# Levels of urotensin-II in obese children and adolescents

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# LEVELS OF UROTENSIN-II IN OBESE CHILDREN AND ADOLESCENTS

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**LIST OF ABBREVIATONS**

| | |
|---|---|
| **BMI** | **Body mass index** |
| **WHO** | **World Health Organization** |
| **MS** | **Metabolic syndrome** |
| **IDF** | **International Diabetes Federation** |
| **T2DM** | **Type 2 Diabetes Mellitus** |
| **SD** | **Standard deviation** |
| **POMC** | **Pro-opiomelanocortin** |
| **LEPR** | **Leptin receptor** |
| **LEP** | **Leptin gene** |
| **MC4R** | **Melanocortic 4 receptor** |
| **AHA** | **American Heart Association** |
| **HDL** | **High density lipoprotein** |
| **LDL** | **Low-density lipoprotein** |
| **VLDL** | **Very low-density lipoprotein** |
| **WC** | **Waist circumference** |
| **HC** | **Hip circumference** |
| **SNPs** | **Single Nucleotide polymorphisms** |
| **RAAS** | **Renin-angiotensin-aldosterone system** |
| **ROS** | **Reactive oxygen species** |
| **TNF-α** | **Tumor necrosis factor alpha** |
| **IL-6** | **Interleukin 6** |
| **CRP** | **C-reactive protein** |

| | |
|---|---|
| **OSA** | **Obstructive sleep apnea** |
| **NAFLD** | **Nonalcoholic fatty liver disease** |
| **U-II** | **Urotensinogen II** |
| **UT** | **Urotensin receptor** |
| **NADPH** | **Nicotinamide adenine dinucleotide phosphate** |
| **ACTH** | **Adrenocorticotropic hormone** |
| **HOMA IR** | **Homeostatic Model Assessment of Insulin Resistance** |
| **IR** | **Insulin resistance** |
| **CV** | **Coefficient of variation** |
| **BP** | **Blood pressure** |
| **SBP** | **Systolic blood pressure** |
| **DBP** | **Diastolic blood pressure** |
| **HT** | **Hypertension** |
| **CVD** | **Cardiovascular disease** |

# 1. INTRODUCTION

## 1.1. Obesity in population of children and adolescents

### 1.1.1. Definition of obesity

As the World Health Organization (WHO) defines: obesity is abnormal or excessive fat accumulation that presents a risk to health (1). Obesity can be defined based on the body mass index (BMI), which is determined by dividing the weight (kg) by the height ($m^2$): BMI = $kg/m^2$ (1). Based on these measurements in adults WHO have further classified obesity into three groups presented in Table 1 (1).

**Table 1.** Classification of obesity in adults (1).

| Classification | BMI (kg/m2) | Risk of comorbidities |
|---|---|---|
| **Obese class I** | 30.0-34.9 | Moderate |
| **Obese class II** | 35.0-39.9 | Severe |
| **Obese class III** | >40.0 | Very severe |

BMI, body mass index.

The classification of obesity in the pediatric population has been problematic considering that the body is continually changing and the height continues to rise (1). Before puberty, children's skeletal and muscle mass decreases, and girls mature before boys (1). Sex and age correction for obesity in children aged 2 to 18 is therefore recommended (2).

BMI z-scores, also known as BMI standard deviation (SD) scores, are weight-relative metrics adjusted for child age and gender (3). A BMI z-score (or its equivalent BMI-for-age percentile) can be calculated using a child's age, sex, BMI and an appropriate reference standard (Figure 1) (3). According to WHO more than 2 SD are defined as obese, 2-3 SD is moderate obese while more than 3 SD is considered severely obese (4).

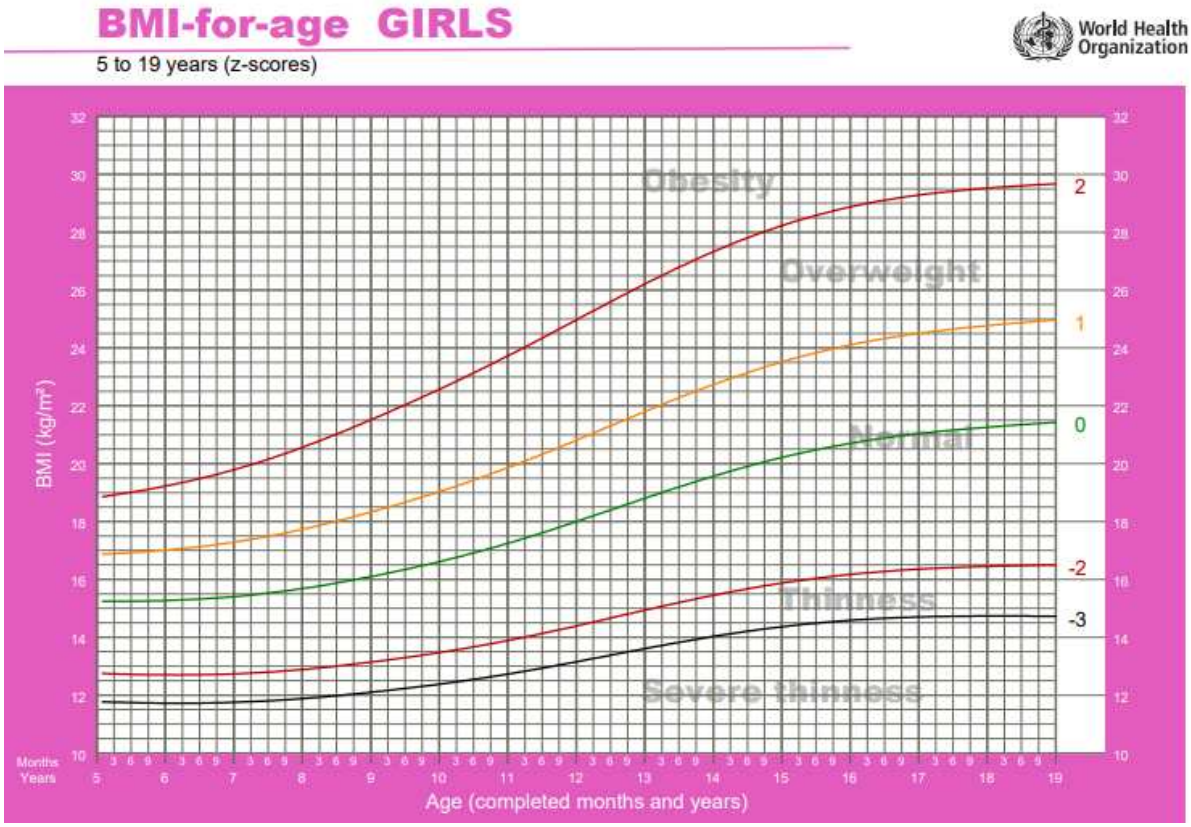


Figure 1. BMI z-scores chart for girls 5 to 19 years (4).

Waist measurements offer a more accurate image of body fat distribution than BMI and, therefore, can provide more insight into the likelihood of cardiovascular disease later in life (5). However, while age and gender-adjusted reference tables have been published in many countries, the threshold values for obesity in children based on waist measurements have not been established nationally or internationally (6).

