Diplomarbeit

Cardiovascular risk factors and blood pressure monitoring in South African adolescents.

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Graz, am 21.09.2020 *Fabian Rainer Klosz eh*

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Zusammenfassung

Hintergrund: Übergewicht ist eine der wichtigsten Ursachen für den Anstieg von Herz-Kreislauf-Erkrankungen. Mittlerweile sind auch viele Kinder und Jugendliche von Übergewicht und Adipositas betroffen. Das metabolische Syndrom beschreibt eine Häufung von kardiometabolischen Risikofaktoren. Betroffene Personen haben ein deutlich erhöhtes Risiko für Herz-Kreislauf-Erkrankungen oder Diabetes Mellitus. Darum ist die Identifikation von gefährdeten Personen besonders wichtig, um geeignete Präventionsmaßnahmen und Therapien einleiten zu können.
Zielsetzung: Wir untersuchten die Prävalenz von kardiometabolischen Risikofaktoren in 13-16-jährigen Jugendlichen in Mthatha, Südafrika.
Methoden: 244 Jugendliche wurden in diese Studie aufgenommen. Anthropometrische Daten wurden erhoben. Der systolische und diastolische Blutdruck wurde gemessen. Blutabnahmen wurden durchgeführt und Nüchternglukose, Triglyceride und HDL-Cholesterin analysiert. Nach dem Ausschluss von 8 Teilnehmern wurde die statistische Analyse für 236 Teilnehmer durchgeführt.
Ergebnisse: Übergewicht und Adipositas zeigten sich in der Studienpopulation hochverbreitet, 30,5% der Teilnehmer wurden als übergewichtig/adipös klassifiziert. 37,7% zeigten hochnormalen oder erhöhten Blutdruck. In der übergewichtigen/adipösen Gruppe waren hochnormaler Blutdruck und Hypertension mit 61,1% hochprävalent und signifikant häufiger als bei untergewichtigen und normalgewichtigen Proband*innen. Jedoch wurde auch bei den normalgewichtigen Proband*innen eine Prävalenz von 27,3% beobachtet. Beinahe die Hälfte der Kohorte (45%) zeigte zumindest einen Risikofaktor. Es zeigte sich ein Clustering von Risikofaktoren, wobei die übergewichtigen/adipösen Proband*innen mehr Risikofaktoren aufwiesen. In einer Subgruppe mit 94 Proband*innen wurde das metabolische Syndrom untersucht. Hier zeigte sich eine Prävalenz von 7,4%, wobei die Prävalenz bei Proband*innen mit Übergewicht/Adipositas bei 26,1% lag.
Schlussfolgerung: Kardiometabolische Risikofaktoren haben eine hohe Prävalenz in der untersuchten Population. Die Jugendlichen haben dadurch ein hohes Risiko zukünftig an Herz-Kreislauf-Erkrankungen zu erkranken. Weitere Untersuchungen auf diesem Gebiet sind notwendig und Präventionsstrategien sollten etabliert werden, um dieses wachsende Problem zu adressieren.

Abstract

Background: Overweight is major factor contributing to the rise of cardiovascular diseases (CVDs). This has also been shown to be prevalent in children and adolescents. The metabolic syndrome (MetS) describes a clustering of cardiometabolic risk factors and individuals classified with MetS have a critically increased risk of suffering cardiovascular events or develop diabetes mellitus (DM). Therefore, identification of individuals at risk, developing prevention programs and initiating therapies is crucial.

Aims: We examined the prevalence of MetS and cardiometabolic risk factors in 13-16-year old adolescents in Mthatha, South Africa.

Methods: 244 adolescents were included in this study. Anthropometric measurements were performed. Systolic and diastolic blood pressure was measured. Blood samples were drawn and fasting glucose (FG), triglycerides (TGs) and high-density lipoprotein cholesterol (HDL-C) were measured. After exclusion of 8 participants, statistical analysis was conducted for 236 participants.

Results: Overweight and obesity (O/O) was highly prevalent in the study population with 30.5% being classified as O/O. 37.7% of were classified as pre-hypertensive (pre-HT) or hypertensive (HT). In the O/O group, pre-HT/HT was highly prevalent with 61.1%, thus being significantly higher as compared to the lean group. Nonetheless, also healthy weight participants showed a prevalence of 27.3%. Risk factor clustering was present, with nearly half (45%) scoring at least one out of four risk factors (RFs). Participants with O/O showed more RFs than lean participants. In a subgroup of 94 participants MetS showed a prevalence of 7.4%, while in O/O it was 26.1%.

Conclusion: Cardiometabolic risk factors have a high prevalence in South African adolescents, thus putting them at a high risk for CVDs in later life. More research in this field is indicated, and prevention strategies to tackle the growing burden of disease should be established.

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Abbreviations

CVDs: Cardiovascular diseases

MetS: Metabolic syndrome

DM: Diabetes mellitus

FG: Fasting glucose

TGs: Triglycerides

HDL-C: High-density lipoprotein cholesterol

O/O: Overweight and obesity

pre-HT: pre-hypertension

HT: Hypertension

RF(s): Risk factor(s)

NO: Nitric oxide

WHO: World Health Organization

T2DM: Type 2 diabetes mellitus

SSA: Sub-Saharan Africa

SA: South Africa

BMI: Body Mass Index

SD: Standard deviation

IOTF: International Obesity Task Force

MHO: Metabolically healthy obesity

VAT: Visceral adipose tissue

IR: Insulin resistance

WC: Waist circumference

NC: Neck circumference

WHtR: Waist-to-height-ratio

BP: Blood pressure

SBP: Systolic blood pressure

DBP: Diastolic blood pressure

MAP: Mean arterial blood pressure

CO: Cardiac output

TPR: Total peripheral resistance

SV: Stroke volume

RAAS: Renin-Angiotensin-Aldosteron-System

ED: Endothelial dysfunction

ROS: Reactive oxygen species

CEs: Cholesterol esters

PLs: Phospholipids

LPL: Lipoprotein-lipase

FFAs: Free fatty acids

VLDL: Very-low-density lipoprotein

IDL: Intermediate-density lipoprotein

LDL: Low-density lipoprotein

LDL-C: Low-density lipoprotein cholesterol

GLUT-4: Glucose transporter type 4

OGTT: Oral glucose tolerance test

IGT: Impaired glucose tolerance

IFG: Impaired fasting glucose

TNF-α: Tumor necrosis factor alpha

IL-6: Interleukin 6

NAFLD: Non-alcoholic fatty liver disease

OSAS: Obstructive sleep apnea syndrome

MUG: Medical University of Graz

WSU: Walter Sisulu University

WHR: Waist-to-hip-ratio

ANOVA: Analysis of variance

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1 Introduction

1.1 The Cardiovascular System

1.1.1 Overview

The cardiovascular System consists of the heart and the vasculature and enables the circulation of the blood. Its main task is to ensure the circulation of oxygen and nutrients, and the transport of metabolites, thermal energy, and chemical signals. The central organ of the cardiovascular system is the heart, which can be separated into two halves and pumps the blood into two circulatory systems. The right heart into the pulmonary circulation, where the blood is oxygenated before it returns to the left heart and enters the systemic circulation. Here it will exchange the nutrients and the wastes in the peripheral tissues. The arterial system is a high-pressure system, while the venous system has low pressure, but has a high capacity of storing the blood. The adequate function of the heart and vasculature is necessary to maintain blood pressure and keep the supply of the organs stable. In the following chapters, the anatomy of this components as well as the physiological principles will be elaborated on shortly (1).

1.1.2 The Heart

The heart is a muscular organ, located in the mediastinum. It is divided into the left and right heart, which simultaneously pump blood into the two circulation systems. Each halve contains an atrium and a chamber. Valves that prevent reverse blood flow are located between each atrium and chamber, as well as between the chambers and the aorta and the pulmonary trunk, respectively. The wall of the heart consists of three layers: The innermost is known as the *endocardium*. The middle layer, the *myocardium* consists of the heart muscle cells and is much thicker in the left heart. The *epicardium* is the outermost layer and contains the coronary arteries, which originate in the aorta and are essential for the supply of the heart with nutrients and oxygen. It is embedded in the pericardium, which functions as a protective layer (2).

1.1.3 Vasculature

The vasculature ensures the blood flow from the heart to peripheral tissues and back. Blood vessels can be classified by their structural characteristics into arteries, arterioles, capillaries, venules, and veins. They consist of up to three distinct layers, which can be differentiated histologically:

The *tunica intima* is the innermost layer and consists of endothelial cells. Depending on tissue type, the permeability of the endothelium differs: While the cells in the brain have little to no permeability, forming the so known blood brain barrier, the endothelium of hepatic or splenic vessels has very loose connections and enables the transit between the blood and tissue. The endothelium plays a key role in the vasomotoric, as vasodilatators like nitric oxide (NO) are produced in the endothelial cells.

The *tunica media* is the muscular layer of the vessels and conists of smooth muscle cells and elastic fibres. The big arteries near the heart, especially the aorta, contain a lot of elastic tissue and form a reservoir that smoothens the shape of the arterial pulse emitted by the heart. This is known as the Windkessel effect. The arterioles have less elastic tissue and contribute mostly to BP regulation. Compared to veins and venoles, arteries and arterioles have a thicker tunica media. The microvasculature, which includes small arterioles, cappilaries and venoles, only has a very thin media, or none at all. This simplifies the diffusion of nutrients and oxygen that takes place in the capillary system.

The *tunica adventitia* is the outermost layer and consists of connective tissue to stabilize the vessel. It also contains the vasa vasorum, small arteries and veins that supply the bigger vessels they surround (3).

1.1.4 Blood

Blood is a suspension of cells, organic and inorganic substances, and plasma. In healthy adults, blood makes up approximately 8% of the body weight, which corresponds to 5-6 liters in average males and 4-5 liters in women. Around 55% of blood is attributed to plasma. It consists of 90% water, and contains proteins, hormones, antibodies, enzymes, inorganic and organic substances, and the respiratory gases. Its organic components include lipids and glucose. The other 45% are the blood cells. The primary component of cellular blood are erythrocytes, which transport oxygen. White blood cells, leucocytes, are a key component of the immune system. Thrombocytes play a crucial part in hemostasis, and are essential for the integrity of the vascular system after injury (4).

1.2 Cardiovascular Diseases

Cardiovascular diseases are a group of illnesses affecting the heart or vasculature. This category includes a variety of different pathologies, such as hypertensive or rheumatic heart disease, heart failure, atrial fibrillation or aneurysms (5). Vascular damage can lead to peripheral, coronary, and cerebrovascular artery disease, decreasing the blood supply to the affected region. This can cause acute cardiovascular events, such as myocardial infarction or strokes, which are the cause for 85% of CVD-related deaths (5, 6).

CVDs are the leading cause of death worldwide, taking an estimated 17.9 million lives each year (7) (**Figure 1**).

More than three quarters of the deaths caused by CVDs happen in low- and middle-income countries (6). To tackle this, the World Health Organization (WHO) developed the *"Global action plan for the prevention and control of NCDs 2013-2020"* (8). It names nine global targets, with the goal to reduce deaths caused by noncommunicable diseases (NCDs) by 25% before 2025. One aim is to improve access to drug therapy and counseling, to prevent heart attacks and strokes, another is to reduce global hypertension. These are directly aimed at the prevention and control of CVDs (6, 8).

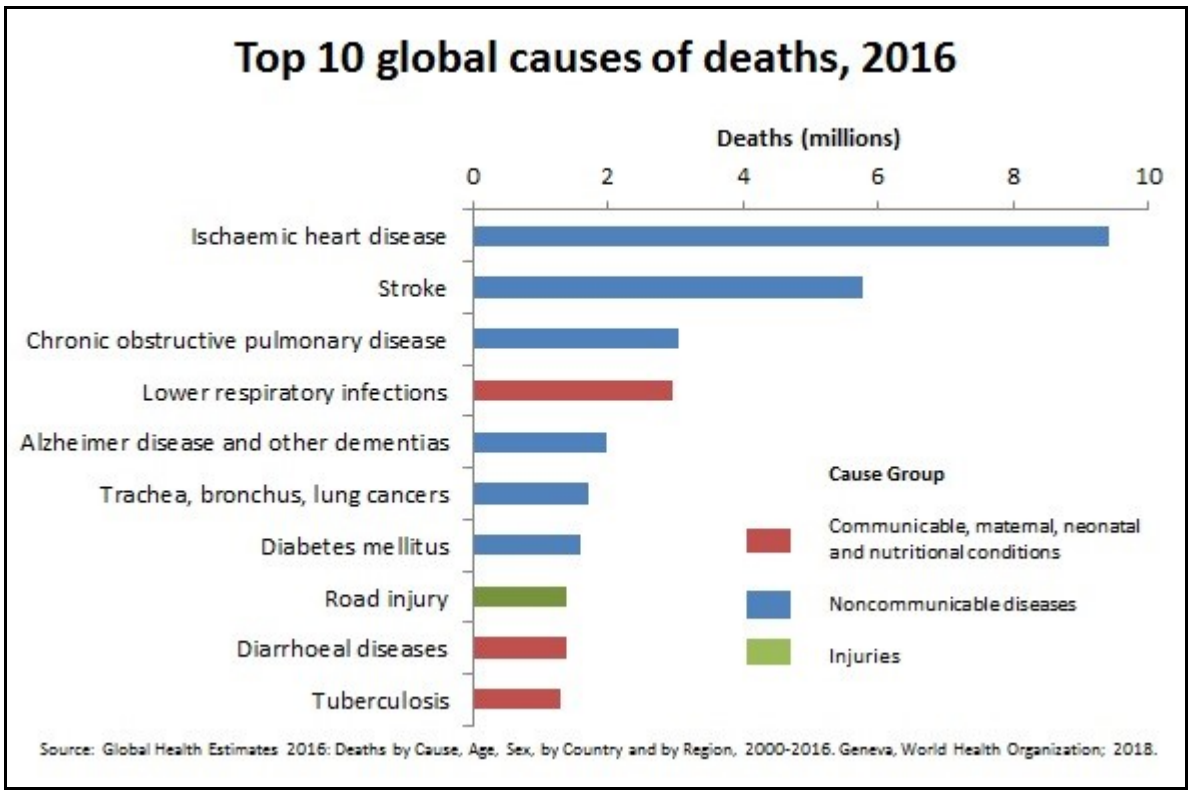

Figure 1: The most important causes of global mortality (9).

