Risk of cardiovascular diseases and diabetes:
a cross-sectional study from North-eastern Brazil

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Doctoral Thesis

University of Oslo

Faculty of Medicine

Institute of Health and Society

Department of Community Medicine and Global Health

Series of dissertations submitted to the
Faculty of Medicine, University of Oslo

ISBN 978-82-348-0096-2

Cover: UiO.
Print production: Graphics Center, University of Oslo.

Acknowledgements

This Ph.D. work would not have been possible without the contribution and support of a great number of people. I am especially grateful to all participants in the study for their time and cooperation.

I would like to express my sincere appreciation and deepest gratitude to my main supervisor, Professor Haakon E. Meyer, for his constructive guidance, critical supervision, valuable support and encouragement throughout my study and research process.

I am also profoundly grateful to my other two supervisors, Professor Renan Magalhães Montenegro Júnior and late Professor Emeritus Bjørgulf Claussen, for their professional guidance, useful critiques, and constant interest in my work throughout the years.

I would like to acknowledge all my co-authors. I am sincerely thankful for all their contribution to this work. A special thanks to Ibrahimu A. Mdala for valuable assistance with all statistical analyses. I am particularly grateful to Professor Akhtar Hussain for his continuous support, guidance, valuable and constructive suggestions during the planning and development of this research work. His willingness to give his time so generously has been very much appreciated.

I wish to express my heartfelt gratitude and appreciation to the Secretary of Health of the city of Pindoretama, Valéria Maria Viana Barbosa, who provided the necessary support and logistics during data collection. My special thanks to all community health workers and my research team for their hard work and endurance.

I am thankful to the Health Department of the city of Pindoretama and Ivar Helles Foundation for their financial assistance.

Finally, I would like to thank my family for their unconditional love, care, and endless support for all these years. I particularly thank my husband for his support and never-ending encouragement; I could not have done this without him. I would also like to express my genuine gratitude to my parents, for their continuous help and encouragement throughout my study.

Oslo, January 2022

Nayla Cristina do Vale Moreira

Dedication

To my son, Andrew A. Moreira, who is a true blessing in my life and the source of my strength and inspiration.

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Summary

Background

Noncommunicable diseases are the leading cause of death globally and disproportionately affect people in lower resource settings. Chronic conditions as cardiovascular diseases (CVDs), type 2 diabetes mellitus (T2DM), as well as metabolic syndrome (MS) are related to reduced life expectancy, increased functional disability and significant socioeconomic burden. Pathophysiological and epidemiological links have been demonstrated among these conditions since they share many of the same risk factors. MS increases the risk of T2DM, cardiovascular events and deaths, whereas T2DM alone confers a 2-fold increase in CVD risk. Racial/ethnic disparities have been reported in the risk of CVDs, T2DM, and in the occurrence of MS. Data from Brazil for the related topics are scarce.

Aims

The main objective was to investigate the risk of CVDs, T2DM, MS, their associations, and determinants in a Brazilian population. We assessed the glycated haemoglobin (HbA1c) as a diagnostic tool for diabetes and pre-diabetes, the prevalence of MS following different definitions, and the risk of CVDs by socioeconomic and demographic characteristics.

Methods

A total of 714 randomly selected subjects aged ≥ 20 years were included in a population-based, cross-sectional study from North-eastern Brazil. Structured questionnaires were used to collect socioeconomic, demographic, and clinical information. Anthropometric and blood pressure measurements were recorded. HbA1c, fasting and 2 h plasma glucose, lipids and insulin levels were measured. The receiver operating characteristic curve assessed the performance of HbA1c. The prevalence of MS was determined following the diagnostic criteria as suggested by the International Diabetes Federation (IDF), modified National Cholesterol Education Program Adult Treatment Plan III (Modified NCEP) and Joint Interim Statement (JIS). CVD risk was assessed by the Framingham risk score. Logistic regression analysis estimated the relationship between MS and pre-diabetes, T2DM, and CVD risk. Multiple linear and Poisson regression analyses evaluated the relationship between some anthropometric markers (body mass index [BMI], waist circumference [WC], waist-to-hip ratio [WHR], and waist-to-height ratio [WHtR]), and CVD risk. The prevalence of high CVD risk among different sociodemographic groups was compared by two-sample test of proportions.

Results

The age- and gender-adjusted prevalence of T2DM was 14.7% and pre-diabetes was 14.2%. The prevalence of MS was 36.1% applying the JIS criteria, 35.1% the IDF and 29.5% Modified NCEP. High CVD risk (Framingham risk score ≥ 10%) was observed in 18.9% of the population. The optimal HbA1c cut-off value was ≥ 6.8% for the diagnosis of diabetes and ≥ 6.0% for pre-diabetes. The area under the curve applying HbA1c was 0.85 (95% CI: 0.80–0.90) for detecting diabetes and 0.61 (95% CI: 0.55–0.67) for pre-diabetes. MS was significantly associated with pre-diabetes, T2DM and CVD risk. Depending on which definition of MS was used, the adjusted odds ratios in those with MS compared with those without the condition ranged from 3.6 to 3.9 for pre-diabetes, from 5.0 to 6.4 for T2DM and from 5.6 to 7.1 for high CVD risk. The JIS and IDF definitions had higher sensitivity than the Modified NCEP to identify pre-diabetes (JIS: 58.2%; IDF: 57.1% vs. Modified NCEP: 46.9%), T2DM (JIS: 76.1%; IDF: 74.3% vs. Modified NCEP: 70.8%) and CVD risk (JIS: 57.1%; IDF: 54.8% vs. Modified NCEP: 48%). Central obesity measures were more strongly associated with predicted CVD risk than BMI. In females, WHR and WHtR were statistically significant predictors of CVD risk, whereas in males only WHtR was significant. Males and older subjects (age ≥ 45 years) showed significantly higher CVD risk, whereas those employed in manual labour had lower risk.

Conclusions

T2DM, MS and increased CVD risk were highly prevalent in this population. Our results may suggest that an HbA1c cut-off point of ≥ 6.8% could be a sensitive marker for the diagnosis of diabetes. However, HbA1c levels might be a weak parameter to identify pre-diabetes. MS showed a significant association with T2DM, pre-diabetes and CVD risk. The IDF and JIS definitions of MS may be better suited in the Brazilian population to predict pre-diabetes, T2DM and CVD risk. Central obesity parameters were strongly associated with predicted CVD risk and might be useful in the clinical assessment of patients. Males and older people showed an increased risk of CVDs, whereas manual labour seems to provide a protective effect. Further large prospective studies in Brazil are warranted to confirm our findings and develop targeted strategies for screening, prevention, and treatment of T2DM, MS, and CVDs.

Sammendrag

Bakgrunn

Ikke-smittsomme sykdommer er den ledende dødsårsaken globalt og påvirker i uforholdsmessig grad mennesker i lav- og mellominntektsland. Kroniske tilstander som hjerte- og karsykdommer (CVDs), type 2 diabetes mellitus (T2DM), samt metabolsk syndrom (MS) fører til redusert levealder, økt funksjonshemming og til en betydelig sosioøkonomisk byrde. Det er påvist en patofysiologiske og epidemiologiske koblinger mellom disse tilstandene siden de deler mange av de samme risikofaktorene. MS øker risikoen for T2DM, kardiovaskulære hendelser og dødsfall, mens T2DM alene gir en dobbel økning i CVD-risiko. Etniske forskjeller er rapportert innen risikoen for hjerte- og karsykdommer, T2DM og forekomst av MS. Data fra Brasil for de relaterte emnene er begrensede.

Mål

Hovedmålet var å undersøke risikoen for CVDs, T2DM, MS, og deres assosiasjoner og determinanter i en brasiliansk befolkning. Vi benyttet glykosylert hemoglobin (HbA1c) som et diagnostisk verktøy for diabetes og pre-diabetes, forekomsten av MS etter forskjellige definisjoner, og risikoen for hjerte- og karsykdommer i forhold til sosioøkonomiske og demografiske kjennetegn.

Metoder

Totalt 714 tilfeldig (randomiserte) utvalgte personer i alderen ≥ 20 år ble inkludert i en populasjonsbasert, tverrsnitts-studie fra Nordøst-Brasil. Strukturerte spørreskjemaer ble brukt til å samle inn sosioøkonomisk, demografisk og klinisk informasjon. Antropometriske målinger og blodtrykksmålinger ble registrert. HbA1c, fastende og 2 timers plasmaglukose, lipider og insulinnivåer ble målt. ROC-kurver ble benyttet til å beregne bidraget fra HbA1c. Prevalensen av MS ble fastsatt etter de diagnostiske kriteriene som foreslått av International Diabetes Federation (IDF), modifisert National Cholesterol Education Program Adult Treatment Plan III (Modified NCEP) og Joint Interim Statement (JIS). CVD-risiko ble vurdert ved hjelp av Framingham risk score. Logistisk regresjonsanalyse beregnet forholdet mellom MS og pre-diabetes, T2DM og CVD-risiko. Lineære og Poisson-regresjonsanalyser evaluerte forholdet mellom antropometriske markører (kroppsmasseindeks [BMI], midje omkrets [WC], midje-til-hofte-ratio [WHR] og midje-til-høyde-ratio [WHtR]), og CVD-risiko.

Resultater

Den alders og kjønn justerte prevalensen var 14,7% for T2DM og 14,2% for pre-diabetes. Prevalensen av MS var 36,1 % ved å anvende JIS-kriteriene, 35,1 % for IDF og 29,5 % for modifisert NCEP. Høy CVD-risiko (Framingham risk score ≥ 10 %) ble observert hos 18,9 % av befolkningen. Den

optimale grenseverdien for HbA1c var ≥ 6,8 % for diagnosen diabetes og ≥ 6,0 % for pre-diabetes. Arealet under kurven for bruk av HbA1c var 0,85 (95 % KI: 0,80–0,90) for påvisning av diabetes og 0,61 (95 % KI: 0,55–0,67) for pre-diabetes. MS hadde en signifikant assosiasjon med pre-diabetes, T2DM og CVD-risiko. Avhengig av hvilken definisjon av MS som ble benyttet, varierte de justerte oddsratioene for MS som eksponeringsvariabel fra 3,6 til 3,9 for pre-diabetes, fra 5,0 til 6,4 for T2DM og fra 5,6 til 7,1 for høy CVD-risiko. JIS- og IDF-definisjonene hadde høyere sensitivitet enn Modifisert NCEP for å identifisere pre-diabetes (JIS: 58,2 %; IDF: 57,1 % vs. Modifisert NCEP: 46,9 %), T2DM (JIS: 76,1 %; IDF: 74,3 % vs. Modifisert NCEP: 70,8 %) og CVD-risiko (JIS: 57,1 %; IDF: 54,8 % vs. Modifisert NCEP: 48 %). Sentrale fedmemål var sterkere assosiert med predikert CVD-risiko enn BMI. Hos kvinner var WHR og WHtR statistisk signifikante prediktorer for CVD-risiko, mens hos menn var kun WHtR signifikant. Menn og eldre personer (alder ≥ 45 år) viste signifikant høyere predikert CVD-risiko, mens de som var ansatt i manuelt arbeid hadde lavere risiko.

Konklusjoner

T2DM, MS og økt CVD-risiko var svært utbredt i denne populasjonen. Resultatene våre kan tyde på at et HbA1c-grensepunkt på ≥ 6,8 % kan være en sensitiv markør for å diagnostisere diabetes. Imidlertid synes HbA1c-nivå å være et svakt parameter for å identifisere pre-diabetes. MS viste en signifikant assosiasjon med T2DM, pre-diabetes og CVD-risiko. IDF- og JIS-definisjonene av MS kan være bedre egnet for å forutsi pre-diabetes, T2DM og CVD-risiko i den brasilianske befolkningen . Sentrale fedmeparametere var sterkt assosiert med predikert CVD-risiko og kan være nyttige i den kliniske vurderingen av pasienter. Menn og eldre viste økt risiko for hjerte- og karsykdommer, mens manuelt arbeid ser ut til å gi en beskyttende effekt. Ytterligere store prospektive studier i Brasil er nødvendig for å bekrefte funnene våre og utvikle målrettede strategier for screening, forebygging og behandling av T2DM, MS og CVDs.

Abbreviations

ADA	American Diabetes Association
AIDS	Acquired Immunodeficiency Syndrome
AUC	Area Under the Curve
AVP	Arginine Vasopressin
BF%	Body Fat Percentage
BMI	Body Mass Index
BP	Blood Pressure
CE	Ceará
CHOD-PAP	cholesterol oxidase - phenol + aminophenazone
CI	Confidence Interval
COVID-19	Coronavirus Disease 2019
CRH	Corticotropin Releasing Hormone
CVD	Cardiovascular Disease
DALYs	Disability Adjusted Life Years
DBP	Diastolic Blood Pressure
DCCT	Diabetes Complications and Control Trial
DM	Diabetes Mellitus
DMT2	Diabetes Mellitus type 2
EASD	European Association for the Study of Diabetes
FPG	Fasting Plasma Glucose
GDM	Gestational Diabetes Mellitus
GDP	Gross Domestic Product
GLM	Generalized Linear Model
GPO-PAP	Glycerol-3-Phosphate Oxidase - Phenol + Aminophenazone
HbA1c	Glycated Haemoglobin
HC	Hip Circumference
HDL-C	High-Density Lipoprotein Cholesterol
HIV	Human Immunodeficiency Virus
HOMA-IR	Homeostasis Model Assessment of Insulin Resistance
HPA axis	Hypothalamic-Pituitary-Adrenal Axis
11β-HSD	11β-hydroxysteroid dehydrogenase
HTN	Hypertension
IBGE	Brazilian Institute of Geography and Statistics

IDF	International Diabetes Federation
IFG	Impaired Fasting Glucose
IGT	Impaired Glucose Tolerance
IPAQ	International Physical Activity Questionnaire
IR	Insulin Resistance
JIS	Joint Interim Statement
LDL-C	Low-Density Lipoprotein Cholesterol
LMICs	Low- and Middle-Income Countries
MET	Metabolic Equivalent of Task
MS	Metabolic Syndrome
MW	Minimum Wage
NCDs	Noncommunicable Diseases
NCEP	National Cholesterol Education Program Expert Panel
NEFA	Non-Esterified (free) Fatty Acids
NF- κB	Nuclear Factor κB
NGSP	National Glycohemoglobin Standardization Program
NPV	Negative Predictive Value
OGTT	Oral Glucose Tolerance Test
OR	Odds Ratio
PAI-1	Plasminogen Activator Inhibitor 1
PPV	Positive Predictive Value
PR	Prevalence Ratio
REK	Regional Committee for Medical and Health Research Ethics
RNA	Ribonucleic Acid
ROC	Receiver Operating Characteristic
SARS-CoV-2	Severe Acute Respiratory Syndrome Coronavirus 2
SBP	Systolic Blood Pressure
SCORE	Systematic Coronary Risk Evaluation
SD	Standard Deviation
SUS	Unified Health System
TC	Total Cholesterol
TG	Triglycerides
TNFα	Tumour Necrosis Factor α
T1DM	Type 1 Diabetes Mellitus

T2DM	Type 2 Diabetes Mellitus
VLDL	Very-Low-Density Lipoprotein
WC	Waist Circumference
WHO	World Health Organisation
WHR	Waist-to-Hip Ratio
WHtR	Waist-to-Height Ratio

List of Papers

Paper I

Glycated Hemoglobin in the Diagnosis of Diabetes Mellitus in a Semi-Urban Brazilian Population.

Nayla Cristina do Vale Moreira, Renan M Montenegro Jr, Haakon E Meyer, Bishwajit Bhowmik, Ibrahimu Mdala, Tasnima Siddiquee, Virgínia Oliveira Fernandes, Akhtar Hussain. Int J Environ Res Public Health. 2019;16(19). Epub 2019/09/29. doi: 10.3390/ijerph16193598. PubMed PMID: 31561434; PubMed Central PMCID: PMCPMC6801550.

Paper II

Prevalence of Metabolic Syndrome by different definitions, and its association with type 2 diabetes, pre-diabetes, and cardiovascular disease risk in Brazil.

Nayla Cristina do Vale Moreira, Akhtar Hussain, Bishwajit Bhowmik, Ibrahimu Mdala, Tasnima Siddiquee, Virgínia Oliveira Fernandes, Renan Magalhães Montenegro Júnior, Haakon E Meyer. Diabetes Metab Syndr. 2020;14(5):1217-24. Epub 2020/07/19. doi: 10.1016/j.dsx.2020.05.043. PubMed PMID: 32682310.

Paper III

Cardiovascular risk, obesity, and sociodemographic indicators in a Brazilian population.

Nayla Cristina do Vale Moreira, Ibrahimu Mdala, Akhtar Hussain, Bishwajit Bhowmik, Tasnima Siddiquee, Virgínia Oliveira Fernandes, Renan M. Montenegro Jr. and Haakon E. Meyer. Front Public Health. 2021;9:725009. Epub 2021/12/18. doi: 10.3389/fpubh.2021.725009. PubMed PMID: 34917567; PubMed Central PMCID: PMCPMC8669243.

Chapter 1: Introduction

1.1 Country Profile

The Federative Republic of Brazil is the fifth largest country in the world, and by far the largest and most populous in South America (1). Initially inhabited by indigenous people, Brazil was discovered by the Portuguese in 1500 and remained under their dominance until 1822. A military regime ruled the country from 1822 to 1985, when it became a democratic nation (2). Located in eastern South America along the Atlantic Ocean, Brazil shares borders with all countries in South America, except for Ecuador and Chile (Figure 1). It is officially divided into five regions (North, Northeast, Centre-West, Southeast and South), and is composed of 26 states and 1 federal district, the capital, Brasília. Geographically diverse, the country presents a wide range of weather conditions, topographies and natural resources (3).

Figure 1: Map of Brazil (Copyright © Google image)

According to 2020 estimates, Brazil has a population of more than 211 million people, who predominantly live in urban areas (87.1%) in the South-eastern and North-eastern regions (1, 4). Highly heterogenous, the Brazilian society is composed of an admixture of ethnic groups that include

Portuguese settlers and native Brazilians initially, and later, other European immigrants (mainly Spanish, German and Italians), African slaves, and a small proportion of Asians, mostly Japanese (2). Despite criticism, the Brazilian Institute of Geography and Statistics (IBGE), classifies the different ethnic categories according to the self-perception of the skin colour. As reported by the 2010 Demographic Census, individuals self-categorized their race/colour as 47.7% white, 43.1% mulatto (mixed white and black), 7.6% black, 1.1% Asian, and 0.4% native Brazilians (4).

Lately Brazil has experienced a rapid sociodemographic transition (5, 6). Total fertility rates have declined steadily, from 6.0 births/woman in 1960 to 1.7 in 2019 (7). The population growth rate has reduced substantially (from 2.9% in 1961 to 0.7% in 2020) (8), and the age pyramid has weighted more towards the adults and elderly (5). According to the 2010 Demographic Census, the literacy rate for the total population was 90.4% (90.1% among males and 90.7% among females). However, among those aged 65 and over, approximately 29.4% were illiterate. The highest rates of illiteracy were reported in the Northeast region (19.1% in 2010), and the lowest in the South (5.1%) (4).

Although Brazil is the eighth-largest economy in the world, the country has been struggling to recover after the severe 2015-16 recession. In 2017, Brazil`s Gross Domestic Product (GDP) grew 1%, and inflation fell to historic lows of 2.9% (1). Nevertheless, the coronavirus disease 2019 (COVID-19) pandemic has strongly upended the lives and livelihoods of the Brazilians. In the first half of 2020, economic activity reduced by 7%, the unemployment rate rose to 14.4%, with 11 million workers leaving the labour force. The level of poverty and income inequality have increased and disproportionately affected women, and some ethnic groups (black, brown, and indigenous populations) in the Northeast, North, and Centre-West regions. Moreover, disparities in opportunities have promoted social exclusion and contributed to higher rates of crime in the country (1, 9).

Since 1988, Brazil has developed a dynamic and complex national health system (the Unified Health System; SUS). Funded by taxes and social contributions, the system is based on the principle that health is a citizen’s right and the state’s duty. It aims to provide comprehensive, universal preventive and curative care through decentralised management and provision of health services, with strong social participation. A 2008 survey showed that about 93% of people seeking health care received treatment (10).

Significant improvements in health status and life expectancy have been reported in Brazil lately. However, because of urbanisation and social and environmental change, new health problems have emerged, whereas some old health issues remain unabated. Maternal mortality has been declining by about 4% annually, and the under-5 mortality by 4.8% a year since 1990. Even though Brazil has exceled in control of vaccine-preventable diseases and human immunodeficiency virus/acquired immunodeficiency syndrome (HIV/AIDS), efforts to control dengue fever and visceral leishmaniasis have repeatedly failed (11). Environmental changes have been associated with the emergence of new

infectious diseases, e.g., Brazilian haemorrhagic fever, hantaviruses, chikungunya and Zika viruses (11, 12). Moreover, hypertension, obesity, diabetes and neuropsychiatric disorders have risen at epidemic levels (11). Currently, with the worldwide spread of the severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), Brazil has been severely affected and is facing one of the worst public health crises in its history. By failing to implement a thorough national response plan to control the pandemic, the health system has collapsed and thousands of Brazilians have died so far (13).

1.2 Noncommunicable Diseases

Noncommunicable or chronic diseases, including cardiovascular diseases (CVDs), diabetes, cancers, chronic respiratory diseases and neuropsychiatric disorders, have reached epidemic proportions, posing a significant threat to human health and socioeconomic development (14).

Noncommunicable diseases (NCDs) are the leading cause of death worldwide and disproportionately affect people in low- and middle-income countries (LMICs). Every year, approximately 41 million people die from NCDs, which corresponds to 71% of all deaths globally. Of them, nearly 80% occur in LMICs (15). In Brazil, NCDs are the main source of disease burden and mortality. In 2016, around 74% of all deaths were due to NCDs, while only 14% were attributed to communicable, maternal, perinatal and nutritional conditions (16).

NCDs are usually associated with four main behavioural risk factors (tobacco use, harmful use of alcohol, unhealthy diet, and physical inactivity), which may lead to key metabolic changes: raised blood pressure, overweight/obesity, hyperglycaemia, and hyperlipidaemia. The rapidly growing burden of NCDs in developing countries has been linked to the negative effects of globalisation, such as unfair trade and irresponsible marketing, urbanisation, industrialisation, and population aging. Large proportions of global marketing target women, and adolescents in LMICs to foster tobacco smoking, consumption of unhealthy food and alcohol. Rapid unplanned urbanisation has promoted a profound change in nutritional and lifestyle patterns, increasing the exposure to several NCDs risk factors (14).

The costs of NCDs to the health systems, businesses, and individuals, are significant and growing. Over the period 2011-2030, it has been estimated that NCDs will cost the global economy more than US$ 30 trillion (17). The socioeconomic consequences of the NCDs epidemic are devastating everywhere, but most strikingly in the less privileged groups in the developing world. Vulnerable and disadvantaged people get sicker and die sooner than those of higher socioeconomic status. Poverty exposes people to preventable risk factors for NCDs, and, in turn, the resulting NCDs may lead to decreased household income from high costs of health care, loss of employment or premature death. In this vicious cycle, NCDs are a driving force to the downward spiral that pushes families into impoverishment (14).

NCDs pose a significant economic burden that can be felt far beyond the health sector. It has been estimated that a 10% increase in NCDs is linked to 0.5% lower rates of annual economic growth (14). Furthermore, NCDs threaten progress towards the 2030 Agenda for Sustainable Development, which includes a target of a one-third reduction in premature deaths from NCDs by 2030 (17). Abundant evidence has shown that effective, feasible, and affordable interventions exist and can be successfully implemented in a wide range of resource settings. Strengthening of health systems, political commitment and full engagement of non-health sectors and key stakeholders are also fundamental to promote stronger national and international responses to fight this epidemic (14).

1.3 Diabetes Mellitus and Pre-Diabetes

1.3.1 Definition and Classification

Diabetes mellitus (DM) is a heterogeneous metabolic disease characterized by chronic elevated blood glucose levels with abnormalities of carbohydrate, protein, and fat metabolism. It occurs as a consequence of defects in insulin secretion, insulin action or both (18). The condition can be classified as follows (19):

- **Type 1 diabetes mellitus (T1DM):** responsible for the minority of the total burden of diabetes (5-10% of the cases). It results from the destruction of the pancreatic β-cells, generally leading to absolute insulin deficiency. This destructive process is commonly caused by a cellular-mediated autoimmune attack on the β-cells. Multiple genetic predispositions and environmental factors have been implicated, but their complex interaction is still poorly understood (19).
- **Type 2 diabetes mellitus (T2DM):** accounts for more than 90% of those with diabetes. It encompasses individuals with insulin resistance and usually relative, rather than absolute, insulin deficiency. Although the specific aetiologies remain unknown, autoimmune destruction of β -cells does not occur (19). T2DM is commonly diagnosed among older adults, but it is increasing among children and younger people due to the rising levels of obesity, physical inactivity and unhealthy diet (20).
- **Specific types of diabetes due to other causes:** it includes monogenic diabetes syndromes (such as neonatal diabetes and maturity-onset diabetes of the young), diseases of the exocrine pancreas (such as cystic fibrosis and pancreatitis), and drug- or chemical-induced diabetes (due to glucocorticoid use, treatment for HIV/AIDS, or after organ transplantation) (19).
- **Gestational diabetes mellitus (GDM):** it can be defined as diabetes that was diagnosed in the second or third trimester of pregnancy and was not clearly overt diabetes prior to gestation (19). After a pregnancy with GDM, women have at least a seven-fold increased risk of developing T2DM (21).

Furthermore, infants of a women with GDM are at a greater risk of obesity, impaired glucose tolerance (IGT) or diabetes at an early age (22).

1.3.2 Pre-diabetes

The onset of most T2DM cases is preceded by an intermediate state of abnormal glucose regulation, i.e., impaired fasting glucose (IFG) and IGT, commonly described as “pre-diabetes”. The term is used for individuals whose blood glucose levels are above the normal range but do not meet the criteria for diabetes (20). In addition to mild hyperglycaemia, patients with pre-diabetes present moderate to severe insulin resistance and progressive β-cell failure with impaired insulin secretion. Both IFG and IGT are associated with an increased risk of T2DM and CVDs (23). Strong evidence lends support to the effectiveness of lifestyle interventions in preventing the progression from pre-diabetes to diabetes (20).

1.3.3 Pathogenesis of Type 2 Diabetes

Although the exact aetiology of T2DM is not completely understood, the pathological process underlying the development of T2DM is thought to entail a complex interaction between lifestyle and genetic factors (24). The pathogenesis may take several years to develop, and T2DM may remain asymptomatic and unrecognized for years (20).

T2DM has a strong genetic component, but only few genetic variants have been identified so far. Positive family history has been linked to a 2-4 fold increased risk for T2DM (24). Concordance rates for diabetes in monozygotic twins have been found as 35-58%, and in dizygotic twins 17–20% (25, 26). However, these rates in monozygotic twins might have underestimated the genetic effects, since the monochorionic intrauterine nutrition of monozygotic twins may result in growth retardation (27). It has been shown that low birthweight itself is associated with a higher risk of T2DM later in life (28, 29).

The activation of genes that predispose someone to diabetes requires the presence of behavioural and environmental factors. Overweight / obesity and physical inactivity are among the most important factors. The risk factors for T2DM can be classified as non-modifiable and modifiable (Table 1) (30).

Table 1. Modifiable and non-modifiable risk factors for T2DM (24, 30-32)

Modifiable risk factors	Non-modifiable risk factors
Overweight and obesity (general and central)	Age
Physical inactivity	Gender
Dietary factors	Ethnicity
Metabolic syndrome:	Family history of diabetes
Hypertension	Polycystic ovary syndrome
Decreased HDL-C	History of GDM
Increased triglyceride	
Previously identified glucose intolerance (IGT and/or IFG)	
Intrauterine environment and early childhood malnutrition	
Inflammation	
Chronic stress	
Smoking	

GDM: Gestational diabetes mellitus; HDL-C: High-Density Lipoprotein Cholesterol; IFG: Impaired Fasting Glucose; IGT: Impaired Glucose Tolerance; Type 2 Diabetes Mellitus (T2DM).

Insulin is the key hormone involved in the regulation of blood glucose levels. Normally insulin secretion reduces the hepatic endogenous glucose production, enhances glucose disposal in skeletal muscle cells and suppresses fatty acid release from fatty tissue. However, in the initial stages of T2DM, the body's cells develop a partial inability to respond to insulin, which is called insulin resistance. To compensate the reduced insulin action, β-cell function is increased, and blood glucose levels are only mildly elevated. Nevertheless, this is followed by a failure of the pancreas to keep up with the demand over time. At this point, decreasing levels of insulin lead to the development of overt hyperglycaemia and raised concentrations of fatty acids in the bloodstream, which in turn adversely affect β-cell function (Figure 2) (24).

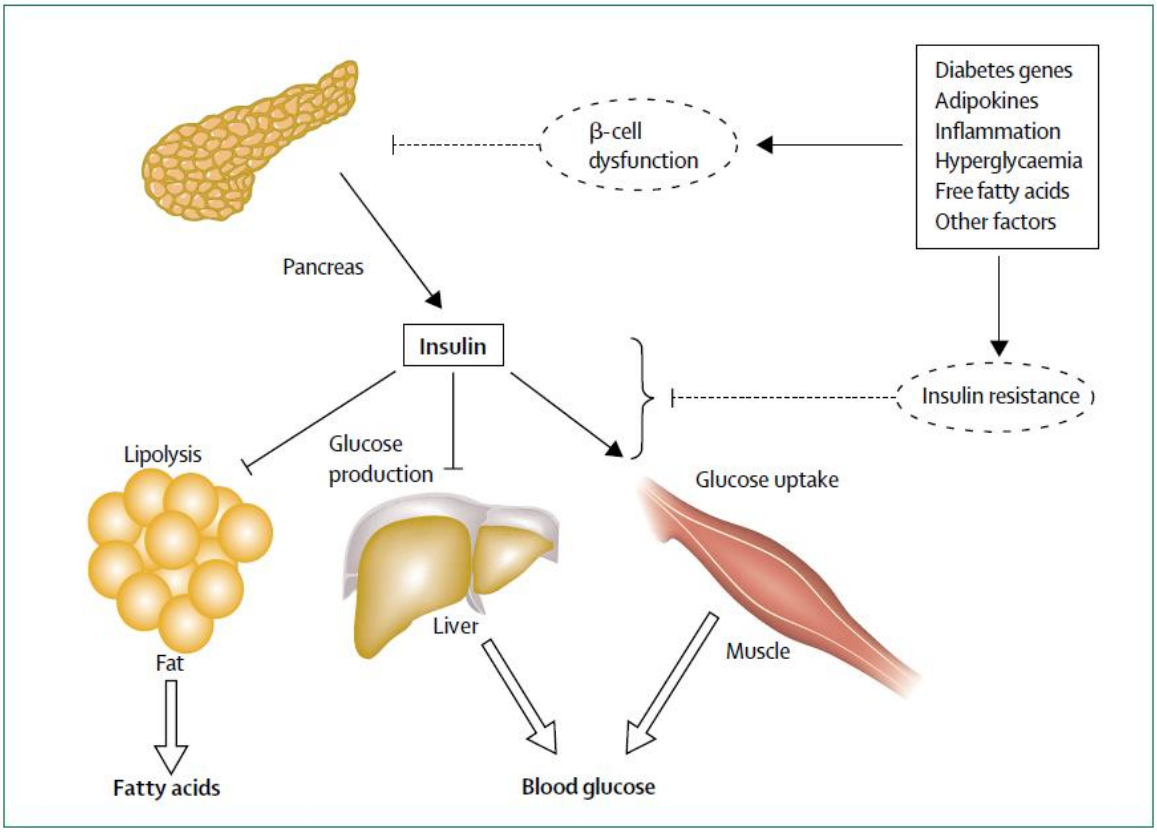

Figure 2. Pathophysiology of hyperglycaemia and increased free fatty acids in T2DM (24)*

1.3.4 Chronic Complications of Type 2 Diabetes

Diabetes can affect several organs and, over time, lead to serious complications. Diabetes-related complications are responsible for significant morbidity and mortality (20). Microvascular complications include damage to small arteries and capillaries in the eyes (retinopathy), kidneys (nephropathy), and nervous system (neuropathy). Diabetes is a major cause of renal failure and blindness throughout the world. It also accounts for more than half of all non-traumatic lower limb amputations (14). Macrovascular complications (damage to medium and large vessels) include coronary artery disease, stroke and peripheral vascular disease (33).

1.3.5 Diagnosis of Diabetes and Pre-diabetes

1.3.5.1 History of the Diagnosis of Diabetes and Pre-diabetes

The recommendations for the diagnosis of DM have been changing over the years. Until the late 1970s, the nomenclature and diagnostic criteria for DM varied substantially. In 1979, the National Diabetes Data Group produced a consensus document (34) standardizing the definition and the criteria for diagnosis, which was later endorsed by the World Health Organization (WHO) (35). In 1997, using

data from three different populations, the American Diabetes Association (ADA) recommended changes to the diagnostic criteria for diabetes and lesser degrees of impaired glucose regulation (IFG/IGT) (36). For epidemiological studies, the 1997 ADA criteria (36) stated that the fasting plasma glucose (≥ 7.0 mmol/l) alone should be used, while the 1985 WHO criteria (35) recommended the 2-hour glucose value alone (2-hour ≥ 11.1 mmol/l). In a reanalysis of European data, when the ADA criteria were applied, changes in the prevalence of diabetes ranged from a reduction of 4.0% to an increase of 13.2%. Moreover, the two recommendations showed a high degree of disagreement in the classification of diabetes status, which was dependent on age and body mass index (BMI) (37). However, in 1999, the WHO proposed that either the fasting or 2-h value after 75g oral glucose might be used alone for epidemiological or population screening purposes (18). Furthermore, the recommended cut-off values for diabetes diagnosis have also changed. In 1997, the ADA proposed that the cut-off point for fasting plasma glucose (FPG) levels should be reduced from 7.8 to 7.0 mmol/l. Additionally, the term impaired fasting glucose (IFG) was applied to identify people whose FPG levels ranged from 6.1 mmol/l to 6.9 mmol/l (36). Later in 1999, the WHO published similar recommendations (18). In 2003, a follow-up report from ADA suggested that the lower cut point for IFG should be further reduced from 6.1 to 5.6 mmol/l (38).