### 1.1.2. Epidemiology

Since 1980 the global prevalence of obesity has more than doubled, with almost a third of the world's population now listed as overweight or obese (7). According to *Chooi et al.,* 609 million adults worldwide were estimated to be obese in 2015 (7). The prevalence was higher in men than women in the age group 25 to 44 years, while after 45 to 49 years, it became higher in women (7). As for adults, obesity is becoming more common in young children and adolescents (8). In the United States, it is estimated that 16% of children and

adolescents are overweight, and 34% are obese (8). Similar trends have been found in numerous European countries, where 31.8% of school-aged children are overweight or obese, in accordance with the latest International Task Force standards (9). A study conducted by *Yang et al.* compared the prevalence of underweight and overweight among young adolescence in low-income countries and found that the prevalence of overweight was higher than those of underweight (10). This was especially seen in countries in Central and South America where the prevalence of obesity was higher (10). In the same study, the prevalence of obesity between boys and girls did not differ significantly (10). Obesity in children has reached epidemic proportions, making it the most common chronic illness in this age group (11). A study conducted by Milanovic et al. showed a 35.9% prevalence of obesity in Croatian children aged 7-9 years old (12). Croatian boys had a higher prevalence than Croatian girls (12). The study also found that the prevalence was higher in children living in Adriatic Croatia and lowest in the City of Zagreb (12). This could be explained by the fact that obesity is associated with lower socioeconomic status, and Zagreb being the capital has the highest proportion of highly educated populations (12).

### 1.1.3. Pathophysiology and risk factors

The causes of obesity are many and vary between individuals (13,14). Several factors act in, and often at the same time (13). To simplify it, one can say that obesity is due to an unbalance between energy intake and energy consumption. The difference in this energy balance will eventually increase the weight of a person (15).

It is difficult to define if the cause of obesity is due to a high intake of food with an increased number of calories or a lifestyle with low physical activity (15). This is due in large part to underreporting of food intake, especially when it comes to patients with obesity (15). Obesity is caused by multifactorial factors like genes, socioeconomic and cultural factors (14). The literature describes several risk factors that are related to the development of overweight and obesity in children.

#### *1.1.3.1. Growth and development*

Puberty is a delicate time, with physiological insulin resistance and hyperinsulinemia being common (11). As a result, the prevalence of obesity at this stage places additional stress on the body, increasing the risk of complications (11). Research has shown that obesity is far more likely to persist through puberty than becoming outgrown (16). Obesity in childhood and adolescence significantly increases the risk of obesity in adulthood, and Must et al. state that obese children make up 85 percent of obese adults (17).

#### *1.1.3.2. Genetics*

It is known that obesity has a strong hereditary component (18). If both parents are overweight, the child has an 80% risk of being overweight as well (18). Obesity has a 10% risk of developing in children with two normal-weight parents (18). There is a 50% risk that the second or third child would be obese if one of the two siblings is obese (18). If one of three siblings is obese, there is an 80% risk that one of the others will be as well (18). Whether this is genetic or the environment is difficult to answer, but it is likely to be a strong hereditary component (19). Obesity due to monogenetic defects is rare (20–22). The majority of the causes are due to polygenic inheritance combined with predisposing environmental factors (23). Monogenetic defects that cause obesity are mainly due to mutations in genes of the leptin/melanocortin axis (21). Pro-opiomelanocortin (POMC), Leptin receptor (LEPR), and leptin gene (LEP) are all involved in the regulation of food intake, and defects in these can be responsible for obesity (21). Melanocortic 4 receptor (MC4R) mutations are the most frequent who have shown an association with obesity and cause 2-3% of obesity in adults and children (21,22). It is also known that many syndromes have obesity as part of their clinical picture, like Bardet-Biedel syndrome and Prader-Willi syndrome (21).

#### *1.1.3.3. Birth weight*

Research conducted tends to focus on the correlation between low-birth-weight and obesity occurring in adulthood rather than during childhood. But some studies that have been conducted on childhood obesity show a strong connection between both high and low-birth -weight and childhood obesity (24). "Catch up" weight in premature infants is associated with persistent weight gain and overweight (25).

#### *1.1.3.4. Adiposity rebounds*

In 1984, a term called "Adiposity rebound" was introduced to describe the time of transition from an increase in muscle mass to an increase in fat mass, which occurs at the age of 5-6 years (26). At this time, the proportion of adipose tissue is the lowest. The study showed that the earlier this adiposity rebound occurred, the greater was the chance for the child to develop obesity (26). In children who developed obesity, adiposity rebound occurred as early as two to three years of age (26). Almost all children who developed obesity will have their adiposity rebound before the age of six (27).

#### *1.1.3.5. Medications*

Contraceptives, antipsychotics, antidiabetics, antihypertensives and certain antidepressants are among the drugs that have been linked to increased body fat (28). If these medications are administered improperly to infants, there is a chance of weight gain. Obesity is one of the side effects of high-dose inhaled corticosteroids in the pediatric population (29).

#### *1.1.3.6. Inactivity*

Studies have concluded that a high degree of physical activity is associated with a low waistline and protects against the development of obesity in children (30). Time spent in front of monitors, televisions, and other portable digital devices is referred to as "screen time" (31). Reduced exercise, increased food consumption, and impact from food ads can all lead to the development of obesity as the result of increased screen time (31). Spending a lot of screen time as a child is a bigger risk for overweight than screen time in adulthood (31). This is because patterns and habits of physical activity are established at a young age, and the earlier one establishes an inactive lifestyle, the greater the risk of obesity (32).

#### *1.1.3.7. Diet*

Longitudinal studies in children have not shown a clear connection between energy intake or composition of the diet and the development of obesity (15). Consumption of sugary drinks is the exception (33). Although fast food and large portion sizes are associated with increased energy and fat intake, have none of these factors alone showed a significant correlation with obesity in cross-sectional studies or longitudinal studies (33). One of the problems, as mentioned earlier, is that overweight and obese individuals tend to underreport their eating habits (15). It is probably a combination of diet and other factors contributing to

the increased prevalence of overweight and obesity among children and adolescence (33). It has been shown that the consumption of sweet drinks is positively correlated to obesity (33). The reason may be that the sugar in sweet drinks causes a rapid rise in blood sugar, a higher insulin reaction, and increased fat deposition through the effect of insulin. An overview study from 2006 claims that there is strong evidence that sugary drinking is directly related to weight gain and obesity in children and adolescents (34). Previous studies have shown that children and adolescents with the largest intake of sweet drinks also have the highest energy intake. One explanation may be that children fail to compensate for the increased energy intake via beverages by reducing the intake of other foods (33). Some studies have found that infants who are breastfed have a protective effect against obesity and that infant formula is one risk factor for the development of obesity (15).

#### *1.1.3.8. Psychosocial factors*

Overeating and inactivity, and thus obesity, can be a reaction to psychological problems and strains of varying severity. Mental disorders such as depression and abuse trauma can in some lead to weight gain due to overeating. However, most obese children and adolescents are not fulfilling the criteria for a psychiatric diagnosis (35). In review research on mental disorders in obese children and adolescents in the period 1993-2003, the authors concluded that there is no higher incidence of mental comorbidity in the general population of obese children and adolescents (35).