1.3 Cardiovascular Risk Factors

Risk factors that promote the development of CVDs can be classified in different ways: Some authors differentiate between risk factors of the individual, and community-based determinants (5) (**Table 1**). Others differentiate between non-modifiable and modifiable risk factors (10). Modifiable risk factors are usually rooted in unhealthy behavior and can thus be addressed. *Cardiometabolic risk factors*, also referred to as intermediate risk factors, such as O/O, elevated glucose levels, dyslipidemia and HT are also often connected to these behavioral risks (10). Elevated parameters may indicate a significantly higher risk. As they are easily measurable, screening is recommended to implement prevention strategies and therapeutic concepts (6).

Table 1: Risk factors and determinants influencing CVDs (5, 10-12).

Individual risk factors	**Community based determinants**
Age, sex, genetic disposition	Air pollution
Tobacco use, excessive alcohol consumption	Housing environment
Dietary habits, salt intake	Accessibility of (un)healthy food
Physical inactivity	Health care accessibility
O/O, HT, dyslipidemia, DM	Health policies

With changes in lifestyle and dietary habits, cardiometabolic risk factors like O/O have become more prevalent all over the world (13-15). Over the last decades, these problems have been increasingly affecting young individuals, with a rising prevalence of childhood obesity and type 2 diabetes mellitus (T2DM) (14, 16). These changes have also affected Sub-Saharan Africa (SSA) (17). South Africa (SA) is one of the countries with the most dramatic rise, with O/O increasing nearly10-fold, from 2.3% in 1990 to 22.9% in 2016 among children aged 5-9 years (16) (**Figure 2**).

Apart from the medical consequences of O/O, increased weight status also has an economic impact. Childhood obesity causes medical costs of approximately $19,000 per child, according to a study from the United States (18).

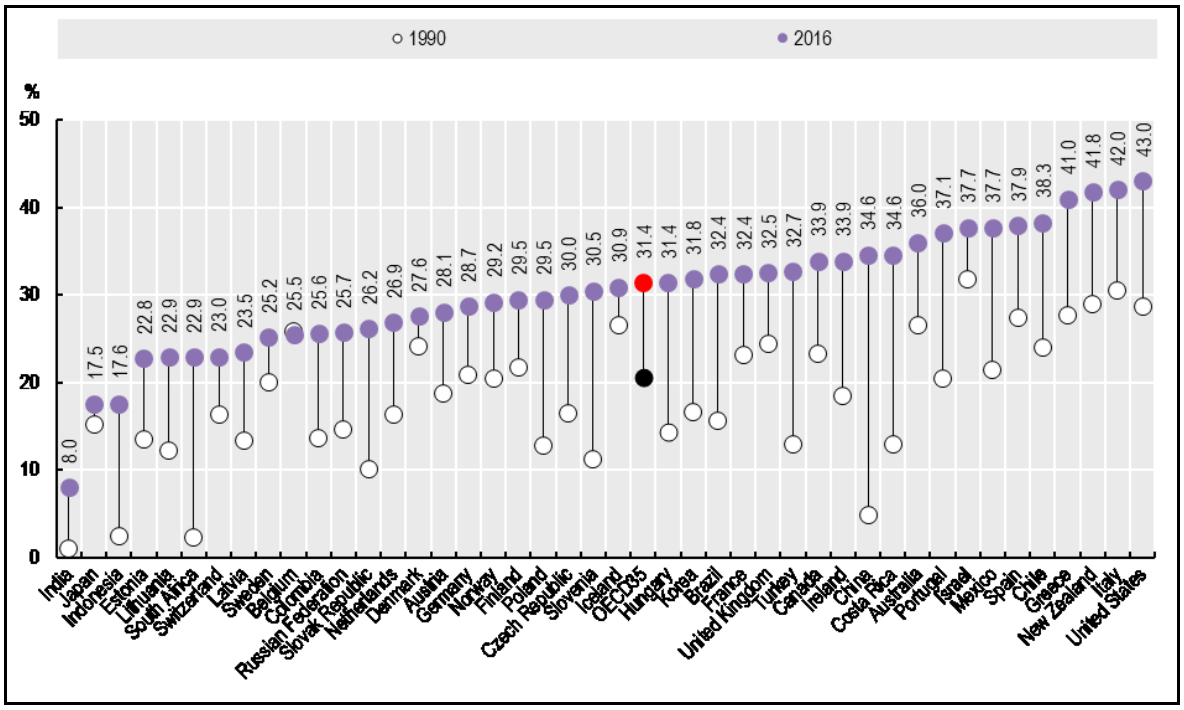

Figure 2: WHO Global Health Observatory data on the rise of childhood O/O (19).

1.4 Overweight and Obesity

1.4.1 Energy Balance and Nutritional Intake

To maintain integrity, the body needs to be supplied with energy. This is accomplished through the intake of food, which contains sources of energy such as fat, carbohydrates, and proteins. In the anabolic state of metabolism excess energy is stored, while in the catabolic state these deposits are consumed (20).

In a state of long-term overnutrition and a chronic positive energy balance, excess energy results in accumulation of fat, leading to O/O. Weight gain involves several factors, such as increased energy intake, low energy expenditure and individual metabolic properties. These may also occur together and are influenced by a variety of causes, making the development of obesity more complex than often thought (13, 21).

1.4.2 Definition of Overweight and Obesity

Adult Definition

The WHO defines overweight and obesity (O/O) as *"abnormal or excessive fat accumulation that may impair health"* (14).

A common way to classify O/O is the Body Mass Index (BMI). The BMI (kg/m^2) is defined as the body weight (kg) divided by the persons height squared (m^2) (22).

The cut off point for overweight is a BMI of 25, while for Obesity it is 30 (14). The WHO classifies nutritional status into *underweight*, *normal weight*, *pre-obesity/overweight* and *obesity*. The latter can further be broken down into different grades (22) (**Table 2**).

Table 2: BMI values and corresponding weight status classification (22).

BMI (kg/m^2)	**Weight status classification**
<18.5	Underweight
18.5-24.9	Normal weight
25-29.9	Pre-obesity/ Overweight
30-34.9	Obesity class I
35-39.9	Obesity class II
≥40	Obesity class III

Children and Adolescent Definition

As the BMI does not factor in age or sex, the criteria for children need to be adjusted. The WHO suggests using z- scores, which represent the standard deviation (SD) from the Growth Standards median. In children younger than 5 years overweight is defined as *"a weight-for-height greater than 2 standard deviations above WHO Child Growth Standards median"* and for obesity 3 SDs above the median. In children and adolescents between 5 and 19 years of age, O/O is defined as values greater than 1 SD for overweight, and greater than 2 SDs for obesity, respectively (14). The WHO Growth charts for girls and boys can be accessed via the WHO website (23).

Another way to classify weight status is through age, sex, and height-specific percentiles (24) (**Table 3**). The International Obesity Task Force (IOTF) publishes internationally applicable cut-offs for childhood and adolescent O/O (25).

Table 3: BMI percentiles and terminology in children and adolescents (24).

BMI percentile	Weight status
<5th	Underweight
≥5th <85th	Healthy weight
≥85th <95th	Overweight
≥95th	Obesity

1.4.3 Pathological Body Fat Distribution and Anthropometric Measurements

The BMI is a widely used surrogate marker for O/O, but as it only takes total body weight and height into consideration, it has no informative value concerning the body composition (26, 27). Several authors demanded additional measurements to accompany BMI, to make more precise statements about O/O (27).

The term metabolically healthy obesity (MHO) has been coined, to differentiate individuals with obesity, who lack metabolic dysregulation like IR, impaired glucose tolerance, or dyslipidemia from individuals who show these characteristics (26, 27). This concept must be used with caution, as it may imply an apparent innocuousness of increased body mass, while individuals with MHO have still shown a higher rate of adverse effects compared to metabolically healthy normal weights. Hence O/O should be taken seriously even without present metabolic abnormalities (26-29).

Visceral adipose tissue (VAT) shows different properties than subcutaneous adipose tissue, resulting in decreased adipose tissue function, storage capacity and insulin resistance (IR),

as well as increased inflammatory activity. Thus, an abdominal fat distribution type poses a higher risk for complications (26) (**Figure 3**).

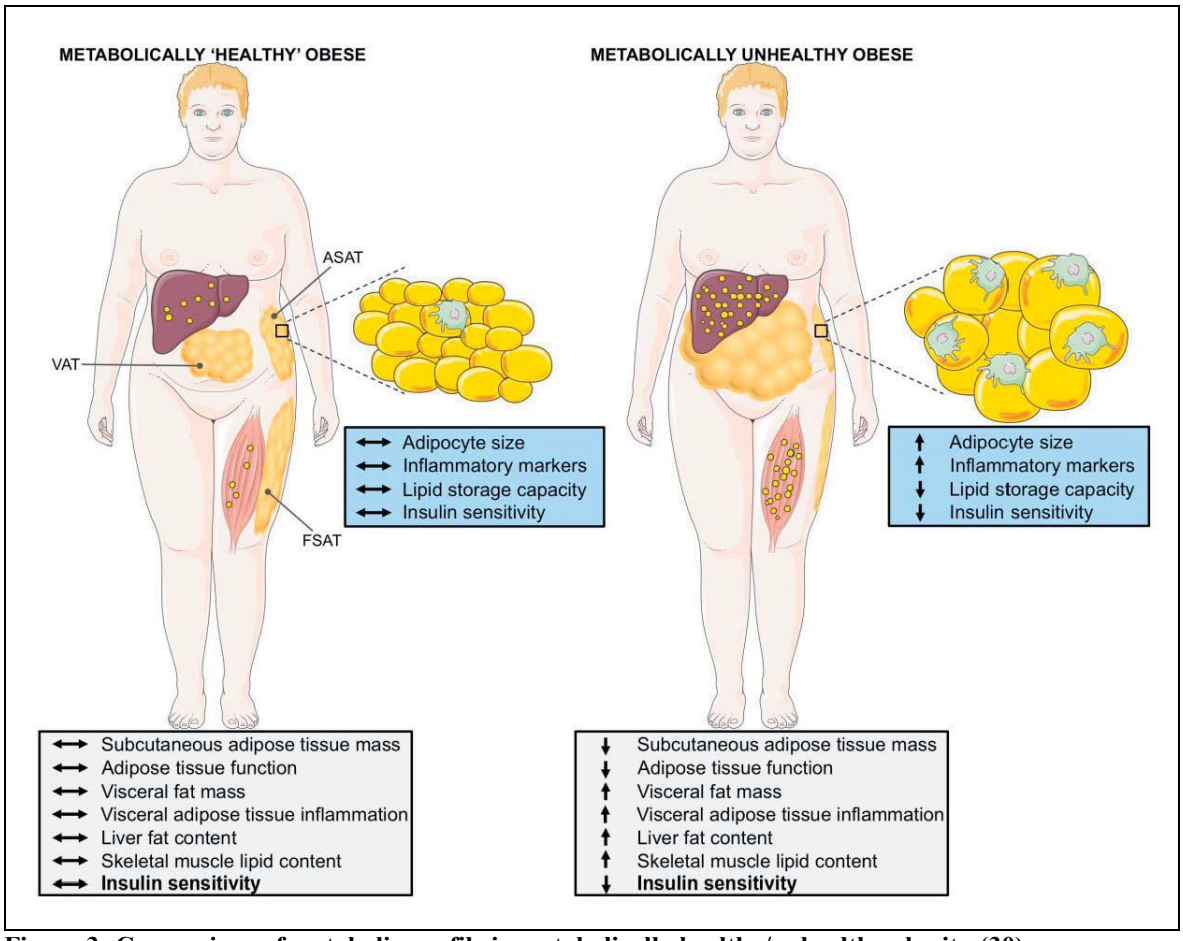

Figure 3: Comparison of metabolic profile in metabolically healthy/unhealthy obesity (30).

A measure to estimate abdominal fat by a noninvasive measurement technique is waist circumference (WC). According to the WHO, measurement should follow several normal breaths and should be taken parallel to the ground at the "*midpoint between the top of the iliac crest and the lower margin of the last palpable rib in the mid axillary line (31).*" More recently neck circumference (NC) has been shown to be another easy obtainable parameter, that can be used to get information on cardiovascular risk (32). Both WC and NC can also be used in clinical practice to assess O/O and cardiovascular risk in children and adolescents (33, 34). When analyzing anthropometric measures, an inclusion of BMI, sex, age and ethnicity improves their accuracy in predicting cardiometabolic risk (35). Waist-to-height ratio (WHtR) has been shown to be an even better predictor for cardiometabolic risk than BMI or WC alone (36).

