The effects of moderate daily red wine intake on arterial stiffness and hemodynamic parameters in type 2 diabetes mellitus

Januš, Anika Katarina

UNIVERSITY OF SPLIT
SCHOOL OF MEDICINE

ANIKA KATARINA JANUŠ

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Assoc. Prof. Ivana Mudnić, MD, PhD

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Table of Contents

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List of Abbreviations

ADA: American Diabetes Association
AGEs: Advanced glycation end-products
AIx: Augmentation Index
ASCVD: Atherosclerotic cardiovascular disease
BMI: Body mass index
CGM: Continuous glucose monitoring
CHD: Coronary heart disease
CKD: Chronic kidney disease
COX-2: Cyclooxygenase-2
CRP: C-reactive Protein
CVD: Cardiovascular Disease
DAG: Diacylglycerol
DKA: Diabetic ketoacidosis
DPP: Diabetes Prevention Program
DSST: Diabetes control and complications trial
eNOS: endothelial Nitric oxide synthase
ER: Endoplasmic Reticulum
ESRD: End-stage renal disease
FFAs: Free fatty acids
FPG: Fasting plasma glucose
GDM: Gestational diabetes mellitus
HbA1c: Hemoglobin A1c
HF: Heart failure
HHS: Hyperglycemic hyperosmolar state
HR: Heart rate
IFP: Impaired fasting glucose
IGT: Impaired glucose tolerance
IPC: Ischemic Preconditioning
I/R: Ischemia/Reperfusion
LV: Left Ventricle
MAP: Mean arterial pressure
MI: Myocardial infarction

NGSP: National Glycohemoglobin Standardization Program

OGTT: Oral glucose tolerance test

PAD: Peripheral artery disease

PAI-1: Plasminogen activator inhibitor-1

PGI_2: Prostacyclin

PKC: Protein kinase C

PR: Peripheral resistance

PWA: Pulse wave analysis

PWV: Pulse wave velocity

ROS: Reactive oxygen species

RW: Red wine

SMBG: Self-monitoring of blood glucose

SV: Stroke volume

T2DM: Type 2 Diabetes Mellitus

TGF-β: Transforming growth factor β

TLR: Toll-like receptors

TXA_2: Thromboxane A2

1. INTRODUCTION AND BACKGROUND

1.1. Type 2 Diabetes Mellitus

1.1.1 Pathogenesis and Pathophysiology

In 2019, around 463 million adult people worldwide were estimated to live with diabetes, of which more than 90% are affected by Type 2 diabetes mellitus (T2DM) (1) . A common characteristic between all entities of the diabetes spectrum is chronic hyperglycemia, with its cause being either a disorder of insulin secretion, a disorder of its effect on target tissues, the so-called insulin-resistance, or a combination of both (2).

Although the exact mechanism of the development of Type 2 DM is not entirely known, there are several risk factors proven to be associated with it (3). These are primarily a family history of diabetes, obesity and physical inactivity. In addition, the prevalence not only varies between different countries, but also between different ethnicities in the same country. For example, in the US, the prevalence is highest in American-Indian and Alaskan natives, followed in descending order by non-Hispanic blacks, Hispanics, Asian Americans, with the lowest prevalence having non-Hispanic whites. Other remaining risk factors to be mentioned are prediabetes, a history of Gestational Diabetes Mellitus (GDM) in females, hypertension and cardiovascular disease, as well as low HDL cholesterol levels and high triglyceride levels. To conclude, the development of T2DM is determined by the interaction between different consequences of a sedentary lifestyle and a genetic predisposition.

Insulin plays a key role in blood glucose control. It is produced by the pancreatic β cells of the islets of Langerhans, and its release is primarily stimulated by hyperglycemia. Glucose freely enters the β-islet cells via GLUT-2 transporters in an insulin-independent manner. Thus, an increase in blood glucose leads to an increase in metabolic flux through glycolysis, the citric acid cycle, leading to the formation of ATP, which then causes the inhibition of ATP-sensitive K^+ channels. This results in cell membrane depolarization, leading to Ca^{2+} influx via voltage-sensitive Ca^{2+} channels, which in turn stimulates the exocytosis of insulin (4). The secreted insulin acts primarily on GLUT-4 transporters in skeletal muscle and adipose tissue by recruiting these transporters from the interior to the cell membrane, actively increasing the glucose transport from the blood stream into the cells (5). The peptide hormone stimulates anabolic processes, like protein and glycogen synthesis and lipogenesis. Additionally, it promotes the formation of fatty acid precursors by increasing glycolysis,

transforming glucose into pyruvate. On the other side, it also inhibits protein and glycogen breakdown, lipolysis, ketogenesis, and suppresses gluconeogenesis from amino acids. Other actions of insulin are cell growth, the increase of renal tubular absorption of Na^+, cellular uptake of Mg^{2+}, and promoting cardiac contractility. On a paracrine level, insulin inhibits the secretion of glucagon, a catabolic hormone with functions opposite to insulin (6).