### 1.1.4. Complications

Childhood obesity is associated with an increased risk for several complications (17,36,37). Studies clearly show that 20% of obese children have at least one cardiovascular risk factor highly related to the early stages of atherosclerosis (hypercholesterolemia, hyperinsulinemia, hypertriglyceridemia, and hypertension) (36). Besides the several metabolic complications, obese children are also at risk for mental health issues, orthopedic and respiratory problems, and certain cancers (17). Obesity in childhood increases the likelihood for obesity in adult age which in turn give complications like cardiovascular disease, joint problems, polycystic ovary syndrome and fertility problems (37).

### 1.1.5. Psychosocial consequences

In one of the first studies of stigma and obesity conducted by Richardson et al. in 1961, where 10-11 years old were to rank pictures of children based on who they wanted as a friend, overweight was ranked as the least attractive compared to normal-weight healthy and disabled children (38). When the study was repeated, the same results were found (38). In a study of 8210 children reported 36% of the overweight boys and 34% of the overweight girls that they are exposed to weight-related teasing and bullying (39). The stigma obese children and young people are exposed to from friends, parents, teachers, and others has significant psychological, social, and health-related consequences for the individual (40). Obesity in children increases the risk for disturbed eating behavior (40). Disrupted eating behavior increases further the likelihood of weight gain. In addition, being overweight and having disturbed eating patterns is a risk factor for eating disorders (40).

## 1.2. Metabolic syndrome

### 1.2.1. Definition

Metabolic syndrome (MS) is a collection of conditions that increase cardiovascular disease risk and include hypertension, impaired glucose metabolism, dyslipidemia, and abdominal obesity (41).

Various public health organizations, i.a, World Health Organization (WHO), American Heart Association (AHA), International Diabetes Federation (IDF), have all tried to present a definition of metabolic syndrome. According to the IDF which is the criteria most used in the literature when describing MS, for a person to be defined as having metabolic syndrome they must have central obesity plus two of the following four factors seen in Table 2 (42).

**Tabel 2.** The new International Diabetes Federation (IDF) definition of MS for adults (42).

| | |
|---|---|
| Elevated triglycerides (or medically induced) | >150 mg/dL (1,7 mmol/L) |
| Reduced HDL-C (or medically induced) | <40 mg/dL (1.0 mmol/L) in males<br><50 mg/dL (1.3 mmol/L) in females |
| Elevated blood pressure (or use of antihypertensive medications) | Systolic >130 and/or diastolic >85 mmHg |
| Elevated fasting glucose (or use of antiglycemic drugs) | >100 mg/dL (>5.6 mmol/L) |

Adapted from: Zimmet et al. The metabolic syndrome in children and adolescents – an IDF consensus report.

While several attempts have been made to describe the MS in children, no accepted definition has yet been reached (43). Indeed, in 2009, an American Heart Association (AHA) writing committee declined to identify it (44). However, in 2007 the international diabetes federation (IDF) proposed a definition of pediatric MS in children aged 6 to 10 years with waist circumference (WC) > 90$^{th}$ percentile and which have other related risk factors (family history of diabetes or cardiovascular disease) and in children aged from 10 to 16 years who are obese with WC > 90th percentile and in addition meet two or more of the parameters for adult criteria (Triglycerides, HDL-cholesterol, blood pressure and glucose concentrations) (45). as presented in Table 3. For children over the age of 16 years, the adult IDF criteria can be used (45).

**Table 3.** The IDF consensus definition of metabolic syndrome in children and adolescents

| Age group (years) | Obesity (WC) | Triglycerides | HDL-C | Blood pressure | Glucose (mmol/L) or known T2DM |
|---|---|---|---|---|---|
| 6 - <10 | >90th percentile | MS cannot be diagnosed, further measurements should be made if family history of MS, T2DM, Dyslipidemia, CVD, HT and/or obesity | | | |
| 10– <16 years | >90% percentile or adult cut-off if lower | >1.7 mmol/L (>150 mg/dL) | <1.03 mmol/L (<40 mg/dL) | Systolic >130/ diastolic >85 mm Hg | >5.6 mmol/L |
| >16 years | Use existing IDF criteria for adults, ie:<br>Central obesity (defined as waist circumference >94 cm for men and >80 cm for woman, with ethnicity specific values for other groups) plus any two of the following four factors:<br>Raised triglycerides >1.7 mmol/L<br>Reduced HDL-cholesterol: <1.03 mmol/L in males and <1.29 mmol/L in females, or specific treatment for these lipid abnormalities<br>Raised blood pressure: systolic = 130 or diastolic = 85 mm Hg, or treatment of previosuly diagnosed hypertension<br>Impaired fasting glycemia (IGF): fasting plasma glucose (FPG) = 5.6 mmol/L (>100 mg/dL) or previosuly diagnosed type 2 diabetes) | | | | |

Adapted from: Zimmet et al. The metabolic syndrome in children and adolescents – an IDF consensus report.

The development of classification for pediatric metabolic syndrome is complicated by a number of limitations. During puberty will children develop transient insulin resistance (46), and normal lipid levels vary by age, sex, and race (47).

### 1.2.2. Epidemiology

It is estimated that 25% of the world's population meets the criteria for metabolic syndrome (45). The prevalence of MS in obese children and adolescents varies widely between publications, ranging from 0% to 60%, and depends on which criteria are used to describe MS (48). Metabolic syndrome is common in obese children and adolescents, and its prevalence rises as obesity worsens (41). In Croatia the prevalence of MS is higher compared to other Mediterranean countries like Greece and Portuguese (48). Of the 210 Croatian children and adolescents conducted in the study, 30.3% had the prevalence of MS when using the IDF criteria (49). According to a study conducted by *Weiss* et al., the incidence of metabolic syndrome increased directly with the degree of obesity, and each component of the syndrome worsens with the obesity – a correlation that is independent of age, sex and race (50). Studies show that metabolic syndrome is associated with a doubled risk of cardiovascular disease and a five-fold increased risk of developing type 2 diabetes mellitus (T2DM) (49).

### 1.2.3. Pathophysiology

#### *1.2.3.1. Insulin resistance:*

The most common metabolic alteration related to obesity is insulin resistance, which is considered one of the main pathophysiological mechanism of MS. (50). Insulin lowers blood sugar by increasing the glucose uptake into muscle and liver and inhibiting lipolysis and hepatic gluconeogenesis (51). Insulin resistance can be seen as a decreased biological response to a normal insulin level in target organs such as muscle, adipose tissue, and liver, resulting in increased secretion of insulin from the pancreas to maintain a normal blood sugar level. An increase in free fatty acids plays a central role in the pathophysiology (52). Insulin resistance in adipose tissue increases the levels of circulating free fatty acids by inhibiting lipolysis. This increase in free fatty acids will help inhibit insulin's inhibition of lipolysis and lead to more free circulating fatty acids (53). Free fatty acids inhibit the activation of protein kinase in muscle tissue, leading to decreased glucose uptake and increasing gluconeogenesis and lipogenesis in the liver (52). Insulin resistance is thought to be caused by a combination of hereditary and environmental factors (51,54). However, other factors like obesity, ethnicity, gender, and puberty can affect insulin sensitivity (54).