1.5 Hypertension

1.5.1 Physiology of Blood Pressure

Arterial blood pressure (BP) is the driving force of blood flow. It changes between the phases of the cardiac cycle: The moment of highest blood pressure is called systole, where the ventricle contracts. Systolic blood pressure (SBP) reaches values of 120 mmHG under normal circumstances. At the diastole, the phase of ventricle relaxation, diastolic blood pressure (DBP) is lowest around 80 mmHG. The average BP over the heart phases is called mean arterial pressure (MAP). The MAP is calculated by multiplication of cardiac output (CO) and total peripheral resistance (TPR). CO is the amount of blood the heart pumps per minute and is calculated by stroke volume (SV) times heart rate (37).
That means, that arterial BP can be adapted in several ways. Firstly, by accelerating or decelerating the heartbeat or changing SV, which alters CO. The second option is adapting TPR, which can be achieved by vasoconstriction or dilation of the peripheral arteries (37). The regulation of BP has several layers. While the short time regulation is mediated via the baro- and chemoreceptors, over time the Renin-Angiotensin-Aldosterone-System (RAAS) is activated, which leads to vasoconstriction as well as renal fluid retention to expand the plasma volume (3, 37). Regulation of BP is crucial, as BP falling to low, leads to an undersupply of organs, which can result in reversible or irreversible tissue damage (37). On the other hand, a pathologic increase of blood pressure, known as hypertension, leads to damage to the vessels and the heart, causing CVDs (10, 38, 39).

1.5.2 Definition of Hypertension

Adult Definition

Hypertension is the elevation of blood pressure above the norm. It is defined as a BP above 140/90 mmHg. Hypertension can further be categorized by its severity (12) (**Table 4**).

Table 4: Categorization of BP (12).

Category	SBP (mmHg)		DBP (mmHg)
Optimal	<120	and	<80
Normal	120-129	and/or	80-84
High normal	130-139	and/or	85-89
HT grade 1	140-159	and/or	90-99
HT grade 2	160-179	and/or	100-109
HT grade 3	≥180	and/or	≥110
Isolated systolic HT	≥140	and	>90

Children and Adolescent Definition

In children, age- sex- and height-specific centile curves are used. Values between the 90^{th} and 95^{th} percentile are considered high normal or pre-hypertension, while values above the 95^{th} percentile are considered hypertension (**Table 5**). At the age of 16 years or older, the adult cut-off values can be used (40).

Table 5: Categorization for HT in pediatrics (40).

Category	SBP or DBP percentile
Normal	$<90^{th}$
High normal/ pre- HT	$\geq 90^{th} < 95^{th}$
Hypertension	$\geq 95^{th}$
Stage 1 HT	$\geq 95^{th} \leq 99^{th}$+ 5 mmHg
Stage 2 HT	$>99^{th}$ + 5 mmHg
Isolated systolic HT	SBP $\geq 95^{th}$ and DBP $<90^{th}$

1.5.3 Pathophysiology of Hypertension and Endothelial Dysfunction

Hypertension can also be classified by its etiology. Secondary HT makes up to 5-10% of all HT cases, and is characterized by its identifiable cause (41). Examples are endocrine, renal, and vascular diseases, as well as obstructive sleep apnea, and medication (12).
For the much more common form, known as primary or essential HT, no single cause can be attributed, although several risk factors for the development are known. Risk is increased by male sex, behavioral factors like smoking, a sedentary lifestyle and salt intake, as well as genetic predisposition and living conditions (12).
Endothelial dysfunction (ED) may play an important role in the development of HT (42, 43). The endothelium, besides its barrier function, also secretes a multitude of molecules impacting inflammation, coagulation, and angiogenesis, as well as vasoactive substances (42, 43). ED is a term describing the endothelium's impaired ability of this mechanisms. NO is a potent vasodilator produced by endothelial cells. In ED, the response to NO is diminished, which can be attributed to a decreased bioavailability, an inactivation through reactive oxygen species (ROS), as well as a higher susceptibility to vasoconstrictive mediators of the endothelium (42-45).
This vulnerability may result in a remodeling of the vascular wall, which leads to arterial stiffness, endothelial damage, atherosclerosis, HT and ultimately to cardiovascular events (12, 42) (**Figure 4**).
Measures to estimate early vascular damages are pulse wave velocity to measure arterial stiffness as well as flow mediated dilatation, which examines the endothelial capability of vasodilatation following sheer stress (46, 47).

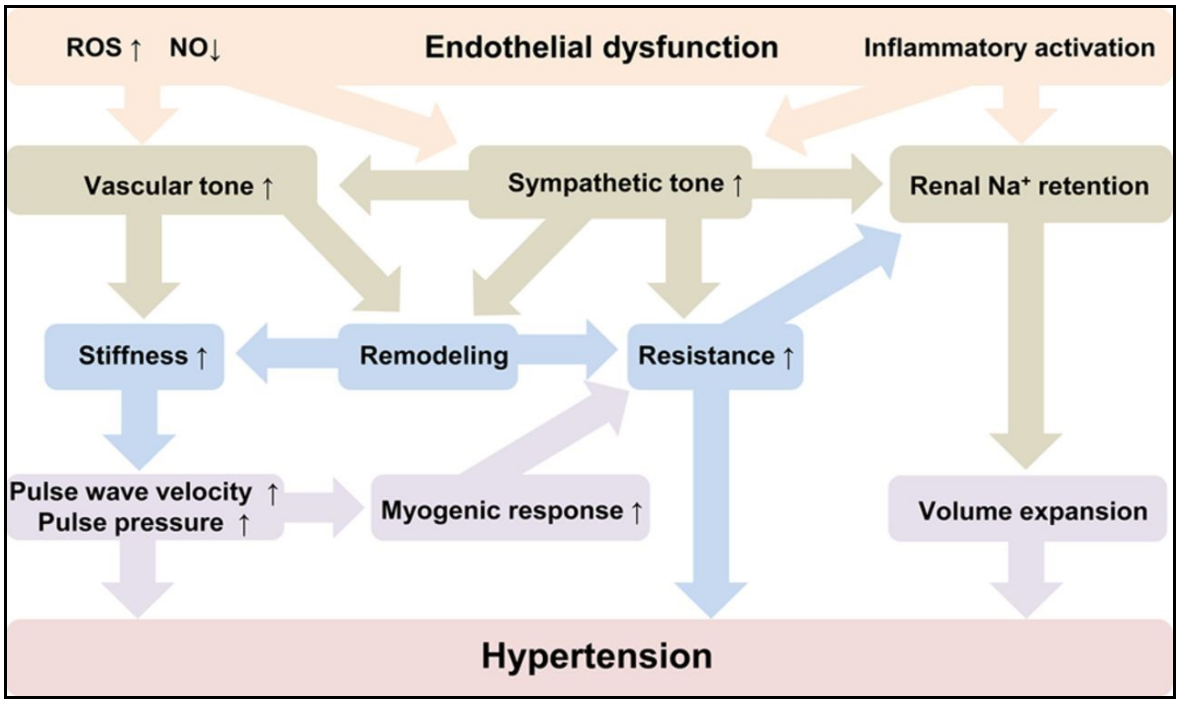

Figure 4: Pathophysiological pathways of ED leading to HT (48).

1.6 Dyslipidemia

1.6.1 Physiology of Lipoproteins

Lipoproteins are the transport vehicles for lipids in the organism. They consist of cholesterol, cholesterol esters (CEs) and TGs. Furthermore, they contain apolipoproteins, which are composed of phospholipids (PLs) and proteins and function as structure giving components, ligands, and activators or inhibitors for enzymes. They can be classified by their composition and character into 6 major groups (11) (**Table 6**).

Table 6: Classification of Lipoproteins (11).

Class	Density (g/mL)	Diameter (nm)	TGs (%)	Cholesterol (%)	CE (%	PLs (%)
Chylomicrons	<0.95	80-100	90-95	1	2-4	2-6
VLDL	0.95-1.006	30-80	50-65	4-7	8-14	12-16
IDL	1.006-1.019	25-30	25-40	7-11	20-35	16-24
LDL	1.019-1.063	20-25	4-6	6-15	34-35	22-26
HDL	1.063-1.210	8-13	7	5	10-20	55
LP(a)	1.006-1.125	25-30	4-8	6-9	35-46	17-24

VLDL: Very low-density lipoprotein, IDL: Intermediate-density lipoprotein, LDL: Low-density lipoprotein, HDL: High-density lipoprotein, LP(a): Lipoprotein (a)

The physiological pathways of lipoproteins can be separated into the exogenous and the endogenous pathway. The exogenous pathway starts with lipids being absorbed from the intestine and transported in triglyceride-rich lipoproteins, called chylomicrons. They are split by the lipoprotein-lipase (LPL), to release free fatty acids (FFAs) that are taken up by skeletal muscles, adipocytes, and the liver. The remnants of the chylomicrons are taken up by the liver as well (49).

In the liver the endogenous pathway begins, with the release of very low-density lipoproteins (VLDL), which are rich in TGs as well. Those are transported to peripheral tissues. Through the LPL they are transformed into intermediate density lipoprotein (IDL), which can further be reduced to low density lipoproteins (LDL). This process leads to a diminishing of TGs, with cholesterol remaining. The LDL transports the cholesterol to the liver and peripheral tissues. Precursor particles of high-density lipoprotein (HDL) are released from the liver and are adapted through the acquisition of various apolipoproteins. HDL transports back cholesterol from peripheral tissues (49) (**Figure 5**).

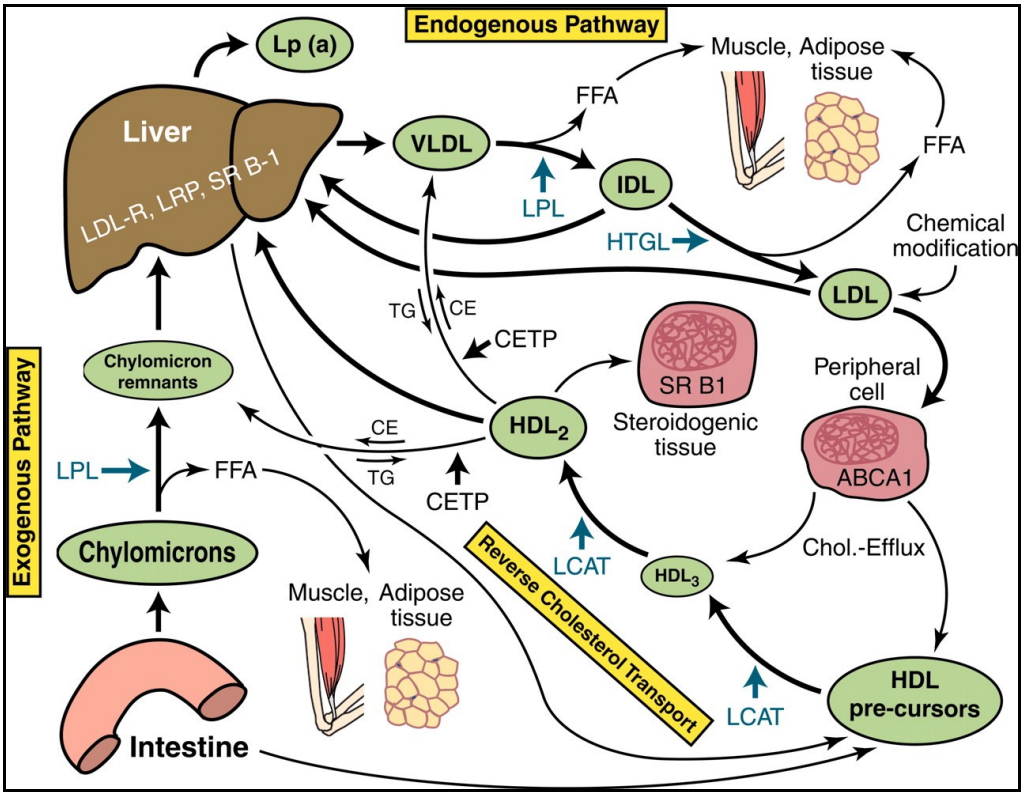

Figure 5: Metabolism and pathways of lipoproteins (49).

1.6.2 Pathophysiological Aspects

A dysregulation of the lipoprotein metabolism can be referred to as dyslipoproteinemia or dyslipidemia and manifests as abnormal levels in the blood. Causes are multifactorial, rooting in a primary genetic predisposition, or having a secondary cause like obesity, endocrine diseases, renal failure, or drug intake (50). Typically, an elevation of LDL-cholesterol (LDL-C), total cholesterol, and TGs, as well as a decrease of HDL-C can be described. These changes can occur together, or single parameters can be changed (51). Dyslipidemia plays a key role in development of atherosclerosis. LDL-C particles are prone to oxidation. They are salvaged by macrophages and taken up into the vessel walls forming atherosclerotic plaques. This process is enhanced, if the endothelium is damaged and vulnerable, therefore ED plays a role in atherogenesis. TGs and $LP_{(a)}$ have been shown to have atherogenic potential as well, while the protective properties of HDL-C are still controversial. Atherosclerotic plaques may undergo an evolution, involving macrophages and smooth muscle cells of the intima. Over time they may become more vulnerable and rupture, leading to cardiovascular events. (52).

1.7 Hyperglycemia

1.7.1 Physiology of Insulin

Insulin is a peptide hormone, which's predecessors are produced in the β-cells of the islets of Langerhans in the pancreas. While the highly glucose dependent brain cells and erythrocytes import glucose through insulin independent transports, the glucose uptake in other tissues is reliant on the presence of insulin. Insulin enhances the insertion of the glucose transporter type 4 (GLUT-4) into the cell membranes. Through GLUT-4 glucose is then transported into the cells of skeletal muscle and adipose tissue, which lowers the plasma glucose levels. Furthermore, it decreases gluconeogenesis in the liver. It stimulates the formation and storage of glycogen in the musculature and liver, which can later be split into glucose again in catabolic state of metabolism. After glycogen storages are filled, glucose is transformed into fatty acids and insulin stimulates the uptake into adipocytes to form fat deposits. Insulin has also a major role in the transport of amino acids to cells, supporting cell growth (53).