Other substances that, in a healthy state, cause insulin secretion are amino acids, free fatty acids (FFAs), ketone bodies, glucagon and secretin.

In diabetic individuals, it is believed that insulin resistance precedes the defect in insulin secretion and that diabetes becomes fully apparent when the insulin secretion becomes inadequate to meet the target tissue´s requirements (3).

Although the exact mechanisms leading to insulin resistance are not known, it is suggested that particularly obesity, visceral adiposity, and a lack of physical exercise are the leading causes. The reduction of insulin receptors and tyrosine kinase activity develops secondary and can be explained by the compensatory hyperinsulinemia resulting from insulin resistance. Hence, the primary defect is suggested to lie in the more downstream insulin-regulated phosphorylation and dephosphorylation steps. Lipid accumulation in skeletal myocytes is thought to impair mitochondrial oxidative phosphorylation, reducing the insulin-stimulated mitochondrial ATP production. Additionally, the lipid accumulation and impaired fatty acid oxidation in skeletal myocytes may lead to the generation of lipid peroxidase, a reactive oxygen species (3,7). Furthermore, the increased amount of FFAs, especially in obesity and visceral adiposity, impairs both glucose utilization in skeletal muscle and β cell function, as well as enhances glucose production by the liver, all contributing to insulin resistance. Another point to mention is, that in obesity, the production of the insulin-sensitizing peptide adiponectin by adipocytes is decreased, which is believed to add to the hepatic insulin resistance. Leptin, another hormone secreted by adipocytes, acts in healthy state to control satiety in the central nervous system and to promote insulin sensitivity and energy expenditure. However, in obesity, there is decreased sensitivity to leptin, leading to the inability to recognize satiety despite high energy stores, thereby aggravating insulin resistance and hyperlipidemia (3,4).

If insulin resistance develops, a higher insulin concentration, and therefore increased secretion by the pancreatic β-islet cells, is needed to cause the aforementioned effects on end-organ tissues. Overload of the β-islet cells causes progressive exhaustion, leading eventually to apoptosis and reduction of the pancreatic β cell mass. With the endoplasmic reticulum (ER) being an essential organelle in insulin secretion, this process is suggested to be mediated by the ER-stress pathway, resulting in nitric oxide-induced apoptosis of β cells (8,9).

With the progressive exhaustion of the β-islet cells and reduced β cell mass, the required hyperinsulinemic levels cannot be met, ensuing in hyperglycemia. In the beginning of diminished insulin secretion, there will still be some residual insulin secretion, although not enough to meet the elevated requirements occurring after meals. This results in postprandial hyperglycemia, as reflected by the oral glucose tolerance test (OGTT) with increased blood glucose levels two hours after a glucose challenge (3).

Glucagon and insulin affect the carbohydrate metabolism as physiologic antagonists. However, when the insulin level is too low to counteract glucagon, its action is uninhibited and continuously stimulates hepatic gluconeogenesis and glycogenolysis, further aggravating the dysfunctional insulin secretion. Since the hepatic glucose output is stimulated even while fasting by the unopposed glucagon, individuals develop a fasting hyperglycemia in addition to the already existing postprandial hyperglycemia (4).

1.1.2 Clinical Findings and Acute Complications

Acute clinical findings concerning diabetes are primarily related to hyperglycemia. When the threshold of renal glucose reabsorption is reached, glucosuria ensues, leading to polyuria and nocturia by osmotic diuresis. As a consequence of polyuria, dehydration follows, and leads to thirst and polydipsia. Glucosuria also increases the risk for candida genitourinary infections. The loss of glucose in urine also means a loss of calories, resulting in hunger and polyphagia. The combination of dehydration and calorie loss also leads to weight loss in some individuals. As a consequence of the change in osmolality that follows hyperglycemia, a change in the water content of the lens in the eye ensues, which may result in blurred vision (4).