For decades, only tests based on blood glucose measurements were applied to diagnose DM, either by the FPG levels or 2-h values in the 75 g oral glucose tolerance test (OGTT) (39). Nevertheless, the FPG and OGTT present several limitations. Both tests require an overnight fasting period of at least 8 hours (40), and abnormal results must be demonstrated on more than one occasion in the absence of unequivocal hyperglycaemia (39). Patient nonadherence to fasting, metabolic disturbances or stresses (e.g., illness, trauma, endocrinopathies, etc), use of certain medications or laboratory error may compromise the accuracy of test results. Although assessing FPG levels is inexpensive and low-risk, measurements may vary substantially within individuals over the long term (40). Additionally, the OGTT is costly, labour intensive, time-consuming, and has low overall test-retest reproducibility (40, 41).

In 2008, an International Expert Committee, with members appointed by the ADA, the European Association for the Study of Diabetes (EASD) and International Diabetes Federation (IDF), recommended the use of the Glycated Haemoglobin (HbA1c) test to diagnose diabetes, with a threshold of ≥ 6.5%. For identifying pre-diabetes cases, the International Expert Committee indicated that individuals with an HbA1C level ≥ 6% but < 6.5% are likely at the highest risk for progression to diabetes (42). On the other hand, based on several prospective studies, the ADA suggested an HbA1c range of 5.7 - 6.4% to detect individuals with pre-diabetes (39). In a 2011 report, the WHO also stated that HbA1c can be used as a diagnostic test for diabetes. Nevertheless, no formal recommendations were made regarding HbA1c levels below 6.5% (43).

Compared with FPG and OGTT, the HbA1c test is more convenient, quicker, stable and less variable biologically (42). Since HbA1c levels represent a 2–3-month average of blood glucose concentrations, the test can be performed at any time of the day regardless of the duration of fasting or the content of the last meal (41). It is also relatively unaffected by acute perturbations in glucose levels during periods of stress and illness (42). Moreover, compared with FPG, HbA1c is better associated with CVDs, and equally related to microangiopathic complications (retinopathy) (44). Nevertheless, haemoglobinopathies, thalassemia syndromes, hyperbilirubinemia, renal failure, laboratory error or use of certain medications may influence the accuracy of HbA1c analysis. Furthermore, any condition that impacts red cell turnover, i.e., haemolytic or iron deficiency anaemia, chronic malaria, major blood loss, or blood transfusions, may lead to spurious HbA1c values (42, 45). Although HbA1c captures chronic hyperglycaemia, it will miss acute hyperglycaemia. It has also been estimated that using HbA1c may delay the diagnosis of diabetes in approximately 60% of incident cases (44).

1.3.5.2 Current Recommended Diagnostic Criteria for Diabetes and Pre-diabetes

According to the current ADA recommendations, diabetes and pre-diabetes can be diagnosed as follows (19):

Table 2. Diagnosis of DM and Pre-diabetes (ADA Criteria)

	Glucose Concentration	
	mmol/l (mg/dl)	**% (mmol/mol)**
Diabetes Mellitus		
FPG [1, 2]	≥ 7.0 (≥ 126)	
Or		
2-h post glucose load [1, 3]	≥ 11.1 (≥ 200)	
Or		
Random Plasma Glucose [4]	≥ 11.1 (≥ 200)	
Or		
HbA1c [1, 5]		≥ 6.5 (≥ 48)
Impaired Glucose Tolerance		
2-h post glucose load	7.8 (140) - 11.0 (199)	
Or		
HbA1c		5.7 - 6.4 (39 - 47)
Impaired Fasting Glucose		
FPG	5.6 (100) - 6.9 (125)	
Or		
HbA1c		5.7 - 6.4 (39 - 47)

ADA: American Diabetes Association. DM: Diabetes Mellitus. FPG: Fasting Plasma Glucose. HbA1c: Glycated Haemoglobin.

[1] *In the absence of unequivocal hyperglycaemia, diagnosis requires two abnormal test results from the same sample or in two separate test samples.*

[2] *Fasting is defined as no caloric intake for at least 8h.*

[3] *The test should be performed as described by the World Health Organization, using a glucose load containing the equivalent of 75 g anhydrous glucose dissolved in water.*

[4] *In a patient with classic symptoms of hyperglycaemia (such as polyuria, polydipsia, weight loss, sometimes with polyphagia, and blurred vision) or hyperglycaemic crisis.*

[5] *The test should be performed in a laboratory using a method that is National Glycohemoglobin Standardization Program (NGSP) certified and standardized to the Diabetes Complications and Control Trial (DCCT) assay.*

On the other hand, following the WHO, the values for the diagnosis of DM and other intermediate states of abnormal glucose regulation are (18, 43, 46):

Table 3. Diagnosis of DM and Pre-diabetes (WHO Criteria)

	Glucose Concentration	
	mmol/l (mg/dl)[1]	**% (mmol/mol)**
Diabetes Mellitus		
FPG	≥ 7.0 (≥ 126)	
Or		
2-h post glucose load [2]	≥ 11.1 (≥ 200)	
Or		
HbA1c		≥ 6.5 (≥ 48)
Impaired Glucose Tolerance		
FPG (if measured)	< 7.0 (< 126)	
And		
2-h post glucose load [2]	≥ 7.8 (≥ 140) and < 11.1 (< 200)	
Impaired Fasting Glucose		
FPG	≥ 6.1 (≥ 110) and < 7.0 (< 126)	
And *(if measured)*		
2-h post glucose load [2]	< 7.8 (< 140)	

DM: Diabetes Mellitus. FPG: Fasting Plasma Glucose. HbA1c: Glycated Haemoglobin. WHO: World Health Organization

[1] *For epidemiological or population screening purposes, the fasting or 2-h value after 75 g oral glucose may be used alone. For clinical purposes, the diagnosis of diabetes should always be confirmed by repeating the test on another day unless there is unequivocal hyperglycaemia with acute metabolic decompensation or obvious symptoms.*

[2] *If 2–h plasma glucose is not measured, status is uncertain as diabetes or Impaired Glucose Tolerance cannot be excluded (46).*

1.3.6 Burden of Diabetes and Pre-diabetes Worldwide and in Brazil

The diabetes epidemic has reached alarming levels, affecting nearly half a billion people globally, which represents 9.3% of the world's population. It is estimated that around 50% of those living with DM are unaware that they have the condition. Over the past decades, both the number of cases and the prevalence of diabetes have been rising steadily, particularly in developing countries. Previously regarded as "a disease of the wealthy", LMICs are now facing a firestorm of diabetes and its disabling and life-threatening complications. As a result of demographic ageing, and profound environmental, lifestyle and occupational changes, approximately 80% of those with diabetes live in LMICs (20).

The IDF has projected that approximately 578 million individuals (10.2% of the world population) will have diabetes by the year 2030, and 700 million (10.9%) by 2045, corresponding an

increase of 51%. Additionally, around 374 million people (7.5%) have IGT, and this number is predicted to rise to 548 million (8.6%) by 2045. A total of 4.2 million people died in 2019 due to DM and its complications, accounting for 11.3% of global deaths from all causes among individuals aged 20-79 years. Almost 50% of those deaths occurred in the working age group, resulting in a substantial negative economic impact for countries (20).

An increasing prevalence of DM with age was observed in 2019, and a similar trend is predicted for the years 2030 and 2045. While the prevalence of DM was 1.4% among adults aged 20-24 years in 2019, it was 19.9% among those aged 75-79 years. A slightly lower prevalence was found among females in 2019 (9.0% vs 9.6%). More people with diabetes live in urban (310.3 million) than in rural areas (152.6 million). Due to global urbanisation, the number of people with diabetes in urban areas is expected to increase more markedly by 2030 and 2045, widening the difference in relation to rural settings (20).

In South and Central America, around 32 million people (20-79 years) had diabetes in 2019 (age-adjusted comparative prevalence of 8.5%), and this number is projected to reach 49 million by 2045. Of those with DM, approximately 13.3 million (41.9%) are undiagnosed. In this region of the world, most adults with the condition live in urban areas (85.5%) and in middle-income countries (87.5%). Approximately 33.9 million adults aged 20-79 years had IGT in 2019 (age-adjusted comparative prevalence of 9.7%), with projections to reach 48.1 million by 2045 (20).

Like other developing countries, Brazil has experienced a marked epidemiologic and economic transition over the past years. Greater income, industrialisation, urbanisation, and globalisation of unhealthy habits have fostered sedentary lifestyles and higher intake of energy dense foods, ultimately contributing to increasing rates of obesity and diabetes (47). According to IDF estimates, Brazil had around 16.8 million adults (20–79 years) with diabetes in 2019 (age-adjusted comparative prevalence of 10.4%), ranking the country in 5th place among those with the highest numbers of people with diabetes in the world (Table 4). In South and Central America, Brazil has the highest number of adults with diabetes, followed by Colombia (2.8 million) and Argentina (1.8 million) (20).

Table 4. Top 10 countries for numbers of adults aged 20-79 years with diabetes in 2019 and 2045 (20)

2019			2045		
Rank	**Country**	**Number of people with diabetes (millions)**	**Rank**	**Country**	**Number of people with diabetes (millions)**
1	China	116.4	1	China	147.2
2	India	77.0	2	India	134.2
3	United States of America	31.0	3	Pakistan	37.1
4	Pakistan	19.4	4	United States of America	36.0
5	Brazil	16.8	5	Brazil	26.0
6	Mexico	12.8	6	Mexico	22.3
7	Indonesia	10.7	7	Egypt	16.9
8	Germany	9.5	8	Indonesia	16.6
9	Egypt	8.9	9	Bangladesh	15.0
10	Bangladesh	8.4	10	Turkey	10.4

From 1986 to 1988, a multicentre study on diabetes was conducted in nine Brazilian state capitals, including a representative sample of 21,847 subjects aged 30-69 years. The prevalence of DM was estimated at 7.6 and that of IGT 7.8%, without significant differences between genders (48). Since then, although several cross-sectional analyses have been conducted, no strong, nationwide, and consistent data are available to evaluate the trends over time (49, 50). A meta-analysis of articles published between 1980 and 2015, identified an increase in the prevalence of DM from 7.4 % in the 1980s to 15.7 % in the 2010s, when the diagnosis was made by a combination of FPG, OGTT, and self-report. Even though the prevalence was similar among the five different Brazilian macro-regions, it was higher in females and older people (49). Data from the IBGE and Ministry of Health have also shown an increasing trend in diabetes-related mortality in most state capitals between 1950 to 2000, particularly in the Northeast region (51). It has been estimated that diabetes is responsible for 278,778 years of potential life lost for every 100,000 people, and for 5.1% (6.0% among females and 4.4% among males) of the total disability adjusted life years (DALYs) in the country (50).

Although multiple data sources are available, evidence on diabetes-related complications on a national and regional level is scarce in Brazil. According to the Ministry of Health, diabetic retinopathy is the leading cause of irreversible blindness in Brazil. Based on studies among certain groups and

limited areas, around 20 to 40% of patients with T2DM are affected by diabetic retinopathy (50). However, given the narrow scope and restricted geographical coverage of these studies, estimating the national prevalence of diabetic retinopathy is problematic. Diabetic nephropathy is another important public health problem. It is estimated that at least one third of Brazilians with T2DM have this complication (50, 52). Ischaemic heart disease and hypertension are the most frequent CVDs in those with diabetes (50). A cross-sectional study including 927 people with T2DM in Southern Brazil observed a prevalence of hypertension of 73%, coronary artery disease 36%, and peripheral vascular disease 33% (53). In 2007, another study from Southern Brazil with 340 patients found a prevalence of diabetic peripheral neuropathy of 22% (54).

In addition to the impact caused by premature mortality and lower quality of life due to its complications, DM imposes a large economic burden on individuals, national healthcare systems, and countries. In 2019, the total diabetes-related health expenditure was estimated as USD 760 billion globally, and it is expected to reach USD 845 billion by 2045 (11.2% increase). In South and Central America, the total health expenditure on diabetes was USD 69.7 billion in 2019, with projections to be USD 85.7 billion by 2045 (22.9% increase). In this region of the world, the mean annual health expenditure per person with diabetes was highest in Brazil (USD 3,117), and lowest in Nicaragua (USD 564). After United States of America and China, Brazil was the third country in 2019 with the highest diabetes-related health expenditures in the world (USD 52.3 billion) (20).

The Brazilian government has introduced several policies and programmes to improve access to diabetes care and reduce the prevalence of the disease (50). In 2001, the first national diabetes screening campaign was implemented by the public health services (55). The Primary Health Care Department has organized basic health services including the Family Health Programme, and the hypertension and diabetes management (HiperDia) (50). Introduced in 1994, the Family Health Strategy, through multi-disciplinary professional teams, has focused on health promotion actions, prevention, rehabilitation, and maintenance of the community health (10). Free essential medicines and the necessary equipment to monitor capillary glycaemia are provided to people with diabetes by the Brazilian Unified Health System (56). Preventive efforts include anti-tobacco programs, healthy nutrition policies (regulation of food marketing and advertising), school health promotion, and programs to encourage healthy lifestyles by providing free-of-charge spaces for physical activity practices. Nevertheless, better allocation of resources to provide good quality care for patients with diabetes in all regions of Brazil is urgently needed. Nationwide data on the prevalence and incidence of diabetes and its complications, as well as information regarding the quality and access to health care are largely lacking, which prevent the formulation of more appropriate policies and strategies (50).

1.4 Metabolic Syndrome

1.4.1 Overview – Definitions and Contributing Factors

Metabolic syndrome (MS) is defined as a clustering of interrelated risk factors including central obesity, atherogenic dyslipidaemia, hyperglycaemia and hypertension (57). Insulin resistance, genetic predisposition, physical inactivity, smoking, unhealthy diet, ageing, proinflammatory state and hormonal changes are among the main contributing factors (58). The MS is an asymptomatic disorder, therefore its clinical significance relies on its ability to identify people for preventive interventions that they might otherwise not receive (59).

Several definitions of MS have been suggested so far, but the most widely used have been produced by the National Cholesterol Education Programme Adult Treatment Panel III (NCEP) in 2001 (60), which was updated in 2005 by the American Heart Association/National Heart, Lung, and Blood Institute (Modified NCEP) (61), and the IDF (57). Recently, different organizations have issued a Joint Interim Statement (JIS) as an attempt to develop a unifying definition of MS (62). Table 5 shows the measurements and diagnostic criteria applied in different definitions.

Table 5: Criteria for clinical diagnosis of the MS following different definitions

	Risk Factors	IDF (57)	Modified NCEP (61)	JIS (62)
Criteria for Diagnosis of MS		**Abdominal obesity plus 2 or more risk factors**	**Any 3 or more of 5 risk factors**	**Any 3 or more of 5 risk factors**
1	**Central Obesity**	WC ≥ 90 cm in males, ≥ 80 cm in females	WC ≥ 102 cm in males, ≥ 88 cm in females	WC ≥ 90 cm in males, ≥ 80 cm in females
2	**TG**	≥ 1.7 mmol/l (150 mg/dl) or on specific treatment for elevated TG	≥ 1.7 mmol/l (150 mg/dl) or on drug treatment for elevated TG	≥ 1.7 mmol/l (150 mg/dl) or on drug treatment for elevated TG
3	**HDL-C**	< 1.03 mmol/l (40 mg/dl) in males, < 1.30 mmol/l (50 mg/dl) in females or on drug treatment for reduced HDL-C	< 1.03 mmol/l (40 mg/dl) in males, < 1.30 mmol/l (50 mg/dl) in females or on drug treatment for reduced HDL-C	< 1.03 mmol/l (40 mg/dl) in males, < 1.30 mmol/l (50 mg/dl) in females or on drug treatment for reduced HDL-C
4	**Blood Pressure**	SBP ≥ 130 mmHg or DBP ≥ 85 mmHg or treatment of previously diagnosed hypertension	SBP ≥ 130 mmHg or DBP ≥ 85 mmHg or current use of antihypertensive drugs in a patient with a history of hypertension	SBP ≥ 130 mmHg or DBP ≥ 85 mmHg or antihypertensive drug treatment in a patient with a history of hypertension
5	**FPG**	≥ 5.6 mmol/l (100 mg/dl) or previously diagnosed Type 2 diabetes	≥ 5.6 mmol/L (100mg/dl) or on drug treatment for elevated glucose	≥ 5.6 mmol/L (100mg/dl) or on drug treatment for elevated glucose

DBP: Diastolic Blood Pressure. FPG: Fasting Plasma Glucose. HDL-C: High-Density Lipoprotein Cholesterol. IDF: International Diabetes Federation. JIS: Joint Interim Statement. MS: Metabolic Syndrome. NCEP: National Cholesterol Education Program Expert Panel. SBP: Systolic Blood Pressure. TG: Triglycerides. WC: Waist Circumference.

1.4.2 Burden of the Metabolic Syndrome Worldwide and in Brazil

The prevalence of the MS is steadily increasing worldwide. Although its prevalence varies across populations due to the diverse sociodemographic characteristics and different definitions applied, it is estimated that about 20-25% of the world's adult population has the MS (63).

In developing countries, particularly in South America, the MS has become a major public health concern. Rapid socioeconomic and demographic transitions have contributed to changes in lifestyle and dietary patterns, as well as to increasing rates of obesity (64). Following the IDF criteria (57), in a survey conducted in 2003 including 1,833 adults, the prevalence of the MS in Chile was 36.8% (65). According to the same definition, a 2007 report showed a prevalence of 32.9% in Colombia (66), while in Mexico, the 2006 National Health and Nutrition Survey found a prevalence of 49.8% (67). Applying the Modified NCEP criteria (61), Peru presented one of the lowest prevalence in the region (18.8%) (68), while Puerto Rico one of the highest (43.3%) (69).

In Brazil, few studies have been conducted about the prevalence of the MS and its determinants. A systematic review from 2013 showed that the weighted mean prevalence of the MS was 29.6% (range: 14.9% - 65.3%) (58). The MS was most prevalent (65.3%) in a study conducted in an indigenous population from southern Brazil, using the NCEP criteria (2001) (60). On the other hand, the lowest prevalence (14.9%) was found in a rural area in the Southeast region, following the Modified NCEP criteria (2005) (61). In this review, most studies reported a higher prevalence of the MS among women, with a gender difference ranging between 0.2 to 44.7%. Furthermore, the prevalence of the MS typically increased with age, and was highest among those older than 50 years. The most frequent components of the MS found in the review were low HDL-cholesterol (59.3%) and hypertension (52.5%) (58).

1.5 Cardiovascular Diseases

1.5.1 Overview – Definition and Contributing Factors

CVDs are a group of disorders of the heart and blood vessels, including ischaemic heart disease or coronary artery disease (e.g., heart attack), cerebrovascular disease (e.g., stroke), diseases of the aorta and arteries (e.g., hypertension and peripheral vascular disease), congenital heart disease, rheumatic heart disease, cardiomyopathies, and cardiac arrhythmias. Most of them are related to behavioural risk factors such as unhealthy diet, physical inactivity, tobacco use and harmful use of alcohol, which may lead to hypertension, overweight/obesity, diabetes, and dyslipidaemia. Furthermore, globalisation, urbanisation, population ageing, poverty, stress and hereditary factors are among the key underlying determinants of CVDs (70).

1.5.2 Burden of Cardiovascular Diseases Worldwide and in Brazil

CVDs are the leading cause of death worldwide, and are associated with increased disability, poor quality of life and high socioeconomic burden (71). CVD mortality rates are equivalent to the combined number of deaths from nutritional deficiencies, infectious diseases, and maternal and perinatal conditions (72). In the last decade, the remarkable growth of CVDs can be mainly attributable to the increasing incidence in LMICs. In 2016, about 17.9 million people died from CVDs, mainly due to heart attack and stroke. Of these deaths, more than 75% occurred in LMICs (70).

In South America, aging, rapid economic development, globalisation, and urbanisation have contributed to the increasing burden of CVDs and unfavourable trends of major cardiovascular risk factors. From 1990 to 2010, CVD mortality due to ischemic heart disease has increased 59%, and from stroke 39% in the region. Guyana, Trinidad and Tobago, Paraguay, Venezuela, and Brazil have the highest CVD death rates, whereas Puerto Rico, Costa Rica, Chile, and Peru the lowest (73).

In Brazil, CVDs are the main cause of death. The mortality burden, especially premature deaths, disproportionately affects the poorest. In addition, CVDs are responsible for the highest healthcare expenditure for hospital admissions. Although Brazilian cardiovascular mortality is high, a recent decline in CVD death rates have occurred in the country. A similar trend has also been observed in Argentina, Chile, and Cuba. In Brazil, better tobacco control and access to primary care have been pointed out as potential explanatory factors for this reduction (47).

1.5.3 Cardiovascular Risk Factors

The main underlying pathological mechanism of a large proportion of CVDs is the development of atherosclerosis. Atherosclerosis is a complex process that develops over many years since childhood and adolescence. Fatty material and cholesterol are deposited inside the medium- and large-sized blood vessels (arteries), making them narrower and less pliable. These deposits (atheromatous plaques) can eventually rupture, triggering the formation of a blood clot. If the blood clot develops in a coronary or a cerebral artery, this may lead to a heart attack or stroke (71).

As previously mentioned, the main risk factors for atherosclerosis and ultimately CVDs include both behavioural and metabolic factors. These factors often coexist in the same individual and act synergistically to increase his/her total risk of developing an acute vascular event. If those at risk of CVDs are early identified and measures are taken to address the risk factors, a vast majority of fatal and non-fatal cardiovascular events can be prevented (71).

In the following paragraphs, the main behavioural and metabolic risk factors for CVDs are described.

1.5.3.1 Tobacco Use

The tobacco epidemic is a major public health threat, killing more than 8 million people per year globally. According to the WHO, nearly 80% of the 1.1 billion smokers worldwide live in LMICs, where the burden of tobacco-related illness and death is greatest. Tobacco use contributes to poverty given the substantial health care costs for treating tobacco-related diseases, and lost human capital resulting from tobacco-attributable morbidity and mortality (74).

Significant progress has been made in tobacco control in Latin American, with the establishment of legislations for smoke-free public places and workplaces, higher taxation, and restrictions in advertising. Nevertheless, the prevalence of smoking remains high in the region (73). A population-based study from 2008, including 11,550 individuals living in 7 Latin America cities (Barquisimeto, Bogota, Buenos Aires, Lima, Mexico City, Quito, and Santiago), found an overall smoking prevalence of 30% (range: 22%-45%) (75). In Brazil, the prevalence has declined from 34.8% in 1989 to 22.4% in 2003. However, around 13.6% of all deaths in adults living in 16 Brazilian capitals in 2003 were attributable to tobacco use, probably because of the previous higher smoking prevalence (47).

1.5.3.2 Physical Inactivity

Physical inactivity is one of the leading risk factors for global mortality. It contributes to the rising prevalence of obesity, hypertension, and diabetes, in addition to being an independent risk factor for CVD itself (73). It has been estimated that 1 in 4 adults, as well as more than 80% of the adolescents aged 11-17 years worldwide are insufficiently physically active (76).

According to a worldwide report, about 43% of adults are physically inactive in the Americas (77). In Latin America, physical inactivity has been identified as the 10th leading risk factor for disability-adjusted year loss. Moreover, it is estimated that around 60,000 deaths due to CVDs in Latin America could have been prevented in 2008 solely by increasing the level of physical activity (73). In Brazil, patterns of physical activity have only been assessed recently. Self-reports from 2009 showed that only 14.7% Brazilian adults performed the minimum recommended level of 30 min of leisure-time physical activity at least 5 days per week (47).

1.5.3.3 Unhealthy Diet

High dietary intake of saturated fat, trans-fat cholesterol and salt, and low consumption of fruits, vegetables and fish are associated with increased cardiovascular risk. It is estimated that around 16 million (1.0%) DALYs and 1.7 million (2.8%) deaths globally are due to low intake of fruits and vegetables. On the other hand, a healthy diet can contribute to a healthy body weight, and desirable lipid profile and blood pressure levels, thus reducing the risk of CVDs (71).

Latin America has recently experienced a significant nutritional transition, with increased consumption of high-energy-density foods and reduced intake of grains, legumes, and other sources of fibre (78). In Brazil, no repeated national surveys have been performed on dietary patterns. Four large representative surveys of family food expenditure in Brazilian metropolitan areas from mid-1970s to mid-2000s have shown a reduction in household purchase of basic traditional foods (rice, beans, and vegetables), and substantial increase (up to 400%) in the purchase of processed foods (cookies and biscuits, soft drinks, processed meat, and ready meals) (47).

1.5.3.4 Harmful Use of Alcohol

Approximately 3 million deaths each year and 5.1 % of the global burden of disease and injury are attributable to the harmful use of alcohol worldwide. Causal relationships have been established between alcohol consumption and several mental and behavioural disorders, liver cirrhosis, cancers, CVDs, infectious diseases like tuberculosis and HIV/AIDS, as well as injuries resulting from traffic accidents and violence. In addition to the health consequences, harmful drinking also produces substantial socioeconomic losses to individuals and society at large (79).

Evidence from Latin America and the Caribbean suggests that excessive drinking is a relevant component of the global burden of disease, with a significant impact on NCDs and injuries in the region (80). In Brazil, the prevalence of excessive alcohol consumption and dependence is high and continues to rise. Alcohol dependence is estimated as 9-12% of the adult population, affecting more males than females, young people, and those with intermediate levels of education and income. Furthermore, age-adjusted mortality from mental and behavioural disorders due to excessive drinking increased 21% from 1996 to 2007 (47).

1.5.3.5 Obesity

Obesity is a chronic, stigmatized, and costly disease, that has become a worldwide public health problem affecting millions of people (81). The condition can be described as the excessive accumulation of adipose tissue to an extent that may impair physical and psychosocial health and well-being (82). It is fundamentally caused by excess energy consumption relative to energy expenditure. However, the aetiology of obesity involves a complex interaction of genetic, physiologic, environmental, psychological, social, economic, and even political factors. Physical inactivity, certain medications, endocrine and sleep disorders, psychiatric illnesses, intrauterine effects, smoking cessation are among the contributing factors for its development (83). Obesity has been recognised as a major underlying factor in the pathogenesis of several diseases, including T2DM, hypertension, dyslipidaemia, CVDs, kidney disease, and cancer (84).

Since measuring body fat distribution directly is expensive and time-consuming, several indirect markers have been applied in clinical practice and research (82). BMI is a common measure that reflects total body fat amounts, whereas the waist circumference (WC), waist-to-hip ratio (WHR), waist-to-height ratio (WHtR), and body fat percentage (BF%) are used as indicators for intra-abdominal adiposity or central obesity (85, 86). Conflicting evidence has been reported regarding the usefulness of these different anthropometric indices (87-89). Some studies have shown that BMI is a comparatively poor predictor of CVD risk, all-cause mortality, and deaths due to CVDs and cancer (86). Nevertheless, other reports have found that BMI either was a better predictor (88) or identified individuals at increased risk of CVD as effectively as WC (87). Recently anthropometric measurements of central obesity have been emphasized as more useful markers of obesity-related health burden (86). As shown previously in Table 4, the latest definitions of the MS have intra-abdominal obesity and specifically WC as one of the main components (57, 61, 62).

In Brazil, obesity is a growing concern. According to WHO estimates from 2016, around 22% of adults aged ≥ 18 years and 9% of adolescents aged 10-19 years were obese (16). Between 1989 and 2003, the prevalence of overweight adolescents more than doubled in boys, especially in those of lower income. From 2006 to 2009, obesity in adults increased from 11.4% to 13.9%, with higher increments among females of lower socioeconomic status. Brazil lacks reliable data on socioeconomic-specific trends in patterns of food intake and physical activity. Thus, it is unknown the impact of diet and physical activity on the higher obesity increases among those with lower-income (47).

1.5.3.6 Dyslipidaemia

Elevated total cholesterol (TC), low-density lipoprotein cholesterol (LDL-C), triglycerides, low levels of high-density lipoprotein cholesterol (HDL-C), and their combination are important risk factors for heart disease and stroke. In 2008, the global prevalence of high TC (≥ 5.0 mmol/L) among adults was 39% (37% for males and 40% for females). Overall, raised cholesterol is estimated to cause 2.6 million deaths and 29.7 million DALYS globally (71).

A study published in 2009 with data from eight Latin American countries (Argentina, Chile, Colombia, Costa Rica, Dominican Republic, Peru, Puerto Rico and Venezuela), including 32,462 subjects reported a prevalence of raised cholesterol of 9%, low HDL-C of 76.9% among females and 32.8% among males, and hypertriglyceridemia 25% (90). In Brazil, a 2004 study, involving a representative sample of adults from cities with 100, 000 inhabitants or more, showed that 22% of the subjects had hypercholesterolaemia (TC ≥ 5 mmol/L) (47).

1.5.3.7 Hypertension

Hypertension is one of the major modifiable CVD risk factors and an important cause of premature death worldwide. According to the WHO, around 1.13 billion people have hypertension globally, with two-thirds living in LMICs (91).

The prevalence of hypertension varies across Latin America countries, possibly reflecting the different stages of epidemiological transitions between them (73). In 2013, the hypertension prevalence in adults in Santiago (Chile), Buenos Aires (Argentina), and Barquisimeto (Venezuela), varied from 24% to 29%, whereas in Quito (Ecuador), Bogotá (Colombia), Mexico City (Mexico), and Lima (Peru) ranged from 9% to 13% (90). In Brazil, nationally representative data from 2008 found that 24.0% of females and 17.3% of males, aged ≥ 20 years were hypertensive. Furthermore, about 50% of males and more than half of females aged 60 years or older reported a previous diagnosis of hypertension (47).

1.6 Framingham Risk Score Model

Predicting CVD risk in individual patients may be particularly useful for implementing preventive treatments in those who are asymptomatic but at sufficiently high risk for developing CVDs. Cardiovascular risk factors usually cluster and interact multiplicatively to promote vascular risk (92). Therefore, several algorithms have been developed to assess this risk, including, for instance, the Framingham risk score (93-95), QRISK risk score (QRESEARCH cardiovascular risk algorithm) (96, 97), the Systematic Coronary Risk Evaluation (SCORE) project (98), Reynolds risk score (99), etc.

Initiated in 1948, the Framingham Heart Study was the first long-term study of its kind to contribute significantly to a better understanding of CVD predisposing factors and determinants. As a result of the Framingham Heart Study, different multivariable mathematical functions have been developed for predicting the risk of CVD events and guide preventive care. For a person free of CVD, his/her risk factors are entered into the function to estimate the probability of developing a cardiovascular event within a certain period. Based on these estimations, more effective and comprehensive prevention strategies can be implemented (100).

The Framingham Heart Study produced sex-specific prediction algorithms for assessing risk of developing incident coronary heart disease based on a cohort of a white middle-class population. Nevertheless, these functions have been used in ethnically diverse cohorts including whites, blacks, Native Americans, Japanese American men, and Hispanics, and have shown to perform well in different settings (101, 102). Recent models are easy to apply and remain a valuable tool for CVD risk prevalence and prognosis. They usually include age, gender, lipid and blood pressure levels, treatment for hypertension, smoking, and diabetes status (93).

1.7 Epidemiological and Pathophysiological Link between Metabolic Syndrome, Type 2 Diabetes, and Cardiovascular Diseases

People with the MS are twice as likely to die from and three times as likely to have a heart attack or stroke compared with those without the condition. Additionally, the MS confers a 5-fold increase in risk for T2DM (59). On the other hand, T2DM confers a 2-fold increase in CVD risk, equivalent to that of a previous myocardial infarction. After an acute coronary syndrome, people with T2DM present poorer outcomes and higher rates of reinfarction and heart failure (103).

Obesity and insulin resistance have been identified as the core factors in the pathophysiology of MS and T2DM. Insulin resistance is highly associated with obesity and physical inactivity, and multiple mechanisms for this interaction have been identified. Adipose tissue is involved with the secretion and activation of various inhibitory triggers of insulin, including inflammatory cytokines (e.g., tumour necrosis factor α [TNFα] and interleukin 6), non-esterified (free) fatty acids (NEFAs), leptin, resistin, nuclear factor κB (NF- κB) and angiotensin II (24). Further, visceral fat tissue also secretes plasminogen activator inhibitor 1 (PAI-1), which is related to thrombogenic vascular diseases. In the presence of insulin resistance, NEFAs are more mobilized from stored fat tissue triglycerides. In the liver, due to hepatic insulin resistance, NEFAs induce an increased production of glucose and triglycerides and secretion of very-low-density lipoprotein (VLDL), sustaining a vicious cycle. In the muscles, they reduce insulin sensitivity by inhibiting insulin-mediated glucose uptake and increasing fibrinogen PAI-1 production (32).

Previous studies have linked intrauterine undernutrition with later obesity, suggesting a foetal/developmental origin of metabolic diseases. In addition to central obesity, intrauterine and early childhood undernutrition may lead to reduced pancreatic ß-cells growth, and subnormal insulin secretory responses, which, in turn, may increase susceptibility to insulin resistance, MS, T2DM and ultimately CVDs (32, 104). Additionally, evidence suggests that chronic hypersecretion of stress mediators like cortisol may lead to visceral fat accumulation and directly cause insulin resistance, with reactive insulin hypersecretion. Cellular oxidative stress, with higher production of reactive oxygen species, has also been associated with the activation of the renin-angiotensin-aldosterone system, which may induce resistance to insulin. Finally, micro ribonucleic acids (RNAs) appear to play an important regulatory role in adipocyte differentiation, metabolic integration, insulin resistance and appetite regulation (32) (Figure 3).