1.7.2 Insulin Resistance

Insulin Resistance (IR) describes a state, with an inadequate peripheral tissue response to insulin (54). The sensitivity to insulin can change physiologically over lifetime, with higher grades of IR being reported in puberty (55). IR is multifactorially caused, with O/O being a key factor in the development and progression, while being also influenced by genetic predispositions (56). The pathogenesis involves chronic inflammation, as well as endocrine pathways affected by the adipose tissue (56-60). In earlier stages IR is often compensated by an adaptation of the β-cells and reactive hyperinsulinemia, maintaining normal levels of plasma glucose (54, 56). Over time these mechanisms may become exhausted, with a development of β-cell-dysfunction and insulin secretion going down. With the failure of this compensatory mechanism, impaired glucose tolerance and DM can develop (56).

1.7.3 Diabetes Mellitus

Diabetes mellitus describes a group of diseases, which are characterized by chronic hyperglycemia, due to insufficient effects of insulin. DM can be classified by its pathogenetic pathway. Common forms are autoimmune type I diabetes, T2DM, and gestational diabetes in pregnancy. Several other, rarer forms are known, including DM in the context of other metabolic diseases, chronic pancreatitis, genetic disorders, or drug intake (61). T2DM is the most common form, making up for over 90% of DM cases. It is closely linked to obesity and insulin resistance, being associated with genetic and behavioral factors (61, 62).

DM can be diagnosed by documenting elevated plasma glucose levels (**Table 7**). The cut-offs differ, depending on the conducted test. It is possible to measure FG, A1c, which is an indicator for plasma glucose over the last months, or plasma glucose after an oral glucose tolerance test (OGTT) (61).

Table 7: Criteria for the diagnosis of diabetes mellitus (61).

Criterion	Cut-off
Fasting glucose	≥126 mg/dL
2-hour glucose after OGTT	≥200 mg/dL
Hyperglycemic symptoms+ random glucose	≥200 mg/dL
A1c	≥6.5 mg/dL

Individuals who show plasma glucose levels above the norm, that do not fulfill the criteria for diagnosis DM, are at a higher risk of developing it. This condition can be described as pre-diabetes, which is defined by an A1c from 5.7-6.4%, impaired glucose tolerance (IGT), or impaired fasting glucose (IFG). IGT is defined by a 2-hour OGTT result in the range of 140-199 mg/dL. Depending on which institutions definition is used, IFG is defined by FG between 100-125 mg/dL or 110-125 mg/dL (62).

Diagnosis and treatment of DM is crucial to prevent its acute and chronic complications. Acute complications include ketogenic and hyperosmolar coma. Chronic hyperglycemia damages the peripheral nerves, leading to peripheral polyneuropathy and diabetic foot syndrome. Microvascular complications include retinopathy and nephropathy, while the macrovascular complications are cardiovascular events like myocardial infarction or strokes (63-65).

1.8 The Metabolic Syndrome

1.8.1 History

Nearly 100 years ago research papers on the association between obesity, hypertension, diabetes, and hyperuricemia were published (66, 67). Over the course of the 20th century several authors described different constellations of cardiometabolic risk factors and diseases. Some of them also used the term *Metabolic Syndrome* (68-71).
In 1988 Reaven reported on the *Syndrome X*, which included resistance to insulin-stimulated glucose uptake, glucose intolerance, hyperinsulinemia, increased VLDL, TGs, decreased HDL and HT (72). The syndrome has also been referred to by *Syndrome of Insulin Resistance* (73) or *Deadly Quartet* (74). Since the late 1990s, the syndrome has gotten into focus of research, with several large international organizations publishing criteria for the definition of MetS (63, 75-79).

1.8.2 Definitions

Adult Criteria

Over the last years a multitude of definitions for the MetS have been proposed. This includes contributions by the WHO (63), the National Cholesterol Education Program Adult Treatment Panel III (75), the American Heart Association/National Heart, Lung and Blood Institute (76), the American Association of Clinical Endocrinology (77), the International Diabetes Federation (79), and the European Group for the Study of Insulin Resistance (78).
While they have commonalities, they differentiate in relation to their cut-off-points, included parameters and prerequisite factors. While some definitions have their focus on IR, others value the aspect of central obesity most (80).
In 2009 a joint interim statement has been published by the big contributing societies, which includes an agreement on a consensus definition of the MetS (**Table 8**). It can be diagnosed, if 3 out of the 5 criteria are fulfilled. The issue of applicability to different ethnic groups and recommendation of an update of the criteria in regard of ethnic-specific cut-offs was also discussed (80, 81).

Table 8: The consensus definition of MetS (81).

Criterion	Cut-off	Notes
Central obesity	WC> 94cm (males) WC> 80cm (females)	Ethnic specific cut-offs should be used if available.
Dyslipidemia (TGs)	TGs≥ 150 mg/dl or Drug treatment	
Dyslipidemia (HDL-C)	HDL-C < 40 mg/dl (males) HDL-C < 50 mg/dl (females) or Drug treatment	Cut-off for females may have to be adapted in certain populations.
Hypertension	SBP≥ 130 mm or DBP≥ 85 mmHg or Drug treatment	
Hyperglycemia	FG≥100 mg/dl or Diagnosed T2DM	

Children and Adolescent Criteria

While the adult criteria for defining MetS have been highly varied in the past, a commonly accepted consensus definition has been published. The definitions for children and adolescents are still highly variable and differ between different organizations. Until today, several different definitions are in use (82, 83) (**Table 9**).

Table 9: Common classifications for pediatric MetS.

Publication	Criteria for diagnosis	Central obesity	Hypertension	Dyslipidemia: TGs	Dyslipidemia: HDL-C	Fasting glucose
Cook et al. (84)	3/5	WC ≥90th pc	≥90th pc	≥110 mg/dl	≤40 mg/dl	≥110 mg/dl
Ford et al. (85)	3/5	WC ≥90th pc	≥90th pc	≥110 mg/dl	≤40 mg/dl	≥110 mg/dl (+additional diagnostics ≥100 mg/dl)
De Ferranti et al. (86)	3/5	WC ≥75th pc	≥90th pc	≥100 mg/dl	≤50 mg/dl	≥110 mg/dl
Alberti et al. (87)	WC+ 2/4	10-15 years: WC ≥90th pc ≥16 years: WC ≥94 cm (males) WC ≥80 cm (females)	SBP ≥130 mmHg or DBP ≥85 mmHg or Medication	≥150 mg/dl or Medication	<40 mg/dl (males) <50 mg/dl (females)	≥100 mg/dl or Diagnosed T2DM

1.8.3 Pathophysiology

The pathogenesis of the MetS shows great complexity and is still not fully understood (81, 88). As reflected in the different definitions, abdominal obesity and IR are key contributors to the development of MetS (83-89) (**Figure 6**). Furthermore several neurohormonal, endocrine, and inflammatory pathways have been identified (83, 88, 89).

A proposed starting point is the amassment of adipose tissue, that shows different gene expression, metabolic properties, and signaling that may lead to IR (90-92). In lean individuals, IR may also be the beginning of this cascade (93).
With impaired insulin function, lipolysis in the adipocytes is exacerbated, resulting in the release of free fatty acids (FFAs) (94). FFAs are further processed in the liver, leading to stimulated production of VLDL, resulting in a decrease in HDL and increase of TGs, leading to dyslipidemia (95-97). Excess lipids are stored in the liver, leading to ectopic fat depots and in succession to nonalcoholic fatty liver disease (98). Hepatic lipase fissures LDL into small dense LDL particles, which are easier oxidized and have increased atherogenic potential (95, 96).

Pancreatic beta cells are stimulated to increase insulin secretion in response to the resistance of peripheral tissues. As these mechanisms can be exhausted, decompensation may occur, resulting in a relative hypoinsulinemia, despite high blood levels of the hormone (89). FFAs also show a negative impact on insulin signaling resulting in a vicious cycle (99). Stimulation of gluconeogenesis further worsens hyperglycemia, which in combination with peripheral IR leads to manifestation of T2DM (89).

In the presence of FFAs the genesis of ROS is increased, damaging the endothelium, and leading to ED. ED is also mediated by the atherogenic properties of LDL and the sympathetic inhibition of NO caused by hyperinsulinemia (89). Furthermore, in obesity the RAAS is upregulated, which additionally contributes to the development of HT (100, 101).

Adipose tissue secretes a high number of metabolically impactful substances called adipokines. Two impactful adipokines are leptin and adiponectin (58). Adiponectin downregulates inflammation and shows a positive impact on IR on cardiometabolic risk (58, 102, 103). Leptin plays an important role in food intake and saturation (104).

It has been shown to correlate with cardiovascular risk (105). In obesity adiponectin levels have shown to be are decreased, while leptin levels are increased (106)

Inflammation seems to play an important role in the pathogenesis of the MetS and CVDs, as multiple inflammatory markers are elevated in metabolic dysregulation (60, 107). Elevated levels of tumor necrosis factor alpha (TNF-α) have shown to be associated with abdominal obesity and insulin resistance (108). TNF-α phosphorylates and inactivates insulin receptors in peripheral tissues, induces lipolysis, and inhibits the release of adiponectin (109). TNF-α also contributes to the increase of leptin levels (110, 111). Furthermore, TNF-α, Interleukin-1β, and Interleukin-6 (IL-6) have shown to be associated with sleeping disorders, which play an important role in the development of obesity (112).

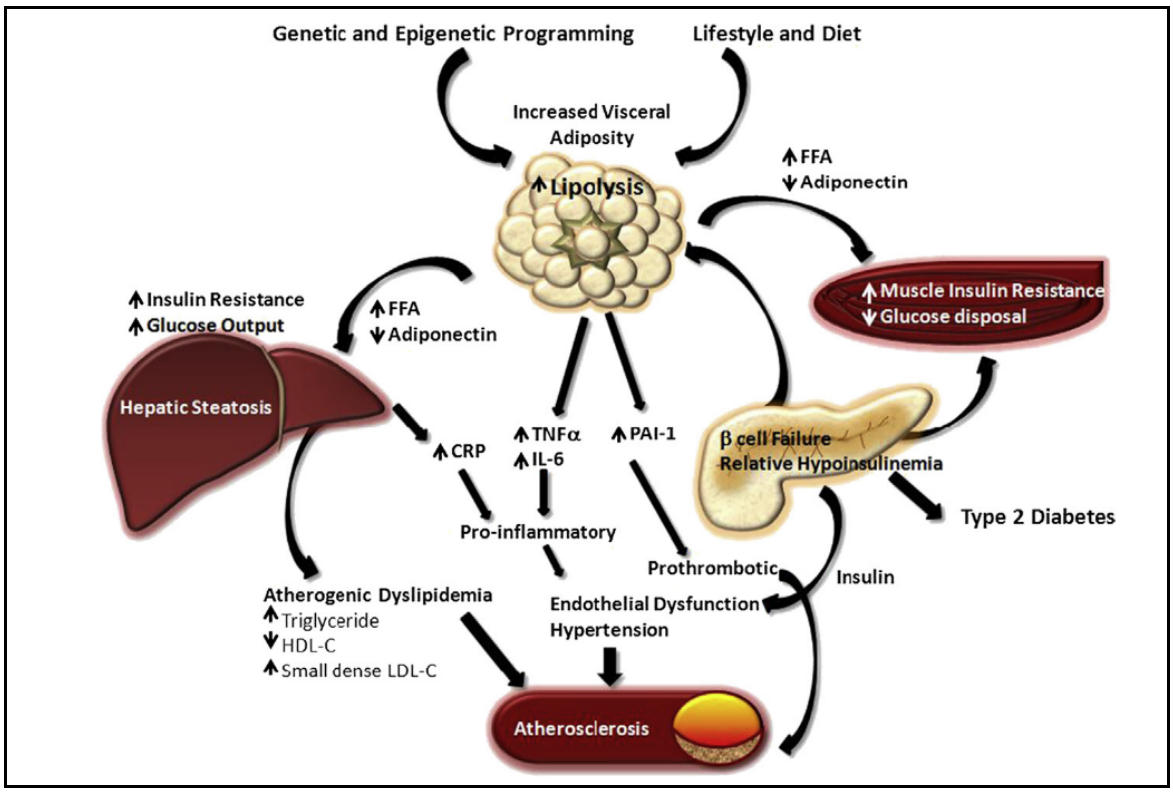

Figure 6: The pathophysiology of MetS (113).

1.8.4 Predisposing Factors

Several studies on the predisposing factors for the development of MetS have been conducted. These showed an association with a sedentary lifestyle (114), increased television consumption (115), high caloric intake and a "western-style diet" (116), as well as the consumption of sugary drinks (117, 118). Furthermore, individuals with a family history of MetS (119) and with low socioeconomic status (120) have been shown to be at higher risk. A low cardiorespiratory fitness is also a factor in the development of MetS, which implies the importance of physical constitution in prevention programs (121). Literature also mentions the impact of medication, as psychiatric patients treated with clozapine, a typical antipsychotic, show an increased risk for MetS (122). HIV-patients, on antiviral therapy also show a high prevalence of metabolic abnormalities, putting them at higher risk of CVDs (123). This must not be neglected, as SSA has the highest prevalence of HIV worldwide (124).