Although more common in Type 1 Diabetes Mellitus, diabetic ketoacidosis (DKA) is also observed in some Type 2 diabetics. This occurs especially under conditions of stress, such as infections or severe trauma, which cause a rise in the hormones opposing the effects of insulin. In this state of decreased insulin, but increased glucagon levels, severe hyperglycemia develops with values ranging from 13 mmol/l to 33 mmol/l (3). Consequently, polyuria ensues but cannot be compensated adequately by oral intake, since DKA also leads to nausea and vomiting associated with further fluid losses. Hypovolemia again aggravates hyperglycemia as it stimulates glucagon, leading to further glucose production, and moreover causes a decrease in renal clearance of glucose. Sodium is lost by osmotic diuresis, and serum levels are typically low because hyperglycemia leads to osmosis drawing water into the extracellular space, further diluting sodium concentration in this compartment. Hyperosmolality in DKA may cause coma in some patients, as it leads to a loss of intracellular fluid in the brain. The combination of insulinopenia and an increase in other hormones associated with stress, such as catecholamines, lead to excessive lipolysis and the release of free fatty acids. In hyperglucagonemia, the hepatic metabolism is shifted towards ketogenesis, with the main ketone bodies being the organic acids acetoacetate and β-hydroxybutyrate, causing an increased anion gap-metabolic acidosis. Acidosis triggers Kussmaul breathing, and the ketone body acetone, present in small amounts, causes the characteristic fruity smelling breath in DKA. Another symptom suggestive of DKA is abdominal pain (3,4).

Hyperglycemic Hyperosmolar State (HHS), seen more often in Type 2 diabetics, is also caused by insulinopenia. The hyperosmolar state occurring due to hyperglycemia is typically precipitated by decreased fluid intake associated with a concurrent disease process such as myocardial infarction, stroke, or an infection. Limited access to water or renal insufficiency, typically seen in elderly debilitated individuals, worsens hyperglycemia and dehydration. In HHS, there is usually a small residual amount of insulin, which is able to inhibit lipolysis and thereby ketogenesis as well. Hyperglycemia typically exceeds 33.3 mmol/l, with hyperosmolality also being more profound in HHS, compared to DKA. Patients often present late due to the absence of symptoms associated with ketoacidosis, like nausea and vomiting, abdominal pain, and Kussmaul respirations (3,4).

1.1.3 Chronic Complications

Chronic complications of diabetes mellitus can be divided into vascular and non-vascular, with the vascular complications further being subdivided into micro- and macrovascular. Prevention or delay of these complications can be accomplished with early detection of diabetes or prediabetes and aggressive glycemic control. Microvascular complications are pathognomonic for diabetes. They include retinopathy, multiple types of neuropathy, and nephropathy (3).

There are at least four main mechanisms on how chronic hyperglycemia may cause microvascular damage. One is the increased formation of advanced glycation end-products (AGEs) caused by excessive intracellular glucose. This leads to alterations in endothelial gene expression, cross-linking of proteins, which promotes vascular thickness and stiffness, and also the binding to AGE receptors on macrophages and endothelium stimulating inflammation and resulting in vascular dysfunction. Another suggested mechanism is that hyperglycemia leads to an increased flux through the sorbitol pathway, supposedly causing NADPH consumption during glucose reduction by the enzyme aldose reductase. Due to decreased availability of NADPH, there is a decrease of free radical clearance, with the resultant damage being primarily related to nerve cells. Third, an increase of diacylglycerol (DAG), caused by hyperglycemia-stimulated glycolysis, activates protein kinase C (PKC). Excess PKC, by altering gene transcription for various proteins, changes endothelial permeability and leads to a thickening of the extracellular matrix. The last pathway, concerning hexosamine and its substrate fructose-6-phosphate, leads to increased glycosylation, and with this, altered function of proteins like endothelial nitric oxide synthase. Furthermore, the expression of transforming growth factor β (TGF-β) or plasminogen activator inhibitor-1 (PAI-1) is stimulated, both aggravating microvascular damage. All of these pathways are thought to be connected to the same underlying mechanism, which is oxidative stress caused by hyperglycemia. The reactive oxygen species are thought to cause a diversion away from glycolysis by glyceraldehyde-3-phosphate dehydrogenase (GADPH) towards the four pathways mentioned above (3,4).

Diabetes-related retinopathy is a leading cause of blindness in middle-aged and elderly people (10). The duration of diabetes and the level of glycemic control are important prognostic factors for the development of retinopathy (3,4).