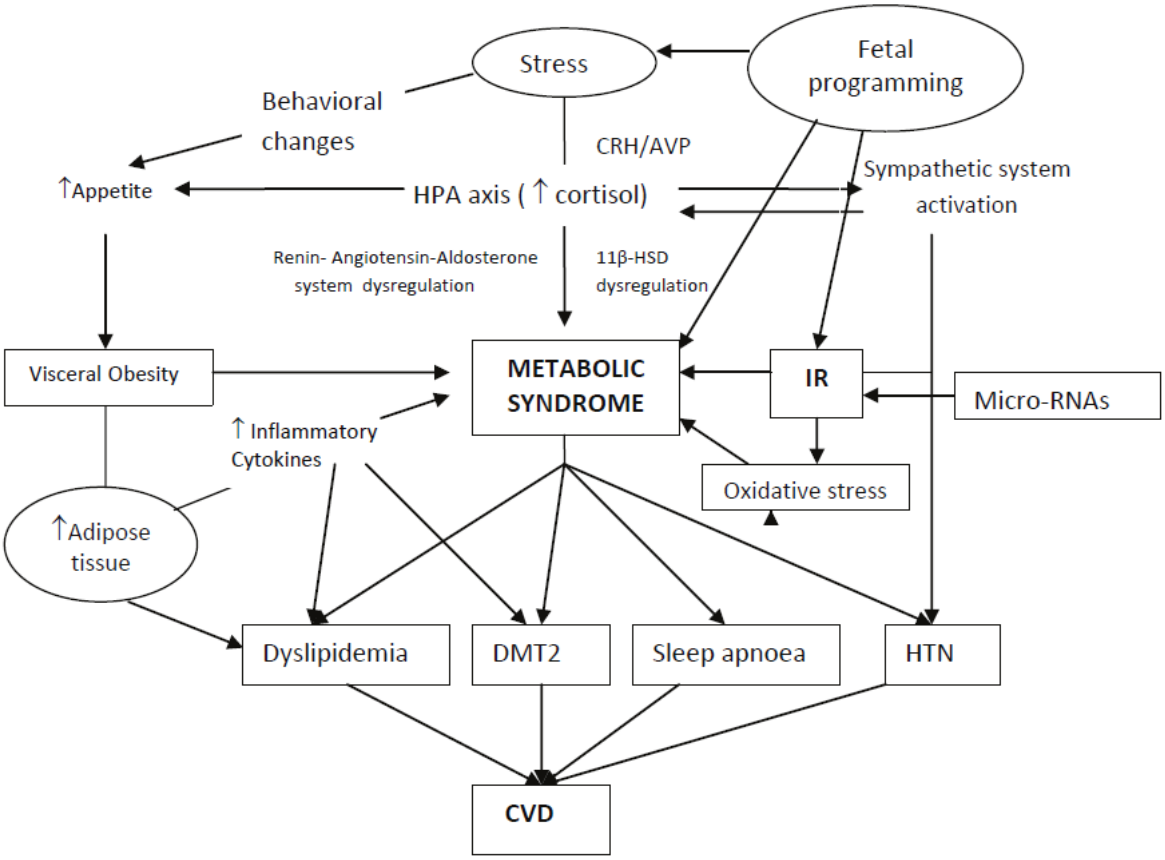

Figure 3: Schematic image of the conditions implicated in the pathophysiology of the metabolic syndrome, type 2 diabetes, and cardiovascular diseases. RNA: Ribonucleic Acid; IR: Insulin Resistance; HTN: Hypertension; 11β-HSD: 11β-hydroxysteroid dehydrogenase; HPA axis: Hypothalamic-Pituitary-Adrenal Axis; DMT2: Diabetes Mellitus type 2; CVD: Cardiovascular disease; CRH: Corticotropin Releasing Hormone; AVP: Arginine Vasopressin (32)*

Combined with other factors such as genetic predisposition, age, gender, and smoking, all the alterations described above may lead to dyslipidaemia, hypertension and T2DM, which are associated with atherosclerosis, endothelial dysfunction, vascular inflammation, and fibrosis, as well as arterial remodelling. These abnormalities compose the pathway to the development of macrovascular and microvascular disease, and ultimately CVDs. Vascular damage and endothelial dysfunction are amplified when diabetes and hypertension co-occur (Figure 4) (32, 103).

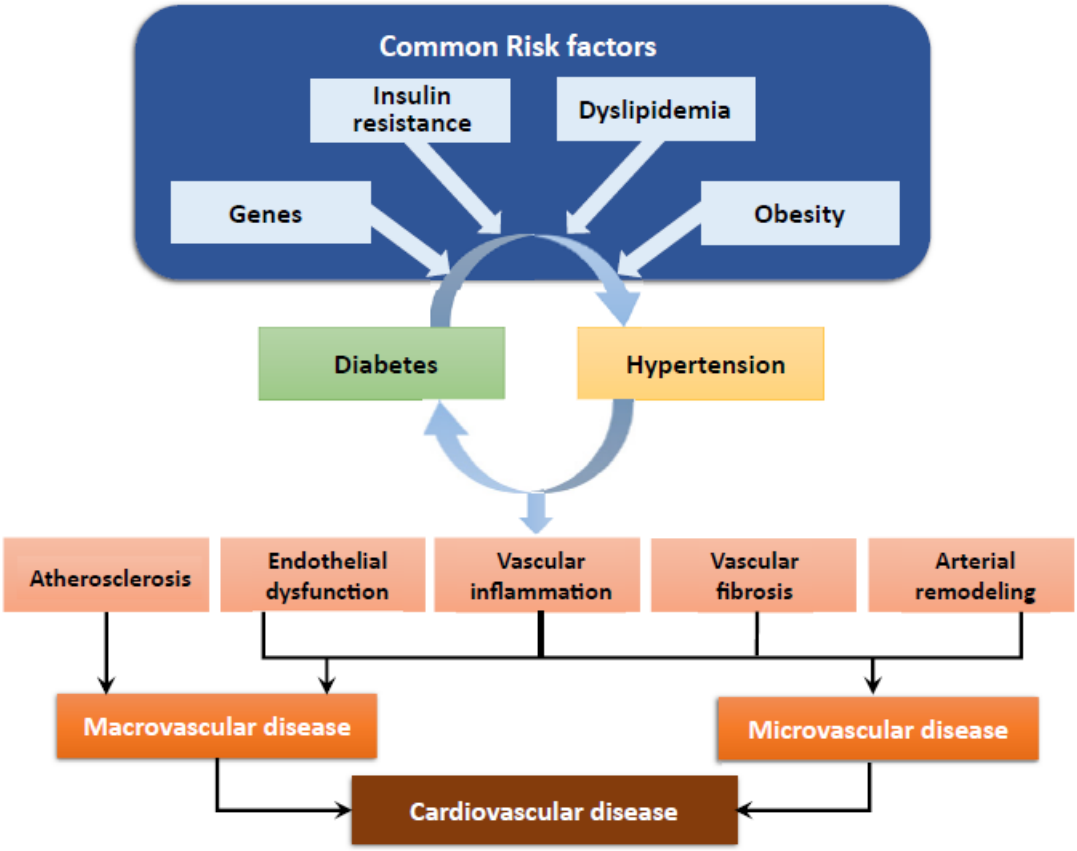

Figure 4: Vascular processes whereby diabetes and hypertension predispose to cardiovascular disease (103)*

Chapter 2: Rationale and Objectives

2.1 Rationale

As previously mentioned, a radical shift in the disease burden has occurred in Brazil during the last decades. In 1930, around 46% of all deaths were due to infectious diseases (47), whereas, in 2016, more than 70% were attributable to NCDs, and only less than 10% to infectious or parasitic disorders (16). This profound change took place within the context of economic and social development, population aging, more mechanisation and industrialisation, rapid urbanisation, improved access to food, and dissemination of unhealthy habits. Therefore, the Brazilian population has been increasingly exposed to higher risks of chronic diseases and cardiovascular risk factors (47).

Although the MS has been recognized as a major public health problem worldwide, several questions and controversies persist. Its value as a tool to assess the risk of future CVD has been claimed as weak (105) or no better than the sum of its components (106). Additionally, even though the syndrome seems to be effective in predicting diabetes, its predictive ability beyond that of glucose intolerance has been challenged (107). It has been argued that the MS is an ill-characterized entity, with no clear rationale for thresholds (108). Different definitions of MS have been suggested so far by several scientific organizations (57, 60-62). Despite sharing common features, these definitions present different parameters or cut-off values, which produce discrepancies in applicability, uniformity, and positive predictive value (109). In Brazil, few studies have been conducted regarding the prevalence of MS and its determinants. Moreover, scarce evidence exists about the applicability and agreement of different definitions of MS, as well as their predictive value in the estimation of T2DM, pre-diabetes, and CVD risk in the Brazilian population.

As formerly discussed, T2DM can remain undiagnosed for years and cause several chronic complications. Pre-diabetes increases the risk for T2DM and CVDs (20). On the other hand, CVDs are responsible for about half of the total mortality among those with diabetes (110). Therefore, early diagnosis of pre-diabetes and diabetes may reduce long-term complications, healthcare costs, and premature death (111-113). The HbA1c test has been widely applied in diagnosing diabetes and has shown several advantages (42). Nevertheless, racial and ethnic disparities have been described in the relationship between HbA1c and glucose levels (45). Concerns remain about misdiagnosing people with diabetes when using the recommended HbA1c cut-off of 6.5%. Thus, several cut-off levels of HbA1c have been suggested to diagnose diabetes in different ethnic groups (41). Few studies in Brazil have assessed the efficiency of HbA1c in diagnosing diabetes and pre-diabetes, and usually involved small sample sizes or a clinically targeted population. It is still unknown whether the recommended HbA1c cut-off of 6.5% is suitable for the Brazilian population. Furthermore, since insulin resistance is

possibly the main pathophysiological link between DM and CVDs, it would be of interest to investigate estimates of insulin resistance in relation to HbA1c values.

In Brazil, CVDs are the main cause of death and account for the highest healthcare expenditure for hospital admissions (47). Most CVDs are associated with a complex interaction of modifiable risk factors, and, among them, overweight and obesity are an increasing concern (71). Several anthropometric markers of general and central obesity have been used to investigate adiposity-related risk (85). However, conflicting results have been produced regarding the usefulness of these parameters (87-89). Adiposity is highly heterogeneous with age, gender, and ethnicity (114), therefore it remains uncertain which anthropometric indicators correlate better with CVD risk factors and subsequent CVD risk in different populations (115). Brazil is a continental country with marked socioeconomic and cultural differences across the five regions, which may influence the individual cardiovascular profiles (4, 116). Although the prevalence of cardiovascular risk factors has been reported by several, most studies have limitations, including potential selection bias and the use of self-reported data (116). Most importantly, scarce evidence from the Northeast region of Brazil exists regarding CVD risk, sociodemographic and anthropometric characteristics of the population.

2.2 Study Aims

The overall aim of this study was to produce a better understanding of CVD risk, MS, diabetes and their determinants and associations, which in turn may lead to a better management of these conditions. Further, identifying more suitable cut-off values of HbA1c for diagnosing diabetes in this population may decrease the number of false positive and negative cases. Therefore, it may potentially reduce morbidity, mortality, and healthcare costs. Finally, the findings may also give grounds to the development of new hypotheses for exploration and new management and preventive guidelines.

2.3 General Objective

- To investigate CVD risk, diabetes, MS, their associations, and determinants.

2.4 Specific Objectives

- To determine the suitable cut-off values of HbA1c for the diagnosis of diabetes and pre-diabetes, as well as the cut-off points of Homeostasis Model Assessment of Insulin Resistance (HOMA-IR) to predict the risk of diabetes.
- To compare the prevalence of MS following different definitions (Modified NCEP, IDF and JIS), their agreement, and the association of MS with CVD risk, diabetes, and pre-diabetes.

- To study CVD risk following the Framingham risk score by socioeconomic and demographic characteristics and investigate the association between the predicted risk of CVDs and obesity.

Chapter 3: Methods

3.1 Study Site, Study Design and Population

This study was conducted in the city of Pindoretama, situated 40 km from the capital, Fortaleza, in the state of Ceará (CE), in the North-eastern region of Brazil (Figure 5). According to the latest demographic census conducted by the IBGE in 2010, the total population of Pindoretama was approximately 18,683 inhabitants (117). Following the national trend, Pindoretama has experienced increased industrialisation, population aging, and steady and rapid urbanisation, with a decreasing portion of the population dependent on agricultural activities (118).

Figure 5: Study Site. Geographical Location of Pindoretama-CE, Brazil

This population-based cross-sectional study was conducted from August 2012 to January 2013. The survey took place in the six main community health centres located throughout the city. Pindoretama was selected as the study site given the availability of nearby laboratory facilities and adequate centres to perform examinations and investigations; support from the local health and governmental authorities; availability of nurse assistants and health volunteers willing to be trained and be part of the research team, etc.

3.2 Sample Selection

3.2.1 Sample Size

The minimum required sample size was calculated by the following formula:

$n = 4 (z_{crit})^2 p (1 - p) / D^2$ (119), where "n" is the total sample size, "z_{crit}"= 1.96 (Standard Normal Deviate for a Significance Criterion = 0.05 and a Confidence Interval = 0.95), "*p"* = 0.051 (prevalence estimate from a previous study of high/intermediate risk of CVD according to the Framingham risk score (120), and "*D*" = 0.0454 (total width of the expected confidence interval). Two-tailed statistical analyses were used. Thus, $n = 4 \times (1.96)^2 \times 0.051 \times 0.949 / (0.0454)^2 \rightarrow n = 360.83$.

The eligible subjects were selected based on a simple random procedure, using a registry list provided by the health authorities, with the names of the citizens in alphabetic order. Random numbers were produced with the statistical software R (121), and subsequently matched with the names in the list. Around 1,000 subjects, both males and females, aged ≥ 20 years, were selected randomly based on the list. Of these, one hundred and sixty-three subjects (127 males) were not found by the community health workers and, therefore, could not be invited to join the study. Thirty-one were excluded. Thus, eight hundred and six randomly selected subjects (300 males and 506 females) were invited to participate. Out of these, seven hundred and fourteen (242 males and 472 females) agreed to join the study, corresponding to a total response rate of 88.6% (males: 80.7%; females: 93.3%) (Figure 6).

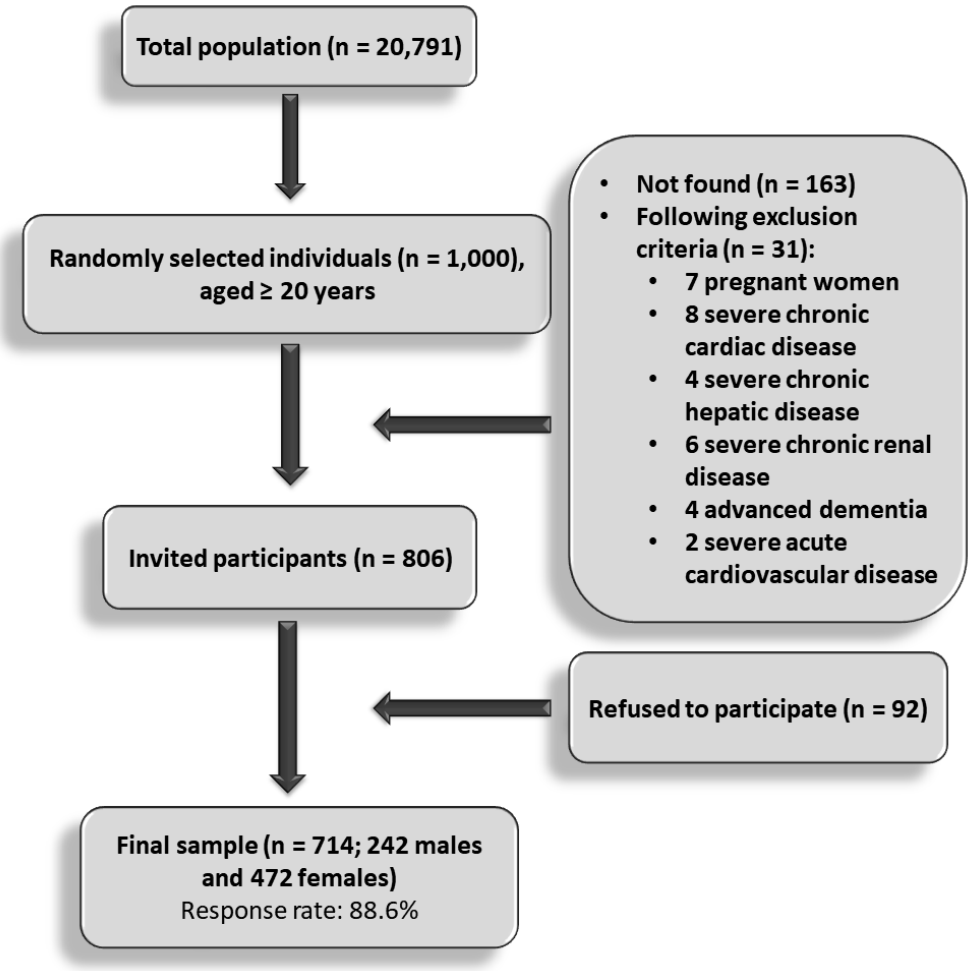

Figure 6: Recruitment of the study subjects

3.2.2 Inclusion Criteria

Subjects of both genders, aged ≥ 20 years.

3.2.3 Exclusion Criteria

Pregnant women, those with an acute or chronic severe cardiac, renal, or hepatic illness, as well as physically or mentally disabled subjects unable to follow simple questions and examinations.

3.3 Data Collection

3.3.1 Training of the Research Team

The PhD candidate, under the guidance of the local supervisor (Professor Renan Magalhães Montenegro Júnior) was responsible for recruiting and training the research team members. To ensure quality control, intensive training was provided for the investigators before the start of the study to

reduce bias as much as possible. All procedures and methods of efficient data collection were discussed among the team members. One research assistant was responsible to collect socioeconomic, demographic, and physical activity data through face-to-face interviews with the subjects. Anthropometric, blood pressure (BP) and BF% measurements were taken by another investigator, whereas one nurse assistant conducted the capillary HbA1c assessment. During the fieldwork, the PhD candidate encouraged the research team members to discuss all problems faced and their potential solutions.

3.3.2 Survey Procedures

The randomly selected subjects were contacted and invited to the study by local community health workers and the PhD candidate. By the time of invitation, the research purposes and methods of investigation were explained. The subjects were instructed twice (personally by the community health workers and by a phone call on the previous day of the survey) regarding an overnight fasting of at least 8 hours. Those who failed to fast were rescheduled for another appointment. Upon arrival at the field sites, the study objectives, investigation procedures and examinations were discussed again. The subjects willing to participate were given informed consent. At first, an initial fasting blood sample was taken. Then the subjects were given a 75-gram oral glucose drink and requested to wait for 2 hours to collect a second blood sample. During this waiting time, they were also interviewed with pre-tested questionnaires regarding socioeconomic, demographic, clinical, and physical activity information. Capillary HbA1c measures, anthropometric measurements, BP, and BF% were taken. Details of questionnaires, investigations and physical examinations are described below.

3.3.3 Interviewer-Administered Questionnaire

Before the study, a structured questionnaire was developed to assess socioeconomic and demographic information, lifestyle behaviours and clinical data (appendix I). Its content and questions were elaborated considering surveys conducted in previous studies, by consulting with existing information and clinical practices. The questionnaire was developed in the local language, i.e., Portuguese. It included questions on sociodemographic characteristics (age, sex, marital and work status, income, and education), lifestyle behaviours (smoking and alcohol consumption), personal and family history of CVDs, diabetes, hypertension, and use of medications.

Pretesting was conducted to assess the questionnaire's feasibility and catch and solve unforeseen problems. As a result, the wording was improved, unnecessary questions were crossed out, and others were added. Adjustments were also made to reduce the time required to complete all the questions.

3.3.4 International Physical Activity Questionnaire

Physical activity information was obtained by the interview administered International Physical Activity Questionnaire (IPAQ) short form (Appendix II), which was designed primarily for population surveillance among adults. The IPAQ assesses the time spent on walking, in vigorous-, moderate-intensity, and sedentary activities. A comprehensive set of domains is evaluated, including leisure time activities, domestic and gardening, as well as work- and transport-related physical activities (122). The total score is computed by summing up the duration and frequency of walking, moderate- and vigorous-intensity activities (123). A study conducted in 12 countries including Brazil showed that the IPAQ instruments exhibited acceptable reliability and validity, comparable to other established self-report physical activity measures (124).

3.3.5 Anthropometric Measurements

Anthropometric measurements, including weight, height, waist, and hip circumferences were taken with the subjects standing in bare feet and in light clothing. Weight was recorded using a portable digital scale (OMRON medical scale) to the nearest 0.1 kg, placed on a flat surface, and calibrated daily before use. Height was taken using a stand-alone stadiometer, recorded to the nearest 0.1 cm, with each subject in erect position and their head in the Frankfurt plane. BMI was estimated as the weight (kg) divided by the square of the height (m^2). WC was taken by placing a non-stretchable tape horizontally on the midpoint between the lower border of the ribs and the top of the iliac crest, on the mid-axillary line. Hip circumference (HC) was measured with a similar tape at the greatest protrusion of the buttocks. WC, and HC were recorded to the nearest 0.1 cm. WHR was calculated as the WC divided by the HC, whereas the WHtR as the WC divided by the height.

3.3.6 Measurement of Body Fat Percentage - Bioelectrical Impedance Method

BF% was measured by a portable bipolar body fat analyser (Omron®, Model HBF-306, Omron Healthcare, Inc., Illinois, United States), with the subjects standing upright, holding the hand grips on both sides, with arms slightly outstretched and making a 90-degree angle with the chest. Considering gender, age, weight, and height, the device works by a formula that calculates the fat percentage given the electric resistance encountered by the micro currents (500 μA, 50 kHz), which are emitted from one hand grip to the other. The fluctuation in the value is recorded and the BF% measurement is displayed within 7 seconds (125).

3.3.7 Measurement of Blood Pressure

BP was measured twice at a 10-minute interval, after a resting time of at least 15 minutes, using a validated automatic sphygmomanometer (Omron® BP785 IntelliSense® Automatic Blood Pressure Monitor with ComFitTM Cuff, Omron Healthcare, Inc., Illinois, United States). The measurements were taken in the left arm, with appropriate size cuffs, and the subjects were sitting with legs uncrossed. The mean value of the two measurements was used for analysis.

3.3.8 Biochemical Assessments

After an overnight fasting of at least 8 hours, a sample of 10ml of peripheral venous blood was collected on arrival for FPG, lipid profiles, and insulin measurements. Another 3ml of venous blood were taken two hours after a 75-gram glucose load for the OGTT. For plasma glucose tests, the blood was drawn in tubes containing sodium fluoride and potassium oxalate (1:3). The collected samples were transferred to a sterile container, stored immediately over ice, and then centrifuged within approximately 1 hour of collection. Plasma was frozen and transported on dry ice in vaccine containers within 2 hours to the laboratory where the samples were stored at – 20° Celsius until the assays were performed. Glucose oxidase method was used for measuring fasting and 2-h plasma glucose levels, whereas chemiluminescence for fasting insulin. Capillary HbA1c levels were measured by A1CNow® Multi-Test A1C System (Bayer). TC was estimated by the cholesterol oxidase - phenol + aminophenazone (CHOD-PAP) method, while HDL-C was determined by a homogenous enzymatic colorimetric method. Triglycerides were measured by the glycerol-3-phosphate oxidase - phenol + aminophenazone (GPO-PAP) method. The Friedewald's formula estimated the LDL-C levels (126). All these biochemical analyses were carried out by the same laboratory technician teams using the same methods throughout the study period.

3.3.9 Estimating the Framingham Risk Score

The predicted 10-year risk for an incident cardiovascular event was estimated using the Framingham 10-year risk score model, as published by D'Agostino et al in 2008 (93). The model predictors included age, systolic blood pressure (SBP), use of antihypertensive medication, smoking status, diabetes, TC, and HDL-C. Thirteen subjects reported a previous history of stroke and/or myocardial infarction and were excluded from the analyses. Furthermore, owing to missing values, the Framingham risk score was calculated only for 693 subjects (229 males and 464 females).

3.4 Definition of Terms

- Subjects with a Framingham 10-year predicted risk of 10% or above were defined as having a high CVD risk (93).
- Diabetes mellitus and pre-diabetes were defined according to the diagnostic criteria recommended by the WHO in 1999 (18):
  - Diabetes was defined as FPG ≥ 7.0 mmol/L (≥ 126 mg/dL), or 2-h post glucose load ≥ 11.1 mmol/L (≥ 200 mg/dL), or both.
  - Pre-diabetes:
    - IFG: FPG ≥ 6.1 mmol/l (≥ 110 mg/dL), but <7.0 mmol/L (<126 mg/dL), and 2-h post glucose load <7.8 mmol/L (<140 mg/dL).
    - IGT: FPG <7.0 mmol/L (<126 mg/dL), and 2-h post glucose load ≥ 7.8 mmol/L (≥ 140 mg/dL), but < 11.1 mmol/L (< 200 mg/dL).
- MS was defined following the diagnostic criteria as suggested by the Modified NCEP (61), IDF (57) and JIS (62) (Table 5). For the Modified NCEP, the WC cut-off points were ≥ 102 cm in males and ≥ 88 cm in females, whereas for the IDF and JIS definitions, the cut-points were WC ≥ 90 cm in males and ≥ 80 cm in females.
- HOMA-IR was calculated by using the method described by Matthews et al. ([fasting insulin (mU/l) × FPG (mmol/L)] / 22.5) (127).
- Overweight / general obesity for both genders was defined as a BMI of ≥ 25 kg/m^2 (128).
- According to the WHO recommendations, for males, a WHR ≥ 0.90 cm was considered "high" (a substantially increased risk of metabolic complications), whereas, for females, a WHR ≥ 0.85 cm was classified as "high" (128). A cut-off of ≥ 0.50 defined a high WHtR (129).
- Hypertension was defined as SBP ≥ 140 mmHg and/or diastolic blood pressure (DBP) ≥ 90 mmHg and/or on current treatment with antihypertensive medication (130).
- Dyslipidaemia was defined as triglycerides ≥ 1.7 mmol/L for both genders and HDL-C < 0.9 mmol/L for males, and < 1.0 mmol/L for females (18).
- Ethnicity was based on the subjects' self-perception of their skin colour. The different ethnic groups were categorized into "white", "brown", and "black" in paper II, and "white" and "non-white" in paper III (4).
- Smoking habit was categorized as either "yes" or "no". Those who self-reported as being smokers or had stopped smoking for less than 1 year were classified as "yes".
- Physical activity was categorized as "low", "moderate" or "high". "Low" was applied when no activity was reported, or some activity was reported but not enough to meet the other two categories. "Moderate" was used when either of the following 3 criteria was met: 1) Three or more

days of vigorous activity of at least 20 minutes/day, or 2) Five or more days of moderate-intensity activity and/or walking of at least 30 minutes/day, or 3) Five or more days of any combination of walking, moderate-intensity or vigorous-intensity activities achieving a minimum of at least 600 metabolic equivalent of task (MET)-minutes/week. "High" was considered when any one of the following 2 criteria was present: 1) Vigorous-intensity activity on at least 3 days and accumulating at least 1500 MET-minutes/week, or 2) Seven or more days of any combination of walking, moderate- or vigorous-intensity activities accumulating at least 3000 MET-minutes/week (123). For data analysis, these results were transformed into a binary variable, i.e., either "low" or the combination of the categories "moderate" and "high".

3.5 Data Management and Storage

All the information materials including informed consents, questionnaires, clinical examination sheets, and biochemical analyses results were kept in a secure and locked cabinet. The data were only accessible by the PhD candidate and local supervisor. The information collected was registered in a computer database by using SPSS (131). Missing values and data entry errors were checked carefully. Recently the data set has been only stored and analysed at the University of Oslo's platform for the processing of sensitive research data (TSD) (http://www.uio.no/english/services/it/research/sensitive-data/). All biological materials have been destroyed.

3.6 Statistical Methods

In all three papers, continuous variables were expressed as means and 95% confidence intervals (CIs), whereas categorical data as percentages and 95% CIs. Generalized linear regression models (GLMs) were fitted to the data after adjusting for age/gender. We fitted GLMs with linear link function for comparing differences between adjusted means, whereas GLMs with the logit link function were applied for differences between proportions. Based on the estimations of the adjusted logistic regression models, the prevalence of diabetes, pre-diabetes, MS, and those with a predicted 10-year CVD risk of ≥ 10% were obtained as predictive margins. In paper III, to control confounding by age in the predicted means and proportions, we fixed age at 45 years. In paper I, the receiver operating characteristic (ROC) curve analysis was applied to evaluate the discriminatory ability of the HbA1c test for detecting diabetes mellitus and pre-diabetes, as well as to identify the most suitable cut-off points of HOMA-IR to predict the risk of diabetes. In addition, optimal HbA1c and HOMA-IR cut-off points were obtained based on the highest Youden index (132). In paper II, Kappa statistics was used to evaluate the agreement between three definitions of MS (Modified NCEP, IDF and JIS). Multiple logistic

regression analysis adjusted for age, gender, and BMI assessed the association of MS with pre-diabetes, T2DM, and CVD risk. In papers I and II, diagnostic test properties including sensitivity, specificity, positive predictive value (PPV) and negative predictive value (NPV) were calculated using contingency tables. In paper III, a two-sample test of proportions was used to compare the prevalence of high CVD risk among the different sociodemographic groups. In addition, anthropometric measurements were converted to z-scores (original value subtracted by the mean and divided by the standard deviation [SD]) to represent the number of SDs above and below the mean for each subject. Multiple linear regression analysis investigated the relationship between the standardized anthropometric markers and CVD risk. Further, we calculated crude and adjusted prevalence ratios (PRs) of the anthropometric indicators for detecting high CVD risk using Poisson regression analysis. The Akaike Information Criteria was applied to compare nested models. Data were analysed using Stata 15th edition (133), SPSS 25th (134) and 26th (131) versions, and R for Windows (121). The significance level was set at 0.05, and all tests were two-sided.

3.7 Ethical Considerations

The study was carried out following the ethical principles outlined in the Helsinki Declaration (135). Prior to any investigation, the research protocol was approved by the local Ethical Committee in Brazil (Protocol Number: 045.06.12) and the Regional Committee for Medical and Health Research Ethics (REK) in Norway (Reference: 2012/779/REK sør-øst D). Written or verbal consent was sought from all subjects. To secure the free participation of illiterate subjects, verbal consent was assured by a local witness, who signed the informed consent. The subjects received all information regarding the study investigations and procedures, anonymity, and confidentiality of the data, as well as their right to withdraw from the study at any stage or withhold their data from the analysis. Written feedback from examinations and blood tests were given and explained to all subjects. Those diagnosed with any clinical condition were referred to the nearest health centre for further treatment and follow up.

Chapter 4: Results

The main results are described below.

4.1 Diabetes and Pre-diabetes (Paper I)

4.1.1 Prevalence of Diabetes and Pre-diabetes

The overall prevalence of diabetes mellitus adjusted for age and gender was 14.7% (95% CI: 12.2–17.2), and pre-diabetes 14.2% (95% CI: 11.6–16.7). The crude prevalence of diabetes was 16.1% (95% CI: 13.0–19.7%) among females, and 12.0% (95% CI: 8.4–16.7%) among males. The crude prevalence of pre-diabetes was 15.7% (95% CI: 12.7–19.3%) among females, and 11.2% (95% CI: 7.8–15.8%) among males. Both the prevalence of diabetes and pre-diabetes were not significantly different between males and females.

4.1.2 Use of HbA1c as a Diagnostic Tool of Diabetes and Pre-diabetes

According to the Youden index, the optimal HbA1c cut-off value was ≥ 6.8% for the diagnosis of diabetes, and ≥ 6.0% for pre-diabetes. The area under the curve (AUC) was 0.85 (95% CI: 0.80–0.90) for detecting diabetes, whereas for pre-diabetes was 0.61 (95% CI: 0.55–0.67) (Figure 7). For the proposed cut-off point of capillary HbA1c ≥ 6.8%, the sensitivity was 69.2%, specificity 92.1%, PPV 60.2%, and NPV 94.6%. At the suggested cut-off for pre-diabetes, i.e., capillary HbA1c ≥ 6.0%, the sensitivity was 67.3%, specificity 52.0%, PPV 18.7%, and NPV 90.6%.

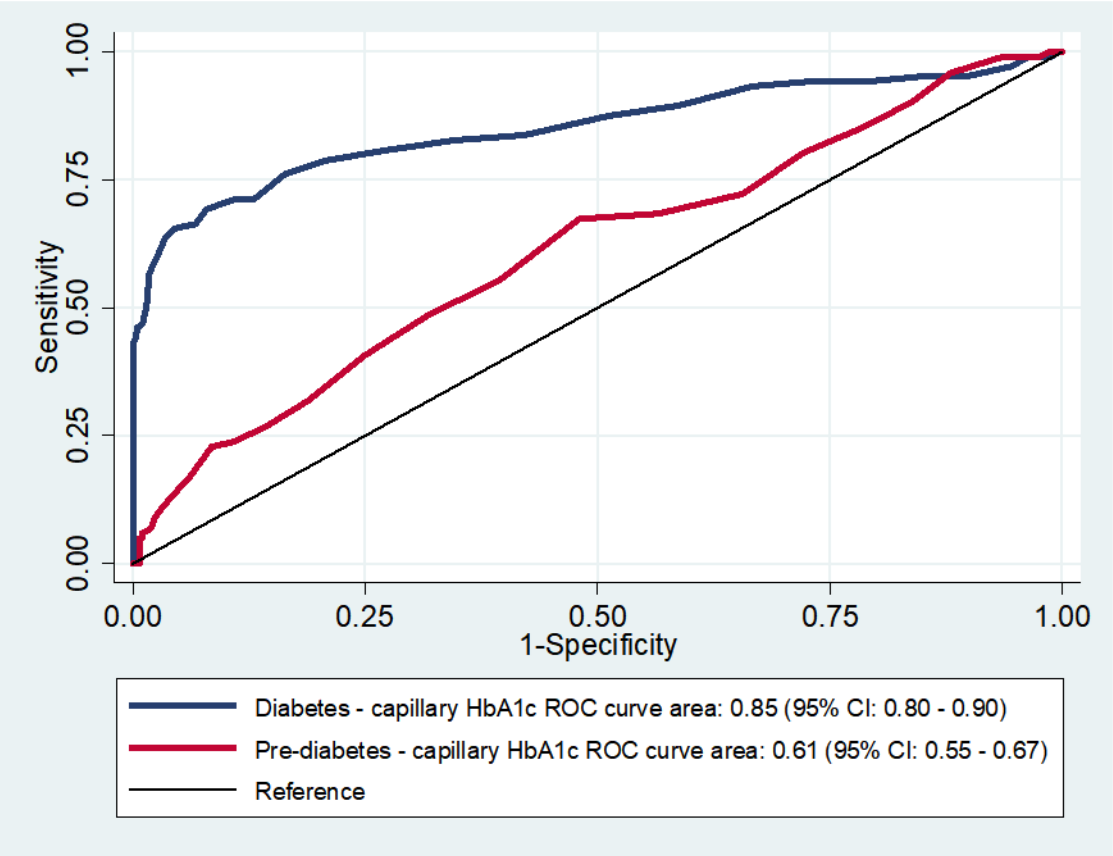

Figure 7: Receiver operating characteristics (ROC) curves for HbA1c (glycated hemoglobin) to diagnose diabetes and pre-diabetes

4.1.3 HOMA-IR Cut-off Points and the Risk of Diabetes

Figure 8 shows the ROC curves to identify the most suitable cut-off value for HOMA-IR against the recommended cut-off point of HbA1c ≥ 6.5%, and the proposed cut-off value of ≥ 6.8% from our data. Following the highest Youden index, the optimal HOMA-IR cut-off value was 1.81 for HbA1c at 6.5%, and 2.06 for HbA1c at 6.8%. The AUC for HbA1c at 6.5% was 0.66 (95% CI: 0.61–0.71), and for HbA1c at 6.8% was 0.74 (95% CI: 0.68–0.79). At the HOMA-IR cut-off point of 1.81, the sensitivity was 52.0% (95% CI: 44.4–59.5) and specificity 78.0% (95% CI: 74.2–81.5), whereas at the point of 2.06, the sensitivity was 59.7% (95% CI: 50.3–68.6) and specificity 81.9% (95% CI: 78.5–84.9).