1.8.5 Consequences and Comorbidities

MetS is associated with a multitude of complications, concerning different organ systems (15, 83, 89, 125, 126). Patients diagnosed with MetS have a significantly higher mortality from cardiovascular events, than healthy individuals (127, 128). The chance of suffering cardiac events in succession to other medical procedures or hospitalization is increased (129) and cardiac events seem to be more severe, as Clavijo et al. reported a bigger infarction size and a higher rate of complications in patients with MetS (130).
It also increases the risk of developing diabetes mellitus, with studies reporting an increase in risk of up to 5-fold comparing to healthy individuals (131).
Furthermore, several types of cancer are associated with obesity and the MetS (132-134).
Nonalcoholic fatty liver disease is also strongly associated with MetS and insulin resistance, thus making liver screening useful for predicting cardiovascular risk (135).
Obesity and the MetS are also associated with the polycystic ovary syndrome, which features multiple ovarian cysts and hormone dysregulation, leading to hyperandrogenism, irregularities in the menstrual cycle, ovulatory dysfunction and fertility issues (136).
The obstructive sleep apnea syndrome (OSAS) can also be observed more often in obese individuals, as increased masses of fat in abdominal an thoracic areas impair the physiologic breathing process and lead to sleep fragmentation and temporary hypoxia

(137). Vice versa it is also reported that the treatment of severe OSAS shows a positive effect on insulin sensitivity and reduces the cardiovascular risk (138).
Studies support that depressive symptoms are more prevalent in patients diagnosed with DM or obesity, recommending the screening for depression in affected individuals. (139, 140).

1.8.6 Therapeutic Possibilities

As there is no single drug that targets the complex pathways of MetS, the most important measure for prevention and treatment remains a change in diet and lifestyle (141).
Lowering of caloric intake and therefore a reduction in substrate lowers the development of obesity, IR, and accumulation of ectopic fat deposits (141, 142). Also, an adaptation in composition of the nutriment essential, as a high fiber content extenuates the rise of serum glucose and therefore the discharge of insulin. High fiber intake also shows positive effects on the hepatic lipogenesis and lipid export (141, 143, 144).
In addition to nutritional measures, the second big intervention is to increase physical exercise. In exercise substrate is used and the genesis of fatty acids is lowered, which has a positive effect on IR. It furthermore increases the mitochondrial substrate metabolism in the liver and the sympathetic activation triggers several pathways that lead to genesis of mitochondria (141, 145-147).
Lifestyle modifications are also the key measures in the treatment and prevention of MetS components. Renowned societies for HT, dyslipidemia and DM, all list a reduction of O/O as the primary aim, before starting pharmaceutical treatment (11, 12, 40, 62).
For treatment of HT, DM, and reduction of atherogenic lipid, different drug options are available. Rask et al. recommend statins for dyslipidemia , RAAS-blockers for HT, and Metformin, SGLT2-inhibitors or GLP1-agonists for IGT as first line therapies in MetS patients (148). In high risk patients, thrombocyte aggregation inhibitors are indicated to reduce the risk of cardiovascular events (11, 12).
In Patients with severe obesity, a surgical approach is an option. In the field of bariatric surgery, procedures like partial gastrectomy or gastric bypass can be performed, which have been shown leading to significant loss of weight in the follow up. They can also be performed in morbidly obese adolescents, with an improvement in weight, cardiovascular health, and quality of life being reported (149).
There is also research going on in the field of nutraceuticals, which are dietary supplements that add other health benefits in addition to their nutritional value, examples being herbs, extracts, or essential oils. While some studies have demonstrated their positive effects on IR and obesity, or anti-inflammatory properties, they are still under investigation and not recommended to be used instead of the pharmacotherapy recommended to treat MetS (88).

1.9 Aims and Objectives

With a shift in dietary habits and lifestyle, obesity has been rising in children and adults in SSA (16, 150). Prevention and treatment of excessive body weight is crucial, as it is a key contributor to cardiovascular risk, and low-income countries suffer over proportionally from CVDs (6).

While the data available on cardiovascular risk in children of underprivileged areas is still low, several studies have been conducted by researchers of the Walter Sisulu University (WSU) in Mthatha over the last years. Based on those studies (151, 152), which have shown that the prevalence of cardiovascular risk factors in SSA adolescents is high and childhood obesity rising, we hypothesized that there will be a similarly high prevalence of overweight and obesity, accompanied by a high prevalence of cardiovascular risk factors in this population of adolescents as well. Based on the current literature we expected individuals with O/O to be more susceptible for cardiovascular risk factors (151-153).

The aim of this study was to assess the prevalence of MetS and cardiometabolic risk factors in adolescents from the secondary grade schools of Mthatha. Therefore, anthropometric and blood pressure data, as well as blood parameters to assess dyslipidemia and impaired fasting glucose were collected.

To carry out the measurements, a team of medical students from the Medical University of Graz (MUG) was formed, which travelled to Mthatha in February 2018 to conduct the measurements together with a team from the WSU. After our departure, data acquisition was to be continued by the South African colleagues.

2 Methodology

Study Design

This study was part of a project investigating cardiovascular risk in adolescents and young adults SSA. It was conducted as a cross-sectional cohort study. 244 students aged between 13 and 16 years, from four secondary schools in Mthatha, Eastern Cape, SA, were recruited. Recruitment was carried out in cooperation between the WSU, teachers, and parents or legal guardians of participants.

Criteria of Inclusion and Exclusion

Included were healthy male and female adolescents of African ancestry between 13 and 16 years of age. Adolescents with any cardiovascular, pulmonary, renal, or orthopedic disease, as well as individuals with physical handicaps that would impair the anthropometric measurements were not eligible for this study. Also excluded were pregnant or lactating females, patients on blood pressure lowering medication and endurance athletes.

Ethics

The study followed the principles stated in the Declaration of Helsinki (revised version, 2008) and South African regulations. Clearance was given by the Ethic Committee of the WSU (Reference Number: 014/2014, **Appendix 7.1**). The study protocol and measurements were explained to the adolescents and their parents or legal guardians and given in writing. Written informed consent was obtained from the adolescents and their legal guardians (**Appendix 7.2**). Participant data was anonymized, using codes that were assigned before the start of data collection.

Data Collection and Anthropometric Measurements

All measurements were conducted before midday, after an overnight fast. Anthropometric measurements were conducted, as proposed in the International Standards for Anthropometric Assessments (154) by trained medical and physiology students from MUG and WSU. Results where documented on data sheets (**Appendix 7.3**). Measurements were performed in light clothing, without shoes. Measurements on male participants were conducted by male fieldworkers, female participants by females. During measurements participants had their feet positioned together, standing straight with their arms at the side.

Using a stadiometer, height was measured to the nearest 0.1 cm. An anthropometric tape was used to measure the various circumferences (neck, chest, mid-upper-arm, waist, hip, thigh, calf, ankle). Waist to height ratio (WHtR) and waist to hip ratio (WHR) were calculated. Body weight was measured using a scale (BC-1000, Tanita, Amsterdam, NL), that also calculated BMI and body fat percentage (**Figure 7**). Participants were assigned a weight status classification, using age- and sex adjusted BMI percentiles by the IOTF (25): They were classified as underweight (BMI $<5^{th}$ percentile), healthy weight ($\geq5^{th}$ $<85^{th}$ percentile), overweight ($\geq85^{th}$ $<95^{th}$ percentile) or obese ($\geq95^{th}$ percentile).

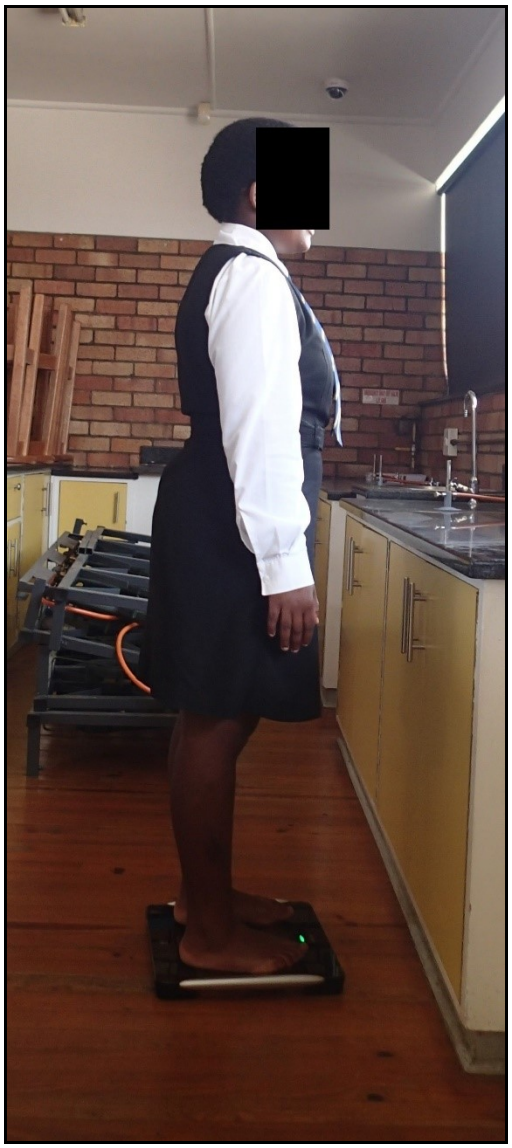

Figure 7: Demonstration of the Tanita scale.

Blood Pressure Measurements

All Participants were asked to abstain from physical activity for 48 hours, as well as from caffeine and any other stimulants, for 24 hours prior to the measurements. Cuffs of appropriate size were fitted to the upper arms of participants. Following five minutes of rest, three measurements in two-minute intervals were conducted using an automated oscillometric sphygmomanometer (HBP-1100; Omron Healthcare Co. Ltd., **Figure 8**). The average of the three results for SBP and DBP were calculated. Using percentile curves for age, sex and height, participants were classified as normotensive (SBP and DBP <90^{th} percentile), pre-hypertensive (SBP and/or DBP ≥ 90^{th} <95^{th} percentile) or hypertensive (SBP and/or DBP ≥ 95^{th} percentile) according to children BP-guidelines (40, 155).

Figure 8: Omron Healthcare automated sphygmomanometer.

Biochemical Parameters

Venous blood samples were collected after an overnight fast by a trained nurse. After proper storage, plasma was obtained through centrifugation. FG, TGs, and HDL-C were measured.

Classification of MetS

The classification by Cook et al. (84) was used to diagnose MetS. Criteria were met when three out of five parameters were above their threshold. This included WC (≥90^{th} percentile), BP (≥90^{th} percentile), TGs (≥110 mg/dl, ≥1.24 mmol/l), HDL-C (≤40 mg/dl, ≤1.03 mmol/l), and FG (≥110 mg/dl, 6.1 mmol/l).

Statistical Analysis

The statistical package for social sciences (SPSS) Version 26.0 (IBM Corp. Armonk, NY, USA) was used to analyze all data. Descriptive statistics were computed for sex, age, BMI, anthropometric data, BP, and blood parameters and shown as mean ± SD. Data was tested for normality using the Shapiro-Wilks test. Analyses of variances (ANOVA/MANOVA) or student's t-test where calculated to show differences between groups, with a significance level of $\alpha = 0.05$. Chi-square test was performed for categorical data. The relationships between anthropometric data and hemodynamic- or serum parameters were displayed using Pearson-correlations.

3 Results

3.1 Baseline Characteristics

Recruitment included a total of 244 adolescents (188 females, 56 males). After the exclusion of participants with values (hemodynamic or anthropometric) ± 3 SDs of the mean, 236 (181 females, 55 males) remained for statistical analysis (**Table 10**).

Table 10: Characteristics of the study population.

Parameter	n	mean	SD
Age (y)	236	14.36	1.00
BMI (kg/m²)	236	21.82	4.57
Waist circumference (cm)	234	72.01	9.80
Systolic Blood Pressure (mmHg)	236	115.19	11.50
Diastolic Blood Pressure (mmHg)	236	72.82	8.10
Fasting glucose (mmol/L)	207	4.78	0.50
Triglycerides (mmol/L)	204	0.85	0.35
HDL-C (mmol/L)	102	1.24	0.57

3.2 Weight Status

Applying BMI percentiles, 15.7% (37/236) of participants were classified as overweight and 14.8% (35/236) as obese, leading to a prevalence of O/O of 30.5% (72/236). 65.3% (154/236) were classified as healthy weight, and 4.2% (10/236) as underweight, leading to a total of 69.5% (164/236) being classified as lean (underweight+ healthy weight).

The prevalence of O/O was 32.0% (58/181) in females, while it was 25.4% (14/55) in males (**Table 11**). Chi-square test of independence showed no significant relation between weight status and sex ($\chi^2(1, n=236) = 0.864$, $p=0.353$).

Figure 9 shows the distribution of BMI percentiles among the study population and its skewness to the right.