#### *1.2.3.2. Visceral fat*

Visceral fat is believed to be a strong contributor to insulin resistance and metabolic syndrome (50). It contributes to insulin resistance to a greater extent than subcutaneous fat (51). This is because visceral lipolysis provides an increased amount of free fatty acids to the liver via the portal vein, "portal theory" (51). This will increase the synthesis of triglycerides and apolipoprotein B, which contains very-low-density lipoprotein (VLDL). Increased low-density lipoprotein (LDL) cholesterol and decreases in high-density lipoprotein (HDL) cholesterol are indirect effects of insulin resistance caused by altered lipid metabolism in the liver (53).

Adipose tissue secretes cytokines and proteins that are of great importance to developing both cardiovascular disease and metabolic syndrome (55). The most common is adiponectin which has an insulin-sensitizing effect (55). It has been reported that single nucleotide polymorphisms (SNPs) in the adiponectin gene are seen in T2DM patients around the world, which indicates that the cytokine plays a crucial role in glucose and lipid metabolism (56). Adiponectin has an anti-inflammatory and protective effect against atherosclerosis, as it accumulates in the subendothelial region of wounded vascular walls, lowering adhesion molecule expression and macrophage recruitment (57). It is shown that levels of this cytokine are decreased in obese children and adolescents and are inversely related to the degree of obesity (41).

Angiotensin II is produced by adipose tissue and, through the activation of renin-angiotensin-aldosterone-system (RAAS) plays an important role in the development of metabolic syndrome (58). Reactive oxygen species (ROS) causes oxidation of LDL, endothelial damage, and platelet aggregation. ROS contributes to the synthesis of a number of proteins on the endothelium and smooth muscle cells (58). Together with the RAAS cascade, this is likely to cause inflammation, endothelial damage, and production of fibroblast, which in turn is associated with high blood pressure and dyslipidemia, and ultimately the development of diabetes and cardiovascular disease (59).

#### *1.2.3.3. Inflammation*

Adipose tissue also produces inflammatory cytokines like Tumor Necrosis Factor alpha (TNF-α) and interleukin-6 (IL-6). TNF-α can modify insulin action at many levels in the intracellular pathway (55). IL-6 stimulates the production of C-reactive protein (CRP) from the liver, which can give an understanding of the subclinical chronic inflammation seen in obesity. The balance between these cytokines is disrupted with obesity, with bigger adipocytes and macrophages producing more inflammatory cytokines like TNF-α and IL-6 and less anti-inflammatory peptides like adiponectin (60).

A study conducted by *Weiss et al.* showed that CRP and IL-6 are increased in normal upper levels in the population of obese children and adolescents (41). Catestatin, a peptide generated from chromogranin A has a wide range of biological functions, including suppressing catecholamine release, lowering blood pressure, reducing beta-adrenergic stimulation and regulating oxidative stress (61). The serum concentration of this peptide has been identified to be lower in obese children and adolescents (62). In the study conducted by *Simunovic et al.* it was suggested that low catestatin levels can aggravate hypertension and that the peptide could be used as a predictor of insulin resistance and low-grade inflammation (62). According to these findings, catestatin may be a new link in the pathophysiological mechanism of MS in obese children and adolescents (62).

### 1.2.4. Etiology

Metabolic syndrome is in the same manner as obesity caused by a combination of genetic and environmental factors. However, only 10% of metabolic syndrome is due to genetic predisposition (62). Many mechanisms have been proposed without a clear answer.

Cortisol is an essential component of developing visceral adiposity and has been linked to the development of the metabolic syndrome (44).

Sleep deprivation has been shown to have a connection to obesity among children. Obesity is linked to obstructive sleep apnea (OSA), which are shown to have an association with insulin resistance and low-grade inflammation, both of which are involved in the development of metabolic syndrome (63).

Dietary factors like low fat intake compensated with high carbohydrate intake have also been speculated as a cause for the development of metabolic syndrome in children (43). Also, fructose, which is the most used sweetener in the United States, has been demonstrated in animal studies to increase calorie intake, decrease resting energy expenditure, excess fat deposition, and lead to insulin resistance, implying that fructose consumption is contributing to insulin resistance, obesity, and T2DM (64).

Obesity appears to be linked to changes in branched-chain amino acids, just like it seems to be connected to changes in adipokines and cardiovascular risk factors. Valine, a highly gluconeogenic amino acid, is elevated in obese children and may contribute to increased hepatic glucose output (65).

### 1.2.5. Complications

Besides cardiovascular disease and T2DM other clinical conditions are associated with a metabolic syndrome like chronic-low grade inflammation, oxidative stress, reduced glucose tolerance, hyperandrogenism, and polycystic ovary syndrome, Alzheimer's disease, obstructive sleep apnea (OSA), and nonalcoholic fatty liver disease (NAFLD) (49).

## 1.3. Urotensin-II

Urotensinogen II (U-II) is an 11 amino acid long cyclic peptide (Figure 2) that is encoded by the *UTS2* gene located at 1p36 and was first discovered in the spinal cord in a telofish in 1980 (66–68).