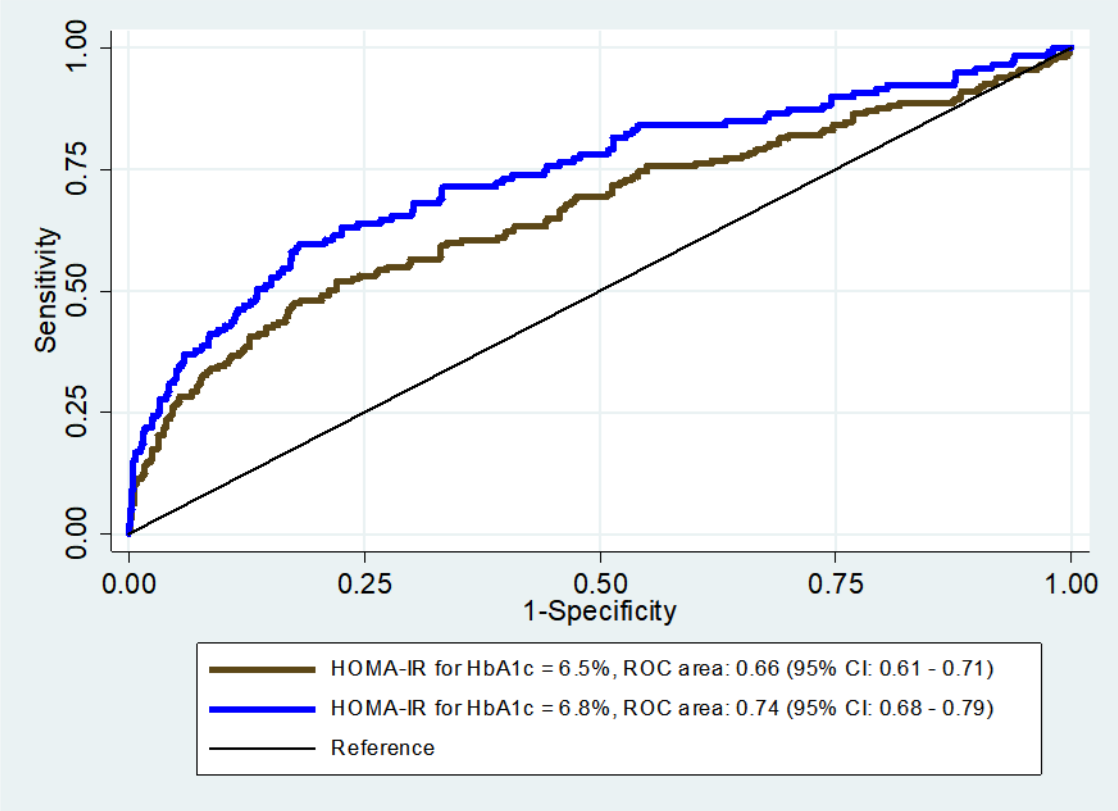

Figure 8: Receiver operating characteristics (ROC) curves for the homeostasis model assessment of insulin resistance (HOMA-IR) for cut-off values of HbA1c at 6.5% and 6.8%

4.2 Metabolic Syndrome (Paper II)

4.2.1 Prevalence of MS Applying the Modified NCEP, IDF and JIS Definitions, and their Agreement

The age- and gender-adjusted prevalence of MS was 36.1% (males: 18.9%, females: 44.8%) applying the JIS criteria, 35.1% (males: 18.3%, females: 43.7%) following the IDF definition and 29.5% (males: 12.6%, females: 38.2%) the Modified NCEP. These estimates of the MS prevalence were not significantly different between the definitions. The highest agreement was found between the IDF and JIS definitions (kappa: 0.98, p-value: <0.001), and the lowest between the IDF and Modified NCEP criteria (kappa: 0.83, p-value: <0.001). As can be shown in Figure 9, most participants with MS had the condition according to all three definitions (n=202) (additional data).

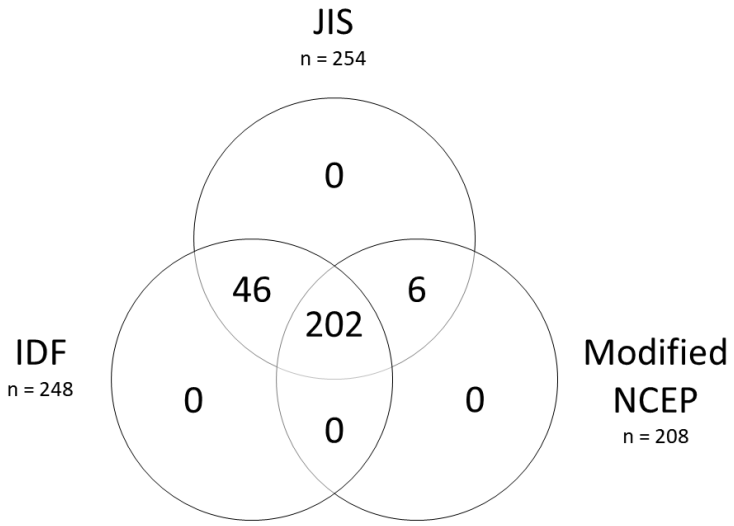

Figure 9: Venn diagram showing the overlapping of subjects with metabolic syndrome following the diagnostic criteria as suggested by the International Diabetes Federation (IDF), modified National Cholesterol Education Program Adult Treatment Plan III (Modified NCEP) and Joint Interim Statement (JIS) (additional figure).

4.2.2 MS and Sociodemographic Characteristics

According to all three definitions, the prevalence of MS among females was significantly higher than in males. Additionally, the prevalence increased significantly with age, BMI status, and level of income. However, it did not differ significantly among the ethnic groups.

4.3 CVD Risk (Paper III)

4.3.1 CVD Risk and Sociodemographic Characteristics

The estimated overall prevalence of high CVD risk (predicted 10-year CVD risk of ≥ 10%) adjusted for age and gender was 18.9% (14.3-23.6) (Table 6). It was significantly higher among males (31.9% vs. 12.5%; p-value: < 0.001), and those with more than 45 years of age (68.9% vs. 4.2%; p-value: < 0.001). Further, the prevalence of high CVD risk was significantly lower among those with an occupation requiring manual labour (7.6% vs. 21.7%; p-value: 0.008), defined as jobs in agriculture and construction.

Table 6: Predicted proportions of subjects with 10-year CVD risk of ≥ 10% using the Framingham Risk Score by sociodemographic characteristics

Characteristics	n	Predicted 10-year risk ≥ 10% *% (95% CIs)*	p-value
Overall	693*	18.9 (14.3-23.6)	
Gender			
Male	229	31.9 (21.8-42.0)	< 0.001
Female	464	12.5 (8.0-17.0)	
Age groups			
< 45 years	388	4.2 (2.2-6.2)	< 0.001
≥ 45 years	305	68.9 (63.8-74.0)	
Ethnicity			
White	116	20.8 (9.4-32.2)	0.58
Non-white	577	18.6 (13.7-23.5)	
Education			
< 10 years	489	19.2 (14.1-24.4)	0.60
≥ 10 years	204	17.5 (7.4-27.7)	
Monthly Income			
< 2MW	623	17.7 (12.8-22.7)	0.11
≥ 2MW	68	25.6 (11.4-39.9)	
Occupation**			
Non-manual Labour	629	21.7 (16.4-27.1)	0.008
Manual Labour	64	7.6 (1.3-13.9)	

Data are percentage (95% confidence intervals), adjusted for age (at age fixed to 45 years) and gender. *The study collected data from 701 subjects in total, but due to some missing values, the Framingham Risk Score was calculated for 693 subjects (229 males and 464 females). **Manual Labour: jobs in agriculture and construction. Non-manual Labour: other occupations. CIs: Confidence Intervals. CVD: Cardiovascular Disease. MW: Minimum Wage in 2012.

4.4 Association between CVD Risk, Diabetes, Pre-diabetes, Metabolic Syndrome, and Related Cardiometabolic Risk Factors (Papers II and III)

4.4.1 Association between MS and Pre-diabetes, T2DM, and CVD Risk

According to all definitions of MS, the prevalence of MS was higher among those with pre-diabetes, T2DM, and high CVD risk. Following the JIS criteria, MS was present in 58.2% of subjects with pre-diabetes, 76.1% of those with T2DM, and 57.1% of those with high CVD risk. When the IDF definition was used, the respective parameters were 57.1, 74.3, and 54.8%, whereas for the Modified NCEP, the values were 46.9, 70.8, and 48.0%.

After controlling for age, gender, and BMI, MS was significantly associated with pre-diabetes, T2DM and high CVD risk, following all defining criteria of MS. However, the adjusted odds ratios (OR)

for pre-diabetes (ranging from 3.6 - 3.9), T2DM (5.0 - 6.4) and high CVD risk (5.6 - 7.1) were not significantly different between the different definitions of MS (Table 7).

Table 7: Odds ratios (OR) for pre-diabetes, T2DM, and people with high CVD risk in those with MS compared with those without MS

	Pre-Diabetes	T2DM	High CVD Risk
	OR (95% CI)	OR (95% CI)	OR (95% CI)
IDF	3.9 (2.3-6.5)[a]	5.0 (3.0-8.5)[a]	5.6 (2.9-10.9)[a]
Modified NCEP	3.6 (2.0-6.2)[a]	6.4 (3.7-11.1)[a]	5.7 (2.9-11.3)[a]
JIS	3.9 (2.3-6.5)[a]	5.4 (3.2-9.3)[a]	7.1 (3.6-14.2)[a]

Adjusted for age, gender, and body mass index.
[a] p <0.001; CI: Confidence Interval. CVD: Cardiovascular Disease. IDF: International Diabetes Federation. JIS: Joint Interim Statement. MS: Metabolic Syndrome. NCEP: National Cholesterol Education Program Expert Panel. T2DM: Type 2 Diabetes Mellitus.

The JIS definition had higher sensitivity than the Modified NCEP to identify subjects with pre-diabetes (58.2% vs. 46.9%), T2DM (76.1% vs. 70.8%) and high CVD risk (57.1% vs. 48%). Nevertheless, for the Modified NCEP definition, its specificity (pre-diabetes: 83.4%; T2DM: 78.3%; high CVD risk: 81.2%) and PPV (pre-diabetes: 31.5%; T2DM: 38.4%; high CVD risk: 59.1%) were higher than for the JIS definition. The IDF and JIS definitions showed similar sensitivity, specificity, PPV and NPV.

4.4.2 Association between Anthropometric Markers (WC, BMI, WHR and WHtR) and the Predicted Risk of CVDs

Multiple linear regression evaluated the age-adjusted associations between 1 SD increment in each anthropometric marker and the predict risk of CVDs. In females, WHR and WHtR were statistically significant predictors of CVD risk, whereas in males only WHtR was significant, followed by a borderline-significant association for WC. WHtR had the highest slope coefficient in males (1.82; 95% CI: 0.09 – 3.56), while WHR presented the highest slope in females (1.13; 95% CI: 0.14 – 2.11).

Poisson regression with robust variance was used to calculate crude and adjusted prevalence ratios (PRs) of the different anthropometric measures for identifying high CVD risk (Table 8). Adjusted PRs were obtained after controlling for age, level of physical activity, family history of cardiac disease and stroke. We included an interaction term between age and the corresponding anthropometric indicator in some adjusted models, based on their statistical significance and the Akaike Information Criteria. In males, all anthropometric markers were significant. In females, significant positive associations were found between all anthropometric parameters and high CVD risk in the adjusted

models, except for WC. WHtR showed the highest adjusted PR for males (9.9, 95% CI: 2.8-34.8, p-value < 0.001) and females (43.4, 95% CI: 2.6-716.8, p-value 0.002).

Table 8: Crude and adjusted prevalence ratios (PRs) of anthropometric indices for identifying high CVD risk (≥ 10% using the Framingham Risk Score).

Characteristics	***Crude PR[a]* *(95% CIs)***	**p-value**	***Adjusted PR[b]* *(95% CIs)***	**p-value**
Males				
WC (> 102 cm)[c]	1.9 (1.4-2.5)	< 0.001	7.5 (2.1-27.0)	0.002
BMI (≥ 25 kg/m^2)[c]	1.2 (0.8-1.6)	0.366	4.9 (1.6-14.9)	0.005
WHR (≥ 0.90)[c]	2.7 (1.7-4.2)	< 0.001	8.7 (2.4-31.5)	0.001
WHtR (≥ 0.50)[c]	2.3 (1.4-3.7)	0.001	9.9 (2.8-34.8)	< 0.001
Females				
WC (> 88 cm)	1.7 (1.2-2.3)	0.001	1.3 (1.0-1.7)	0.087
BMI (≥ 25 kg/m^2)	1.1 (0.8-1.5)	0.565	1.4 (1.1-1.9)	0.008
WHR (≥ 0.85)[c]	4.1 (2.4-7.3)	< 0.001	11.0 (2.8-43.6)	0.001
WHtR (≥ 0.50)[c]	3.5 (1.6-7.6)	0.002	43.4 (2.6-716.8)	0.008

[a] Crude prevalence ratio after univariable Poisson regression analysis. [b] Adjusted prevalence ratios for age, level of physical activity, family history of cardiac disease and stroke. [c] An interaction term between each anthropometric marker and age was included in the adjusted models. The Akaike Information Criteria was used to compare nested models. BMI: Body Mass Index. CIs: Confidence Intervals. CVD: Cardiovascular Disease. WC: Waist Circumference. WHR: Waist-to-Hip Ratio. WHtR: Waist-to-Height Ratio.

Chapter 5: Discussion

5.1 Methodological Discussion

5.1.1 Study Design

This research study applied a cross-sectional design. Cross-sectional studies are commonly conducted to assess the prevalence of a disease and its association with risk indicators in relation to socioeconomic, demographic, and health-related characteristics of certain population. They are specially used to describe subjects in the population and the mean levels and distributions of exposures at one point in time. Furthermore, cross-sectional studies are relatively easy and quick to carry out, usually not very resource-intensive, and numerous variables can be collected at once (136).

Since our study aimed to assess the occurrence of MS, the CVD risk profile of the population, and the suitable cut-off values of HbA1c for diagnosing diabetes and pre-diabetes, the cross-sectional design was appropriate for our descriptive and analytical purposes. Nevertheless, considering that in cross-sectional studies the exposures and effects are measured simultaneously, a cause-effect relationship or the sequence of the events cannot be determined (136). Therefore, a long-term prospective study would be more appropriate for investigating individual risk factors for an incident CVD event. Similarly, the usefulness of our proposed cut-off value of HbA1c for diagnosing diabetes would be better assessed through a follow-up study. By examining the rate of diabetes-related complications developed over time, more accurate estimations could be reached. Despite the limitations of the cross-sectional design, the data produced are valuable in presenting prevalence data and gaining insight in the associations between exposures and outcomes. They may serve as a foundation for developing or strengthening hypotheses about related risk factors, as well as promoting the development of new management and preventive guidelines.

5.1.2 Strengths of the Study

To the best of our knowledge, this was the first population-based study from Brazil to investigate the performance of HbA1c in diagnosing T2DM and pre-diabetes. It was one of the few to compare the prevalence of MS among subjects with pre-diabetes, T2DM and high CVD risk, following the recent JIS definition in relation to other more established definitions. Further, it was also one of the rare population-based studies from Brazil to investigate the CVD risk by sociodemographic characteristics, as well as the association between different obesity markers and the risk of a cardiovascular event. Although the final sample was somewhat small, it was large enough to meet the

required sample size for analysis. The subjects were selected through random sampling in a well-defined population. The participation rate was high, reducing the risk of non-respondent biases.

The survey was performed by trained and highly motivated staff. For the interviews, research team members were recruited from the local community to prevent any social or linguistic barriers. The instruments for data collection were based on previous surveys with pre-tested questionnaires. Biological samples were collected, handled, and transported following standard protocols. The fasting state of the subjects was secured at three times: 1) orientation at inclusion, 2) telephone call the night before the blood test, and 3) on-site investigation. All analyses were performed in a certified laboratory. Quality control of the laboratory was assessed internally and externally, during the analysis phase. To minimize the risk of misclassification errors due to poor recall, the actual measurements of anthropometric parameters and blood pressure were carried out instead of using self-reported measures, which is common in population-based studies.

5.1.3 Limitations of the Study

This study was based on a cross-sectional design, and therefore cannot determine causality between exposure and outcome. Nevertheless, the results were of scientific importance to establish baseline data for policy implications and further research. Despite random selection procedures, the data were collected from one semi-urban area in the Northeast region of Brazil. Considering the large ethnic, cultural, and socioeconomic disparities across the five regions of Brazil (4), generalization of our findings should be done with caution. Information bias may have taken place as interview responses may be subjected to personal interpretation. Nevertheless, we minimized the possibility of reporting bias by pretesting the questionnaires, training the interviewers, and recruiting them from the local community, as well as through internal scrutiny.

The American Diabetes Association recommends that, in the absence of unequivocal hyperglycaemia, an abnormal HbA1c, FPG, or OGTT result should be confirmed by repeat testing before making a diagnosis of diabetes (19). In our study, we were unable to retest and confirm blood glucose abnormalities. However, for epidemiological purposes, the WHO considers that fasting or 2-h value after 75 g oral glucose may be used alone (18). Although HbA1c was measured from the whole capillary blood, it has been shown a high degree of sensitivity, specificity, and PPV between capillary and venous blood (137).

5.1.4 Validity

5.1.4.1 Internal Validity

Internal validity refers to which degree the results of an observation are correct for the population under study. Although it can be threatened by all sources of systematic error, appropriate design and attention to detail can minimize the biases (136). Most violations of the internal validity can be classified into the following categories:

5.1.4.1.1 Selection Bias

Selection bias corresponds to systematic errors in procedures applied to select the study subjects or the factors that might influence participation (136). We used random procedure for the selection of subjects, and the response rate was high.

In this study, we had an overrepresentation of females (females 472 vs. males 242). As previously mentioned, out of 1,000 names randomly selected from the registry list, around 163 subjects (78% males) were not found and therefore not invited to join the study. Among 92 subjects who refused to participate, about 63% were males. Population-based surveys conducted during the day may be a hindrance to male participation. Males are often engaged in income-generating work and therefore may not have been able to participate in the study. Although more females participated in the study, the number of males was large enough to provide adequate statistical power for analysis. In addition, the overrepresentation of females was dealt with by adjusting for gender in the multivariable analyses or through stratification by gender. However, considering that we had a lower participation rate among male subjects (females: 93.3 %; males: 80.7%), selection bias cannot be excluded.

Selection bias may have also taken place given that the subjects at higher risk for diabetes or contrarily those with positive health behaviour were more likely to respond. It is also possible that male participation was affected by poor health status or unemployment. To reduce the possibility of selection bias of illiterate subjects, a witnessed verbal consent was taken before their inclusion in the study.

5.1.4.1.2 Measurement Bias

Measurement bias takes place when the individual measurements or classifications of disease or exposure are inaccurate. Several sources of measurement bias exist, and their effects have varying importance (136).

Information bias refers to systematic errors in the information obtained from study subjects (136). Compared to self-reported questionnaires, interviewer-guided questionnaires generally result in higher response rates and more complete answers. Nevertheless, the interviewers may influence the subjects. Asking questions in different ways or interpreting, or coding information differently may generate information bias (138). To avoid information biases, well-trained interviewers from the local community were employed to conduct pre-tested questionnaires. Repetitive checks were executed by the PhD candidate after the questionnaires were completed, and double entry was performed when the questionnaires were put into the database. However, we cannot exclude the possibility of some interference of the interviewers on the subjects' responses. Furthermore, recall and reporting biases might also have occurred. Recall bias, described as differential recall of information by subjects with or without a disease of interest (136), might have been present in variables like family history of disease, use of medications, etc. Alcohol consumption, smoking habits and monthly income might have been misreported as subjects may conceal the actual responses due to embarrassment or other reasons.

Although the Framingham risk score has been used and validated in ethnically diverse cohorts including whites, blacks, Native Americans, and Hispanics (101), it was not recalibrated in our population. This might have entailed some uncertainty in the CVD risk estimation. However, it was beyond the scope and available resources of this study to validate the Framingham risk score. Additionally, to reach the required sample size and adequate statistical power, we included subjects aged ≥ 20 years instead of those aged 30-74 years, as published by D' Agostino et al in 2008 (93). Although our sample had a broad age range (minimum: 20 years – maximum: 97 years), analyses were also performed within those aged 30-74 years, and the results were not significantly different compared with the total sample.

Anthropometric, BP and BF% measurements were assessed by only one research team member after proper training. The weighing device was calibrated against a standard (15Kg) daily. The automatic electronic sphygmomanometer was properly validated, checked, and calibrated. Nevertheless, it is acknowledged that electronic sphygmomanometers may produce systematic errors in some patients (139). To minimize potential errors, the BP levels were measured twice in all subjects, after a resting time of at least 15 minutes.

Blood analyses were performed in a certified laboratory. To prevent glycolysis, the samples for plasma glucose measurements were collected in tubes containing sodium fluoride and potassium oxalate (1:3) and centrifuged within approximately 1 hour of collection. Separated plasma samples

were sent to the laboratory on dry ice in vaccine containers and stored at – 20° Celsius until the assays were performed. Laboratory results underwent internal and external scrutiny. To avoid misclassification errors when estimating LDL-C levels by the Friedewald formula (126), levels of triglycerides exceeding 400mg/dl were excluded and taken as missing values.

5.1.4.1.3 Confounding

A confounder can be defined as a third factor or another exposure that affects both the exposure under investigation and the outcome variable. Thus, confounding may take place when the effects of two exposures have not been separated and the analysis concludes that the effect is due to one variable rather than the other. The influence of confounding factors can be substantial, and may even change the apparent direction of an association (136).

Considering that the problem may arise if this extraneous factor is unequally distributed between the exposure subgroups, the study subjects were selected randomly. Furthermore, control of confounding was done by stratification and adjustments in multivariable analyses. The selection of confounders was based on previous literature and knowledge and included the following: age, gender, ethnicity, years of education, income, family history of cardiac disease and stroke, physical activity, BMI. Nevertheless, we cannot rule out the possibility of remaining confounding that was not controlled for. Future studies in this field should apply new confounding-selection methods (e.g., Directed Acyclic Graphs) to explore other potentially relevant confounders.

5.1.4.2 External Validity

External validity or generalizability refers to which extent the results of a study are applicable to other contexts and populations (136). Due to five centuries of miscegenation, the Brazilian population presents a high degree of heterogeneity, with admixtures of people from European, African, and native American origins (140, 141). As mentioned previously, Brazil is a continental country with relevant socioeconomic, ethnic, and regional disparities. Furthermore, a semi-urban area was selected for the study. Therefore, caution should be taken when generalizing the results. However, since Brazilians compose a mixed society in general and more than 80% of the population live in urban / semi-urban areas (4), our sample might possibly be representative of a great portion of the country's population.

All laboratory investigations in the three papers were measured following international guidelines. Overrepresentation of females is less likely to have influenced the external validity of the results since all analyses were performed after stratification or adjusting for gender in multivariable

models. Although the response rate among males was lower, it was larger than what is usually considered acceptable (142).

5.2 Discussion of the Main Findings

5.2.1 Diabetes and Pre-diabetes

5.2.1.1 Prevalence of Diabetes and Pre-diabetes

We found a higher prevalence of diabetes and pre-diabetes than some previous large studies conducted in Brazil (48, 143, 144). On the other hand, baseline data from the Brazilian Longitudinal Study of Adult Health, a cohort study of 15,105 civil servants aged 35-74 years, showed higher frequencies of diabetes (19.7%) and pre-diabetes (ranging from 16.1% to 52.6% according to different criteria) (145). Nevertheless, in this cohort study, random sampling procedures were not applied, and the subjects were not selected from the general population.

In our study, both the prevalence of diabetes and pre-diabetes were not significantly different between males and females. Conflicting results have been reported in Brazil (48, 50, 145) and elsewhere (20, 146, 147) regarding gender-related disparities in the prevalence of diabetes. In line with our findings, some studies did not find any differences (48, 147), whereas others either showed that females were more affected (49, 50) or males (20, 145).

5.2.1.2 Use of HbA1c as a Diagnostic Tool of Diabetes and Pre-diabetes

Recently, several HbA1c cut-off points have been suggested to diagnose diabetes in different populations (41). Even though the International Expert Committee (42) and ADA (19) recommend a HbA1c threshold of ≥ 6.5%, our study found an optimal cut-off value of ≥ 6.8%, which was higher than what has been reported by other countries (148-153). One of the very few studies from Brazil about the topic has suggested a cut-off of HbA1c ≥ 6.0%, however its sensitivity was as low as 51.3% (154). Compared to the cut-off of ≥ 6.5%, our proposed threshold showed a somewhat lower sensitivity, but a substantially higher PPV (60.2% vs. 44.5%). Racial and ethnic disparities have been described in the relationship between HbA1c and glucose levels and might explain the differences in optimal cut-off values. Although the reasons for such disparities remain unknown, potential underlying factors are differences in red blood cell survival, extracellular-intracellular glucose balance, and nonglycemic genetic determinants of haemoglobin glycation (45). Since Brazil has one of the most heterogeneous populations globally, with striking socioeconomic, ethnic, and regional disparities, the findings in the present study may not be representative for the whole country. Caution should be taken when

generalizing the results. Long-term prospective studies are required to confirm our findings in such a multi-ethnic and multi-cultural society like Brazil.

For identifying pre-diabetes, a HbA1c cut-off of ≥ 6.0% was found in this study, which was higher than that recommended by the ADA, i.e., ≥ 5.7% (19), but similar to the cut-off point suggested by the International Expert Committee for high-risk groups, i.e., ≥ 6.0% (42). In our study, the AUC for detecting pre-diabetes was considerably low (0.61) and a great portion of those diagnosed with pre-diabetes using the WHO criteria had normal HbA1c levels. Therefore, our results do not support HbA1c as a suitable diagnostic tool for pre-diabetes in this population.

5.2.1.3 HbA1c and Insulin Resistance

The homeostatic model assessment (HOMA) is a robust tool for assessing insulin resistance and has been widely applied in research (127, 155). However, the cut-off points of HOMA-IR greatly vary according to different races, age groups, genders, diseases, etc (156). In our study, we found that the AUC for HbA1c at 6.8% (0.74) was higher than for HbA1c at 6.5% (0.66). Furthermore, the sensitivity and specificity were also higher for the HOMA-IR at 2.06 for the best fit of HbA1c at 6.8%, compared with those for the HOMA-IR cut-off at 1.81 and HbA1c at 6,5%. These findings may indicate an improved assessment of HbA1c at 6.8% and a cut-off value of 2.06 for HOMA-IR for the risk of diabetes and CVD in this population.

5.2.2 Metabolic Syndrome

5.2.2.1 Comparing the Prevalence of MS Applying the Modified NCEP, IDF and JIS Definitions

In this study, we found a high prevalence of MS following all definitions applied. The age- and gender-adjusted prevalence of MS was 36.1% using the JIS definition, 35.1% the IDF and 29.5% the Modified NCEP criteria. Compared to other South American countries, our estimates were higher than those reported from Peru (Modified NCEP: 18.8%) (68), lower than Mexico (IDF: 49.8%; Modified NCEP: 41.6%) (67), Puerto Rico (Modified NCEP: 43.3%) (69) and Ecuador (IDF: 40%) (157), and similar to Chile (IDF: 36.8%; Modified NCEP: 31.6%) (65) and Colombia (IDF: 32.9%; Modified NCEP: 34.8%) (66). These differences might be explained by genetic, environmental, and sociodemographic factors in the countries and/or their populations (158). However, they could also be due to systematic errors.

Even though the prevalence of MS did not differ significantly between the three definitions, it was highest when using the JIS criteria, followed by the IDF and the Modified NCEP. The higher prevalence of MS obtained using the JIS and IDF criteria compared to Modified NCEP may be due to the lower WC cut-off values applied by both the JIS (62) and IDF (57).

5.2.2.2 MS and Sociodemographic Characteristics

Consistent with other studies (67, 159, 160), females had a significantly higher prevalence of MS according to all three definitions. This was especially evident for the IDF (females 43.7% vs. males 18.3%; p-value < 0.001) and JIS (females 44.8% vs. males 18.9%; p-value < 0.001) criteria. Considering that central obesity has been strongly correlated with insulin resistance and MS (57), this gender difference might be partly a result of greater central obesity in females. In our sample, females had a significantly higher prevalence of abdominal obesity when applying both the WC cut-off points of ≥ 80 cm for females; ≥ 90 cm for males (81.7% vs. 52.1%) and of ≥ 88 cm for females; ≥ 102 cm for males (56.9% vs. 14.9%). In addition, females had more abnormalities in glucose metabolism (27.8% vs. 18.7%) and low HDL-C levels (73.5% vs. 4.1%). In this study, approximately 33% of females were ≥ 50 years of age. Metabolic changes accompanying menopause have been linked to increased risk of MS and CVD (161). Therefore, this might also explain our higher prevalence of MS among females, which is in line with other studies (160).

The prevalence of MS increased significantly and progressively with age and BMI status, which has also been described by several (67, 162-164). Contrarily to other reports from Southern and South-eastern Brazil (165, 166), but in line with a study from India (167), our findings showed a higher occurrence of MS with greater income. Furthermore, as reported by a study from the Southeast region of Brazil including 1,507 subjects (165), ethnicity was not a significant predictor of MS. This may possibly be due to the mixed genetic composition of the Brazilian population (140, 141).

5.2.3 CVD Risk

5.2.3.1 CVD Risk and Sociodemographic Characteristics

Our results showed a high prevalence of increased CVD risk, i.e., Framingham risk score ≥ 10%. The prevalence was higher than that reported in other South American countries (168, 169) and Southern Brazil (120, 170), similar to India (171), but lower than Honduras (172) and China (173). These disparities might be due to genetic, racial, sociodemographic, and cultural diversity, as well as the use of other versions of the Framingham risk score. Furthermore, the recent rapid industrialisation and urbanisation of Pindoretama (118), resulting in lifestyle and dietary changes, might explain the high occurrence of several cardiovascular risk factors and subsequent increased Framingham risk score in our population.

In line with other studies (169, 171, 174), although the prevalence of diabetes was not significantly different between males and females, our data showed a greater prevalence of high CVD risk among males. This finding might be explained by the significantly higher SBP, and proportion of tobacco use

among males. As expected and observed by others (170, 174), the Framingham risk score increased significantly with age. Further, those employed in agriculture or construction presented lower CVD risk, which might be related to the protective effect of physical activity (71). Nevertheless, the CVD risk did not differ significantly by ethnicity. It is likely that the extensive miscegenation of the overall Brazilian population (140, 141) may have reduced the differences among the ethnic groups. Additionally, we did not find a significant association between CVD risk and education. In our data, only 4% of the subjects had a university degree or higher (data not shown). It is likely that the overall low level of education may explain the lack of significant results.

5.2.4 Association between CVD Risk, Diabetes, Pre-diabetes, Metabolic Syndrome, and Related Cardiometabolic Risk Factors

5.2.4.1 Association between MS and Pre-diabetes, T2DM, and CVD Risk

In agreement with current scientific evidence (160, 175, 176), a significant association was found between MS and pre-diabetes, T2DM and high CVD risk. We observed a higher prevalence of MS among those diagnosed with pre-diabetes, T2DM and high CVD risk than in the overall population. The prevalence of MS was highest when the JIS criteria was used, possibly because central obesity is not a mandatory component in this definition. Furthermore, the JIS and IDF definitions presented higher sensitivity in the identification of subjects with these three conditions, which may be due to the lower WC cut-off point applied in these criteria.

Diabetes and CVDs are conditions of high morbidity and mortality, accounting for large portions of the health care budgets in developing countries, including Brazil (14, 47). Screening of MS in primary care, specially using the IDF and JIS criteria, may timely identify patients at higher risk of pre-diabetes, T2DM and CVDs in the Brazilian population. Intensive interventions involving multiple cardiovascular risk factors can potentially benefit these patients and contribute to health cost savings. Nevertheless, prospective studies are warranted to confirm our results.

5.2.4.2 CVD Risk and Obesity Measures

In relation to increased CVD risk, the adjusted PRs for WHR and WHtR were the highest in both genders. In females, the association between the WHR and Framingham risk score was higher than that of WC, BMI and WHtR. In males, WHtR had the highest slope coefficient, followed by WC. These findings might suggest that central obesity measures were more predictive of CVD risk than a general obesity measure like BMI. Therefore, in our population, it is likely that BMI alone is insufficient to

account for the association between CVD risk and obesity. However, follow-up studies are still needed to investigate future risk of CVDs and their relationship with obesity in Brazil.

Growing evidence has shown that abdominal adiposity is more strongly associated with metabolic and cardiovascular problems than total obesity (177, 178). Even within normal ranges of BMI, high visceral fat remains an independent cardiovascular risk factor (178). Accumulation of abdominal fat is related to insulin resistance, increased systemic inflammation, accelerated progression of atherosclerosis and endothelial dysfunction, contributing to CVD risk (85, 178). This might explain the stronger association between abdominal obesity measures and CVD risk found in our study.

Chapter 6: Conclusions and Implications

We found that T2DM, MS and increased CVD risk were highly prevalent in this population.

Our data may suggest that an HbA1c threshold of ≥ 6.8% could be considered a sensitive marker for the diagnosis of diabetes, and for insulin resistance which is linked to CVD. Nevertheless, HbA1c levels might be a weak parameter to identify pre-diabetes. Early detection of diabetes through HbA1c is likely to be cost-effective as timely initiation of treatment will prevent complications, thereby reducing the burden on many and the pressure on the health budget.