Table 11: Weight status, split by sex

			Weight status				Total (n)
			Underweight	Healthy weight	Overweight	Obesity	
Sex	Female	n	5	118	31	27	181
		%	2.8%	65.2%	17.1%	14.9%	100.0%
	Male	n	5	36	6	8	55
		%	9.1%	65.5%	10.9%	14.5%	100.0%
Total		n	10	154	37	35	236
		%	4.2%	65.3%	15.7%	14.8%	100.0%

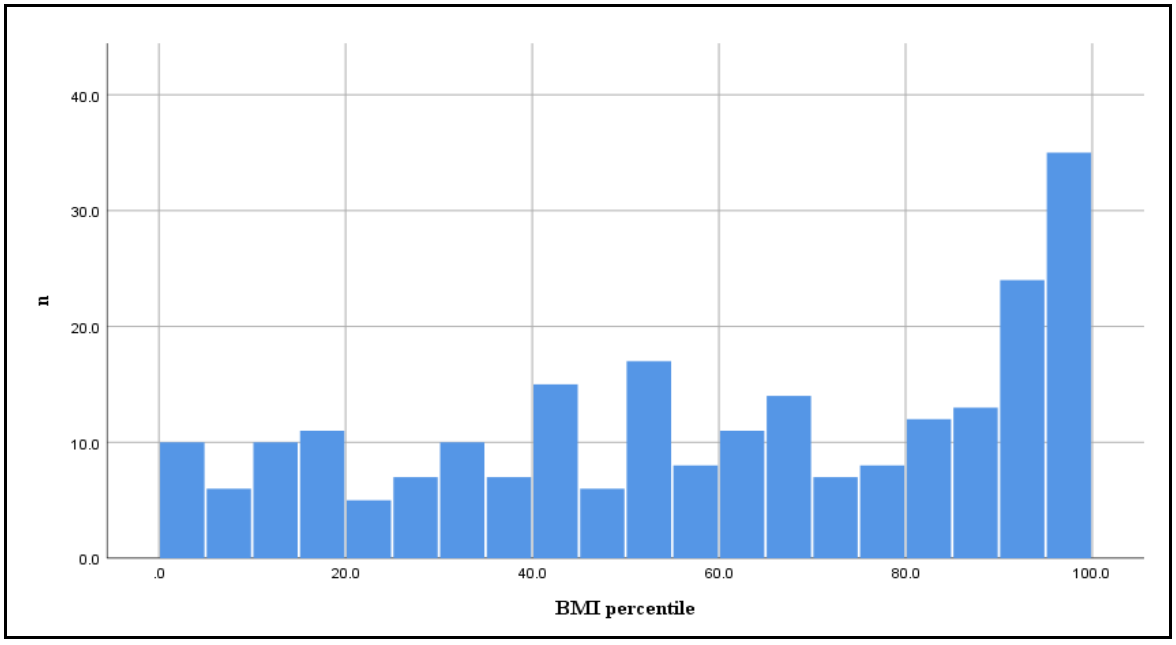

Figure 9: Distribution of BMI percentiles.

3.3 Blood Pressure

After assignment of BP percentiles, 14.8% (35/236) were classified as pre-HT, while 22.9% (54/236) were classified as HT, resulting in a total prevalence of pre-HT/HT of 37.7% (89/236).

Males showed a prevalence of pre-HT/HT of 34.5%, while females showed a prevalence of 38.7% (**Table 12**). Chi-square test of independence showed no significant relation between BP-status and sex ($\chi 2(1, n=236) =0.306, p=0.58$).

In the aspect of weight status, 61.1% (44/72) of students with O/O were classified with pre-HT/HT. In the adolescents with obesity, the prevalence of pre-HT/HT was even higher at 71.4% (25/35). Chi-square test of independence showed a significant relation between weight status and BP-status ($\chi 2(1, n=236) =24.150, p<0.001, \varphi= 0,32$). Participants with O/O were more likely to be pre-HT/HT. Nonetheless also in the healthy weight adolescents 43 27.3% (42/154) where classified as pre-HT/HT (**Table 13**, **Figure 10**).

Weight and BP-status combined, 49.7% (117/236) of the study cohort were classified as O/O, pre-HT/HT, or both (**Figure 11**).

Table 12: BP-status, by sex.

			BP-status			Total (n)
			NT	pre-HT	HT	
Sex	Female	n	111	27	43	181
		%	61.3%	14.9%	23.8%	100.0%
	Male	n	36	8	11	55
		%	65.5%	14.5%	20.0%	100.0%
Total		n	147	35	54	236
		%	62.3%	14.8%	22.9%	100.0%

Table 13: BP-status, by weight status.

			BP-Status			Total (n)
			NT	pre-HT	HT	
Weight status	Underweight	n	7	1	2	10
		%	70.0%	10.0%	20.0%	100.0%
	Healthy weight	n	112	20	22	154
		%	72.7%	13.0%	14.3%	100.0%
	Overweight	n	18	6	13	37
		%	48.6%	16.2%	35.1%	100.0%
	Obesity	n	10	8	17	35
		%	28.6%	22.9%	48.6%	100.0%
Total		n	147	35	54	236
		%	62.3%	14.8%	22.9%	100.0%

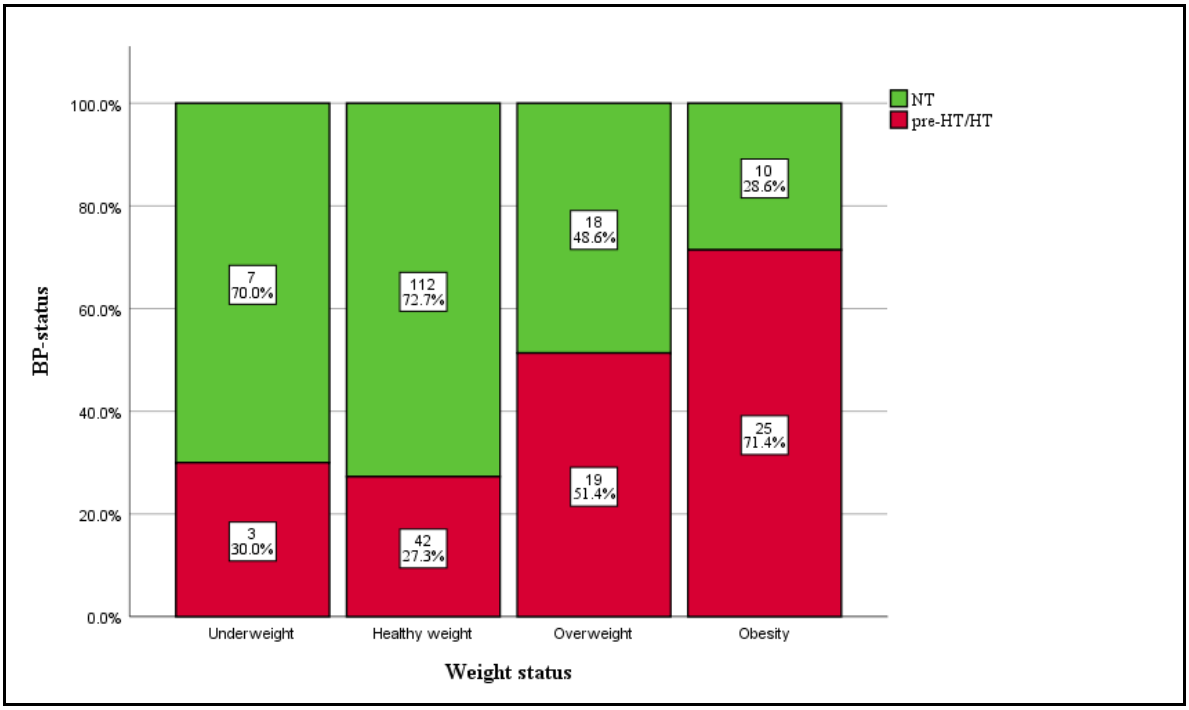

Figure 10: Distribution of BP-status, by weight status.
Percentages display the prevalence in each weight group.

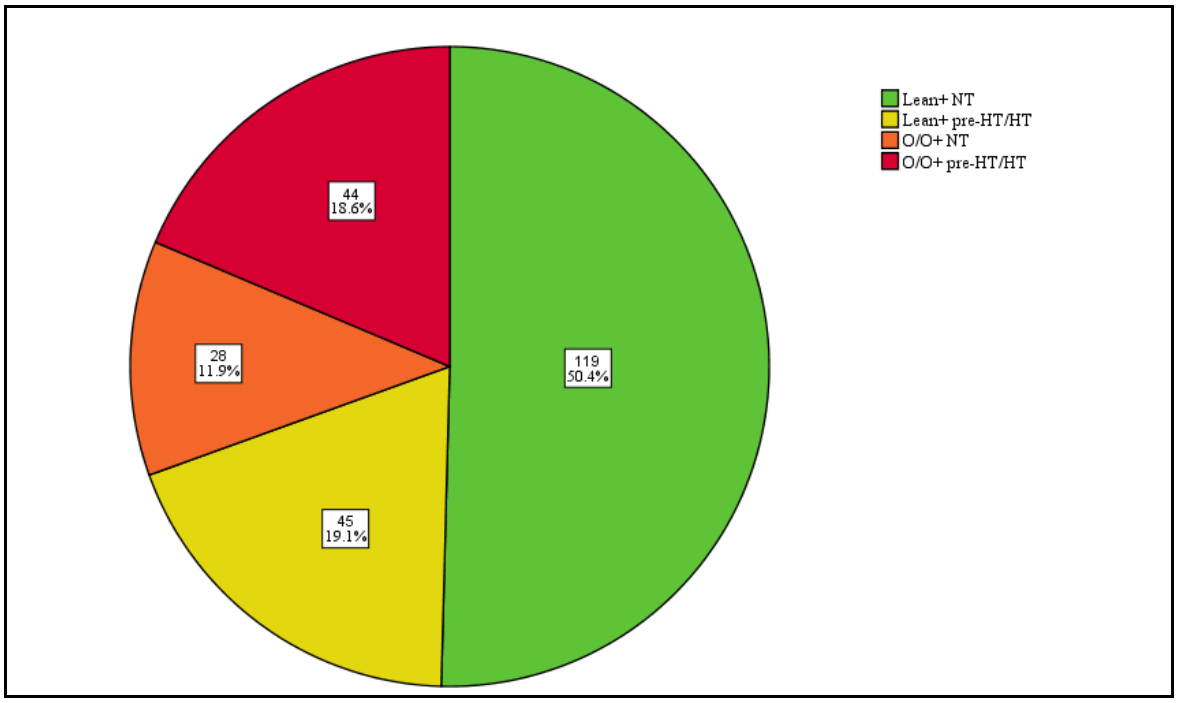

Figure 11: Cohort divided into groups, classified by weight- and BP-status.
Nearly half (49.6%) of the study population were either O/O (orange) or pre-HT/HT (yellow) or both (red). 50.4% were classified as lean and normotensive (green).

3.4 Anthropometric Measurements

Several anthropometric measures differed between the sexes ($F_{(7, 223)}$=12.36, p<0.001; Wilks' λ=0.72). Males had larger NC ($F_{(1, 229)}$=2.89, p≤0.001) and AC ($F_{(1, 229)}$=0.04, p=0.026), while females had larger HC ($F_{(1, 229)}$=3,59; p=0,059) and a greater amount of total body fat ($t_{(231)}$=9.94, p<0.001) (**Table 14**).

Comparing pre-HT/HT and NT participants, MANOVA showed significant differences in all anthropometric measures, with the exception of AC (females: $F_{(7, 169)}$=3.41, p=0.002, Wilks' λ=0.88, males: $F_{(7, 46)}$=5.92, p<0.001, Wilks' λ=0.53), total body fat (females: $F_{(1,178)}$=16.8, p<0.001; males: $F_{(1,53)}$=9.0, p=0.004), and BMI (females: $F_{(1,179)}$=12.7, p<0.001, males: $F_{(1,53)}$=12.2, p=0.001) in both sexes (**Table 14**).

Table 14: Anthropometric values split by BP-status.

	Female			**Male**	
Parameter	**NT** (n=111)	**Pre-HT/HT** (n=70)		**NT** (n=36)	**Pre-HT/HT** (n=19)
Age	14.3 (1.0)	14.1 (0.9)		14.8 (1.0)	14.7 (1.2)
Neck Circumference	2.7 (2.1)	30.3 (1.8) *	f<m	31.3 (1.9)	32.7 (3.2) *
Mid upper arm Circumference	25.2 (3.4)	26.9 (3.8) **		24.8 (2.9)	28.5 (5.0) **
Waist Circumference	69.6 (8.7)	75.3 (9.9) **		68.9 (7.6)	79.5 (12.2) **
Hip Circumference	90.3 (10.8)	96.3 (11.4) **	f>m	85.7 (7.9)	95.7 (14.7) *
Thigh Circumference	47.3 (7.2)	51.5 (7.6) **		43.6 (5.1)	53.6 (10.3) **
Calf Circumference	32.6 (4.3)	34.6 (3.9) **		32.1 (3.5)	35.2 (5.8) *
Ankle Circumference	22.8 (2.2)	23.0 (1.8)	f<m	23.2 (2.3)	24.3 (2.6)
WHtR	0.44 (0.05)	0.49 (0.07) **		0.42 (0.05)	0.48 (0.07) **
Fat (%)	25.0 (7.5)	29.8 (7.9) **	f>m	12.4 (6.5)	18.7 (9.0) *
BMI (kg/m^2)	21.1 (4.2)	23.5 (4.7) **		19.6 (3.6)	23.9 (5.5) **

Values presented as mean± SD. Significant differences are flagged by * for p<0,05 and ** for p<0,01. All circumferences in cm.

Pearson correlations were calculated to show the relationship between anthropometric measurements and cardiovascular risk factors. All anthropometric measurements correlated significantly with BMI ($p<0.01$).

SBP correlated with all anthropometric measurements as well as BMI and WHtR ($p<0.01$ for all), while DBP only correlated with BMI, MUAC, chest circumference, waist circumference, hip circumference, thigh circumference and WHtR.

Significant correlations for triglycerides were found for BMI, WHtR, and all anthropometric measurements but neck circumference and ancle circumference.

For fasting glucose, only thigh circumference and WHtR showed significant correlations, while for HDL-C no significant correlations were found (**Table 15**).

Table 15: Correlations between anthropometric measurements and risk factors.