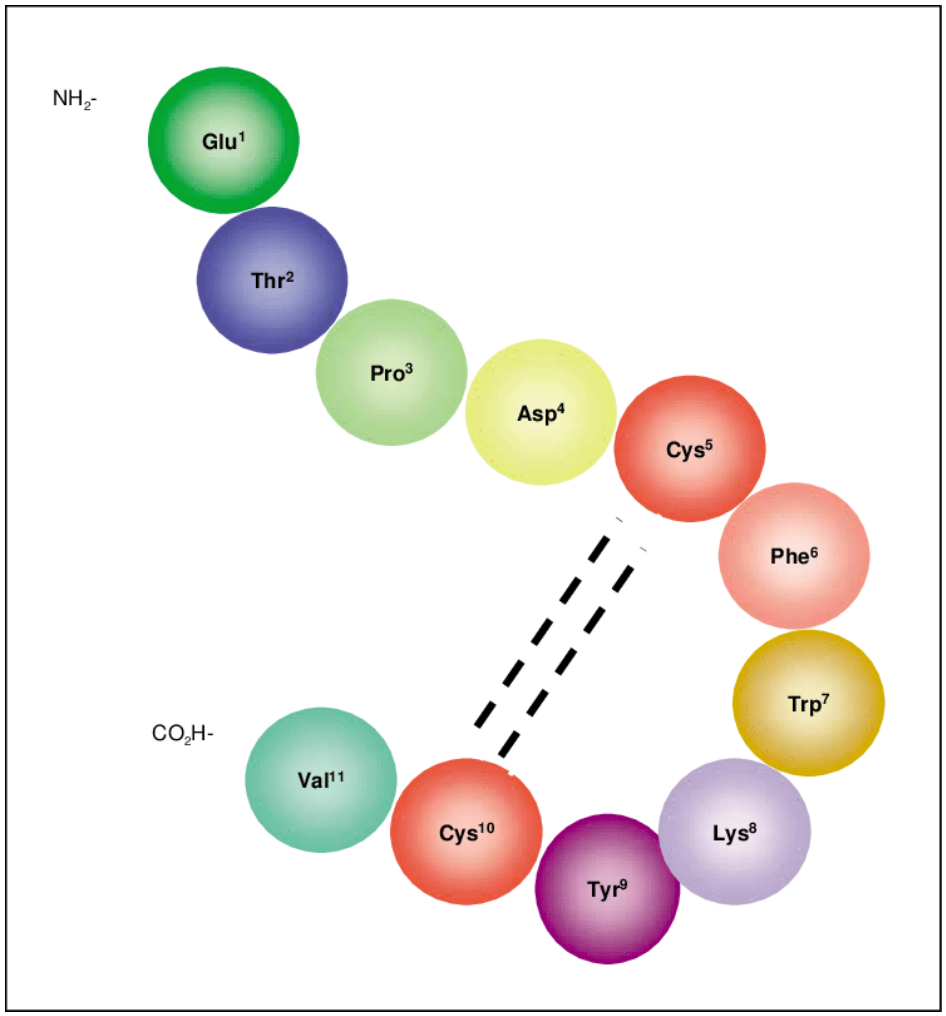


**Figure 2.** Structure of Urotensin II, 11- amino acid long peptide (69).

The main tissues producing U-II are vascular wall cells, the heart, liver, and kidneys (70). However, it has also been identified in the gastrointestinal and central nervous systems (71). U-II has been shown to be the most potent vasoconstrictor peptide by pharmacological studies. It acts on the urotensin receptor (UT), which is a G-coupled receptor (66).

Many studies have linked U-II to the development of diabetes due to its effect on glucose metabolism and insulin resistance (67). In humans, high levels of U-II have been found in diabetic patients, regardless of the fasting plasma glucose or glycosylated hemoglobin level, which suggests that the release of U-II is not due to hyperglycemia (72).

U-II has also been shown to stimulate the sympathetic nervous system and release epinephrine and ACTH in conscious, unstressed sheep (73). This increase in ACTH could contribute to increased insulin secretion and then the progression of insulin resistance and diabetes through its sympathomimetic effects (74). As mentioned above, the gene encoding U-II is the *UTS2* gene, certain polymorphisms in this gene are in the Japanese population associated with diabetes and insulin resistance (68).

U-II has shown to be increased in both human and experimental atherosclerosis (75). Both U-II and UT are identified in high concentrations within monocyte/macrophage infiltration of atherosclerotic plaque from human coronary and carotid arteries (76). It has been shown that U-II accelerates human macrophage foam cell formation and potentiate oxidized LDL, thereby contributing to the development of atherosclerosis and angiogenesis (77). In fish, U-II is showed to cause enhanced lipogenesis through glucose-6-phosphate dehydrogenase activity and NADPH production in addition to enhance the depot lipase activity, which may lead to hyperlipidemia (74).

Due to its high levels in diabetic patients and the effect of atherosclerosis, U-II has been found to have a critical role in the micro complications of diabetes mellitus (78). In patients with diabetic nephropathy are high levels of U-II and UT are located in the tubular epithelial cells, which also increase with renal damage (72). It is found that the mRNA expression of U-II and UT increases many folds in patients with diabetic nephropathy. Also, a study conducted by *Suguro et al.* found a strong correlation between U-II and diabetic retinopathy in addition to atherosclerosis in patients with T2DM (78).

Plasma U-II levels are high in hypertensive patients and have shown a strong correlation with its development, possibly through its vasoconstrictor properties (75). However, it has later been found that this peptide has both vasodilator and vasoconstrictor property, depending on the anatomic location of the vessel and the state of the endothelium. If the endothelium is dysfunctional, the U-II will directly stimulate vascular smooth muscle cells and cause vasoconstriction (79). Elevated U-II levels are therefore observed in conditions with endothelial dysfunctions like essential hypertension, myocardial infarction, heart failure, renal failure, T2DM, and portal hypertension caused by cirrhosis (80). Several studies, including *You et al*., found that blocking the U-II both diastolic and systolic blood pressure decreased significantly in obese mice (81).

U-II plasma levels correlate positively to body weight (75). U-II serum levels are associated with body mass index, and deletion of U-II is related to lean phenotype (81). Administration of U-II has been shown to increase appetite and food intake in mice (82).

# 2. OBJECTIVES

The main aims of this study are:

1. Determine serum U-II levels in obese children and adolescents with MS in comparison with obese controls without MS.
2. Examine the association of serum U-II levels with hypertension.

Hypothesis of this study are:

1. Serum urotensin-II levels will be higher in obese children and adolescents with MS in comparison with obese children and adolescents without MS.
2. Serum urotensin-II levels will be higher in obese children and adolescents with hypertension in comparison with obese children and adolescents without hypertension.

# 4. MATERIALS AND METHODS

## 4.1. Subjects

In this cross-sectional study, 40 obese children and adolescents were enrolled as part of clinical research on early cardiovascular risk factors in obese children and adolescents at the University Hospital of Split, Division of pediatric endocrinology (61,83). This study was approved by Ethical Committee of University Hospital of Split (500-3/ 17-01/10, 2181-147-01/06/ M.S.-17-2) and was performed in accordance with the Declaration of Helsinki. All monogenic and secondary causes of obesity, disorders, other diseases, and/or drugs that affect hypertension, glucose and lipid metabolism was exclusion criteria for this study. Every parent signed an informed written consent form, and the child gave his or her verbal approval.

## 4.2. Anthropometric characteristics of study participants

A thorough clinical examination was undertaken, as well as a family history, with the goal of detecting acanthosis nigricans. Body height and weight were measured using a stadiometer and an electronic scale (both from Seca, Hamburg, Germany), and BMI was determined by multiplying the patient's body weight in kilograms by the square of his or her body height in meters. While the participant was standing, the waits circumference (WC) and hip circumference (HC) were measured in centimeters with a non-elastic measuring tape. The Tanner stage was used to determine pubertal status; those subjects classified as Tanner stage I was considered prepubertal, while those classified as Tanner stages II to V were considered pubertal (84,85).

## 4.3. Collection of data

Blood samples were taken from patients after they had fasted for at least 12 hours the night before. The concentrations of insulin were determined using an electrochemiluminescence immunoassay (COBAS e601, Roche Diagnostics GmbH, Mannheim, Germany), and the concentrations of plasma hemoglobin A1c were determined using high-performance liquid chromatography (HumaNex A1c, HUMAN, Wiesbaden, Germany).

The concentrations of aspartate transaminase, alanine transaminase, and gamma-glutamyl transferase, as well as fasting glucose, total cholesterol, triglyceride, HDL cholesterol, low-density lipoprotein cholesterol, aspartate transaminase, alanine transaminase, and gamma-glutamyl transferase, were measured using routine laboratory methods (ARCHITECT ci16200; Abbott, Chicago, Illinois). Serum urotensin-II concentrations were measured using an enzyme-linked immunosorbent assay kit for human U-II (EIA kit, Phoenix Pharmaceuticals Inc., Burlingame, California) according to the manufacturer's instructions.