The prevalence of MS showed a significant association with T2DM, pre-diabetes and CVD risk. The IDF and JIS definitions of MS may be better suited in the Brazilian population to predict pre-diabetes, T2DM and CVD risk. Screening of MS in primary care centres may identify patients at higher risk of these conditions, and early intensive multifactorial interventions could benefit this population.

A high risk of CVDs according to the Framingham risk score was found particularly among men and older people. Central obesity parameters, specially WHR and WHtR, were strongly associated with predicted CVD risk and might be useful in the clinical assessment of patients. Targeted strategies for screening, prevention and treatment of CVDs may likely decrease disease burden and health expenditure in Brazil.

Our data warrant screening of diabetes and MS in primary care as a routine check-up to detect people at risk at its earliest stage. Preventive policies should include public awareness programs and health promotion measures. Cardiovascular risk factors like hypertension, dyslipidaemia, obesity, unhealthy diet, physical inactivity, and tobacco use should be addressed from a clinical and public health perspective. Social media, newspapers, TV channels, and local organizations should be used for highlighting the severity of diabetes, pre-diabetes, metabolic syndrome, and CVDs.

Chapter 7: Future Research

- Prospective follow-up studies including all possible risk factors for diabetes and CVDs are required to confirm our findings.
- Long-term prospective studies are warranted to investigate the factors affecting the use of HbA1c as a diagnostic tool for diabetes and pre-diabetes, such as ethnicity, biological mechanisms, food habits, and lifestyle.
- Prospective studies are also needed to further elucidate the future risk of CVDs and their relationship with obesity in Brazil.
- Nationally representative studies are required for a comprehensive understanding regarding CVDs and all risk factors.
- An intervention study is recommended to identify effective, practical, and acceptable methods for the prevention of T2DM and CVDs and their related risk factors, including hypertension, dyslipidaemia, and MS in this population.
- Further research is warranted to assess the genetic influence for the development of diabetes, MS, and CVDs in Brazil.
- Future studies to monitor trends in risk factors, as well as the incidence of CVDs and diabetes are needed.

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Appendix I:

Questionnaire - General Information, Sociodemographic, Economic and Medical Data

Questionnaire - General Information, Sociodemographic, Economic and Medical Data

ID Number: **Date:**

Interviewer:

1. Gender: ……
   (Male = 1, Female = 2)
2. Age: ……..
3. Place of birth: ……..
4. Race: ……
   (White = 1, Pardo (Brown) = 2, Black = 3, Yellow (i.e., East Asian) = 4, Indigenous = 5, Other = 6)
5. Marital Status: ……
   (Married = 1, Single = 2, Divorced / Separated = 3, Cohabitant = 4, Widow / er = 5, Other = 6)
6. Level of Education: .....
   (Illiterate = 1, Primary School = 2, High School = 3, University or Higher = 4)
7. Years of Education completed: .....
8. Occupation:
   a. Current Status: ……..
      (Unemployed = 1, Part Time = 2, Full Time = 3, Retired = 4, Sick Benefit* = 5)
   b. In case of being currently at work → Type of Occupation: ……..
      (Student =1, Agriculture = 2, Industry and Services = 3, Domestic Labor** = 4, Construction = 5, Other = 6)

*Those who were away from work due to some temporarily disabling condition. ** Housewives or those who worked in other houses performing domestic tasks, such as cleaning, cooking, etc.

9. What is your monthly income? ……
10. How many members are there in your family?
    A) > 18 years: …… B) < 18years: ……
11. Health
    a. What is your present state of health? ……..
       (Poor = 1, Not so Good =2, Good = 3, Very Good = 4)
    b. Do you have any of these illnesses or have you suffered from them in the past? Are you on treatment? How long? What type of treatment? Regularly?

	Has the Disease	Had the Disease	Age of Onset	On Treatment	How Long	Type of Treatment	Regular Treatment
Diabetes Type 1							
Diabetes Type 2							
Heart Disease							
Hypertension							
Stroke							
Depression							
Kidney Disorders							
Liver Diseases							

c. Are you in use of any medication for conditions not mentioned previously? ……
(Yes = 1, No = 2)
   - If Yes, which types? ……..

d. For females → Are you pregnant? ……. / Have you ever received a diagnosis of Gestational Diabetes Mellitus? ……..
(Yes =1, No = 2)

12. Family History
    a. Have your parents or any of your siblings suffered from the following illnesses?

	Mother		Age of Onset	Father		Age of Onset	Siblings		Age of Onset
	Yes	No		Yes	No		Yes	No	
Type 2 Diabetes									
Heart Disease									
Hypertension									
Stroke									
Depression									

13. Smoking
    a. Smoking History***: ……..
    (Never = 0, Previous Mild = 1, Previous Heavy = 2, Current Mild = 3, Current Heavy = 4)
    b. If you smoke daily now, what do you smoke?

	Yes	No
Cigarettes		
Cigars		
Other		

    c. If you have smoked before, how long is it since you stopped smoking? ……..
    d. If you smoke now, or have smoked before:
       - How many cigarettes do you or did you usually smoke daily? ……
       - How old were you when you started smoking? ……..
       - How many years altogether have you smoked? ……..

***Previous: those who have stopped smoking for more than 1 year. Current: those who currently smoke or have stopped smoking for less than 1 year. Mild: less than 20 cigarettes/day. Heavy: more than 20 cigarettes/day

14. Alcohol Consumption

    a. How often have you consumed alcohol in the course of the past year? (Low alcohol beer and non-alcoholic beer are not included)

Never	About once pr. month	2-4 times pr. month	ca. 2-3 times pr. week	ca. 4 or more times pr. week

To be filled by the Investigators

15. Fasting Blood Sample →Date and Time……
    Time of Last Meal……..
16. Glucose Drink →Time: …….
17. Anthropometrics
    Height (cm): …….. Weight (Kg): …… Hip circumference (cm): …….
    Waist circumference (cm): ……
18. Body Fat Percentage: .....
19. Blood pressure (Systolic Blood Pressure/Diastolic Blood Pressure) →
    First Measure: ……. Second Measure: ……..
20. Second Blood Sample →Time: …….
21. HbA1c: .....

Appendix II:

International Physical Activity Questionnaire (IPAQ) - Short Form

International Physical Activity Questionnaire (IPAQ) - Short Form

I am going to ask you about the time you spent being physically active in the last 7 days. Please answer each question even if you do not consider yourself to be an active person. Think about the activities you do at work, as part of your house and yard work, to get from place to place, and in your spare time for recreation, exercise, or sport.

Now, think about all the *vigorous* activities which take *hard physical effort* that you did in the last 7 days. Vigorous activities make you breathe much harder than normal and may include heavy lifting, digging, aerobics, or fast bicycling. Think only about those physical activities that you did for at least 10 minutes at a time.

1. During the **last 7 days**, on how many days did you do **vigorous** physical activities?

   _____ Days per week

   ( ) Don't Know/Not Sure

   ( ) Refused

   [**Interviewer clarification**: Think only about those physical activities that you do for at least 10 minutes at a time.]

   [**Interviewer note**: If respondent answers zero, refuses or does not know, skip to Question 3]

2. How much time did you usually spend doing **vigorous** physical activities on one of those days?

   __ __ Hours per day
   __ __ __ Minutes per day

   ( ) Don't Know/Not Sure

   ( ) Refused

   [**Interviewer clarification**: Think only about those physical activities you do for at least 10 minutes at a time.]

   [**Interviewer probe**: An average time for one of the days on which you do vigorous activity is being sought. If the respondent can't answer because the pattern of time spent varies widely from day to day, ask: "How much time in total would you spend **over the last 7 days** doing vigorous physical activities?"

   __ __ Hours per week
   __ __ __ __Minutes per week

   ( ) Don't Know/Not Sure

   ( ) Refused

Now think about activities which take *moderate physical effort* that you did in the last 7 days. Moderate physical activities make you breathe somewhat harder than normal and may include carrying light loads, bicycling at a regular pace, or doubles tennis. Do not include walking. Again, think about only those physical activities that you did for at least 10 minutes at a time.

3. During the **last 7 days**, on how many days did you do **moderate** physical activities?

____ Days per week

( ) Don't Know/Not Sure

( ) Refused

[**Interviewer clarification**: Think only about those physical activities that you do for at least 10 minutes at a time]

[**Interviewer Note**: *If respondent answers zero,* refuses or does not know, skip to Question 5]

4. How much time did you usually spend doing **moderate** physical activities on one of those days?

__ __ Hours per day
__ __ __ Minutes per day

( ) Don't Know/Not Sure

( ) Refused

[**Interviewer clarification**: Think only about those physical activities that you do for at least 10 minutes at a time.]

[**Interviewer probe**: An average time for one of the days on which you do moderate activity is being sought. If the respondent can't answer because the pattern of time spent varies widely from day to day, or includes time spent in multiple jobs, ask: "What is the total amount of time you spent over the **last 7 days** doing moderate physical activities?"

__ __ __ Hours per week
__ __ __ __Minutes per week

( ) Don't Know/Not Sure

( ) Refused

Now think about the time you spent walking in the last 7 days. This includes at work and at home, walking to travel from place to place, and any other walking that you have done solely for recreation, sport, exercise, or leisure.

5. During the **last 7 days**, on how many days did you **walk** for at least 10 minutes at a time?

____ Days per week

( ) Don't Know/Not Sure

( ) Refused

[**Interviewer clarification**: Think only about the walking that you do for at least 10 minutes at a time.]

[**Interviewer Note**: *If respondent answers zero,* refuses or does not know, skip to Question 7]

6. How much time did you usually spend **walking** on one of those days?

__ __ Hours per day
__ __ __ Minutes per day

( ) Don't Know/Not Sure

( ) Refused

[**Interviewer probe**: An average time for one of the days on which you walk is being sought. If the respondent can't answer because the pattern of time spent varies widely from day to day, ask: "What is the total amount of time you spent walking over **the last 7 days**?"

__ __ __ Hours per week
__ __ __ __Minutes per week

( ) Don't Know/Not Sure

( ) Refused

Now think about the time you spent sitting on weekdays during the last 7 days. Include time spent at work, at home, while doing course work, and during leisure time. This may include time spent sitting at a desk, visiting friends, reading, or sitting or lying down to watch television.

7. During the last 7 days, how much time did you usually spend ***sitting*** on a **weekday**?
__ __ Hours per weekday
__ __ __ Minutes per weekday

( ) Don't Know/Not Sure

( ) Refused

[**Interviewer clarification**: Include time spent lying down (awake) as well as

sitting]

[**Interviewer probe**: An average time per day spent sitting is being sought. If the respondent can't answer because the pattern of time spent varies widely from day to day, ask: "What is the total amount of time you spent *sitting* last **Wednesday**?"

__ __ Hours on Wednesday
__ __ __ Minutes on Wednesday

( ) Don't Know/Not Sure

( ) Refused

Appendix III:

Paper I – Paper III

I

International Journal of
Environmental Research and Public Health

MDPI

Article

Glycated Hemoglobin in the Diagnosis of Diabetes Mellitus in a Semi-Urban Brazilian Population

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Received: 28 August 2019; Accepted: 20 September 2019; Published: 26 September 2019

Abstract: The study evaluated glycated hemoglobin (HbA1c) as a diagnostic tool for diabetes and pre-diabetes in the Brazilian population. Further, the homeostasis model assessment of insulin resistance (HOMA-IR) was also examined against HbA1c values to identify the most suitable cut-off points for HOMA-IR to predict the risk of diabetes. A cross-sectional study was conducted among 714 randomly selected subjects. HbA1c, fasting, and 2 h plasma glucose values were measured. Insulin resistance estimates were calculated with HOMA-IR. The receiver operating characteristic curve assessed HbA1c performance. The adjusted prevalence rate of diabetes mellitus was 14.7%, and pre-diabetes 14.2%. The optimal HbA1c cut-off value was ≥6.8% for the diagnosis of diabetes, and ≥6.0% for pre-diabetes. The area under the curve using HbA1c was 0.85 (95% CI: 0.80–0.90) for detecting diabetes and 0.61 (95% CI: 0.55–0.67) for pre-diabetes. The optimal HOMA-IR cut-off value was 2.06 for HbA1c at 6.8%. The HbA1c cut-off value of ≥6.8% may be suitable for diagnosing diabetes in the Brazilian population. Our results do not support the use of HbA1c to diagnose pre-diabetes. A HOMA-IR cut-off point of 2.06 was a sensitive marker to assess the risk of diabetes.

Keywords: diabetes mellitus; glycated hemoglobin; diagnosis; insulin resistance; Brazil

1. Introduction

Diabetes mellitus (DM) and pre-diabetes (impaired glucose tolerance and impaired fasting glucose) are huge-scale pandemics. Estimates from 2015 showed that 415 million adults had diabetes worldwide, and this number is projected to increase to 642 million people by 2040. According to the International Diabetes Federation (IDF), Brazil has an estimated prevalence of DM among adults of approximately 10.2%. Brazil is the country in South America with the highest number of people with the disease [1].

Type 2 DM, responsible for approximately 90–95% of those with diabetes, is often asymptomatic in its early stages and can remain undiagnosed for years [2]. Around 193 million people are unaware that they have the condition [1]. Uncontrolled DM is associated with dysfunction and failure of several organs, which may result in blindness, limb amputations, peripheral neuropathy, and kidney failure [3–5]. Among those with diabetes, cardiovascular disease (CVD) accounts for about half of the

total mortality, and the risk of a first myocardial infarction (MI) is similar to that for reinfarction among nondiabetic patients that suffered a previous MI [6,7]. Insulin resistance (IR) has been addressed as the main pathophysiological link between DM and cardiovascular diseases (CVDs), even in the absence of glucose intolerance for a long time [8]. Pre-diabetes greatly increases the risk of developing type 2 DM and is also associated with the occurrence of CVDs. Thus, the early diagnosis of pre-diabetes and diabetes could reduce long-term complications, healthcare costs, and premature death [9–11].

The recommended tests and cut-off points for the diagnosis of DM have been changing over the years. Yet no consensus has been reached on the most effective screening test for its detection. Blood glucose measurements have been applied to define diabetes, either by Fasting Plasma Glucose (FPG) levels or the 2 h values in the 75 g Oral Glucose Tolerance Test (OGTT) [12]. Both tests require patients to fast overnight for at least 8–12 h, and their accuracy may be compromised by patient nonadherence to the fasting period, laboratory error or use of certain medications [13,14]. Although measuring FPG is inexpensive and relatively risk-free, the results may vary substantially within individuals over the long term [13], and must be confirmed with a second test on a subsequent day, in the absence of unequivocal hyperglycemia [12]. On the other hand, OGTT is expensive, time-consuming, labor-intensive, and has shown a low overall test-retest reproducibility [13,15].

Screening or diagnosing DM by using glycated hemoglobin (HbA1c) levels has been extensively discussed [15,16]. In 2008, an International Expert Committee recommended the use of the HbA1c test for diagnosing diabetes, with a threshold of ≥6.5% (48 mmol/mol) [17]. The HbA1c test is more convenient (fasting or timed samples are not required), stable and less variable biologically than FPG or OGTT. In addition, the HbA1c is a better indicator of chronic glycemic levels, has a better index of the risk for long-term complications and is less affected by acute perturbations in glucose levels during periods of stress and illness. Nevertheless, some limitations still persist for using the HbA1c test to diagnose diabetes [12,17]. Haemoglobinopathies or renal failure, as well as laboratory error or the use of certain medications, could influence the accuracy of HbA1c analysis [13]. Moreover, conditions that interfere with the red cell turnover, such as hemolytic or iron deficiency anemia, chronic malaria, major blood loss, or blood transfusions, may result in spurious HbA1c values [17].

Recently, racial and ethnic disparities have been reported in the relationship between glucose levels and HbA1c [18]. Although the reasons for those differences are still unknown, several cut-off levels of HbA1c have been suggested to screen for diabetes in different ethnic populations. Since the etiology and significance of such disparities remain unclear, race-specific diagnostic values are currently not recommended [17]. Few studies have been conducted in Brazil to evaluate the efficiency of HbA1c in diagnosing diabetes and pre-diabetes. It is still largely unknown whether the performance of the recommended HbA1c cut-off value of ≥6.5% (48 mmol/mol) is suitable for the Brazilian population. Therefore, we conducted a population-based survey to examine the suitable cut-off values for HbA1c in the Brazilian population. Further, the homeostasis model assessment of insulin resistance (HOMA-IR) was also examined against HbA1c values to identify the most suitable cut-off points for HOMA-IR to predict the risk of diabetes.

2. Materials and Methods

2.1. Study Population

A cross-sectional study was conducted between August 2012 and January 2013 in the city of Pindoretama, in the state of Ceará (CE), Northeastern Brazil. Subjects of both genders and age ≥20 years, who were able to communicate and wanted to join the study were considered eligible to be included. Pregnant women, those below 20 years of age, with an acute or chronic severe cardiac, renal, or hepatic illness, as well as physically or mentally disabled individuals unable to follow simple questions and examinations were excluded.

The health registry list with the names of Pindoretama's citizens in alphabetic order was applied to select eligible participants. Random numbers were generated with the statistical software R [19] and

matched with the names in the list thereafter. Eight hundred and six randomly selected subjects were invited to participate. Out of these, seven hundred and fourteen agreed to join the study, corresponding to a participation rate of 88.6%.

2.2. Ethics

The study was conducted in accordance with the ethical principles outlined in the Helsinki Declaration [20]. Prior to any investigation, written or verbal consent was obtained from all subjects. In the case of illiteracy, verbal consent was assured by a local witness, who signed the informed consent, to secure the free participation of the subjects. The participants were informed of their rights to withdraw from the study at any stage or withhold their data from analysis. The privacy of the participants regarding the results of the tests and the collected data was assured. Those who were diagnosed with any clinical condition were referred to the respective health center for further follow-up. Before undertaking the research, the protocol was approved by both the local Ethical Committee in Brazil (Protocol Number: 045.06.12) and the Regional Committee for Medical and Health Research Ethics (REK) in Norway (Reference: 2012/779/REK sør-øst D).

2.3. Survey Procedures and Data Collection Tools

The selected subjects were invited to join the study by the local Community Health Workers (CHWs). By the time of invitation, the CHWs briefly informed the potential participants about the study purposes and methods of investigation. The data collection took place in the six main community health centers located throughout the city. Approximately one month was spent on the fieldwork in each center. Thus, the subjects could choose the most suitable day of the week to attend the survey. The participants were instructed twice (by the CHWs in person and by a phone call on the previous day of the data collection) to start fasting from 8 pm of the night before the survey procedures. After the participants' arrival at the study place, the details about the research objectives and investigations were explained again. Those willing to participate were given informed consent. Ten milliliters of peripheral venous blood was collected for measuring FPG and lipids levels. Then, the subjects took a 75 g oral glucose load (according to the World Health Organization guidelines) to be prepared to the OGTT. Two hours after the glucose load, another venous sample was drawn. During these 2 h, the participants were questioned by trained interviewers, with pre-tested questionnaires regarding socio-demographic, economic, clinical, and nutritional data. Anthropometric measurements, blood pressure, and body fat percentage (BF%) were registered.

The weight was taken with the subject standing in bare feet and in light clothing, by using a portable digital scale, calibrated before use and recorded to the nearest 0.1 kg. The height was measured by using a wall-mounted stadiometer, recorded to the nearest 0.1 cm, with the participant looking straight and in the erect position. The body mass index (BMI) was calculated as the weight in kilograms divided by the square of height in meters (kg/m^2). The BF% was measured by a portable bipolar body fat analyzer (Omron®, Model HBF-306, Omron Healthcare, Inc., Illinois, United States). The waist circumference was taken by using a non-stretchable tape, placed horizontally on the midpoint between the lower part of the 12th rib and the top of the iliac crest, under the mid-axillary line. To the hip girth measurement, a similar tape was positioned to the maximum circumference around the buttocks. Waist and hip circumference were recorded to the nearest 0.1 cm. The waist-to-hip ratio (WHR) was calculated as the waist divided by the hip measurement. Following the recommendations of the World Health Organization (WHO), for males, a WHR $\geq$ 0.90 cm was considered "high" (a substantially increased risk of metabolic complications), whereas, for females, a WHR $\geq$ 0.85 cm was classified as "high" [21]. Blood Pressure was measured twice, within a 15 min rest interval, by using an electronic sphygmomanometer (Omron® BP785 IntelliSense® Automatic Blood Pressure Monitor with ComFitTM Cuff, Omron Healthcare, Inc., Illinois, United States). The mean value of the two measurements was applied in the analyses.

The blood samples were transferred to a sterile container, stored immediately over ice and centrifuged within approximately 1 h of collection. Plasma was frozen and transported to the laboratory, where the samples were stored at −20 °C until the analyses were performed. Fasting and 2 h plasma glucose levels were analyzed by the glucose oxidase method. Fasting insulin levels were determined by chemiluminescence. Total cholesterol (TC) was estimated by the cholesterol oxidase − phenol + aminophenazone (CHOD-PAP) method. High-density lipoprotein cholesterol (HDL-C) was determined by a homogenous enzymatic colorimetric method. The glycerol-3-phosphate oxidase − phenol + aminophenazone (GPO-PAP) method assessed the levels of triglycerides (TG). Low-density lipoprotein cholesterol (LDL-C) was estimated by the Friedewald Formula [22]. Capillary HbA1c levels were measured by A1CNow® Multi-Test A1C System (Bayer). Quality control of the laboratory was assessed internally and externally.

Diabetes mellitus, impaired fasting glycemia (IFG), and Impaired glucose tolerance (IGT) were defined according to the 1999 WHO criteria. Thus, diabetes cases were those with fasting (venous) plasma glucose value ≥7.0 mmol/L (≥126 mg/dL), or the plasma glucose value 2 h after a 75 g oral glucose load ≥11.1 mmol/L (≥200 mg/dL), or both. Additionally, IGT was defined as fasting plasma glucose <7.0 mmol/L (<126 mg/dL), and 2 h plasma glucose ≥7.8 mmol/L (≥40 mg/dL), but <11.1 mmol/L (<200 mg/dL). IFG cases were defined as fasting plasma glucose ≥6.1 mmol/L (≥110 mg/dL), but <7.0 mmol/L (<126 mg/dL), and 2 h plasma glucose <7.8 mmol/L (<140 mg/dL). Subjects with IFG and IGT were classified as pre-diabetes cases. Dyslipidemia was defined as TG ≥1.7 mmol/L and HDL-C <0.9 mmol/L for men, and <1.0 mmol/L for women [23]. Insulin resistance in the fasting state was estimated with HOMA-IR ([insulin (mU/l) × glucose (mmol/L)]/22.5) [24].

2.4. Statistical Analysis

Statistical analyses were performed by using SPSS 25th version [25], R for Windows [19], and Stata 15th edition [26]. Numerical data were presented as means and 95% CI (confidence interval), while categorical data as numbers or percentages, and 95% CI. The overall prevalence rates of diabetes and pre-diabetes were adjusted for age and gender. Differences between the groups of means and proportions adjusted for age and gender were tested by logistic regression. The receiver operating characteristic (ROC) curve analysis was used to assess the discriminatory ability of the HbA1c test for detecting diabetes mellitus and pre-diabetes, given the WHO criteria as the gold standard. Additionally, ROC curves were also applied to compare the performance of HbA1c, FPG, and 2 h post-glucose load measurements for diagnosing diabetes. The ROC curves were achieved by using Stata [26]. Optimal cut-off points were obtained based on the highest Youden index [27]. The agreement for classification of diabetes using different cut-off points of HbA1c and the WHO criteria was assessed by the kappa statistic [28]. Bivariate Pearson's correlation coefficient and bootstrapped 95% CI with 1000 replications was applied to evaluate the strengths of pairwise associations between HbA1c, FPG, and 2 h post-glucose load. Diagnostic test properties including sensitivity, specificity, positive predictive value (PPV), and negative predictive value (NPV), with 95% CI were also calculated for different cut-off points of HbA1c, FPG, and 2 h post-glucose load [29]. A p-value < 0.05 was considered statistically significant and all p-values presented were two-tailed.

3. Results

The clinical characteristics of 714 subjects classified as normal, pre-diabetics, or diabetics according to the WHO criteria are described in Table 1.

Table 1. Characteristics of 714 subjects classified as normal, pre-diabetics, or diabetics, according to the WHO criteria.

Characteristics	Normal	Pre-Diabetes (IFG and/or IGT)	Diabetes Mellitus
% (95% CI)	71.15 (67.95–74.35)	14.15 (11.61–16.68)	14.71 (12.17–17.24)
Age (years)	42.54 (41.19–43.89)	49.58 (46.55–52.60) *	53.25 (50.29–56.22) *
Sex (female), % (*n*)	63.4 (322)	73.3 (74) *	72.4 (76)
Waist (cm)			
Male	87.77 (86.07–89.48)	94.42 (89.92–98.93) *	95.95 (91.63–100.27) *
Female	88.29 (86.93–89.66)	90.87 (88.04–93.70)	98.45 (95.58–101.32) *,†
Hip Circumference (cm)			
Male	94.74 (93.48–96.00)	98.17 (94.83–101.50)	98.13 (94.94–101.33) *
Female	99.18 (98.04–100.33)	100.91 (98.54–103.28)	103.50 (101.10–105.91) *
WHR, Mean (95% CI)			
Male	0.93 (0.91–0.95)	0.96 (0.92–1.00)	0.98 (0.94–1.02) *
Female	0.89 (0.88–0.90)	0.90 (0.88–0.92)	0.96 (0.94–0.98) *,†
WHR (high), % (95% CI)	63.48 (59.66–67.30)	79.04 (70.98–87.10) *	95.49 (90.61–100.37) *,†
BMI (kg/m^2)	26.20 (25.76–26.64)	27.34 (26.36–28.32) *	29.67 (28.69–30.65) *,†
BF%, mean (95% CI)	32.03 (31.43–32.63)	33.64 (32.31–34.96) *	35.70 (34.35–37.06) *,†
Family History of DM, % (95% CI)	35.66 (31.45–39.87)	36.37 (26.95–45.79)	63.90 (54.49–73.30) *,†
SBP (mmHg)	125.09 (123.45–126.73)	134.04 (130.38–137.69) *	133.80 (130.16–137.43) *
DBP (mmHg)	75.43 (73.94–76.93)	79.61 (76.27–82.94)	80.58 (77.27–83.90) *
FPG (mmol/L)	4.61 (4.48–4.76)	5.46 (5.10–5.81) *	9.73 (9.38–10.09) *,†
2 h Post-Glucose Load (mmol/L)	6.06 (5.82–6.30)	8.21 (7.66–8.76) *	16.23 (15.69–16.77) *,†
Capillary HbA1c, Mean (95% CI)	5.98 (5.90–6.07)	6.13 (5.94–6.31) *	8.11 (7.92–8.29) *,†
Fasting Insulin (micro UI/mL)	6.14 (5.71–6.57)	8.00 (7.03–8.96) *	8.63 (7.69–9.58) *
Total Cholesterol (mmol/L)	4.62 (4.54–4.71)	4.81 (4.62–4.99)	5.04 (4.86–5.23) *
Triglycerides (mmol/L)	1.32 (1.17–1.47)	1.88 (1.54–2.21) *	2.35 (2.01–2.68) *
HDL (mmol/L)	1.23 (1.22–1.24)	1.22 (1.20–1.24)	1.20 (1.18–1.23)
LDL (mmol/L)	2.84 (2.76–2.91)	2.91 (2.73–3.09)	2.90 (2.73–3.08)
Dyslipidemia, % (95% CI)	19.69 (16.17–23.21)	27.59 (18.80–36.37)	49.44 (39.60–59.27) *,†

Data are provided as mean (95% confidence interval) or percentage (95% confidence interval), adjusted for age and gender. Comparisons between the groups were performed adjusting for age and gender. * Significantly ($p < 0.05$) different from Normal. † Significantly ($p < 0.05$) different from Pre-Diabetes. WHO: World Health Organization. IFG: Impaired Fasting Glycemia. IGT: Impaired Glucose Tolerance. CI: Confidence Interval. WHR: Waist-to-Hip Ratio. BMI: Body Mass Index. BF%: Body Fat Percentage. DM: Diabetes Mellitus. SBP: Systolic Blood Pressure. DBP: Diastolic Blood Pressure. FPG: Fasting Plasma Glucose. HbA1c: Glycated Hemoglobin. HDL: High-Density Lipoprotein. LDL: Low-Density Lipoprotein.

In the study population, the adjusted prevalence rate of diabetes mellitus was 14.7% (95% CI: 12.2–17.2), and pre-diabetes 14.2% (95% CI: 11.6–16.7). The crude prevalence of diabetes was 16.1% (95% CI: 13.0–19.7%) among females, and 12.0% (95% CI: 8.4–16.7%) among males. The crude prevalence of pre-diabetes among females was 15.7% (95% CI: 12.7–19.3%), while among males was 11.2% (95% CI: 7.8–15.8%). Individuals with diabetes were more dyslipidemic and presented significantly higher WHR, BMI, BF%, systolic, and diastolic blood pressure than normal subjects.

The ROC curves for HbA1c to diagnose diabetes and pre-diabetes, considering the WHO criteria as the gold standard, are shown in Figure 1.

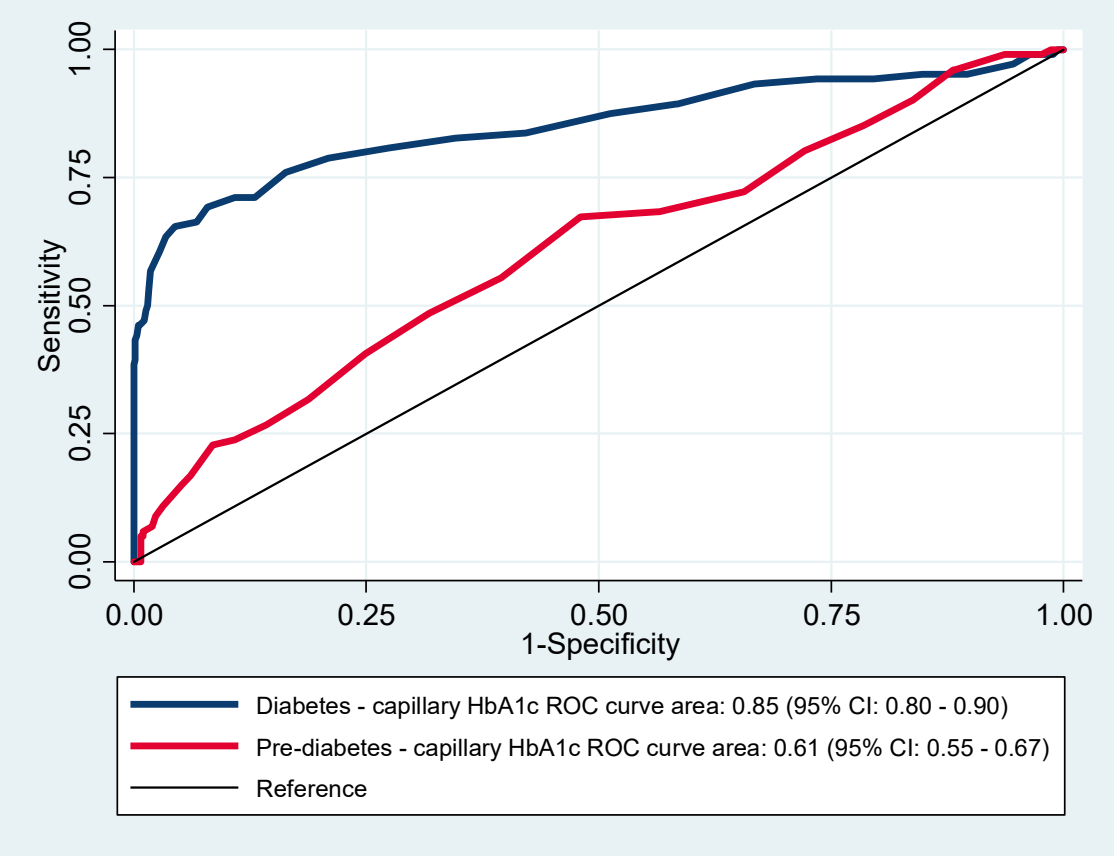

Figure 1. Receiver operating characteristics (ROC) curves for HbA1c (glycated hemoglobin) to diagnose diabetes and pre-diabetes.

The area under the curve (AUC) for detecting diabetes was 0.85 (95% CI: 0.80–0.90), and for pre-diabetes was 0.61 (95% CI: 0.55–0.67). According to the Youden index, the optimal HbA1c cut-off value for diagnosing diabetes was ≥6.8% (51 mmol/mol), and for pre-diabetes was ≥6.0% (42 mmol/mol).

As described in Table 2, at the proposed point for diagnosing diabetes, i.e., capillary HbA1c ≥ 6.8% (51 mmol/mol), the sensitivity was 69.2%, specificity 92.1%, PPV 60.2%, and NPV 94.6%. At the cut-off for detecting pre-diabetes, i.e., capillary HbA1c ≥ 6.0% (42 mmol/mol), the sensitivity was 67.3%, specificity 52.0%, PPV 18.7%, and NPV 90.6%.

Table 2. Diagnostic properties of different HbA1c cut-off levels for detecting diabetes and pre-diabetes, applying the WHO criteria as the gold standard.

	Diabetes				Pre-Diabetes			
HbA1c %, (mmol/mol)	**Sensitivity% (95% CI)**	**Specificity% (95% CI)**	**Positive Predictive Value % (95% CI)**	**Negative Predictive Value % (95% CI)**	**Sensitivity% (95% CI)**	**Specificity% (95% CI)**	**Positive Predictive Value % (95% CI)**	**Negative Predictive Value % (95% CI)**
≥5.7 (39)	94.2 (87.9–97.9)	26.5 (23.1–30.2)	18.1 (17.1–19.1)	96.4 (92.4–98.3)	80.2 (71.1–87.5)	27.9 (24.0–32.0)	15.4 (14.0–16.9)	89.6 (85.0–92.9)
≥6.0 (42)	87.5 (79.6–93.2)	48.8 (44.7–52.8)	22.7 (20.9–24.7)	95.8 (93.1–97.4)	67.3 (57.3–76.3)	52.0 (47.5–56.4)	18.7 (16.3–21.3)	90.6 (87.9–92.8)
≥6.4 (46)	78.8 (69.7–86.2)	79.1 (75.6–82.2)	39.4 (35.1–43.8)	95.6 (93.7–96.9)	31.7 (22.8–41.7)	81.2 (77.5–84.5)	21.7 (16.5–28.0)	87.9 (86.3–89.3)
≥6.5 (48)	75.9 (66.6–83.8)	83.7 (80.5–86.5)	44.5 (39.4–49.8)	95.3 (93.5–96.6)	26.7 (18.4–36.5)	85.8 (82–4–88.7)	23.6 (17.3–31.2)	87.7 (86.3–89.0)
≥6.8 (51)	69.2 (59.4–77.9)	92.1 (89.7–94.1)	60.2 (52.8–67.1)	94.6 (92.9–95.9)	16.8 (10.1–25.6)	93.9 (91.4–95.8)	31.1 (20.6–43.9)	87.3 (86.3–88.3)

HbA1c: Glycated Hemoglobin. WHO: World Health Organization. CI: Confidence Interval.