	BMI	SBP	DBP	TGs	FG	HDL-C
BMI	1	0.382**	0.175**	0.270**	-0.112	-0.028
n	236	236	236	204	207	102
Neck circumference	0.591**	0.400**	0.065	0.136	-0.079	-0.009
n	234	234	234	202	205	101
Mid-upper-arm circumference	0.870**	0.365**	0.132*	0.244**	-0.083	0.052
n	232	232	232	201	204	101
Chest circumference	0.867**	0.436**	0.239**	0.283**	-0.077	0.095
n	231	231	231	199	202	100
Waist circumference	0.862**	0.402**	0.203**	0.294**	0.029	-0.031
n	234	234	234	202	205	101
Hip circumference	0.887**	0.423**	0.212**	0.213**	-0.116	0.037
n	234	234	234	202	205	101
Thigh circumference	0.840**	0.451**	0.234**	0.266**	-0.172*	0.011
n	233	233	233	201	204	100
Calf circumference	0.840**	0.403**	0.119	0.251**	-0.134	0.012
n	234	234	234	202	205	101
Ankle circumference	0.655**	0.264**	-0.039	0.120	0.004	-0.065
n	233	233	233	201	204	100
Waist-to-hip-ratio	0.062	0.029	0.021	0.121	0.222**	-0.07
n	234	234	234	202	205	101
Waist-to-height-ratio	0.822**	0.344**	0.268**	0.308**	0.028	-0.044
n	234	234	234	202	205	101

Significant values are flagged with * for $p<0.05$ and ** for $p<0.01$.

3.5 Risk Factor Clustering

Blood parameters showed high inter-individual variability and they were only available for a subset of the study cohort. HDL-C, TGs, and FG were analyzed separately by a two-way ANOVA to differentiate between lean and O/O adolescents as well as NT and pre-HT/HT participants. Two significant effects and their interaction ($F_{(1, 200)}$=4.46, p=0.036) showed, that both a high weight status ($F_{(1, 200)}$= 5.76, p<0.001) and elevated blood pressure ($F_{(1, 200)}$=11.55, p=0.001) contributed to an elevation of TGs. For FG significantly higher values were found in adolescents with pre-HT/HT compared to NT adolescents ($F_{(1, 203)}$ = 4.52, p=0.035). For HDL-C no significant values where reached (**Table 16**).

Table 16: Association of blood parameters with weight- and BP-status.

	Lean		**Overweight/Obese**	
	NT	pre-HT/HT	NT	pre-HT/HT
	mean (±SD), n	mean (±SD), n	mean (±SD), n	mean (±SD), n
HDL-C (mmol/L)	1.22 (0.57), 54	1.26 (0.55), 21	1.02 (0.19), 11	1.42 (0.73), 16
TGs (mmol/L) *	0.76 (0.21), 104	0.83 (0.31), 43	0.86 (0.33), 24	1.15 (0.55), 33
FG (mmol/L) *	4.75 (0.48), 105	4.94 (0.53), 43	4.66 (0.57), 25	4.79 (0.46), 34

* flag a significance level of p<0.05

Applying the criteria by Cook et al. (84), we examined the prevalence of the risk factors contributing to MetS.
9.4% (22/234) of participants fulfilled the criteria for WC (>90th pc); 37.7% (29/236) for hypertension (>90th pc); 10.3% (21/204) for hypertriglyceridemia (> 110 mg/dl); 3.9% (8/207) for impaired FG (>110 mg/dL); and 56.9% (58/102) for low HDL-C (<40mg/dL).

As there where only sub-samples of the blood parameters available, the prevalence of risk factor clustering was assessed for four risk factors (WC, HT, TGs, FG). A subgroup including participants, with all five MetS components was examined separately.

45% (91/202) of participants that were examined concerning these four risk factors showed at least one elevated parameter. In participants with O/O, 75% (42/56) scored at least one

risk factor. In the O/O group, 12.5% already fulfilled the criteria for MetS (**Table 17**, **Figure 12**).

Table 17: Risk factor clustering in lean and O/O adolescents.

			Number of risk factors				Total (n)
			0	1	2	3	
Weight Status	Lean	n	97	43	6	0	146
		%	66.4%	29.5%	4.1%	0.0%	100.0%
	O/O	n	14	25	10	7	56
		%	25.0%	44.6%	17.9%	12.5%	100.0%
Total		n	111	68	16	7	202
		%	55.0%	33.7%	7.9%	3.5%	100.0%

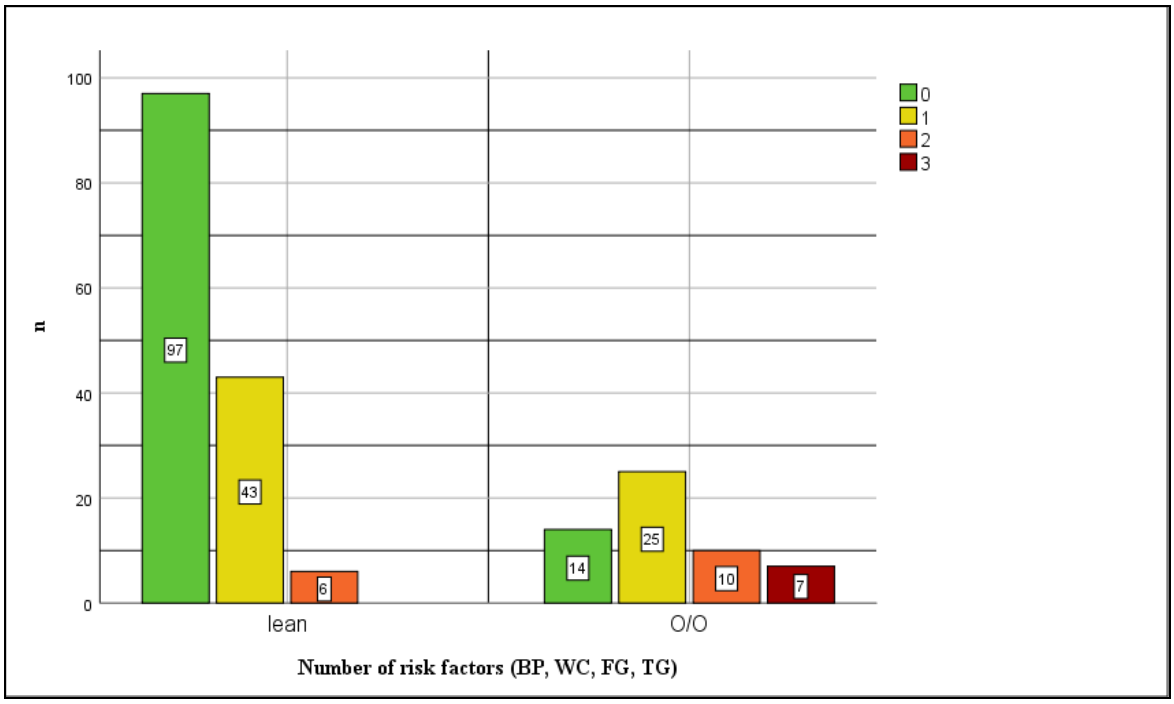

Figure 12: Number of risk factors in lean and O/O participants.

3.6 Metabolic Syndrome

From a total of 94 participants (71 female, 23 male), data for all 5 MetS components (WC, FG, TGs, HDL-C, BP) was collected. 7.5% (7/94) accumulated 3 pathologic components, diagnosing them with MetS. 74.4% (70/94) scored at least 1 risk factor (**Figure 13**).
8.5% (6/71) of females were diagnosed with MetS, while 4.3% (1/23) of males fulfilled the criteria (**Table 18**).
The prevalence of MetS was 1.5% (1/65) in those with healthy weight, and 26.1% (6/23) in the O/O group. In participants with obesity, MetS was diagnosed in 45.5% (5/11) (**Table 19**, **Figure 14**).

Table 18: Number of risk factors, by sex.

			Number of Risk Factors				Total (n)
			0	1	2	3	
Sex	Female	n	17	31	17	6	71
		%	23.9%	43.7%	23.9%	8.5%	100.0%
	Male	n	7	10	5	1	23
		%	30.4%	43.5%	21.7%	4.3%	100.0%
Total		n	24	41	22	7	94
		%	25.5%	43.6%	23.4%	7.4%	100.0%

Table 19: Number of risk factors, by weight status.

			Number of Risk Factors				Total (n)
			0	1	2	3	
Weight Status	Underweight	n	0	3	3	0	6
		%	0.0%	50.0%	50.0%	0.0%	100.0%
	Healthy weight	n	21	30	13	1	65
		%	32.3%	46.2%	20.0%	1.5%	100.0%
	Overweight	n	3	4	4	1	12
		%	25.0%	33.3%	33.3%	8.3%	100.0%
	Obesity	n	0	4	2	5	11
		%	0.0%	36.4%	18.2%	45.5%	100.0%
Total		n	24	41	22	7	94
		%	25.5%	43.6%	23.40%	7.40%	100.0%

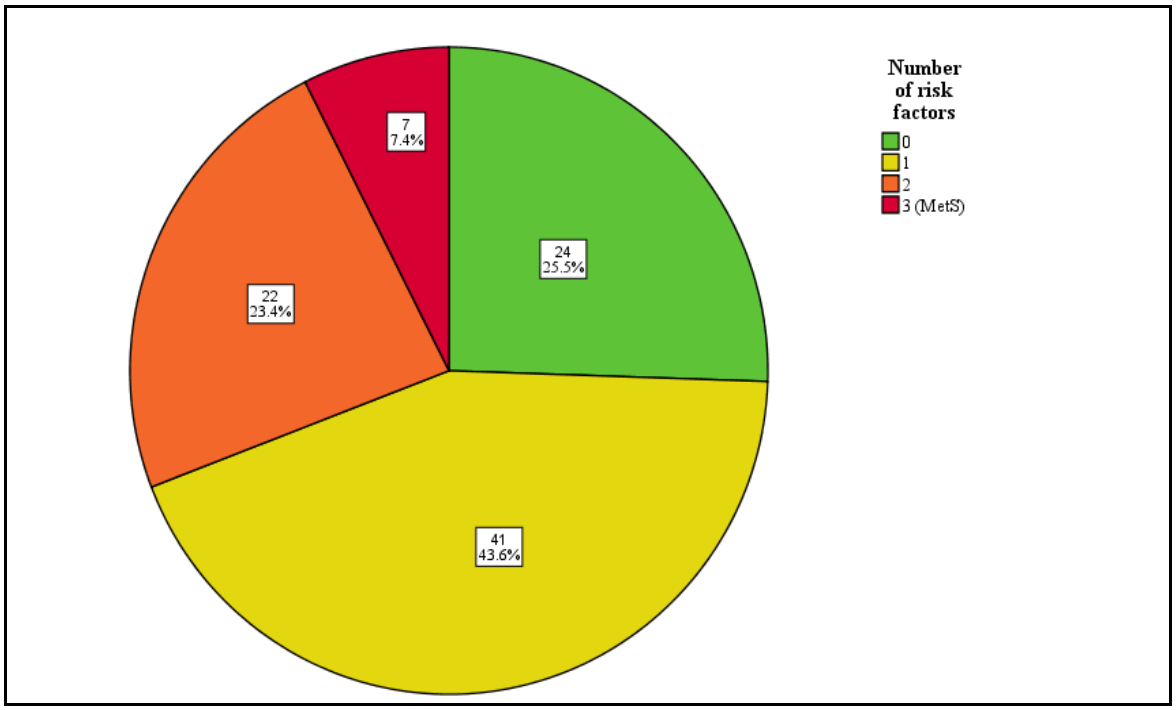

Figure 13: Distribution of risk factors
Only 25.5% (24/94) adolescents showed none of the five risk factors (green). 7.4% (7/94) were diagnosed with MetS (red).

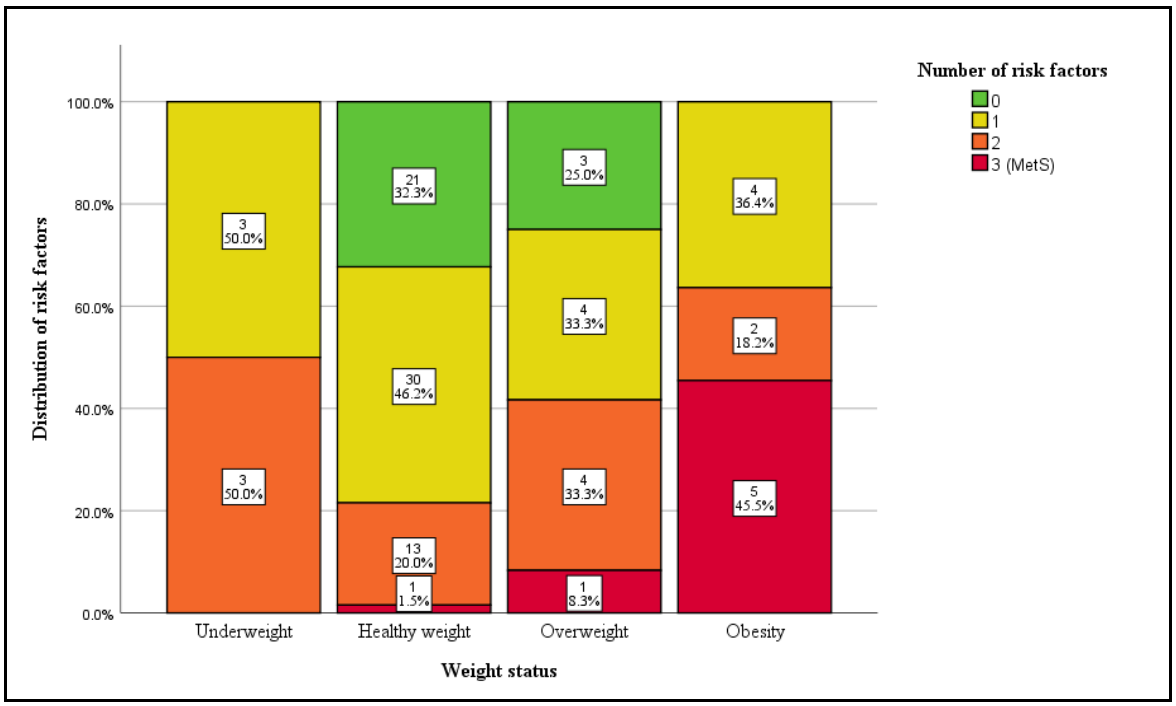

Figure 14: Number of risk factors, by weight status.
While the prevalence of MetS was 1.5% (1/65) in healthy weight participants, it was 45.5% (5/11) in participants with obesity. The prevalence of MetS is displayed in red.