## 4.4. Definitions

Obesity was defined as a BMI z score > 2 after adjusting for age and gender, and the BMI z score was determined for all individuals using the AnthroPlus software, according to the World Health Organization growth charts (WHO, Geneva, Switzerland) (41,86).

Based on the IDF 2007 Guidelines, MS is defined as a WC exceeding the 90$^{th}$ percentile for children and adolescents aged from 10 to <16 years and for adolescents aged from >16 years (males > 94 cm and females > 80 cm) with two or more of the following parameters: systolic blood pressure (SBP) > 130 mm Hg, or diastolic blood pressure (DBP) > 85 mm Hg, HDL cholesterol < 1.03 mmol/L, triglycerides > 1.7 mmol/L (45).

The homeostatic model assessment of insulin resistance (HOMA-IR), which is defined as the product of fasting plasma insulin concentration (mlU/L) and fasting plasma glucose concentration (mmol/L) divided by 22.5, was used to determine the degree of insulin resistance (IR) (87).

High blood pressure was defined with the use of the Fourth Report on the Diagnosis, Evaluation and Treatment of High Blood pressure in Children and Adolescents, where normal blood pressure in children is defined as SBP and DBP below the 90$^{th}$ percentile for age, gender and height, whereas hypertension is defined as SBP and/or DBP consistently above the 95$^{th}$ percentile (88,89).

## 4.5. Statistical analysis

Prism 9 for Mac OS x (version 9.1.0.; GraphPad, La Jolla, CA, USA) was used for statistical analysis. Data were tested for normal distributions using the Kolmogorov-Smirnov test. Comparison of serum U-II levels and other parameters was done by Student t-test or Mann-Whitney U test. the Chi-squared test was used to analyze categorical variables. Statistical significance was defined as $P < 0.05$.

# 5. RESULTS

The study group of 20 obese subjects with MS had the mean age of 17.04 ± 9.18 years, sex distribution was 40% male and 60% female, while in the 20 obese subjects without MS the mean age was 14.36 ± 2.17 years with a sex distribution of 75% male and 25% female (Table 1).

**Table 4.** Demographic characteristics of the subjects enrolled in the study

| Parameter | Obese subjects with MS (N=20) | Obese subjects without MS (N=20) | P* |
|---|---|---|---|
| Sex – N (%) | | | |
| Male | 8 (40) | 14 (75) | 0.057 |
| Female | 12 (60) | 6 (25) | |
| Age (yr) | 17.04 ± 9.18 | 14.21 ± 2.3 | 0.188 |
| Pubertal status – N (%) | | | |
| Tanner 1 | 1 (5) | 3 (15) | 0.772 |
| Tanner 2 | 2 (10) | 1 (5) | |
| Tanner 3 | 4 (20) | 4 (20) | |
| Tanner 4 | 7 (35) | 8 (40) | |
| Tanner 5 | 6 (30) | 4 (20) | |

Data are presented as whole number (percentage) or mean ± standard deviation.

*Groups comparison were performed using the t test or $\chi 2$ test.

The obese subject with MS and the obese subjects without MS did not differ in height (170.4 ± 10.82 vs 169.3 ± 11.93, P = 0.765), weight (91.18 ± 16.98 vs 90 ± 18.21, P=0.833) and BMI (31.32 ± 3.77 vs 31 ± 3.72) (Table 2).

**Table 5.** Baseline anthropometric characteristics of the subjects enrolled in the study

| Parameter | Obese subjects with MS (N=20) | Obese subjects without MS (N=20) | P* |
|---|---|---|---|
| Height (cm) | 170.4 ± 10.82 | 169.3 ± 11.93 | 0.765 |
| Weight (kg) | 91.18 ± 16.98 | 90 ± 18.21 | 0.833 |
| BMI ($kg/m^2$) | 31.32 ± 3.77 | 31 ± 3.72 | 0.787 |
| BMI z score | 2.76 ± 0.55 | 2.81 ± 0.45 | 0.763 |
| Waist circumference | 104.3 ± 9.11 | 103.2 ± 9.24 | 0.700 |
| Hip circumference | 111.4 ± 9.19 | 113 ± 22 | 0.762 |
| Waist to hip ratio | 0.94 ± 0.04 | 0.93 ± 0.09 | 0.701 |

BMI, body mass index.

Data are presented as mean ± standard deviation.

*Groups comparison was performed using the t test.

HDL cholesterol was significantly higher in subject without MS compared to the group with MS (1.16 ± 0.22 vs 0.94 ± 0.11, P < 0.001), and SBP was significantly higher in subject group with MS compared to subjects without MS (132.8 ± 15.18 vs 124.2 ± 10.13) (Table 3).

**Table 6.** Components of metabolic syndrome of the subjects enrolled in the study

| Parameter | Obese subjects with MS (N=20) | Obese subjects without MS (N=20) | P* |
|---|---|---|---|
| HDL cholesterol (mmol/l) | 0.94 ± 0.11 | 1.16 ± 0.22 | <0.001 |
| Triglycerides (mmol/l) | 1.33 ± 0.57 | 1.09 ± 0.38 | 0.137 |
| Fasting glucose (mmol/l) | 5.33 ± 0.93 | 4.96 ± 0.36 | 0.103 |
| SBP (mmHg) | 132.8 ± 15.18 | 124.2 ± 10.13 | 0.042 |
| DBP (mmHg) | 77.73 ± 6.15 | 75 ± 8.63 | 0.236 |

HDL cholesterol, high-density lipoprotein cholesterol; SBP, systolic blood pressure; DBP, diastolic blood pressure.

Data are presented as mean ± standard deviation.

*Groups comparison were performed using the t test.

Additionally, acanthosis nigricans were higher in the subject group without MS compared to the group with MS (70% vs 60%). HOMAR-IR was significantly higher in the group with MS compared to the group without MS (9.34 ± 9.19 vs 6.93 ± 5.24). Table 3 compares additional biochemical markers and clinical characteristics between obese subjects with MS and obese subjects without MS.

**Table 7.** Biochemical and clinical characteristics of the subjects enrolled in the study

| Parameter | Obese subjects with MS (N=20) | Obese subjects without MS (N=20) | P* |
|---|---|---|---|
| Acanthosis – N (%) | 12 (60) | 14 (70) | 0.507 |
| Total cholesterol (mmol/l) | 4.31 ± 0.91 | 4.25 ± 0.69 | 0.816 |
| LDL (mmol/l) | 2.81 ± 0.71 | 2.59 ± 0.61 | 0.315 |
| AST (U/L) | 21.45 ± 7.87 | 21 ± 8.46 | 0.863 |
| ALT (U/L) | 28.65 ± 18.8 | 24.05 ± 17.09 | 0.423 |
| GGT (U/L) | 18.9 ± 6.89 | 21.4 ± 16.19 | 0.528 |
| Fasting insulin (mIU/l) | 36.1 ± 22.41 | 31.21 ± 22.26 | 0.492 |
| HOMA-IR | 9.34 ± 9.19 | 6.93 ± 5.24 | 0.317 |
| Hhemoglobin A1c (%) | 5.45 ± 0.36 | 5.46 ± 0.32 | 0.852 |

LDL cholesterol, low-density lipoprotein cholesterol; AST, aspartate transaminase; ALT, alanine transaminase; GGT, gamma-glutamyl transferase; HOMA-IR, homeostasis model assessment of insulin resistance index.