Figure 2 presents the ROC curves for HbA1c to diagnose diabetes by gender.

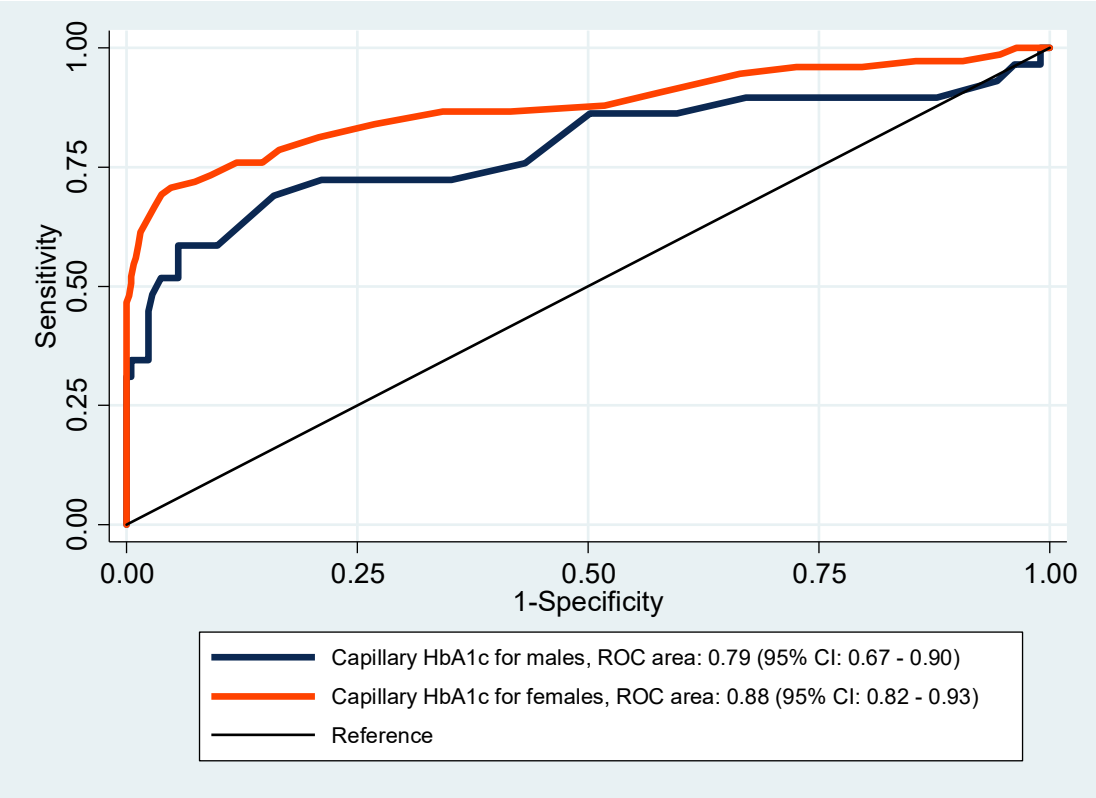

Figure 2. Receiver operating characteristics (ROC) curves for HbA1c to diagnose diabetes by gender.

The AUC for women was 0.88 (95% CI: 0.82–0.93), while for men was 0.79 (95% CI: 0.67–0.90). Nevertheless, there was no significant difference between the two curves (p-value = 0.18). Among women, based on the Youden Index, the most suitable HbA1c cut-off point for diagnosing diabetes was ≥7.0% (53 mmol/mol), while for men was ≥6.5% (48 mmol/mol). With HbA1c ≥ 7.0% (53 mmol/mol) for women, the sensitivity was 70.7% (95% CI: 59.0–80.6), and the specificity 95.2% (95% CI: 92.6–97.1). With HbA1c ≥ 6.5% (48 mmol/mol) for men, the sensitivity was 69.0% (95% CI: 49.2–84.7) and specificity 84.0% (95% CI: 78.4–88.7). A moderate agreement between the WHO criteria and the capillary HbA1c ≥ 6.5% (48 mmol/mol) for the classification of diabetes was found (Kappa = 0.46, $p < 0.001$). A higher agreement was observed between the WHO criteria and capillary HbA1c cut-off ≥6.8% (51 mmol/mol) (Kappa = 0.58, $p < 0.001$).

The diagnostic performances of FPG, 2 h post-glucose load, HbA1c ≥ 6.5% (48 mmol/mol) and ≥6.8% (51 mmol/mol) to diagnose diabetes are described in Table 3.

Table 3. Diagnostic performances of FPG, 2 h post-glucose load and HbA1c to diagnose diabetes, using the WHO criteria as the gold standard.

Diagnostic Criteria	Sensitivity % (95% CI)	Specificity % (95% CI)	Positive Predictive Value % (95% CI)	Negative Predictive Value % (95% CI)	Accuracy % (95% CI)
FPG (≥7 mmol/L)	75.2 (65.9–83.1)	100 (99.4–100.0)	100	95.9 (94.4–97.0)	96.4 (94.7–97.6)
2 h Post-Glucose Load (≥11.1 mmol/L)	77.1 (67.9–84.8)	100 (99.4–100.0)	100	96.2 (94.7–97.3)	96.6 (95.0–97.8)
HbA1c (≥6.5%, ≥48 mmol/mol)	75.9 (66.6–83.8)	83.7 (80.5–86.5)	44.5 (39.4–49.8)	95.3 (93.5–96.6)	82.6 (79.6–85.3)
HbA1c (≥6.8%, ≥51 mmol/mol)	69.2 (59.4–77.9)	92.1 (89.7–94.1)	60.2 (52.8–67.1)	94.6 (92.9–95.9)	88.8 (86.2–91.0)

FPG: Fasting Plasma Glucose. HbA1c: Glycated Hemoglobin. WHO: World Health Organization. CI: Confidence Interval.

The 2 h post-glucose load presented the highest sensitivity (77.1%, 95% CI: 67.9–84.8) among all others. At an HbA1c ≥ 6.5% (48 mmol/mol), the sensitivity (75.9%, 95% CI: 66.6–83.8) was higher than at an HbA1c ≥ 6.8% (51 mmol/mol) (69.2%, 95% CI: 59.4–77.9). However, the specificity (92.1%, 95% CI: 89.7–94.1), PPV (60.2%, 95% CI: 52.8–67.1) and accuracy (88.8 %, 95% CI: 86.2–91.0) were higher for the cut-off of HbA1c ≥ 6.8% (51 mmol/mol) than for the point of HbA1c ≥ 6.5% (48 mmol/mol).

Figure 3 shows the ROC curves for HbA1c, FPG, and 2 h post-glucose load to detect diabetes, given the 1999 WHO criteria as the gold standard.

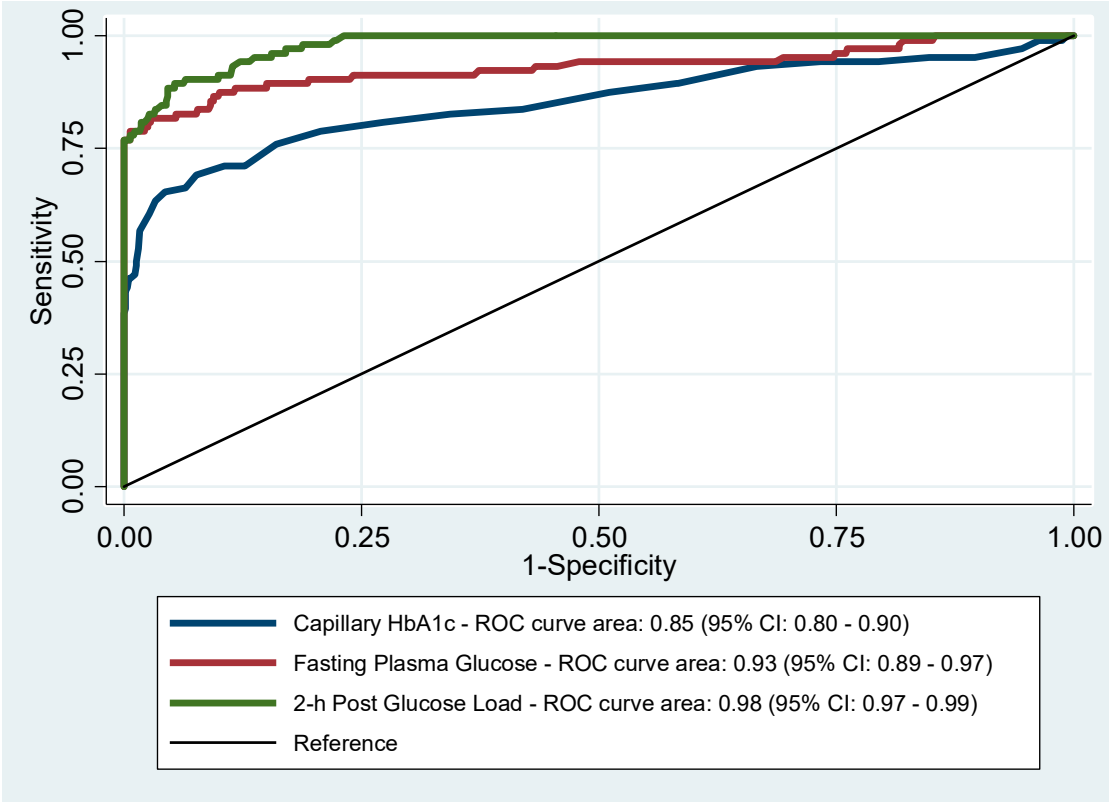

Figure 3. Receiver operating characteristics curves (ROC) for HbA1c, fasting plasma glucose (FPG), and 2 h post-glucose load to diagnose diabetes.

The AUCs were 0.85 (95% CI: 0.80–0.90) for HbA1c, 0.93 (95% CI: 0.89–0.97) for FPG, and 0.98 (95% CI: 0.97–0.99) for 2 h post-glucose load alone. The Pearson's correlation coefficient between HbA1c and FPG was 0.78 (95% CI: 0.70–0.83) and between HbA1c and 2 h post-glucose load was 0.86 (95% CI: 0.81–0.89).

Figure 4 presents the ROC curves to identify the best cut-off value for HOMA-IR against the recommended cut-off point of HbA1c ≥ 6.5% (48 mmol/mol), and the proposed cut-off value of ≥6.8% (51 mmol/mol) from our data.

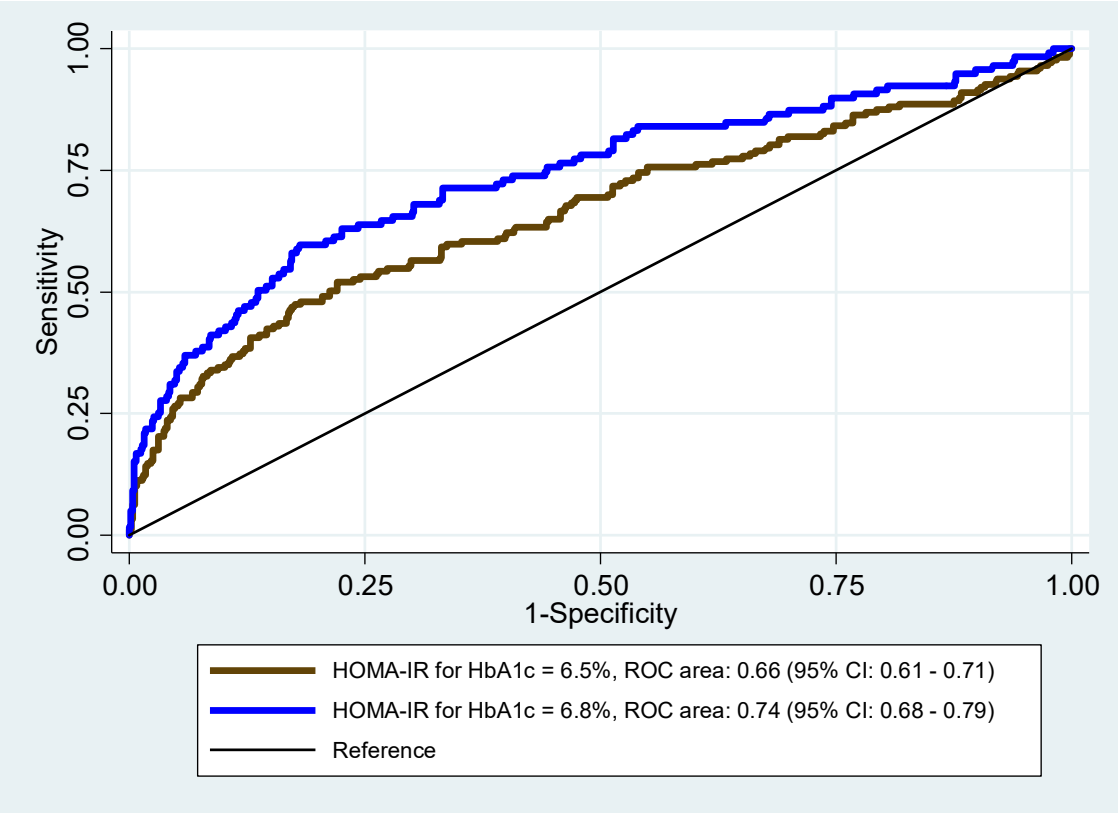

Figure 4. Receiver operating characteristics (ROC) curves for the homeostasis model assessment of insulin resistance (HOMA-IR) for cut-off values of HbA1c at 6.5% and 6.8%.

The AUC for HbA1c at 6.5% (48 mmol/mol) was 0.66 (95% CI: 0.61–0.71), and for HbA1c at 6.8% (51 mmol/mol) was 0.74 (95% CI: 0.68–0.79). According to the Youden Index, the optimal HOMA-IR cut-off value for HbA1c at 6.5% (48 mmol/mol) was 1.81, and at 6.8% (51 mmol/mol) was 2.06. At the HOMA-IR cut-off point of 1.81, the sensitivity was 52.0% (95% CI: 44.4–59.5) and specificity 78.0% (95% CI: 74.2–81.5), while at the point of 2.06, the sensitivity was 59.7% (95% CI: 50.3–68.6) and specificity 81.9% (95% CI: 78.5–84.9).

4. Discussion

We found a higher prevalence rate of diabetes and pre-diabetes than previous large studies conducted in Brazil [30–32]. A large population-based survey, known as the Brazilian Multicenter Study, conducted on a representative sample (n = 21,847) of the urban population aged 30 to 69 years in nine large cities between 1986 and 1988, showed that the prevalence of DM was 7.6 and impaired glucose tolerance (IGT) was 7.8% [31]. More recently, between 1996 and 1997, another cross-sectional study conducted in Southeastern Brazil found that the overall prevalence of DM was 12.1% and IGT 7.7% [32].

Our study suggested that the optimal HbA1c cut-off value for diagnosing diabetes was 6.8% (≥51 mmol/mol), which is higher than the cut-off value of ≥6.5% (≥48 mmol/mol) recommended by the International Expert Committee [17] and the American Diabetes Association [12]. Even though the optimal HbA1c cut-off value for pre-diabetes found in this study, i.e., ≥6.0% (≥42 mmol/mol), was higher than that recommended by the ADA, i.e., ≥5.7% (≥39 mmol/mol) [12], it was similar to the cut-off value suggested by the International Expert Committee for high-risk groups, i.e., ≥6.0% (≥42 mmol/mol) [17]. Although our cut-off value of ≥6.8% (≥51 mmol/mol) showed a somewhat lower sensitivity compared to the recommended cut-off of ≥6.5% (≥48 mmol/mol), the PPV was substantially higher (60.2% versus 44.5%).

Our proposed HbA1c cut-off value to diagnose diabetes was higher than the results found in China [33], Bangladesh [34], South Africa [35], Bulgaria [36], Norway [37], USA [38], and in a South Indian population [39]. To the best of our knowledge, no other previous studies suggested a higher HbA1c cut-off value to identify diabetes and pre-diabetes compared to the suggested guideline by the Expert Committee [17] and the ADA [12]. One of the very few studies conducted in Brazil about the topic has suggested a cut-off of HbA1c ≥ 6.0% (≥42 mmol/mol), which showed a sensitivity of 51.3% to diagnose diabetes [40].

Unlike many other populations, Brazilians compose one of the most heterogeneous societies in the world, as a result of five centuries of miscegenation between European colonizers, slaves from Africa, and autochthonous Amerindians. Since Brazil is a continental country with huge socioeconomic, ethnic and regional disparities, the findings in the present study may not be representative for the whole country. Caution should be taken when generalizing the results. Recently, it has been reported that racial and ethnic variations in HbA1c may impact its use as a diagnostic tool for diabetes. Studies have shown that adjustments for sociodemographic characteristics, access to healthcare, quality of care, and self-management behaviors attenuate, but does not fully explain the racial and ethnic differences in HbA1c. Therefore, some have argued that relying solely or preferably on HbA1c for the diagnosis of diabetes may lead to misclassification and systematic error. Factors such as differences in red blood cell survival, extracellular-intracellular glucose balance, and nonglycemic genetic determinants of hemoglobin glycation are now being investigated as potential contributors for ethnic disparities [18].

Although the International Expert Committee has recommended the use of HbA1c as a diagnostic tool for diabetes, its utility for identifying pre-diabetes has been considered problematic [17]. In our study, a large proportion of the subjects diagnosed with pre-diabetes using the WHO criteria had normal HbA1c levels. Moreover, we found a considerably lower AUC for pre-diabetes. Our results, therefore, do not support HbA1c as an adequate diagnostic tool for pre-diabetes in this population.

In the AUC analysis, we found that 2 h post plasma glucose had the highest AUC in the diagnosis of diabetes, followed by FPG and HbA1c. Additionally, in agreement with previous studies, significant correlation coefficients between HbA1c and FPG, and between HbA1c and 2 h plasma glucose were demonstrated [33,39].

The homeostatic model assessment (HOMA), first described by Matthews et al. in 1985 [24], has been widely used particularly in epidemiological and clinical studies and proved to be a robust tool for assessing insulin resistance (IR) [41]. Nevertheless, the cut-off values of HOMA-IR greatly vary among different races, ages, genders, diseases, complications, and other factors [42]. Therefore, we attempted to measure the cut-off level of HOMA-IR against the recommended cut-off of HbA1c of 6.5% (≥48 mmol/mol) for the diagnosis of diabetes, as well as in relation to the best cut-off value of HbA1c found in the study, which was 6.8% (51 mmol/mol). The AUC for the ROC at HbA1c ≥ 6.5% (≥48 mmol/mol) was 0.66 (95% CI: 0.61–0.71), while for HbA1c ≥ 6.8% (≥51 mmol/mol) was 0.74 (95% CI: 0.68–0.79). The sensitivity and specificity were also higher for the HOMA-IR at 2.06 for the best fit of HbA1c at 6.8% (51 mmol/mol), compared to HbA1c at 6,5% (48 mmol/mol). These findings may also indicate an improved assessment of HbA1c at 6.8% (51 mmol/mol) and a cut-off value of 2.06 for HOMA-IR for the risk of diabetes and CVD in this population.

Among others, the cross-sectional design and sample size are limitations of our study. In order to determine the ideal cut-off points for diagnostic tests of diabetes, the ability of each method to predict the chronic complications of diabetes (such as diabetic retinopathy) should be explored. However, given the cross-sectional nature, we only evaluated the sensitivity and specificity of the various tools. Future follow-up studies are needed to provide more valuable conclusions. The ADA has recommended that, in the absence of unequivocal hyperglycemia, an abnormal HbA1c, fasting plasma glucose, or oral glucose tolerance test result that meets the criteria for diabetes should be confirmed by repeat testing before making a diagnosis of diabetes. Specially, when two different tests are available and the results are discordant, the test with a result above the diagnostic threshold should be repeated, and the diagnosis should be made on the basis of the confirmed test [12]. In our study, we were unable to retest

and confirm blood glucose abnormalities. Further, HbA1c was measured from the whole capillary blood. However, a large body of scientific evidence suggests a high degree of sensitivity, specificity, and PPV between capillary blood and venous blood for HbA1c measures [43]. Known confounding factors such as hemoglobinopathies, severe iron deficiency anemia, hemolytic anemia, and renal or hepatic dysfunction may also have influenced our findings.

Of strength, to the best of our knowledge, this was the first population-based study performed in Brazil to analyze the performance of HbA1c in diagnosing type 2 diabetes. In addition, the survey was conducted by trained and highly motivated staff. The fasting state of the participants was secured at three times: (a) orientation at inclusion, (b) telephone call by the study nurse the night before the test, and (c) on-site investigation. All blood collections, transportation, and storage were performed by trained laboratory personnel, and final analysis was performed at a certified laboratory. Quality control of the laboratory was assessed internally and externally.

5. Conclusions

In conclusion, an HbA1c threshold of ≥6.8% (51 mmol/mol) can be considered a relatively sensitive marker for the diagnosis of diabetes in this population, which differs from the suggested value of ≥6.5% (48 mmol/mol) by the International Expert Committee and ADA [12,17]. However, our data suggest that HbA1c values may be a weak parameter to identify pre-diabetes cases. The debate surrounding the role of HbA1c as a diagnostic test addresses the relative merits and disadvantages of glucose versus HbA1c and brings into focus many biological considerations as well as factors such as cost and accessibility. Early detection of diabetes through HbA1c is likely to be cost-effective as timely initiation of treatment will prevent complications. More studies are required, particularly long-term prospective studies, including all possible factors of influence, such as ethnicity, biological mechanisms, food habits, and lifestyle, in order to confirm our findings in such a multi-ethnic and multi-cultural society like Brazil.

Author Contributions: Conceptualization—N.C.d.V.M., R.M.M.J., A.H. and V.O.F.; methodology,—N.C.d.V.M., R.M.M.J., A.H.; validation—N.C.d.V.M., R.M.M.J., A.H., B.B., T.S. and H.E.M.; formal analysis—N.C.d.V.M., H.E.M., A.H., B.B., T.S. and I.M.; investigation—N.C.d.V.M., R.M.M.J., A.H., and V.O.F.; resources—N.C.d.V.M., R.M.M.J., A.H. and V.O.F.; data curation—N.C.d.V.M., H.E.M., B.B., T.S. and I.M.; visualization—I.M., N.C.d.V.M., B.B., T.S. and H.E.M.; writing—original draft preparation—N.C.d.V.M., H.E.M., A.H. and V.O.F.; writing—review and editing—N.C.d.V.M., R.M.M.J., H.E.M., I.M., B.B., T.S. and A.H.; supervision—H.E.M., R.M.M.J., and A.H.; project administration—N.C.d.V.M., R.M.M.J., and A.H.; funding acquisition—N.C.d.V.M., R.M.M.J., A.H. and V.O.F.

Funding: This research was funded by the Ivar Helles Foundation, and the Health Department of the city of Pindoretama-CE.

Acknowledgments: We acknowledge and thank the valuable contribution of our survey team members and community health workers. We are grateful to all participants in the study for their active cooperation. We thank the Ivar Helles Foundation, Norway, for their financial contribution. We express our appreciation to the Health Department of the city of Pindoretama-CE, in special the Secretary of Health, Valéria Maria Viana Lima, for providing us with local assistance, logistic and financial support.

Conflicts of Interest: The authors declare no conflict of interest. The funders had no role in the design of the study; in the collection, analyses, or interpretation of data; in the writing of the manuscript, or in the decision to publish the results.

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Diabetes & Metabolic Syndrome: Clinical Research & Reviews 14 (2020) 1217–1224

Contents lists available at ScienceDirect

Diabetes & Metabolic Syndrome: Clinical Research & Reviews

journal homepage: www.elsevier.com/locate/dsx

Prevalence of Metabolic Syndrome by different definitions, and its association with type 2 diabetes, pre-diabetes, and cardiovascular disease risk in Brazil

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ARTICLE INFO

Article history:
Received 5 January 2020
Received in revised form
26 May 2020
Accepted 28 May 2020

Keywords:
Metabolic syndrome
Type 2 diabetes mellitus
Pre-diabetes
Cardiovascular disease risk
Brazil

ABSTRACT

Background and aims: Metabolic Syndrome (MS) is increasing in developing countries. Different definitions of MS lead to discrepancies in prevalence estimates and applicability. We assessed the prevalence of MS as defined by the International Diabetes Federation (IDF), modified National Cholesterol Education Program Adult Treatment Plan III (Modified NCEP) and Joint Interim Statement (JIS); compared the diagnostic performance and association of these definitions of MS with pre-diabetes, type 2 diabetes mellitus (T2DM) and cardiovascular disease (CVD) risk.

Methods: A total of 714 randomly selected subjects from Northeastern Brazil were investigated in a cross-sectional study. Sociodemographic, anthropometric, and clinical data were recorded. Diagnostic test performance measures assessed the ability of the different MS definitions to identify those with pre-diabetes, T2DM and increased CVD risk.

Results: The adjusted prevalence of MS was 36.1% applying the JIS criteria, 35.1% the IDF and 29.5% Modified NCEP. Women were more affected by MS according to all definitions. MS was significantly associated with pre-diabetes, T2DM and CVD risk following the three definitions. However, the JIS and IDF definitions showed higher sensitivity than the Modified NCEP to identify pre-diabetes, T2DM and CVD risk. The odds ratios for those conditions were not significantly different when comparing the definitions.

Conclusions: MS is highly prevalent in Brazil, particularly among those with pre-diabetes, T2DM, and high CVD risk. The IDF and JIS criteria may be better suited in the Brazilian population to identify pre-diabetes, T2DM and CVD risk. This may also signify the importance of the assessment of MS in clinical practice.

1. Introduction

Metabolic syndrome (MS) is characterized by a clustering of interrelated risk factors including abdominal obesity, hyperglycemia, hypertension, and dyslipidemia [1]. The condition is associated with an increased risk of type 2 diabetes mellitus (T2DM), cardiovascular events and deaths [2].

The prevalence of MS has grown worldwide, and it is estimated that approximately 20–25% of the world's population has MS [3]. In developing countries, especially in South America, rapid socioeconomic and demographic transitions have fostered great increases in obesity rates, sedentary lifestyles, as well as profound changes in dietary patterns [4]. Studies conducted in Latin American countries as Chile, Colombia, Mexico, Peru and Venezuela showed a high

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https://doi.org/10.1016/j.dsx.2020.05.043

prevalence of MS, ranging from 12.3% to 42.7% [5–9]. In Brazil, according to a systematic review from 2013, the weighted mean prevalence of MS was 29.6% (range: 14.9%–65.3%) [10].

Different definitions of MS have been proposed so far and, therefore, prevalence estimates may vary substantially across populations, depending not only on their characteristics, but specially on the diagnostic criteria applied. The most commonly used definitions have been produced by the National Cholesterol Education Program Adult Treatment Panel III (NCEP) in 2001 [11], which was updated in 2005 by the American Heart Association/National Heart, Lung, and Blood Institute (Modified NCEP) [12], and the International Diabetes Federation (IDF) [13]. Even though the definitions share common features, several parameters differ, which leads to discrepancies in applicability, uniformity, and positive predictive value [14]. More recently, a Joint Interim Statement (JIS) issued by several scientific societies has attempted to develop a unifying definition of MS [2].

Although the MS has been considered a major global health problem, many uncertainties and controversies remain. MS has been pointed out as an ill-characterized entity, with no clear rationale for thresholds [15]. Furthermore, its value as a risk assessment tool for future cardiovascular disease (CVD) has been claimed as weak [16], or no greater than the sum of its components [17]. Although the syndrome is effective in predicting diabetes, its predictive value beyond that of glucose intolerance has also been questioned [15]. In Brazil, few studies have described the prevalence of MS and its determinants. More importantly, there is scarce information about the applicability and agreement of different definitions of MS, as well as their predictive value in the estimation of T2DM, pre-diabetes, and CVD risk in the Brazilian population. Therefore, we aimed to determine the prevalence of MS as defined by the Modified NCEP [12], the IDF [13], and JIS [2]; assess the agreement between the definitions; and investigate the association of MS with pre-diabetes, T2DM and CVD risk. It was hypothesized that the JIS will show a higher prevalence of MS, as well as greater sensitivity to predict the cases of diabetes, pre-diabetes, and high CVD risk.

2. Subjects

This population-based study was conducted in the city of Pindoretama, in the northeast region of Brazil, between August 2012 and January 2013. The recruitment methods and examination procedures have been described beforehand [18]. The data were collected in the six main health centers located throughout the city. Subjects of both genders, aged ≥20 years, able to verbally communicate and willing to participate were eligible to enter the study. Exclusion criteria were those with acute or chronic severe cardiac, renal, or hepatic illness, pregnant women, and physically or mentally disabled individuals.

A registry list with the names of Pindoretama's citizens in alphabetic order was provided by the health authorities and used to select the potential participants. Random numbers were produced with the software R [19] and identified with the names in the list subsequently. Eight hundred and six subjects were randomly selected and of these, 714 agreed to participate (response rate of 88.6%). Owing to the different criteria applied by each MS definition, the total number of recorded observations was n = 707 following the IDF, and n = 704 according to both the Modified NCEP and JIS definitions. On recruitment, participants were requested by the community health workers to visit a nearby health center, after an overnight fast of 8–10 h. Sociodemographic, clinical, and nutritional data were collected by trained interviewers using pre-tested questionnaires. Anthropometric, blood pressure (BP) and body fat percentage (BF%) measurements were also taken.

3. Materials and methods

3.1. Measurements

Anthropometric measurements including height, weight, waist circumference (WC) and hip circumference (HC) were taken with subjects standing in bare feet and with light clothes. Weight was taken by using a portable digital scale, calibrated before use, placed on a flat surface, and recorded to the nearest 0.1 Kg. A well-mounted stadiometer was applied to measure height, with the participant looking straight and in erect position. Height was recorded to the nearest 0.1 cm. The body mass index (BMI) was estimated as the weight in kilograms divided by the square of height in meters (Kg/m^2). The BF% was assessed by a portable bipolar body fat analyzer (Omron®, Model HBF-306, Omron Healthcare, Inc., Illinois, United States). The WC was measured with a non-stretchable tape, positioned horizontally midway between the lower border of the ribs and iliac crest, on the mid-axillary line. The HC was assessed by placing the same tape at the greatest protrusion of the buttocks, with the subject standing straight. WC and HC were registered to the nearest 0.1 cm. The waist-to-hip ratio (WHR) was calculated as the WC divided by the HC. The BP was estimated twice, by using an electronic sphygmomanometer (Omron® BP785 IntelliSense® Automatic Blood Pressure Monitor with ComFitTM Cuff, Omron Healthcare, Inc., Illinois, United States). The first measurement was taken after a resting time of at least 15 min, and the second about 10 min after the first. The mean of the two values was used for analysis.

On arrival at the field center, a 10-mL fasting venous blood sample was collected for measuring fasting plasma glucose (FPG) levels and other relevant laboratory tests. Two hours after a 75 g oral glucose load, another venous sample was drawn for the oral glucose tolerance test (OGTT). Fasting and 2-h plasma glucose levels were assessed by the glucose oxidase method, whereas fasting insulin was determined by chemiluminescence. Total cholesterol (TC) was estimated by the cholesterol oxidase - phenol + aminophenazone (CHOD-PAP) method, while high-density lipoprotein cholesterol (HDL-C) was determined by a homogenous enzymatic colorimetric method. Triglycerides (TG) were determined by the glycerol-3-phosphate oxidase - phenol + aminophenazone (GPO-PAP) method. Low-density lipoprotein cholesterol (LDL-C) was calculated by the Friedewald Formula [20]. Laboratory quality control was assessed internally and externally.

3.2. Definition of variables and outcomes

MS was defined following the diagnostic criteria as suggested by the Modified NCEP [12], IDF [13], and JIS [2]. The three definitions are described in Table 1. Contrary to the Modified NCEP and JIS definitions, IDF considers abdominal obesity a prerequisite for diagnosing MS. Furthermore, the IDF definition applies ethnic-specific WC cut-off points as the measure of central obesity. For South and Central Americans, until population-specific data are available, IDF recommends using South Asian cut-off points, i.e., WC ≥ 90 cm in men and ≥80 cm in women. Therefore, for the Modified NCEP definition, the WC cut-off points applied in this study were ≥102 cm in men and ≥88 cm in women, whereas for the IDF definition were WC ≥ 90 cm in men and ≥80 cm in women. The JIS definition recommends that the IDF cut points for central obesity should be used for non-Europeans in case there is no country-specific data available. Since no WC cut-off points of risk for MS have been established for the Brazilian population, the IDF recommended cut points were also used here for the JIS definition.

Physical activity data were assessed by the International Physical Activity Questionnaire (IPAQ) short form [21]. Following the

Table 1
Criteria for clinical diagnosis of the MS following different definitions.

Risk Factors	IDF	Modified NCEP	JIS
Criteria for Diagnosis of MS	Abdominal obesity plus 2 or more risk factors	Any 3 or more of 5 risk factors	Any 3 or more of 5 risk factors
1 Central Obesity	WC ≥ 90 cm in males, ≥ 80 cm in females	WC ≥ 102 cm in males, ≥ 88 cm in females	WC ≥ 90 cm in males, ≥ 80 cm in females
2 TG	≥1.7 mmol/l (150 mg/dl) or on specific treatment for elevated TG	≥1.7 mmol/l (150 mg/dl) or on drug treatment for elevated TG	≥1.7 mmol/l (150 mg/dl) or on drug treatment for elevated TG
3 HDL-C	<1.03 mmol/l (40 mg/dl) in males, < 1.30 mmol/l (50 mg/dl) in females or on drug treatment for reduced HDL-C	<1.03 mmol/l (40 mg/dl) in males, < 1.30 mmol/l (50 mg/dl) in females or on drug treatment for reduced HDL-C	<1.03 mmol/l (40 mg/dl) in males, < 1.30 mmol/l (50 mg/dl) in females or on drug treatment for reduced HDL-C
4 Blood Pressure	SBP ≥ 130 mmHg or DBP ≥ 85 mmHg or treatment of previously diagnosed hypertension	SBP ≥ 130 mmHg or DBP ≥ 85 mmHg or current use of antihypertensive drugs in a patient with a history of hypertension	SBP ≥ 130 mmHg or DBP ≥ 85 mmHg or antihypertensive drug treatment in a patient with a history of hypertension
5 FG	Fasting plasma glucose ≥ 5.6 mmol/l (100 mg/dl) or previously diagnosed Type 2 diabetes	≥5.6 mmol/L (100 mg/dL) or on drug treatment for elevated glucose	≥5.6 mmol/L (100 mg/dL) or on drug treatment for elevated glucose

DBP: Diastolic Blood Pressure. FG: Fasting Glucose. HDL-C: High-Density Lipoprotein Cholesterol. IDF: International Diabetes Federation. JIS: Joint Interim Statement. MS: Metabolic Syndrome. NCEP: National Cholesterol Education Program Expert Panel. SBP: Systolic Blood Pressure. TG: Triglycerides. WC: Waist Circumference.