4 Discussion

This study assessed the prevalence of the MetS and cardiometabolic risk factors in adolescents aged 13-16 years. We observed a high prevalence of overweight and obesity, with close to a third (30.5%, 72/236) being above the 85th percentile for BMI. These results confirm the trends of rising overweight and obesity that are reported in several studies conducted in SSA and worldwide (16, 151, 152).
Other studies from SSA reported adult and adolescent females being more susceptible to O/O, suggesting a link to social norms and beauty ideals as well as changes of physical activity in puberty (17, 151, 152, 156-158). Our study could not confirm these findings, as both males and females to be both affected from increased body size status.

Previous publications concerning Mthatha have indicated a high prevalence of pre-HT/HT in adults and adolescents, which led to the hypothesis that risk factors are present in these school learners as well, exposing them to a greater risk of suffering from CVDs later in life (151-153). Our study shows very similar results, with the prevalence of pre-HT/HT being 37.7%, in comparison to Nkeh et al. who reported a prevalence close to 33% in 13-17 year olds (152), as well as Sekokotla et al. (151) in 13-18 year olds from Mthatha.
Pre-HT/HT was significantly associated with weight status, supporting the claim that obesity contributes to the development of hypertension (100, 159). While participants with O/O were more likely to be diagnosed with elevated BP, more than a quarter of students with healthy weight were classified as pre-HT/HT (27.3%, 42/154), which indicates further research on the etiology of HT in this population. IR has been suggested as a cause of elevated blood pressure in the lean population (160). Another factor influencing the development of hypertension might be exposure to air pollution (161).
With our study displaying the high prevalence of elevated BP, we want to highlight the importance of educational measures, as the knowledge about CVDs and hypertension, as well as the awareness of affected individuals is low in SSA (162, 163). Furthermore, untreated HT has shown to be high in Mthatha. Adeniyi et al. reported 75.5% of a study population with known HT and T2DM to have uncontrolled hypertension (164). This shows the importance, as uncontrolled hypertension is deemed to be the single most important risk factor for the development of CVDs (10).

Matjuda et al. reported a high prevalence of O/O and pre-HT/HT already in children aged 6-9 years in primary schools of Mthatha and nearby cities. Thus, preventative strategies need to be implemented even earlier (165).

Overall, we observed a high prevalence of cardiometabolic risk factors, with nearly half (45%, 91/202) of the study population showing at least one out of four (WC, BP, FG, TGs) risk factors. In the subgroup where data for all five (WC, BP, FG, TGs, HDL-C) risk factors were available, nearly three quarters (74.4%, 70/94) scored at least one. Risk factor clustering was shown to be present in this cohort, with high numbers of adolescents scoring multiple risk factors. Participants classified as O/O or pre-HT/HT also showed higher values in triglycerides and pre-HT/HT adolescents showed higher values of fasting glucose. These findings are particularly relevant, as Berenson et. al have shown that a higher amount of cardiovascular risk factors is associated with the severity of atherosclerosis in young individuals already (166).

Applying the criteria proposed by Cook et al. (84), we observed a prevalence of 7.4% in a group of 94 adolescents for the MetS, which is comparable, but higher, compared to results from Sekokotla et al. (151), who used the same criteria and reported a prevalence of 6.0% in male and 3.1% in female students in a similar population.
However, as mentioned by different authors, it is questionable if the use of MetS as an identification tool for cardiovascular risk is reasonable in children and adolescents, due to its high instability during adolescence and especially puberty (167, 168). Several researchers therefore suggest to focus on identification and treatment of the individual risk factors instead, as they have been shown to have a higher stability, when observed over time (80, 83).
Another point of criticism is the inconsistent definition of MetS, leading to a low comparability (82, 83). Currently the different cut-off values and criteria result in a different prevalence, even when examining the same collective of subjects, as Magge et al. state referring to publications by Cook et al. and de Ferranti et al. (83, 84, 86). This underlines the importance of unifying the criteria for children and adolescents (80, 82, 83, 169).

Limitations

As this project was conducted as a cross-sectional study, several limitations must be discussed. Firstly, the study cohort was only examined once, giving results from a certain point of time. Thus, longitudinal studies should be conducted, to follow up the participants and observe the development of these risk factors and their health status.

There was a disparity between the number of female and male participants in our study population, leaning female in all four participating schools. Boys have been shown to have higher school dropout rates in SA (170), and the South African education statistics show a slight majority of female students in the secondary grade of Eastern Cape schools (171). Also, more females than males consented to take part, therefore our study population is not ideally representative. This has already been the case in other adolescent studies from Mthatha (151, 152). As males have been shown to be at higher risk of developing CVDs (12), future study designs should aim to include more males.

This being a field study, laboratory conditions could not always be met. Humidity and room temperature varied over the days of measurement, therefore BP values must be interpreted with caution.

As it was a novel situation for many of the participants, blood pressure values may have been elevated due to stress and anxiety. Therefore, future studies should consider following up with ambulatory or home measurements, to confirm results (40, 172-174).

Furthermore, control measurements with the auscultatory method should be conducted, as the oscillometric method has been shown to overestimate BP-values, and percentile curves were developed using auscultatory measurement results (172).

As there are no anthropometric percentile curves for SSA adolescents available, international criteria for anthropometric measurements had to be used (25). While efforts have been made to ensure a global definition of percentiles and cut-off points to be used for all ethnicities, they may not fit the body constitution of adolescents with African ancestry ideally (151). Therefore, we want to emphasize the importance of conducting more extensive studies on anthropometrics in these populations, to develop ethnicity specific percentile curves.

While significant correlations between anthropometric data and cardiovascular risk factors were found, and the development of pre-HT/HT seems to be driven to a large extent by weight status, lean adolescents have been shown to be affected by cardiovascular risk factors as well. Questionnaires concerning the lifestyle and nutrition habits have been conducted prior to the measurements. These are yet to be analyzed and will add additional value to our study. They may show other influences on development of cardiovascular risk, as well as underlying behavior leading to O/O.

Additional Publications

Within the framework this project on cardiovascular risk, three students from MUG where involved with their diploma theses. Besides this thesis, one with the focus on BP has already been published (175). The third diploma thesis is still in progress and will take a closer look into the sex differences, therefore this topic has only been covered superficially in this publication.

Furthermore, a research paper is currently being prepared, which will additionally cover markers of vascular damage and IR.

5 Conclusions and Future Directions

We observed a high prevalence of cardiometabolic risk factors in the study population of 13-16-year-old adolescents. The prevalence of elevated blood pressure and overweight/obesity was particularly high. Therefore, these adolescents are exposed to a higher risk of developing metabolic syndrome, diabetes mellitus and cardiovascular diseases. While participants classified as overweight/obese showed more of the risk factors, lean adolescents were affected as well. This shows the necessity of further research, including longitudinal studies, and exploration of underlying behavioral or environmental risks to enable the development of adequate prevention strategies.

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and CVD. p. 11. Reprinted with permission from Elsevier.

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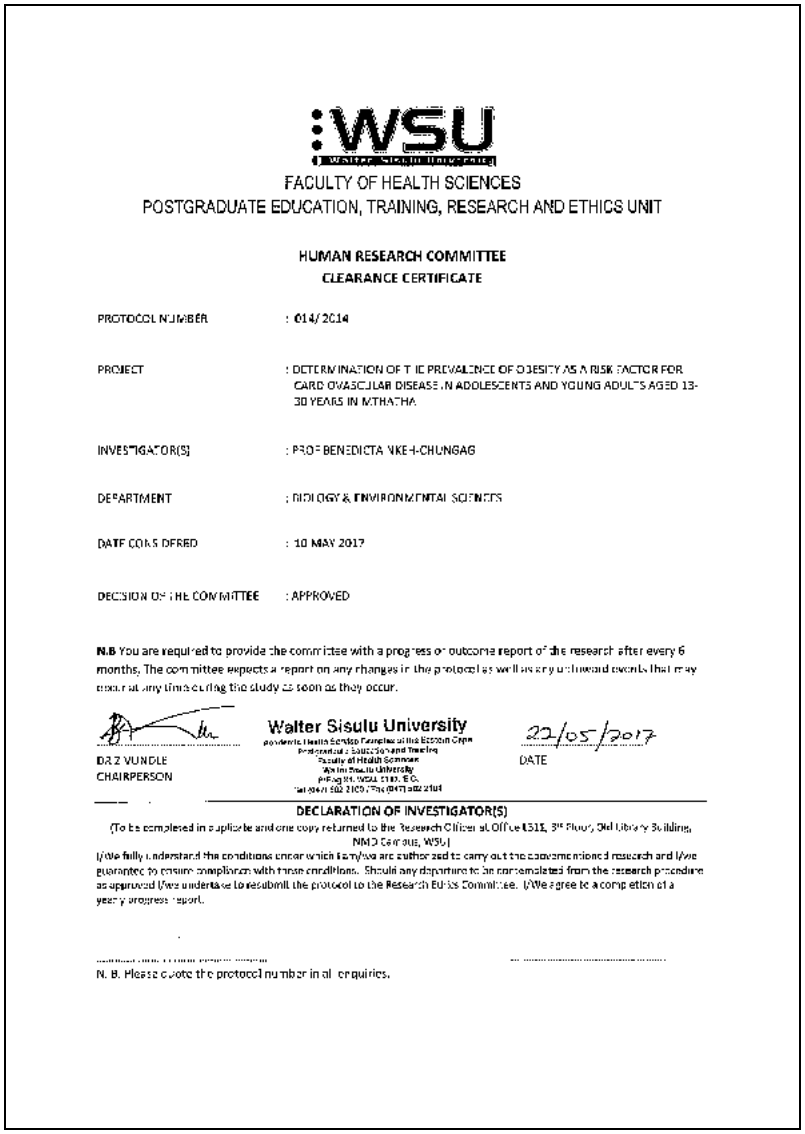
7 Appendices

7.1 Ethical Clearance



WSU
Walter Sisulu University

FACULTY OF HEALTH SCIENCES
POSTGRADUATE EDUCATION, TRAINING, RESEARCH AND ETHICS UNIT

HUMAN RESEARCH COMMITTEE
CLEARANCE CERTIFICATE

PROTOCOL NUMBER	: 014/2014
PROJECT	: DETERMINATION OF THE PREVALENCE OF OBESITY AS A RISK FACTOR FOR CARDIOVASCULAR DISEASE IN ADOLESCENTS AND YOUNG ADULTS AGED 13-30 YEARS IN MTHATHA
INVESTIGATOR(S)	: PROF BENEDICTA NKEH-CHUNGAG
DEPARTMENT	: BIOLOGY & ENVIRONMENTAL SCIENCES
DATE CONSIDERED	: 10 MAY 2017
DECISION OF THE COMMITTEE	: APPROVED

N.B You are required to provide the committee with a progress or outcome report of the research after every 6 months. The committee expects a report on any changes in the protocol as well as any untoward events that may occur at any time during the study as soon as they occur.

DAZ VUNDLE
CHAIRPERSON

Walter Sisulu University
Faculty of Health Sciences
Private Bag X1, MTHATHA, 5099, RSA

22/05/2017
DATE

DECLARATION OF INVESTIGATOR(S)

(To be completed in duplicate and one copy returned to the Research Officer at Office 1511, 3rd Floor, Old Library Building, NMD Campus, WSU)

I/We fully understand the conditions under which I am/we are authorized to carry out the above mentioned research and I/we guarantee to ensure compliance with these conditions. Should any departure to be contemplated from the research procedure as approved I/we undertake to resubmit the protocol to the Research Ethics Committee. I/We agree to a completion of a yearly progress report.

N. B. Please quote the protocol number in all enquiries.

7.2 Consent Form

Consent form (English)

ASSOCIATION OF CARDIOVASCULAR DISEASE RISK FACTORS WITH ENDOTHELIAL FUNCTION IN ADOLESCENTS LIVING IN MTHATHA, SOUTH AFRICA

Consent form

We are hereby requesting for your permission to include your child in this study.

I have read and understand what the study involves and permit mu child/charge (Name of child) _____ Grade _____ to participate in this study, and to donate 5ml of blood and 20 ml of urine samples.

Parent/guardian: Signature _____ Date _____

7.3 Data Sheet

Adolescents study 2017–2018: Cardiovascular disease risk factors

Participants Data sheet

Participant Code: _____

Personal information

Name: _____ Grade: _____

School Name: _____ Sex: Male ☐ Female ☐

Date of birth: _____ Place of birth: _____

Parents' phone number: _____

Anthropometry

Height: _____

Weight: _____ Waist circumference: _____

BMI: _____ Hip circumference: _____

MUAC: _____

Blood pressure

	1	2	3
SBS (mmHg)			
DBD (mmHg)			
Heart Rate			

PWV ☐ **Blood Sample & Urine** ☐