Data are presented as whole number (percentage) or mean ± standard deviation.

*Groups comparison were performed using the t test or $\chi 2$ test.

Measurement of serum U-II levels in obese subject group with MS and obese subject group without MS showed significantly higher levels of U-II in obese children with MS compared to those without MS (4.99 (10.29-3.22) vs 3.38 (5.16-2.03), P = 0,035) (Figure 1).

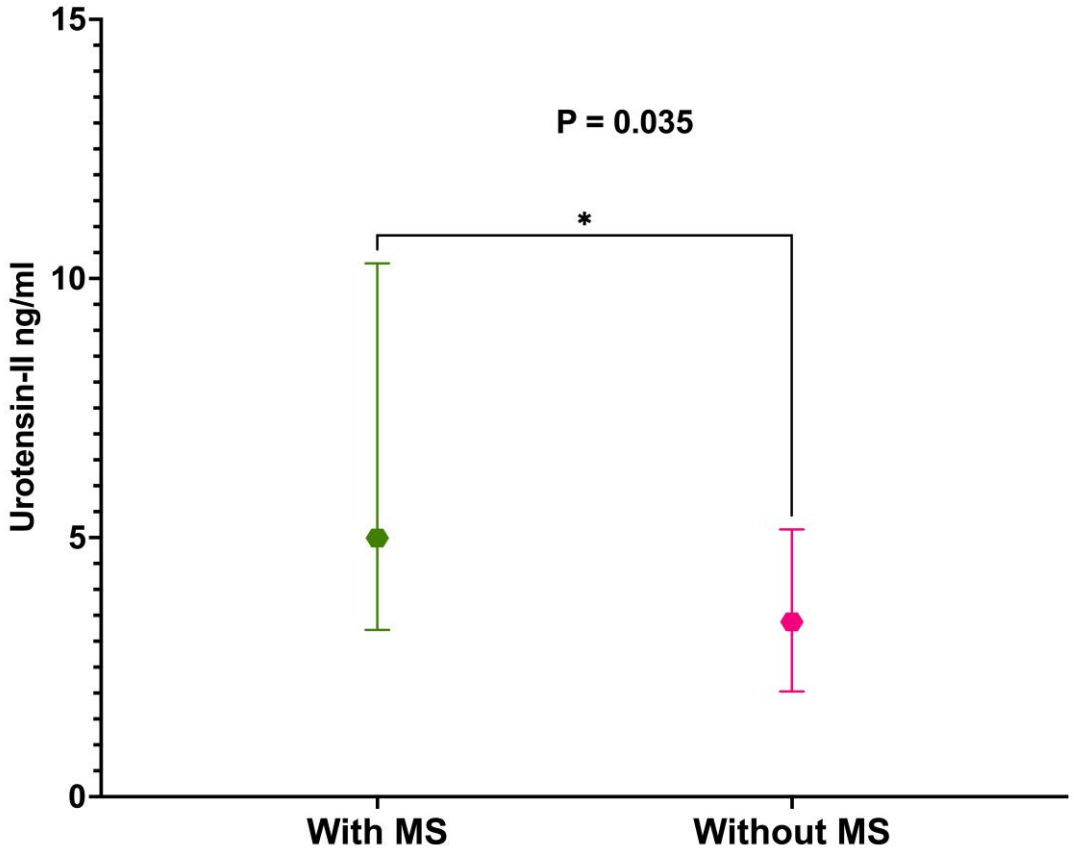


**Figure 3.** Serum concentrations of urotensin-II in children and adolescents with MS compared with children and adolescents without MS.

Data is presented as median (IQR). Differences between subgroups were assessed using Mann-Whitney U test.

Furthermore, after stratification of all subjects depending on the presence of high blood pressure defined as SBP and/or DBP above 95$^{th}$ percentile for age, gender and height and found that the subgroup comprising 23 subjects with high blood pressure presented significantly higher U-II levels compared with the normal blood pressure subgroup (5.06 (9.89-3.38) vs 3.1 (5.12-1.94), P = 0.027) (Figure 2).

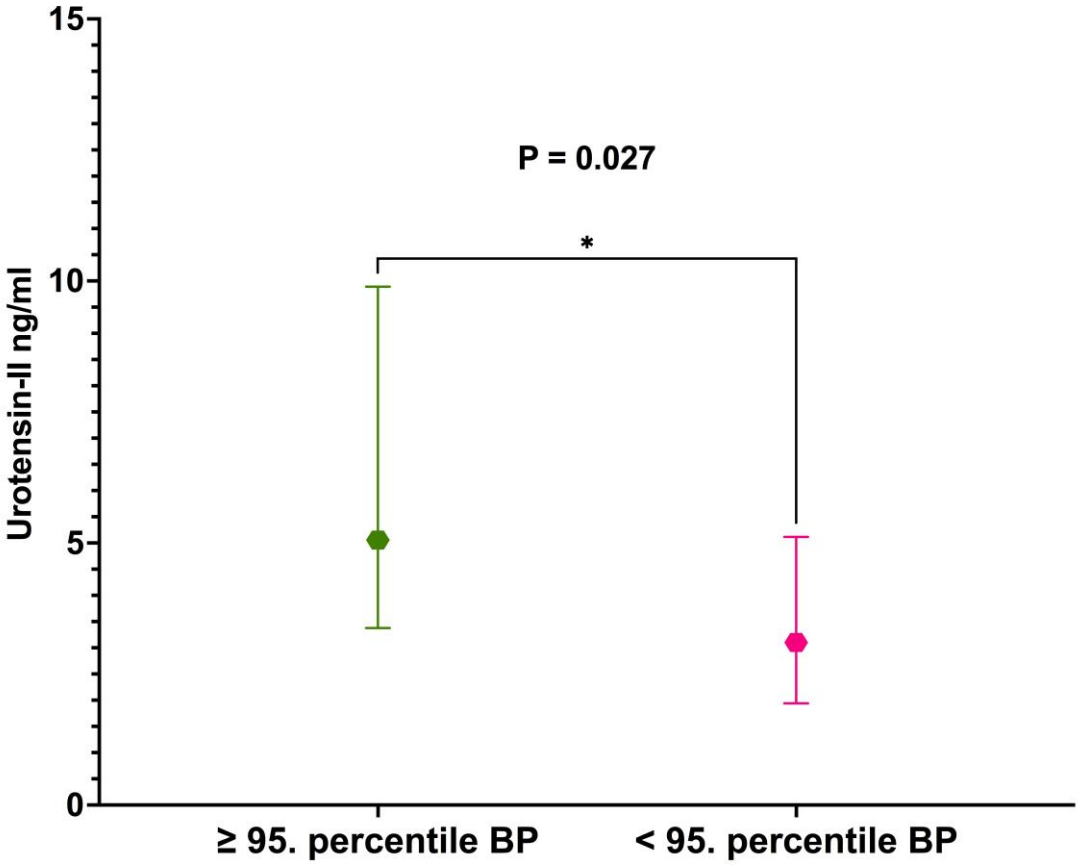


**Figure 4.** Serum concentration of urotensin-II in children and adolescents with >95. percentile for blood pressure compared with children and adolescents with <95. percentile for blood pressure.