Brazilian Institute of Geography and Statistics (IBGE) classification, ethnicity was defined according to the participants' self-perception of their skin color. The different ethnic groups were categorized into "white", "brown", and "black" [22].

The 1999 WHO criteria were applied in diagnosing diabetes mellitus, impaired fasting glycaemia (IFG) and/or impaired glucose tolerance (IGT). Diabetes cases were those who were previously diagnosed, or those with fasting (venous) plasma glucose value ≥ 7.0 mmol/l (≥126 mg/dl), or the plasma glucose value 2 h after a 75 g oral glucose load ≥ 11.1 mmol/l (≥200 mg/dl), or both. IGT was determined when FPG <7.0 mmol/l (<126 mg/dl), and 2-h plasma glucose ≥7.8 mmol/l (≥140 mg/dl), but <11.1 mmol/l (<200 mg/dl). IFG was defined as FPG ≥6.1 mmol/l (≥110 mg/dl), but <7.0 mmol/l (<126 mg/dl), and 2-h plasma glucose <7.8 mmol/l (<140 mg/dl). Individuals with IFG and/or IGT were classified as pre-diabetes cases [23].

Dyslipidemia was described as TG ≥ 1.7 mmol/l and HDL-C < 0.9 mmol/l for men; and <1.0 mmol/l for women [23]. Insulin resistance was calculated with the homeostasis model assessment of insulin resistance (HOMA-IR = [insulin (mU/l) × glucose (mmol/l)]/22.5) [24]. The 10-year risk of CVD was calculated for each participant using a 2008 Framingham risk equation. The model predictors for the gender-specific algorithm included age, TC, HDL-C, systolic BP, antihypertensive medication use, smoking and diabetes status [25]. Individuals with a Framingham predicted risk for an incident cardiovascular event of 10% or above during the next 10 years were described as having high CVD risk. Thirteen subjects reported a history of stroke and/or myocardial infarction and were excluded from the analysis for CVD risk.

3.3. Ethics

The study was carried out according to the guidelines laid down in the Declaration of Helsinki [26], and the protocol was approved by both the local Ethical Committee in Brazil (Protocol Number: 045.06.12) and the Regional Committee for Medical and Health Research Ethics (REK) in Norway (Reference: 2012/779/REK sør-øst D). Written or verbal consent was obtained from each subject before any investigation. The participants were also informed of their right to withdraw from the study at any stage, or to omit their data from the analysis. All the names in the registration list were removed before the analyses were performed. Those diagnosed with any clinical condition were referred to the nearest health center for proper treatment and further follow up.

3.4. Statistical Analysis

Means and 95% confidence interval (CI) were given for numerical data, while percentages and 95% CI for categorical variables. Generalized linear regression models (GLM) were fitted to the data after adjusting for age and gender. In particular, we fitted GLMs with linear link function for comparing differences between adjusted means and GLMs with the logit link function to compare differences between proportions. Following estimation of the adjusted logistic regression models, the prevalence of MS was obtained as margins. Kappa statistics measured the agreement between the three MS definitions [27]. Diagnostic test performance measures including sensitivity, specificity, accuracy, positive predictive value (PPV) and negative predictive value (NPV) were calculated using contingency tables. Adjusted odds ratios (ORs) were calculated based on the IDF, Modified NCEP and JIS definitions for pre-diabetes, T2DM, and CVD risk. Adjusted ORs were obtained by applying logistic regression analysis controlling for age, gender, and BMI. The significance level was set at 0.05. All tests were two-sided. Statistical analyses were performed by using SPSS 25th version [28] and Stata 15th edition [29].

4. Results

As can be seen in Table 2, substantial differences were found in demographic, lifestyle, anthropometric and cardiometabolic characteristics between those with and without MS. Comparing only those with MS, the mean WC (p-value: 0.035) and BMI (p-value: 0.039) were significantly higher using the Modified NCEP criteria than when the JIS definition was applied.

Irrespective of which definition was applied, the prevalence of MS among women (ranging from 38.2 to 44.8%) was significantly higher than in men (ranging from 12.6 to 18.9%) (Table 3). According to all three definitions, the prevalence increased significantly with age, BMI status, and level of income. However, the prevalence did not differ significantly among ethnic groups. Using the Modified NCEP definition, those with ≥10 years of education showed a significantly lower odds for MS than those with <10 years (OR = 0.6, 95% CI: 0.4–0.9; p-value: 0.019).

Table 4 shows the overall prevalence of MS, as well as the prevalence of MS among those with pre-diabetes, T2DM and high CVD risk. The age- and gender-adjusted prevalence was highest applying the definition described by the JIS (36.1%), followed by the IDF (35.1%) and Modified NCEP (29.5%). Nevertheless, these

Table 2
Cardiometabolic, anthropometric and lifestyle characteristics of the study participants with and without MS, applying the criteria as described by the IDF, Modified NCEP and JIS.

Variables	IDF[a]		Modified NCEP[a]		JIS[a]	
	With MS	**Without MS**	**With MS**	**Without MS**	**With MS**	**Without MS**
n	248	459	208	496	254	450
Age (years)	52.4 (50.5–54.3)**	41.1 (39.7–42.5)	53.0 (50.8–55.1)**	41.8 (40.5–43.2)	52.7 (50.8–54.6)**	40.9 (39.4–42.3)
Gender (female), % (95% CI)	84.1 (79.6–88.5)**	55.6 (51.0–60.1)	87.1 (82.7–91.5)**	56.7 (52.3–61.1)	84.2 (79.9–88.6)**	55.1 (50.5–59.8)
Smoking (yes)[b]	36.5 (30.9–42.1)	41.7 (37.4–46.0)	37.7 (31.6–43.9)	40.9 (36.8–45.0)	36.5 (30.9–42.0)	42.1 (37.7–46.4)
Alcohol Consumption (yes)	39.2 (32.9–45.4)	34.9 (30.9–38.9)	39.8 (32.9–46.6)	35.0 (31.2–38.8)	39.0 (32.8–45.1)	35.0 (31.0–39.0)
Physical Activity						
Low	77.2 (71.5–82.8)**	61.1 (56.6–65.7)	82.2 (76.6–87.9)**	60.2 (55.8–64.6)	77.3 (71.7–82.9)**	60.6 (56.0–65.2)
Moderate/High	22.8 (17.2–28.5)	38.9 (34.3–43.4)	17.8 (12.1–23.4)	39.8 (35.4–44.2)	22.7 (17.1–28.3)	39.4 (34.8–44.0)
BMI (kg/m^2)	29.6 (28.9–30.2)**	25.4 (25.0–25.9)	30.4 (29.7–31.0)**	25.4 (25.0–25.9)	29.4 (28.8–30.0)**	25.5 (25.0–25.9)
WC (cm)	97.2 (95.7–98.8)**	86.1 (85.0–87.2)	98.9 (97.3–100.6)**	86.4 (85.3–87.4)	96.7 (95.1–98.2)**	86.4 (85.2–87.5)
WHR, mean (95% CI)	0.95 (0.94–0.96)**	0.90 (0.89–0.91)	0.96 (0.95–0.97)**	0.90 (0.89–0.91)	0.95 (0.93–0.96)**	0.90 (0.89–0.91)
BF%, mean (95% CI)	35.1 (34.2–36.0)**	31.5 (30.9–32.2)	35.6 (34.6–36.6)**	31.7 (31.1–32.3)	34.9 (34.0–35.8)**	31.7 (31.0–32.3)
SBP (mmHg)	137.2 (134.9 –139.6)**	122.5 (120.8 –124.2)	138.4 (135.8 –141.0)**	123.2 (121.5 –124.8)	137.7 (135.4 –140.0)**	122.0 (120.3 –123.7)
DBP (mmHg)	83.9 (81.7–86.1)**	72.9 (71.3–74.4)	83.3 (80.8–85.7)**	74.0 (72.5–75.6)	84.0 (81.9–86.2)**	72.7 (71.1–74.3)
FPG (mmol/l)	6.5 (6.2–6.8)**	4.9 (4.7–5.1)	6.9 (6.5–7.2)**	4.9 (4.7–5.1)	6.6 (6.3–6.9)**	4.8 (4.6–5.1)
2-hour Post Glucose Load (mmol/l)	9.6 (9.1–10.2)**	6.9 (6.5–7.3)	10.3 (9.7–10.9)**	6.8 (6.5–7.2)	9.8 (9.2–10.3)**	6.8 (6.4–7.2)
Fasting Insulin (micro UI/ml)	8.6 (8.0–9.3)**	5.7 (5.2–6.2)	8.9 (8.2–9.6)**	5.8 (5.4–6.3)	8.5 (7.9–9.2)**	5.7 (5.3–6.2)
HOMA-IR	2.4 (2.3–2.6)**	1.2 (1.1–1.3)	2.6 (2.4–2.8)**	1.2 (1.1–1.4)	2.4 (2.2–2.6)**	1.2 (1.1–1.3)
Total Cholesterol (mmol/l)	5.0 (4.9–5.1)**	4.5 (4.4–4.6)	5.1 (4.9–5.2)**	4.6 (4.5–4.6)	5.0 (4.9–5.2)**	4.5 (4.4–4.6)
HDL-C (mmol/l)	1.19 (1.17–1.20)**	1.24 (1.23–1.26)	1.19 (1.17–1.20)**	1.24 (1.23–1.25)	1.18 (1.17–1.20)**	1.25 (1.23–1.26)
LDL-C (mmol/l)	2.9 (2.8–3.0)	2.8 (2.7–2.9)	3.0 (2.8–3.1)	2.8 (2.7–2.9)	2.9 (2.8–3.1)	2.8 (2.7–2.9)
Triglycerides (mmol/l)	2.4 (2.2–2.6)**	1.1 (0.9–1.2)	2.5 (2.3–2.7)**	1.1 (1.0–1.2)	2.4 (2.2–2.6)**	1.0 (0.9–1.1)
Dyslipidemia, % (95% CI)	56.8 (50.3–63.3)**	9.6 (7.0–12.2)	60.5 (53.5–67.5)**	11.3 (8.6–14.1)	58.6 (52.2–64.9)**	8.3 (5.9–10.7)

Data are mean (95% confidence interval) or percentage (95% confidence interval), adjusted for age and gender; [a] The number of observations recorded was 707 for the IDF definition, 704 for both Modified NCEP ATP III and JIS; [b] Included those who self-reported as being smokers or had stopped smoking for less than 1 year; The values were significantly different between those with and without MS at **p < 0.01 or *p < 0.05, by logistic regression analysis; BF%: Body Fat Percentage. BMI: Body Mass Index. CI: Confidence Interval. DBP: Diastolic Blood Pressure. FPG: Fasting Plasma Glucose. HDL-C: High-Density Lipoprotein Cholesterol. HOMA-IR: Homeostasis Model Assessment of Insulin Resistance. IDF: International Diabetes Federation. JIS: Joint Interim Statement. LDL-C: Low-Density Lipoprotein Cholesterol. MS: Metabolic Syndrome. NCEP: National Cholesterol Education Program Expert Panel. SBP: Systolic Blood Pressure. WC: Waist Circumference. WHR: Waist-to-Hip Ratio.

Table 3
Prevalence of MS by demographic and socioeconomic characteristics, age- and gender-adjusted.

	n	IDF	Modified NCEP	JIS
Gender				
Male	242	18.3 (13.6–23.0)**	12.6 (8.5–16.7)**	18.9 (14.2–23.7)**
Female	472	43.7 (39.5–47.9)	38.2 (34.1–42.3)	44.8 (40.7–49.0)
Age Groups				
20–35	235	18.5 (13.6–23.3)**	13.8 (9.4–18.1)**	18.5 (13.6–23.4)**
36–50	241	32.6 (26.9–38.4)	28.7 (23.2–34.2)	33.3 (27.6–39.1)
≥51	238	54.0 (48.1–60.0)	46.0 (40.0–51.9)	56.2 (50.2–62.1)
BMI Status				
<25 kg/m^2	272	16.7 (12.5–20.8)**	9.7 (6.4–13.1)**	18.7 (14.4–23.0)**
25–29.99 kg/m^2	266	38.5 (33.2–43.9)	30.9 (25.9–35.9)	38.9 (33.7–44.2)
≥30 kg/m^2	175	58.3 (51.6–64.9)	57.7 (51.2–64.3)	58.3 (51.7–64.9)
Ethnicity				
White	120	37.8 (29.9–45.8)	32.8 (25.1–40.5)	39.5 (31.6–47.4)
Brown	576	34.7 (31.2–38.3)	29.1 (25.7–32.5)	35.6 (32.1–39.2)
Black	18	27.8 (9.0–46.6)	22.2 (4.7–39.8)	27.8 (9.1–46.5)
Education				
<10 years	508	40.4 (36.5–44.4)	34.8 (31.0–38.6)*	41.8 (37.9–45.7)
≥10 years	206	22.0 (16.5–27.4)	16.7 (11.7–21.6)	22.1 (16.6–27.5)
Monthly Income				
<2 MW	641	34.2 (30.8–37.5)**	28.8 (25.6–31.9)**	35.2 (31.9–38.6)**
≥2 MW	71	44.3 (33.9–54.6)	37.7 (27.6–47.7)	44.9 (34.5–55.3)

Data are provided as % (95% confidence interval) adjusted for age and gender; **p < 0.001 and *p < 0.05, by logistic regression analysis; BMI: Body Mass Index. IDF: International Diabetes Federation. JIS: Joint Interim Statement. MS: Metabolic Syndrome. MW: Minimum Wage in 2012, that corresponds currently to US$ 162.00. NCEP: National Cholesterol Education Program Expert Panel.

estimates were not significantly different. Using the definition recommended by the JIS, MS was present in 58.2% of subjects diagnosed with pre-diabetes, 76.1% of those with T2DM, and 57.1% of those with high CVD risk. Following the IDF definition, the respective parameters were 57.1, 74.3, 54.8%, while for the Modified NCEP, the values were 46.9, 70.8, and 48.0%. The agreement was highest between the definitions described by the IDF and JIS, as measured by the kappa statistics (overall study population and pre-diabetes: 0.98; T2DM and high CVD risk: 0.95). The lowest agreement was observed between the IDF and Modified NCEP definitions, both for overall (0.83) and all other subsets of the study population (pre-diabetes: 0.76; T2DM: 0.82; high CVD risk: 0.77).

Table 4
Adjusted prevalence of MS among overall, subjects with pre-diabetes, T2DM and high CVD risk, as well as the agreement between the definitions of MS as described by the IDF, Modified NCEP and JIS.

	IDF		Modified NCEP	JIS	IDF vs. Modified NCEP	Modified NCEP vs. JIS	IDF vs. JIS
	n	% (95% CI)	% (95% CI)	% (95% CI)	Kappa (p-value)	Kappa (p-value)	Kappa (p-value)
Overall	714	35.1 (31.9–38.3)	29.5 (26.5–32.6)	36.1 (32.9–39.3)	0.83 (<0.001)	0.85 (<0.001)	0.98 (<0.001)
Pre-Diabetes	100	57.1 (48.0–66.3)	46.9 (37.8–56.1)	58.2 (49.1–67.2)	0.76 (<0.001)	0.78 (<0.001)	0.98 (<0.001)
T2DM	114	74.3 (66.9–81.8)	70.8 (63.2–78.4)	76.1 (68.9–83.3)	0.82 (<0.001)	0.86 (<0.001)	0.95 (<0.001)
High CVD Risk[a]	254	54.8 (49.3–60.2)	48.0 (42.6–53.4)	57.1 (51.8–62.5)	0.77 (<0.001)	0.82 (<0.001)	0.95 (<0.001)

Data presented as percentage (95% confidence interval) adjusted for age and gender.
[a] The 10-year risk of CVD was calculated using a 2008 Framingham risk equation. Those with a history of stroke and/or myocardial infarction were excluded from the analysis; CI: Confidence Interval. CVD: Cardiovascular Disease. IDF: International Diabetes Federation. JIS: Joint Interim Statement. MS: Metabolic Syndrome. NCEP: National Cholesterol Education Program Expert Panel. T2DM: Type 2 Diabetes Mellitus.

The diagnostic performances of the different MS definitions to diagnose pre-diabetes, T2DM and high CVD risk are presented in Table 5. The JIS definition showed a greater sensitivity than the Modified NCEP to identify pre-diabetes (58.2% vs 46.9%), T2DM (76.1% vs 70.8%) and high CVD risk (57.1% vs 48%). However, following the Modified NCEP definition, the specificity (pre-diabetes: 83.4%; T2DM: 78.3%; high CVD risk: 81.2%) and PPV (pre-diabetes: 31.5%; T2DM: 38.4%; high CVD risk: 59.1%) were higher than when the JIS definition was applied. The IDF and JIS definitions showed similar results regarding sensitivity, specificity, PPV and NPV.

Table 6 presents the ORs of the IDF, Modified NCEP and JIS definitions for pre-diabetes, T2DM, and high CVD risk using logistic regression analysis after adjustment for age, gender, and BMI. A significant association was found between MS and pre-diabetes, T2DM and high CVD risk, irrespective of which definition of MS was applied. The adjusted ORs for pre-diabetes (ranging from 3.6 to 3.9), T2DM (5.0–6.4) and high CVD risk (5.6–7.1) were not significantly different between the different definitions of MS.

Table 6
Odds rations (OR) for pre-diabetes, T2DM, and people with high CVD risk in those with MS compared with those without MS.

	Pre-Diabetes	T2DM	High CVD Risk
	OR (95% CI)	OR (95% CI)	OR (95% CI)
IDF	3.9 (2.3–6.5)[a]	5.0 (3.0–8.5)[a]	5.6 (2.9–10.9)[a]
Modified NCEP	3.6 (2.0–6.2)[a]	6.4 (3.7–11.1)[a]	5.7 (2.9–11.3)[a]
JIS	3.9 (2.3–6.5)[a]	5.4 (3.2–9.3)[a]	7.1 (3.6–14.2)[a]

Adjusted for age, gender, and body mass index.
[a] $p < 0.001$; CI: Confidence Interval. CVD: Cardiovascular Disease. IDF: International Diabetes Federation. JIS: Joint Interim Statement. MS: Metabolic Syndrome. NCEP: National Cholesterol Education Program Expert Panel. T2DM: Type 2 Diabetes Mellitus.

5. Discussion

To the best of our knowledge, this is one of the first population-based study from Brazil to compare the prevalence of MS among subjects with pre-diabetes, T2DM and high CVD risk, following the recent JIS criteria in relation to other more established definitions. We found a high prevalence of MS in the overall population, and particularly among the participants with pre-diabetes, T2DM, and high CVD risk. Women and those with a higher income were disproportionately affected. The agreement between all three definitions was almost perfect. The JIS and IDF definitions showed a higher sensitivity to identify the subjects with pre-diabetes, T2DM and high CVD risk.

In the current study, the observed prevalence of MS was higher than the estimated prevalence of 20–25% for the global population [3]. Following the Modified NCEP definition, the overall prevalence of MS in our study population (29.5%) was somewhat lower than that in the US population (34.7%), reported by the 2003–2012 National Health and Nutrition Examination Survey (NHANES) [30]. Compared to other middle-income countries, using the IDF

Table 5
Diagnostic performance of the IDF, Modified NCEP and JIS definitions of MS to predict pre-diabetes, T2DM, and people with high CVD risk in an adult Brazilian population.

	Pre-Diabetes	T2DM	High CVD Risk
IDF			
Sensitivity, % (95% CI)	57.1 (46.8–67.1)	74.3 (65.3–82.1)	54.8 (48.4–61.0)
Specificity, % (95% CI)	78.2 (74.3–81.8)	72.4 (68.6–76.0)	76.6 (72.3–80.5)
Positive Predictive Value, % (95% CI)	29.9 (22.9–37.7)	33.9 (28.0–40.2)	57.1 (50.5–63.4)
Negative Predictive Value, % (95% CI)	91.8 (88.8–94.2)	93.7 (91.0–95.7)	74.9 (70.6–78.9)
Accuracy (%)	75.2	72.7	68.7
Modified NCEP			
Sensitivity, % (95% CI)	46.9 (36.8–57.3)	70.8 (61.5–79.0)	48.0 (41.7–54.4)
Specificity, % (95% CI)	83.4 (79.8–86.6)	78.3 (74.8–81.6)	81.2 (77.2–84.7)
Positive Predictive Value, % (95% CI)	31.5 (23.4–40.5)	38.4 (31.7–45.4)	59.1 (52.0–66.0)
Negative Predictive Value, % (95% CI)	90.6 (87.6–93.1)	93.4 (90.8–95.4)	73.3 (69.2–77.2)
Accuracy (%)	78.3	77.1	69.2
JIS			
Sensitivity, % (95% CI)	58.2 (47.8–68.1)	76.1 (67.2–83.6)	57.1 (50.8–63.3)
Specificity, % (95% CI)	77.5 (73.5–81.1)	71.6 (67.8–75.2)	76.6 (72.3–80.5)
Positive Predictive Value, % (95% CI)	29.6 (22.7–37.3)	33.8 (28.0–40.0)	58.0 (51.6–64.3)
Negative Predictive Value, % (95% CI)	91.9 (88.9–94.3)	94.0 (91.4–96.0)	75.9 (71.6–79.8)
Accuracy (%)	74.8	72.3	69.5

CI: Confidence Interval. CVD: Cardiovascular Disease. IDF: International Diabetes Federation. JIS: Joint Interim Statement. MS: Metabolic Syndrome. NCEP: National Cholesterol Education Program Expert Panel. T2DM: Type 2 Diabetes Mellitus.

definition, our estimate of 35.1% (18.3% in men; 43.7% in women) was similar to that reported from Colombia 32.9% [6], lower than Mexico (49.8%) [31], higher than India (25.8%) [32] and China (9.8% in men; 16.6% in women) [33]. According to the JIS definition, 36.1% of the subjects were classified as having MS, which was slightly higher than that observed in central Brazil (32%) [34]. In addition to methodological differences, the varied prevalence rates of MS across populations may be explained by different demographic, epidemiological and nutritional transitions [35], as well as environmental, social [36] and ethnic disparities [37].

Although the prevalence of MS did not differ significantly between the three definitions, it was highest when using the criteria described by the JIS and lowest following the Modified NCEP. The observed higher prevalence of MS obtained using the JIS criteria compared to the Modified NCEP may be due to the higher rate of central obesity identified by the lower WC cut points used for the JIS [2]. Furthermore, the MS prevalence was also somewhat higher according to the IDF definition than the Modified NCEP definition. The IDF criteria places more emphasis on central obesity in the definition of the MS and recommends lower WC cut-off points for South America similarly to the JIS definition [13].

Following all three definitions, the prevalence of MS was significantly higher among women than men, which has also been found elsewhere [31,33,38]. This was especially evident for the prevalence following the IDF (43.7% vs 18.3%; p-value < 0.001) and JIS (44.8% vs 18.9%; p-value < 0.001) definitions. Central obesity has been strongly correlated with insulin resistance and MS [13]. In our study population, as shown in Supplementary Table 1, women had a significantly higher prevalence of abdominal obesity when applying both the different recommended WC cut-off points of $\geq$90 cm for males; $\geq$ 80 cm for females (81.7% vs 52.1%) and of $\geq$102 cm for males; $\geq$ 88 cm for females (56.9% vs 14.9%). Furthermore, women also showed significantly higher rates of abnormalities in glucose metabolism (27.8% vs 18.7%) and HDL-C levels (73.5% vs 4.1%) (Supplementary Table 1). Nevertheless, this gender difference was not observed in another study conducted among 2130 adults in central Brazil [34]. The study involved a younger population, with less than 12,5% of women aged $\geq$50 years, compared to approximately 33% in our data. Furthermore, metabolic changes related to menopause have been linked to an increased risk of MS and CVD [39] and might also explain the higher prevalence of MS among females in our findings.

In this study, the prevalence of MS increased significantly and progressively with age and BMI status, which has been found by several [31,34,40,41]. In contrast with some studies from Southeastern Brazil [42,43], we identified an increasing rate of MS with higher levels of income, following all the definitions. Nevertheless, a study from India among 1178 adults, aged 20–80 years, also found that middle-to-high income significantly contributed to increased risk of MS [44]. In our sample, ethnicity was not an important predictor of MS. One possible reason may be the high degree of heterogeneity and mixed genetic composition of the Brazilian population. Due to five centuries of miscegenation, the country's population consists of interethnic admixtures of people from European, African and native American origins [45]. Although the relative genetic contribution of these diverse ethnic backgrounds may vary across the different regions in Brazil, a study from the Southeast among 1507 individuals found similar results [42].

Consistent with other studies [38,46,47] and as expected, we observed a higher frequency of MS among the subjects diagnosed with pre-diabetes, T2DM and high CVD risk. In our data, the highest prevalence of MS was observed when the JIS definition of MS was applied, possibly because abdominal obesity is not mandatory in this definition. Pre-diabetes and T2DM are known risk factors for atherosclerotic CVD [48], and the MS in T2DM patients is significantly associated with macro- and microvascular complications [46]. Recently Brazil has experienced a growing epidemic of obesity, hypertension, physical inactivity and T2DM. CVDs have become a major public health problem, since they constitute the main cause of death in the country [49]. Diabetes is a costly condition, and a large proportion of these expenditures are related to treating its complications. Intensive interventions involving multiple cardiovascular risk factors should be implemented to prevent or reduce the impact of further complications, which could potentially lead to health cost savings [50].

We examined the diagnostic performances of the different definitions of MS to identify those with pre-diabetes, T2DM, and people with high CVD risk. The JIS and IDF definitions presented higher sensitivity in the identification of participants with these 3 conditions. This difference may be due to the lower WC cut-off point applied by these definitions. These findings may indicate that applying the South Asian WC cut-offs as suggested by the IDF ($\geq$90 cm for males and $\geq$80 cm for females) in the definition of MS may be a better predictor for pre-diabetes, T2DM and CVD risk in our population.

This was a population-based study from a semi-urban area in Northeastern Brazil. The subjects were randomly selected, and the participation rate was high. Although the final sample was relatively small, it was large enough to meet the required sample size for analysis. The survey was performed by thoroughly trained and highly motivated personnel. Collection, transportation, and storage of the blood samples followed standard procedures and the analyses were performed in a certified laboratory. Considering the substantial socioeconomic, ethnic, and regional disparities in Brazil, generalization of our findings should be done with caution. However, since Brazilians have a mixed background in general, our sample might be a good representation of the country's population. Another limitation was the cross-sectional design of the study, as a cause-effect relationship could not be established. Therefore, long-term prospective studies are needed to confirm the association between the aforementioned factors and MS.

In conclusion, our study showed that MS is common in Brazil following the IDF, Modified NCEP and JIS definitions. Although all three definitions may be appropriate to assess the prevalence of MS, the IDF and JIS criteria may be better suited in the Brazilian population to predict pre-diabetes, T2DM and CVD risk. MS is highly prevalent among subjects with pre-diabetes, T2DM and CVD risk. Therefore, screening of MS in primary care centers, especially among women, may identify patients at higher risk of these conditions, and timely intensive multifactorial interventions could benefit this population.

Declaration of competing interest

The authors declare that they have no competing interests. This study received financial sponsorship from the University of Oslo and Ivar Helles Foundation. Nevertheless, the funders had no role in the study design; in the collection, analyses, or interpretation of data; in writing the report, or in the decision to publish the results.

Acknowledgments

We acknowledge the contribution of our study team members, participants, community health workers and lab technicians for their continuous effort and active cooperation in the collection of data. We are thankful for the financial contribution provided by the University of Oslo and Ivar Helles Foundation, Norway. We express our admiration to the Health Department of the city of Pindoretama-CE, particularly the Secretary of Health, Valéria Maria Viana Lima, for all the assistance and logistic support provided.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.dsx.2020.05.043.

Authors' contributions

Nayla Cristina do Vale Moreira contributed with the study concept and design, drafting of the article, as well as acquisition, analysis and interpretation of the data.

Akhtar Hussain contributed with the study concept and design, data analyses, writing the initial draft and revising it critically, study oversight and leadership.

Bishwajit Bhowmik participated in the analysis and interpretation of data, writing the article and revising it critically.

Ibrahimu Mdala contributed with the design of the study, writing the initial draft and revising it critically, as well as organized the database and conducted the statistical analysis.

Tasnima Siddiquee contributed with the design of the study, data curation and analyses, drafting of the article.

Virgínia Oliveira Fernandes contributed with conceptualizing the work and designing the methodology, data collection and analyses, writing the initial draft and revising it critically.

Renan Magalhães Montenegro Júnior contributed with the study concept and design, data analyses, study management and coordination, as well as writing the initial draft and revising it critically.

Haakon E. Meyer contributed with the design of the methodology, data analyses, study oversight and leadership, writing the initial draft and performing critical review.

All authors have read and approved the contents of the final manuscript. Furthermore, they have agreed to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved.

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III

frontiers in Public Health

ORIGINAL RESEARCH
published: 30 November 2021
doi: 10.3389/fpubh.2021.725009

Cardiovascular Risk, Obesity, and Sociodemographic Indicators in a Brazilian Population

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OPEN ACCESS

Edited by:
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Reviewed by:
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Prof Asher Fawwad,
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Specialty section:
This article was submitted to Life-Course Epidemiology and Social Inequalities in Health, a section of the journal Frontiers in Public Health

Received: *14 June 2021*
Accepted: *26 October 2021*
Published: *30 November 2021*

Citation:
Moreira NCdV, Mdala I, Hussain A, Bhowmik B, Siddiquee T, Fernandes VO, Montenegro RM Jr and Meyer HE (2021) Cardiovascular Risk, Obesity, and Sociodemographic Indicators in a Brazilian Population. Front. Public Health 9:725009. doi: 10.3389/fpubh.2021.725009

Background and Aims: Cardiovascular diseases (CVDs) are the leading cause of death globally and in Brazil. Evidence suggests that the risk of CVDs differs by race/ethnicity. Scarce information exists about the association between CVD risk, obesity indicators and sociodemographic characteristics in the Brazilian population.

Objectives: We aimed to assess the CVD risk following the Framingham risk score in relation to the population's sociodemographic profile. Further, we examined the association between anthropometric markers and risk of CVDs.

Methods: A total of 701 subjects aged ≥20 years from North-eastern Brazil were recruited randomly to participate in a population-based, cross-sectional survey. Age-adjusted data for CVD risk, sociodemographic characteristics, and anthropometric indices were assessed, and their relationships examined.

Results: High CVD risk (Framingham risk score ≥10%) was observed in 18.9% of the population. Males (31.9 vs. 12.5%) and older subjects (age ≥45 years: 68.9% vs. age < 45 years: 4.2%) had significantly higher risk of CVDs, whereas those employed in manual labor showed lower risk (7.6 vs. 21.7%). Central obesity measures like waist-to-hip ratio and waist-to-height ratio were more strongly associated with predicted CVD risk than body mass index.

Conclusions: Our population had a high risk of CVDs using the Framingham risk score. Cost-effective strategies for screening, prevention and treatment of CVDs may likely reduce disease burden and health expenditure in Brazil. Central obesity measures were strongly associated with predicted CVD risk and might be useful in the clinical assessment of patients. Follow-up studies are warranted to validate our findings.

Keywords: cardiovascular risk (CVD), Framingham risk score (FRS), obesity, sociodemographic indicators, anthropometric markers

INTRODUCTION

Cardiovascular diseases (CVDs) have reached epidemic proportions worldwide, with a greater impact in low- and middle-income countries (LMICs), including Brazil (1). In 2016, approximately 17.9 million people died from CVDs globally, mostly due to heart attack and stroke. Over 75% of these deaths have taken place in LMICs (2). CVDs are the leading cause of death in Brazil and responsible for the highest healthcare expenditure for hospital admissions (3).

Most CVDs are caused by a complex interaction of several modifiable risk factors, including tobacco use, physical inactivity, unhealthy diet, overweight and obesity, harmful use of alcohol, hypertension, diabetes, and dyslipidaemia (1). Recently, Brazil has experienced a rapid demographic and economic transition, resulting in profound changes in nutritional and lifestyle patterns. Industrialization, urbanization, an aging population, and increased prevalence of unhealthy habits have become root causes of the rising CVD burden in Brazil (3). Amongst these risk factors, obesity is an increasing concern. According to 2016 estimates, around 22% of Brazilian adults aged $\geq$ 18 years and 9% of adolescents aged 10–19 years were obese (4).

Overweight and obesity have been regarded as one of the leading factors for mortality, accounting for around 23% of the ischaemic heart disease burden (5, 6). Several anthropometric measures of general and central obesity have been applied to assess adiposity-related risk, including body mass index (BMI), waist circumference (WC), waist-to-hip ratio (WHR), and waist-to-height ratio (WHtR) (5). However, previous studies have found conflicting results regarding the usefulness of these different anthropometric indices (7–9). Moreover, even though most of the global burden of CVDs is in developing countries, the existing evidence is derived mainly from high-income countries (10). Since adiposity is highly heterogeneous with age, gender, and ethnicity (11), it remains unclear which anthropometric parameters are better correlated with the risk of CVDs in different populations (12).

Evidence suggests that the risk of CVDs differs by race/ethnicity (13). In Brazil, although several studies were conducted for the prevalence of cardiovascular risk factors, most of them have limitations due to potential selection bias and the use of self-reported data in the absence of confirmatory laboratory examinations (14). Moreover, few studies have compared the independent associations between the different anthropometric indices and CVD risk based on the recommended cut-off values (15). Scarce information exists in Brazil about the risk of CVDs and sociodemographic and anthropometric characteristics of the population. Thus, in this cross-sectional, population-based study, we aimed to investigate CVD risk following the Framingham risk score and how it is related to socioeconomic and demographic characteristics. We also studied the association between some anthropometric markers, i.e., WC, BMI, WHR and WHtR, and the predicted risk of CVDs in both genders.

