**
	Three or more of the following features	Central obesity (as defined below) as an essential criterion, plus two or more of the other features	Three or more features. N.B. Waist circumference increase defined by population and country-specific definitions.
Central obesity/ waist circumference	≥ 102cm (men), ≥ 88cm (women)	≥ 94cm (European men), ≥ 90cm (South Asian / Chinese men) ≥ 80cm (women)	Increased waist circumference according to population and country-specific definitions e.g. US ≥ 102cm (men), ≥ 88cm (women) ≥ 94cm (European men), ≥ 90cm (South Asian / Chinese men), ≥ 80cm (non-US women)
Blood pressure	≥ 135/85 mmHg or treated for hypertension	≥130/85 mmHg or treated for hypertension	Increased blood pressure (drug treatment for increased blood pressure is an alternate indicator). Systolic ≥130 mm Hg and/or diastolic ≥85 mm Hg
Triglycerides	≥ 1.7 mmol/l	≥ 1.7 mmol/l or treatment	Increased triglycerides (drug treatment for increased triglycerides is an alternate indicator) ≥ 1.7 mmol/l
HDL-cholesterol	< 1.03 mmol/l (men), < 1.29mmol/l (women)	< 1.03 mmol/l (men), < 1.29mmol/l (women) or treatment	< 1.03 mmol/l (men), < 1.29mmol/l (women) or treatment
Fasting plasma glucose	≥6.1 mmol/l (2001 criterion) ≥5.6 mmol/l (2005 criterion)	≥5.6 mmol/l or diagnosed with type 2 diabetes	Increased fasting glucose (drug treatment for increased glucose is an alternate indicator) ≥5.6 mmol/l