BP, blood pressure. Data is presented as median (IQR). Differences between groups were assessed using Mann-Whitney U test.

# 6. DISCUSSION

The result of our study showed that obese children and adolescents with MS have a higher concentration of serum U-II compared to obese children and adolescents without MS. Furthermore, obese subjects with high blood pressure had higher serum U-II when compared with obese children and adolescents with normal blood pressure. To the best of our knowledge, this is the first study that studied U-II levels in obese children and adolescents, as well as their probable link to MS.

Plasma U-II is known to be elevated in CVD, HT and T2DM (80). Our study supports the possibility that U-II may be significantly elevated in patients with MS and may play a significant role in their pathogenesis. U-II levels have not been studied in obese children and adolescents with MS, but elevated levels of U-II levels have been reported in the adult population of obese subjects with MS in a study conducted by *Gruson et al.* (90).

As mentioned in the introduction, MS consist of a cluster of obesity, hyperlipidemia, HT, hyperglycemia and IR which can lead to the development of T2DM and CVD. The mechanisms that connect the different aspects of MS is mostly based on IR and inflammation (91). IR not only impacts the insufficient inhibition of glucose synthesis in the liver resulting in glucose metabolic homeostasis problems, but it also has a beneficial influence on hepatic lipogenesis (92). Both U-II and UT are found in pancreatic islets, and U-II has been shown in rats to inhibit glucose and arginine-induced insulin response in the pancreas (93). The inhibition of insulin secretion is not due to U-II vasoconstrictive effect but due to the activation of UT receptors (73). The link between IR and U-II was elucidated in a study by *Totsune et al.* where U-II levels were found to be higher in T2DM patients with and without proteinuria when compared to healthy patients (72). The importance of the inflammatory response in MS is linked to the expression of U-II and UT (73). A study conducted by *Segain et al.* found that inflammatory markers like TNF-α, lipopolysaccharides and interferon-γ induce UT expression *in vitro* (94). These findings imply that an inflammatory response to MS and its components may raise U-II levels by boosting the production of the peptide by inflammatory cells (94). Furthermore, a study conducted on woman with gestational diabetes and its association with U-II found a positive correlation between CRP levels and U-II (95).

Additionally, we reported higher U-II levels in obese children and adolescents with BP >95 percentile. Analogously *Cheung et al.* reported elevated levels of plasma -II in patients with hypertension (75). They compared 62 hypertensive subjects and 62 normotensive subjects. Their results showed that patients with hypertension had higher

levels of U-II in their plasma than normotensive controls and that this was linked to systolic blood pressure (75). The author concluded that U-II might play a role in the development of hypertension through its known vasoconstrictor effect (75).

A study conducted by *You et al.* studied the effect of U-II blockade on different components of metabolic syndrome in obese mice (81). They discovered a decrease in free fatty acids, triglycerides, blood pressure, and decreased weight gain (82). They also showed that blocking of U-II improved glucose levels significantly (82). These findings suggest that the blockade of the U-II system either directly or indirectly has a positive impact on the different components of metabolic syndrome and may be an option as a future therapy for obesity and metabolic syndrome (81).

There are some limitations to this study. First, our study's cross-sectional methodology excludes longitudinal follow-up of our individuals, who may have experienced a fall or increase in U-II levels as their obesity progressed. Furthermore, a third component may be linked to both obesity and U-II levels, necessitating more functional research. Finally, this study contains a small sample size which can influence the generalizability of the results.

# 7. CONLUSIONS

In conclusion, obese children and adolescents with MS have higher levels of serum urotensin-II concentrations than obese children and adolescents without MS and possibly could be used as a predictor of MS in pediatric population. U-II represents a potential new link in the complex pathophysiological mechanism of MS in obese children and adolescents, according to our findings. Future study is needed to fully understand the effects of U-II on the start and progression of MS in a population of obese children and adolescents, as well as to identify the clinical implications of U-II.

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# 9. SUMMARY

**Objectives:** The aim of this study is to determine urotensin-II (U-II) levels in obese children and adolescents with metabolic syndrome (MS) compared to obese children and adolescents without MS.

**Patients and methods**: This study enrolled 20 obese children and adolescents with MS and 20 obese children and adolescents without MS. Evaluation included physical examination, medical history, anthropometric measurement, blood pressure measurement and blood sample. IDF guidelines was used for MS criteria. Serum U-II levels were determined with enzyme-linked immunosorbent assay kit.

**Results:** Subjects with MS has significantly higher serum levels of U-II compared to the subjects without MS (9.23 ± 9.69 vs 3.81 ± 1.94). Additionally, we found that obese subjects with high blood pressure had higher serum U-II when compared with obese children and adolescents with normal blood pressure (8.59 ± 9.13 vs 3.68 ± 2.06, P = 0.027).

**Conclusion:** Serum U-II levels is significantly increased in obese children and adolescents with MS compared to children and without MS which implies possible involvement of U-II in MS pathophysiology. To address these findings, larger multicentric investigations are needed in the future.

# 10.CROATIAN SUMMARY

**Naslov:** Razine urotenzina-II u pretile djece i adolescenata

**Ciljevi:** Cilj ovog istraživanja je bilo odrediti razinu urotenzina-II (U-II) u pretile djece i adolescencije s metaboličkim sindromom (MS) u usporedbi s pretilom djecom i adolescenciji bez MS-a.

**Materijali i metode:** Ovo je istraživanje obuhvatilo 20 pretile djece i adolescencije s MS-om i 20 pretile djece i adolescencije bez MS-a. Evaluacija je obuhvaćala fizikalni pregled, anamnezu, antropometrijsko mjerenje, mjerenje krvnog tlaka i uzorkovanje krvi. MS je definiram prema IDF definiciji. Razine U-II u serumu određene su enzim-imunoadsorpcijskom metodom.

**Rezultati:** Ispitanici s MS imali su statistički značajno višu razinu U-II u serumu u usporedbi s ispitanicima bez MS (9,23 ± 9,69 vs 3,81 ± 1,94, P = 0,035). Također, pokazali smo da su pretili ispitanici s visokim krvnim tlakom imali statistički značajno više serumske razine U-II u usporedbi s pretilom djecom i adolescentima s normalnim krvnim tlakom (8,59 ± 9,13 vs 3,68 ± 2,06, P = 0,027).

**Zaključci:** Razina U-II u serumu značajno je povećana u pretile djece i adolescencije s MS-om u usporedbi s djecom i adolescenciji bez MS-a, što implicira moguće sudjelovanje U-II u patofiziologiji MS-a. Potrebna su veća multicentrična istraživanja kako bih se razjasnila uloga U-II.

## 11. CURICULUM VITE

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