MATERIALS AND METHODS

Study Population

This cross-sectional study was carried out between August 2012 and January 2013 in the city of Pindoretama, located in the state of Ceara (CE), North-eastern Brazil. The recruitment and examination procedures have been discussed previously (16). According to the latest demographic census conducted in 2010, the total population of Pindoretama was approximately 18,683 inhabitants (17). The health registry list with the citizens' names in alphabetic order was applied to select the potential study subjects. Random numbers were generated with the statistical software R (18) and identified with the names in the list thereafter. The selected subjects were invited to participate in the survey by local Community Health Workers (CHW). Around 1,000 subjects were randomly selected based on the list. Of these, one hundred and sixty-three were not found by the CHW and, therefore, could not receive the invitation. Thirty-one subjects did not meet the inclusion criteria. Thus, eight hundred and six randomly selected subjects were invited, of whom 714 agreed to participate (a response rate of 88.6%).

Subjects of both genders, aged $\geq$ 20 years who were able to communicate and willing to participate in the study were considered eligible. Those with an acute or chronic severe cardiac, renal, or hepatic illness, as well as physically or mentally disabled subjects unable to follow simple questions and examinations were excluded, as were pregnant women. Since we aimed to assess the CVD risk, those with a previous history of myocardial infarction and/or stroke were considered as having had the condition, and therefore were excluded from the analyses (13 subjects). Seven hundred and one subjects remained. At the time of recruitment, the subjects were requested to visit a nearby health center after an overnight fast of 8–10 h. Pre-tested questionnaires were conducted by trained interviewers to collect sociodemographic and clinical information. Anthropometric measurements, blood pressure, and body fat percentage (BF%) were also registered.

Sample Size Calculation

The required sample size was calculated by the formula: $n = 4\ (z_{crit})^2\ p\ (1–p)/D^2$ (19). The total sample size was represented by "n", "z_{crit}" = 1.96 (Standard Normal Deviate for a Significance Criterion = 0.05 and a Confidence Interval = 0.95), "p" = 0.051 prevalence estimate from a previous study of high/intermediate risk of CVD according to the Framingham risk score (20), and "D" = 0.0454 (total width of the expected confidence interval). Two-tailed statistical analyses were used. Thus, $n = 4 \times (1.96)^2 \times 0.051 \times 0.949/(0.0454)^2$; $n = 360.83$.

Ethics

The study was conducted according to the ethical principles outlined in the Helsinki Declaration (21). The research protocol was approved by the local Ethical Committee in Brazil (Protocol Number: 045.06.12) and the Regional Committee for Medical and Health Research Ethics (REK) in Norway (Reference: 2012/779/REK sør-øst D). Written or verbal consent was sought from each subject prior to any investigation. The subjects were

informed of their right to withdraw from the study at any point or withhold their data from the analysis. Those who were diagnosed with any clinical condition were referred to the nearest health center for treatment and follow-up.

Measurements

Weight, height, WC, and hip circumference (HC) were taken with subjects standing without shoes and wearing light clothing. Body weight (kilograms) was registered to the nearest 0.1 kg using a portable digital scale, calibrated before use and checked every day with a known weight. Height (centimeters) was measured by applying a well-mounted stadiometer, with each subject standing upright with their head in the Frankfurt plane. BMI was calculated as the weight in kilograms divided by the square of the height in meters (kg/m^2). BF% was determined by a portable bipolar body fat analyser (Omron®, Model HBF-306, Omron Healthcare, Inc., Illinois, United States). WC was measured with a non-stretchable tape, positioned horizontally in the middle area between the lower border of the ribs and iliac crest, under the mid-axillary line. HC was assessed with the same tape positioned to the maximum circumference around the buttocks, with the subjects standing straight. WC and HC were recorded to the nearest 0.1 cm. WHR was calculated as the WC divided by the HC, while the WHtR as the WC divided by the height.

Blood pressure (mmHg) was estimated twice at a 10-min interval using a validated automatic sphygmomanometer (Omron® BP785 IntelliSense® Automatic Blood Pressure Monitor with ComFitTM Cuff, Omron Healthcare, Inc., Illinois, United States), with appropriate cuffs, in a sitting position after a resting time of at least 15 min. The mean of the two readings was used for the analysis.

Blood Sampling and Laboratory Assays

On arrival at the data collection center, a 10-mL fasting venous blood sample was taken to determine the concentrations of plasma glucose, insulin, and lipids. Two hours after a 75g oral glucose load, another 3 ml of venous blood was drawn for the oral glucose tolerance test (OGTT). Blood samples were stored immediately over ice and centrifuged after 1 h. Plasma was frozen and transported to the laboratory, where the samples were stored at −20 °C until the analyses were conducted. Quality control of the laboratory was assessed internally and externally.

The glucose oxidase method was applied to estimate fasting and 2-h plasma glucose levels, whereas fasting insulin was determined by chemiluminescence. Total cholesterol (TC) was assessed by the cholesterol oxidase - phenol + aminophenazone (CHOD-PAP) method, while a homogenous enzymatic colorimetric method was used to determine high-density lipoprotein cholesterol (HDL-C) levels. Triglycerides (TG) were assessed by the glycerol-3-phosphate oxidase - phenol + aminophenazone (GPO-PAP) method. The Friedewald Formula (22) was applied to calculate the low-density lipoprotein cholesterol (LDL-C) levels.

Definitions of Variables

The cut-off points for WHR recommended by the World Health Organization (WHO) were applied, i.e., for males, a WHR $\geq$ 0.90 was classified as "high" (substantially increased risk of metabolic complications), whereas, for females, a WHR $\geq$ 0.85 was considered "high". Overweight/obese was defined by a BMI of $\geq$ 25 kg/m^2. A high WC was described as $>$ 102 cm for males, and $>$ 88 cm for females (23). A cut-off of $\geq$ 0.50 was applied to define a high WHtR (24).

Following the definition of the Brazilian Institute of Geography and Statistics (IBGE) classification, ethnicity was assessed according to the subjects' self-perception of their skin color. The different ethnic groups were categorized into "white" and "non-white" (17). Physical activity information was ascertained by the International Physical Activity Questionnaire (IPAQ) short form (25). The IPAQ's total score was computed by summing up the duration and frequency of walking, moderate- and vigorous-intensity activities. Following the guidelines for data processing and analysis, the levels of physical activity were classified into "low", "moderate" and "high" (26). We further categorized them into "low" and "moderate" plus "high" level. Current smoking included those who self-reported as being smokers or had stopped smoking for less than 1 year. Alcohol consumption was ascertained by self-report as yes/no. The occupation of the subjects was categorized into manual and non-manual labor. Manual labor was used to describe jobs in agriculture and construction, whereas non-manual labor described all other occupations.

The 1999 WHO criteria (27) were applied in diagnosing diabetes mellitus. Diabetes cases were defined as those who had a previous diagnosis of type 2 diabetes, or those with fasting (venous) plasma glucose value $\geq$ 7.0 mmol/l ($\geq$ 126 mg/dl), or the 2-h plasma glucose value after a 75 g oral glucose load $\geq$ 11.1 mmol/l ($\geq$ 200 mg/dl), or both. Dyslipidaemia was defined as TG $\geq$ 1.7 mmol/l and HDL $<$ 0.9 mmol/l for males; and $<$ 1.0 mmol/l for females (27). Insulin resistance was estimated by the homeostasis model assessment of insulin resistance (HOMA-IR = [insulin (mU/l) $\times$ glucose (mmol/l)] / 22.5) (28). Hypertension was defined as systolic blood pressure (SBP) $\geq$ 140 mmHg and/or diastolic blood pressure (DBP) $\geq$ 90 mmHg and/or being on blood-pressure-lowering medication (29).

Estimating the Framingham Risk Score

The Framingham 10-year risk score model, as published by D'Agostino et al. (30) in 2008, was applied to estimate the predicted 10-year risk for an incident cardiovascular event. The model predictors included age, gender, SBP, use of antihypertensive medication, TC, HDL-C, smoking and diabetes status (30). Subjects with a Framingham predicted risk of 10% or above during the next 10 years were defined as having high CVD risk. Although data were collected from 701 subjects in total, the Framingham risk score was estimated for 693 subjects owing to missing values (229 males and 464 females). In **Figures 1**, **2**, based on D'Agostino et al. (30) and Chang et al. (31), we presented the mean Framingham risk score estimates for the age range 30–74 years.

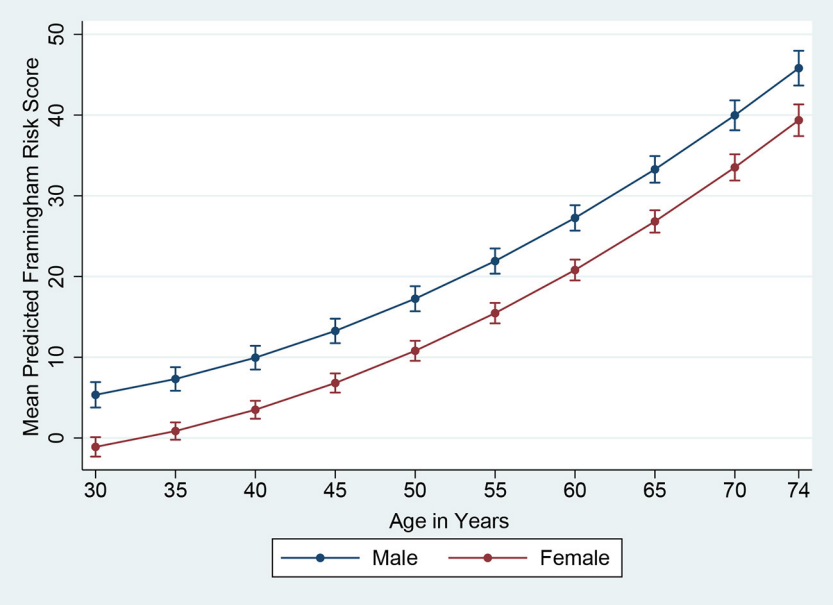

FIGURE 1 | Framingham Risk Score by age and gender (vertical lines are means with 95% CIs).

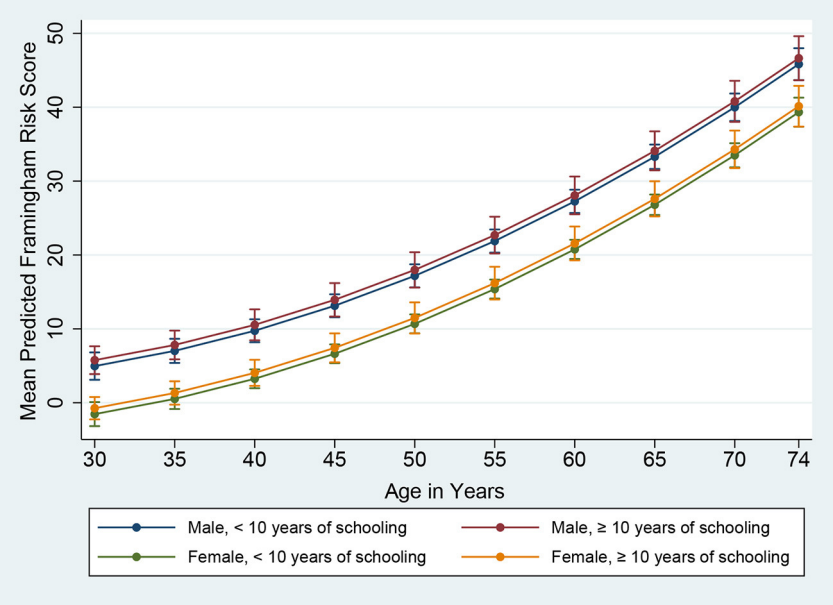

FIGURE 2 | Framingham Risk Score by age, gender, and education (vertical lines are means with 95% CIs).

Statistical Analysis

Continuous data were expressed as means and 95% confidence intervals (CIs), while percentages and 95% CIs were given for categorical variables. Generalized linear regression models (GLM) were fitted to the data after adjusting for age. To compare differences between adjusted means, we fitted GLMs with linear link function, while GLMs with the logit link function were applied to compare differences between proportions. The prevalence of those with a predicted 10-year CVD risk of $\geq$ 10% was calculated as predictive margins, based on the estimation of the adjusted logistic regression models. To control confounding by age in the predicted means and prevalence, we fixed age at 45 years, which was the closest to the mean age in the sample. A two-sample test of proportions was applied to compare the

prevalence of high CVD among the different sociodemographic groups. Anthropometric measurements were converted to z-scores [original value subtracted by the mean and divided by the standard deviation (SD)] to represent the number of SDs above and below the mean for each subject. Multiple linear regression was carried out to investigate the relationship between the standardized anthropometric markers and CVD risk. Further, we calculated crude and adjusted prevalence ratios (PRs) of the anthropometric indicators for detecting high CVD risk using Poisson regression with robust variance, as the prevalence of high CVD risk was above 10%. We tested for two-way interaction between age and the different anthropometric markers. The Akaike Information Criteria (AIC) was used to compare nested models. Data were analyzed using Stata 15th edition (32) and SPSS 26th version (33) statistical software. The results were considered statistically significant with $p < 0.05$, and all tests were two-sided.

RESULTS

A total of 701 subjects (234 males and 467 females, mean age 44.8 $\pm$ 16.0 SD) were included in the analysis. Significant differences were found in sociodemographic, lifestyle, anthropometric and cardiometabolic characteristics between the genders (**Table 1**). Males had a significantly higher proportion of tobacco smoking and alcohol consumption. Females were more physically inactive and showed a higher percentage of overweight/obesity. Age, dyslipidaemia, hypertension, and diabetes status did not differ between the genders. Anthropometric parameters including mean HC, WHtR, BMI and BF% were higher in females, while males had a higher WHR.

The mean predicted Framingham risk score increased substantially with age and was higher among males (**Figure 1**). In addition, the mean predicted risk was not statistically significant between different levels of education in both genders (**Figure 2**).

As shown in **Table 2**, the estimated proportion with a predicted 10-year CVD risk of $\geq$ 10% was significantly higher among males (31.9 vs. 12.5%; p-value: < 0.001), and those with more than 45 years of age (68.9 vs. 4.2%; p-value: < 0.001). Furthermore, it was significantly lower among those with an occupation requiring manual labor (7.6 vs. 21.7%; p-value: 0.008), defined as jobs in agriculture and construction.

Multiple linear regression was carried out to assess the age-adjusted associations between 1 SD increment in each anthropometric marker and the predict risk of CVDs, entered as a continuous variable (**Table 3**). In males, only WHtR was a significant predictor of CVD risk followed by a borderline-significant association for WC, while in females WHR and WHtR were statistically significant. WHtR showed the highest slope coefficient in males, whereas WHR had the highest slope in females.

Table 4 presents the PRs of the different anthropometric measures for identifying a high CVD risk. Univariable and multivariable Poisson regression analyses with robust variance were applied. Adjusted PRs were obtained after controlling for age, level of physical activity, family history of cardiac disease and stroke. An interaction term between age and the corresponding anthropometric parameter was included in some adjusted models, according to their statistical significance and the Akaike Information Criteria (AIC). In females, significant positive associations were found between all anthropometric variables and high CVD risk in the adjusted models, except for WC. In males, all anthropometric markers were significant. WHtR had the highest adjusted PR for males (9.9, 95% CI: 2.8–34.8, p-value < 0.001) and females (43.4, 95% CI: 2.6–716.8, p-value 0.002). This large PR and wide CI in the adjusted model for females and WHtR was due to the few observations of those with high CVD risk and WHtR < 0.50 (6 subjects).

DISCUSSION

To the best of our knowledge, this is one of the few population-based studies from Brazil to investigate the CVD risk by sociodemographic characteristics, as well as the association between different obesity markers and the risk of a cardiovascular event. Males and older people presented higher risk of CVDs in our population, whereas those employed in the manual labor had significantly lower risk. Central obesity measures were more strongly associated with CVD risk than BMI.

We found a high prevalence of increased CVD risk, i.e., Framingham risk score $\geq$ 10%, in this population. Our estimates were higher than those reported in Peru (34), Argentina (35) and Southern Brazil (20, 36), similar to India (37), but lower than Honduras (38) and China (39). These differences might be explained by genetic, racial, sociodemographic, and cultural diversity, as well as the use of other versions of the Framingham risk score, with a varied set of predictors. In our sample, the prevalence of diabetes, smoking, hypertension, and dyslipidaemia was higher than reported in some other Brazilian surveys (3). The recent rapid industrialization and urbanization of Pindoretama (the rural population decreased from 66% to 39% between 1991 and 2010) (40), resulting in lifestyle and dietary changes, might explain the frequent occurrence of these cardiovascular risk factors and subsequent high Framingham risk score in the studied population.

Consistent with previous research (35, 37, 41, 42), males had a higher Framingham risk score than females. This might be due to the significantly higher SBP, and tobacco use among males. As expected, the Framingham risk score increased significantly with age, which is also in line with other studies (36, 41). The subjects employed in in agriculture or construction showed lower CVD risk, possibly reflecting the protective effect of physical activity (1). After controlling for age and gender, among those employed in manual labor, about 60.4% presented a moderate to high level of physical activity, whereas only 30.5% of those in other employment categories were similarly active (data not shown). On the other hand, the CVD risk did not differ significantly among the ethnic groups. This might be explained by the mixed genetic composition of the Brazilian population, essentially formed by an admixture of native Brazilians, Europeans, and Africans (43). It is likely that the extensive miscegenation of the overall Brazilian population may have reduced the differences among the ethnic groups. Although other studies have found an inverse relationship between CVD risk and education, our data did not find significant results. The relationship between education and risk of CVDs has shown great variability across

TABLE 1 | Baseline characteristics of the study subjects.

Characteristics	All ($n = 701$)	Males($n = 234$)	Females ($n = 467$)	p–value*
Age (years)	44.8 (43.6–46.0)	45.6 (43.6–47.7)	44.4 (42.9–45.8)	0.319
Ethnicity (%)				
White	16.6% (13.8–19.3)	10.7% (6.7–14.6)	19.5% (15.9–23.1)	0.003
Non–white	83.4% (80.7–86.2)	89.3% (85.4–93.3)	80.5% (76.9–84.1)	
Education (%)				
<10 years	79.9 (76.0–83.8)	84.7 (79.7–89.7)	77.5 (72.8–82.2)	0.024
≥10 years	20.1 (16.2–24.0)	15.3 (10.3–20.3)	22.5 (17.8–27.2)	
Monthly Income (%)				
<2MW	90.2 (88.0–92.4)	80.6 (75.5–85.7)	95.0 (93.0–96.9)	<0.001
≥2MW	9.8 (7.6–12.0)	19.4 (14.3–24.5)	5.0 (3.1–7.0)	
Manual Labor (%) **	9.5 (7.5–11.4)	27.5 (21.7–33.3)	0.4 (−0.2 – 1.0)	<0.001
Currently Married (%)	66.7 (63.3–70.2)	74.3 (68.8–79.9)	62.9 (58.5–67.3)	0.003
Smoking (yes) (%) ***	38.8 (35.0–42.7)	48.6 (41.7–55.5)	33.9 (29.3–38.5)	<0.001
Alcohol Consumption (yes)	35.1 (31.5–38.6)	54.2 (47.5–61.0)	25.4 (21.3–29.6)	<0.001
Physical Activity (%)				
Low	66.8 (63.4–70.3)	55.4 (49.0–61.8)	72.6 (68.5–76.6)	<0.001
Moderate/High	33.2 (29.7–36.6)	44.6 (38.2–51.0)	27.4 (23.4–31.5)	
WC (cm)	90.1 (89.2–91.0)	89.6 (88.0–91.2)	90.4 (89.3–91.5)	0.415
HC (cm)	98.6 (97.9–99.4)	95.7 (94.4–97.0)	100.1 (99.2–101.0)	<0.001
WHR, mean	0.92 (0.91–0.92)	0.94 (0.93–0.95)	0.90 (0.89–0.91)	<0.001
WHtR, mean	0.57 (0.56–0.58)	0.54 (0.53–0.55)	0.59 (0.58–0.60)	<0.001
BMI (kg/m^2)	26.9 (26.5–27.3)	25.9 (25.3–26.6)	27.4 (26.9–27.8)	<0.001
Overweight/Obese (%)	61.9 (58.4–65.5)	53.4 (47.0–59.8)	66.2 (61.9–70.5)	0.001
BF%, mean	32.8 (32.3–33.4)	24.8 (23.9–25.7)	36.9 (36.2–37.5)	<0.001
SBP (mmHg)	127.6 (126.2–129.0)	132.7 (130.3–135.1)	125.1 (123.4–126.8)	<0.001
DBP (mmHg)	76.8 (75.6–78.1)	77.7 (75.5–79.9)	76.4 (74.8–77.9)	0.326
Hypertension (%)	29.8 (25.8–33.8)	29.3 (22.6–36.1)	30.1 (25.2–34.9)	0.863
Diabetes (%)	14.3 (11.6–17.0)	11.7 (7.6–15.8)	15.6 (12.2–19.0)	0.159
HOMA–IR	1.6 (1.5–1.7)	1.3 (1.1–1.5)	1.8 (1.7–1.9)	<0.001
Total Cholesterol (mmol/l)	4.72 (4.65–4.79)	4.62 (4.50–4.75)	4.76 (4.68–4.85)	0.069
HDL (mmol/l)	1.22 (1.21–1.23)	1.23 (1.21–1.24)	1.22 (1.21–1.23)	0.475
LDL (mmol/l)	2.86 (2.80–2.93)	2.74 (2.63–2.86)	2.92 (2.84–3.00)	0.014
Triglycerides (mmol/l)	1.54 (1.41–1.67)	1.75 (1.52–1.97)	1.44 (1.28–1.60)	0.031
Dyslipidaemia (%)	24.8 (21.6–28.0)	24.2 (18.7–29.8)	25.0 (21.1–29.0)	0.817

*Data are mean (95% confidence intervals) or percentage (95% confidence intervals). Model was evaluated at age 45 years; *p-value for the difference between males and females. **Manual Labor = refers to jobs in agriculture and construction; ***Included those who self-reported as being smokers or had stopped smoking for less than 1 year. BF%, Body Fat Percentage; BMI, Body Mass Index; DBP, Diastolic Blood Pressure; HC, Hip Circumference; HDL-C, High-Density Lipoprotein Cholesterol; HOMA-IR, Homeostasis Model Assessment of Insulin Resistance; LDL-C, Low-Density Lipoprotein Cholesterol; MW, Minimum Wage in 2012; SBP, Systolic Blood Pressure; WC, Waist Circumference; WHtR, Waist-to-Height Ratio; WHR, Waist-to-Hip Ratio.*

populations, depending particularly on the level of health transition and socio-economic development of the country (31, 44). In 2010, the illiteracy rate in Pindoretama among those aged 15 and older was approximately 22%, whereas in Brazil it was 10% (40). In our data, only 4% of the subjects had a university degree or higher (data not shown). Therefore, it is likely that the lack of significant association between education and CVD risk might be due to the overall low level of education in our sample.

We found that the adjusted PR for WHR and WHtR were the highest among the anthropometric indices in relation to increased CVD risk. Further, the association between the WHR and Framingham risk score entered as a continuous variable was higher than that of WC, BMI and WHtR in females, whereas the slope coefficient of WHtR was the highest in males followed by WC. These results may indicate that these central obesity measures were more predictive of CVD risk than the general obesity measure like BMI. Therefore, in line with several others (5, 10), our findings suggest that BMI alone is insufficient to account for the association between CVD risk and obesity in this population. Over recent years, accumulating evidence has shown that abdominal obesity is more strongly associated with metabolic and cardiovascular problems than total adiposity (10, 45). Even within normal ranges of BMI, high visceral fat deposition remains an independent cardiovascular risk factor

TABLE 2 | Predicted proportions of subjects with 10-year CVD risk of ≥10% using the Framingham Risk Score by sociodemographic characteristics.

Characteristics	n	Predicted 10–year risk ≥10% % (95% CIs)	*p*–value
Overall	693*	18.9 (14.3–23.6)	
Gender			
Male	229	31.9 (21.8–42.0)	< 0.001
Female	464	12.5 (8.0–17.0)	
Age groups			
<45 years	388	4.2 (2.2–6.2)	< 0.001
≥45 years	305	68.9 (63.8–74.0)	
Ethnicity			
White	116	20.8 (9.4–32.2)	0.58
Non-white	577	18.6 (13.7–23.5)	
Education			
<10 years	489	19.2 (14.1–24.4)	0.60
≥10 years	204	17.5 (7.4–27.7)	
Monthly income			
<2MW	623	17.7 (12.8–22.7)	0.11
≥2MW	68	25.6 (11.4–39.9)	
Occupation**			
Non-manual labor	629	21.7 (16.4–27.1)	0.008
Manual labor	64	7.6 (1.3–13.9)	

*Data are percentage (95% confidence intervals), adjusted for age (at age fixed to 45 years) and gender. *The study collected data from 701 subjects in total, but due to some missing values, the Framingham Risk Score was calculated for 693 subjects (229 males and 464 females). **Manual Labor: jobs in agriculture and construction. Non-manual Labor: other occupations. CIs: Confidence Intervals. CVD: Cardiovascular Disease. MW: Minimum Wage in 2012.*

TABLE 3 | Association between 1 SD increase in anthropometric markers and CVD risk, using the Framingham Risk Score, age adjusted.

Characteristics	Slope coefficient (β) (95% CIs)	*p*–value*	R square
Males			
WC (per 1 SD)	1.67 (−0.01–3.35)	0.05	0.6754
BMI (per 1 SD)	1.56 (−0.06–3.19)	0.06	0.6750
WHR (per 1 SD)	1.30 (−0.48–3.09)	0.15	0.6728
WHtR (per 1 SD)	1.82 (0.09–3.56)	0.04	0.6760
Females			
WC (per 1 SD)	0.78 (−0.12–1.69)	0.09	0.5811
BMI (per 1 SD)	0.60 (−0.27–1.48)	0.18	0.5801
WHR (per 1 SD)	1.13 (0.14–2.11)	0.03	0.5830
WHtR (per 1 SD)	0.95 (0.01–1.89)	0.04	0.5820

**p-value for each predictor in the regression model, controlling for age. BMI, Body Mass Index; Cis, Confidence Intervals; CVD, Cardiovascular Disease; SD, Standard Deviation; WC, Waist Circumference; WHR, Waist-to-Hip Ratio; WHtR, Waist-to-Height Ratio.*

(45). Although BMI is strongly correlated with gold standard body fat measures, it cannot distinguish between lean and fat mass and does not delineate body fat distribution patterns (46). Whilst BMI would not account for an increase in muscle or fat-free mass, this would be reflected in the central obesity measures (5). Accumulation of visceral fat is related to insulin resistance, increased systemic inflammation, accelerated progression of atherosclerosis and endothelial dysfunction, which contribute to CVD risk (5, 45). This might explain the stronger association between abdominal obesity measures and CVD risk reported in our study. Generally, measures of obesity are not included in the prediction of CVD risk (5). Considering our findings, it might be beneficial to incorporate central obesity indicators such as WHR and WHtR into the clinical assessment of CVD risk.

Some studies have identified WC as the most highly correlated marker with CVD risk factors compared with other central obesity measures and BMI in females (47). Nevertheless, another cross-sectional study from Brazil including 270 women also reported that WHR showed a greater performance than WC in discriminating high coronary risk (15). Although WHR is more difficult to measure than WC, it has been considered a more specific surrogate for fat distribution, presents high precision and no bias over several ethnic groups (5). Our study showed a strong association between WHtR and CVD risk. Contrary to our results, a systematic review and meta-analysis reported that WHtR had the weakest association with CVD risk factors, compared with BMI and other measures of central obesity (47). However, other studies showed an opposite scenario in which WHtR was the most highly correlated obesity marker with CVD risk (5). Compared to WC, studies in different populations have described WHtR as a more sensitive indicator, possibly due to the adjustment to different statures and negative correlation of height to some metabolic risk factors (48).

This study contributes to the limited body of evidence from Brazil on CVD risk and sociodemographic characteristics, as well as on the association between different obesity measures and the risk of CVDs. The subjects were randomly selected, and the participation rate was high. The survey was performed by trained personnel and pre-tested questionnaires were applied. To minimize the risk of misclassification errors due to poor recall, anthropometric parameters were carefully assessed, and no self-reported measures were used. Blood samples were collected, handled, and transported according to standard protocols. Quality control of the laboratory was assessed internally and externally.

Our study had some limitations. It was based on a cross-sectional design and the 10-year CVD risk was calculated instead of using prospective CVD events. Nevertheless, the study generated valuable epidemiological data from Brazil regarding the association between CVD risk, obesity indicators and sociodemographic characteristics. Considering that Brazil is a large country with marked socioeconomic, ethnic, and regional disparities, our findings may not be representative for the whole nation. Caution should be taken when generalizing the results. However, since Brazilians have a mixed background, our sample might be a fair representation of the country's population. Furthermore, we had an overrepresentation of females (females 467 vs. males 234). As previously mentioned, out of 1,000 names randomly selected from the healthy registry list, around 163 subjects were not found by the CHW and therefore could not be invited to participate in the study. Out of these 163, approximately 78% were males. Additionally, among 92 subjects

TABLE 4 | Crude and adjusted prevalence ratios (PRs) of anthropometric indices for identifying high CVD risk (≥10% using the Framingham Risk Score).

Characteristics	Crude PR[a] (95% CIs)	*p*–value	Adjusted PR[b] (95% CIs)	*p*–value
Males				
WC (>102 cm)[c]	1.9 (1.4–2.5)	<0.001	7.5 (2.1–27.0)	0.002
BMI (≥25 kg/m^2)[c]	1.2 (0.8–1.6)	0.366	4.9 (1.6–14.9)	0.005
WHR (≥0.90)[c]	2.7 (1.7–4.2)	<0.001	8.7 (2.4–31.5)	0.001
WHtR (≥0.50)[c]	2.3 (1.4–3.7)	0.001	9.9 (2.8–34.8)	<0.001
Females				
WC (> 88 cm)	1.7 (1.2–2.3)	0.001	1.3 (1.0–1.7)	0.087
BMI (≥25 kg/m^2)	1.1 (0.8–1.5)	0.565	1.4 (1.1–1.9)	0.008
WHR (≥0.85)[c]	4.1 (2.4–7.3)	<0.001	11.0 (2.8–43.6)	0.001
WHtR (≥0.50)[c]	3.5 (1.6–7.6)	0.002	43.4 (2.6–716.8)	0.008

[a]*Crude prevalence ratio after univariable Poisson regression analysis.* [b]*Adjusted prevalence ratios for age, level of physical activity, family history of cardiac disease and stroke.* [c]*An interaction term between each anthropometric marker and age was included in the adjusted models. The Akaike Information Criteria (AIC) was used to compare nested models. BMI, Body Mass Index; Cis, Confidence Intervals; CVD, Cardiovascular Disease; WC, Waist Circumference; WHR, Waist-to-Hip Ratio; WHtR, Waist-to-Height Ratio.*

who refused to participate, around 63% were males. Population-based studies conducted during the day may constitute a hindrance to male participation. Males are often involved in income-generating work and therefore may not have been able to participate in the survey. The overrepresentation of females was dealt with by adjusting the analyses for gender or stratifying by gender. The Framingham risk score was not recalibrated for our population, which might have introduced some uncertainty in the CVD risk estimation. However, this was beyond the scope of the study and our available resources. Furthermore, the Framingham risk score has been widely applied and validated in ethnically diverse cohorts including whites, blacks, Native Americans, and Hispanics (49).

A high risk of CVDs according to the Framingham risk score was found in this population, especially among males and older people. In addition, manual labor seems to provide a protective effect on CVD risk. A timely targeted investment in screening, prevention, and necessary treatment of CVDs could reduce the burden on many and reduce the pressure on the health budget. Central obesity measures are more strongly associated with CVD risk than general obesity indicators. Our data suggest that WHR and WHtR are the best anthropometric markers to identify high CVD risk. Since an increase in muscle mass might not lead to changes in BMI (5), central obesity markers might be more useful to evaluate the effect of lifestyle changes in relation to CVD risk. Therefore, measuring WHR or WHtR might be beneficial in the clinical assessment of CVD risk. Prospective studies are still needed to further elucidate future risk of CVDs and their relationship with obesity in Brazil.

DATA AVAILABILITY STATEMENT

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

ETHICS STATEMENT

The studies involving human participants were reviewed and approved by the Local Ethical Committee in Brazil (Protocol Number: 045.06.12) and the Regional Committee for Medical and Health Research Ethics (REK) in Norway (Reference: 2012/779/REK sør-øst D). The patients/participants provided their written informed consent to participate in this study. In case of illiteracy, verbal consent was assured by a local witness, who signed the informed consent, to secure the free participation of the subjects.

AUTHOR CONTRIBUTIONS

NM contributed to the conceptualisation of research goals and design, drafting of the article, as well as acquisition, analysis, and interpretation of the data. AH contributed to the study concept and design, data analyses, writing the initial draft and revising it critically, study oversight, and leadership. BB participated in the analysis and interpretation of data, writing the article, and revising it critically. IM took part in conceptualizing the study aims and design, writing the initial draft and revising it critically, as well as organizing the database, and conducting the statistical analysis. TS contributed to the design of the study, data curation and analyses, and drafting of the article. VF contributed to conceptualizing the work and designing the methodology, data collection and analyses, writing the initial draft, and revising it critically. RM contributed to the study concept and design, data analyses, study management and coordination, as well as writing the initial draft, and revising it critically. HM contributed to the design of the methodology, data analyses, study oversight and leadership, writing the initial draft, and performing critical review. All authors read and approved the content of the final manuscript. Furthermore, they agreed to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved.

FUNDING

This study was funded by the Ivar Helles Foundation, and the Health Department of the city of Pindoretama-CE.

ACKNOWLEDGMENTS

We acknowledge the valuable contribution of our survey team members, lab technicians and community health workers for their continuous effort in the data collection. We are grateful to all subjects in the study for their active cooperation. We especially appreciate the logistic and financial support of the Health Department of the city of Pindoretama-CE, particularly the Secretary of Health, Valéria Maria Viana Barbosa. We thank Mr. Phil Riley, a native English speaker, and language expert, for editing the manuscript.

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Conflict of Interest: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.