Diabetes-induced nephropathy is the most common cause of chronic kidney disease (CKD) and end-stage renal disease (ESRD) worldwide (11). As glomerular lesions deteriorate and proteinuria increases, clinical diabetic nephropathy, defined by urinary losses of protein of more than 300 mg per day, can be diagnosed. The proteinuria is likely to increase with decreased renal function, which is further aggravated in the case of concomitant hypertension. In type 2 DM, microalbuminuria often comes together with hypertension, or in some cases hypertension is thought to precede and possibly cause albuminuria. Optimally, the concomitant treatment of albuminuria and hypertension with ACE inhibitors or ARBs should be anticipated (3,4).

Diabetic neuropathy occurs in about 50% of both type 1 and type 2 diabetics, thereby being the most common cause of neuropathy worldwide (12).

Although macrovascular complications are not diabetes-specific, these atherosclerotic changes occur with a higher frequency in diabetes (3). They include coronary heart disease (CHD), peripheral artery disease (PAD) and cerebrovascular disease. Type 2 diabetics are especially at risk for cardiovascular disease, which causes 52% of deaths in this group (13). Diabetes itself, by insulin resistance and hyperglycemia, is a known independent risk factor for atherosclerosis.

One way by which insulin resistance is causing vascular dysfunction, is by inactivation of nitric oxide (NO), occurring by two mechanisms. First, the abundance of FFAs, that is related to visceral obesity in type 2 diabetic individuals, causes the FFAs to bind to Toll-like receptors (TLR) and thereby downregulates important pathways like PI3-kinase (PI3K) and Akt (14). By decreased activity of these pathways, endothelial nitric oxide synthase (eNOS) is inactivated, leading to a decrease in NO production. Second, NO is further being inactivated by the increase in ROS that is caused by obesity and diabetes. NO is important for the maintenance of the endothelium, by modulation of vascular tone, regulation of local cell growth, and protection of blood vessels from injurious consequences of platelets and other circulating cells. Hence, a reduction of NO will lead to endothelial dysfunction and atherosclerotic changes. In addition, FFAs binding to TLRs causes nuclear factor NF-κB activation, which stimulates the transcription of inflammatory molecules, further contributing to atherosclerosis (14). Hyperglycemia causes increased ROS production as well. The increase in ROS leads, like in insulin resistance, to NO inactivation and consequently

endothelial dysfunction (15). Furthermore, ROS stimulates PKC activation, which affects endothelial cell growth, and viscosity of the extracellular matrix (16). Moreover, PKC promotes vasoconstriction by stimulating mediators like endothelin-1, which also enhances platelet aggregation; and cyclooxygenase-2 (COX-2), increasing thromboxane A2 (TXA_2) and decreasing prostacyclin (PGI_2) production (16). PKC-induced vasoconstriction adds to vascular dysfunction and atherosclerosis.

Furthermore, diabetes acts synergistically with other risk factors, including hypertension, dyslipidemia and smoking, to prompt cardiovascular disease. In T2DM, hypertension and diabetes are known to be especially correlated. Insulin resistance and resulting activation of the renin-angiotensin system promotes hypertension, and hypertension, in turn, leads to decreased insulin sensitivity (4).

In regard to CHD, diabetic patients, especially in long-standing disease, have a similar risk of myocardial infarction (MI) compared to nondiabetic patients with a prior history of MI (17). Diabetic patients also have an increased mortality following MI, which may be explained by the more severe and diffuse coronary atherosclerosis in these individuals, especially in females (18,19).

For cerebrovascular disease, diabetes is an independent risk factor for both ischemic and hemorrhagic stroke (20). There are some distinct features in diabetes-related ischemic strokes, for example they present more often with limb weakness and dysarthria as signs of lacunar cerebral infarction, when compared to nondiabetic stroke patients (21). Diabetes is also related with a twofold increase in the incidence of dementia, Alzheimer´s disease and vascular dementia, all of which are more likely to show cerebral infarcts rather than β-amyloid and tangles (21).

Diabetes is becoming the major risk factor for PAD as the prevalence of smoking is declining worldwide (22). The incidence and severity of PAD are related to the duration of diabetes and the degree of glycemic control (23). Peripheral neuropathy, that is likely to be present concomitantly, causes a later clinical presentation at a more severe stage (24). Diabetes is related to a worse clinical outcome and an increased risk of mortality in PAD, with an even more pronounced effect in critical limb ischemia, which is the most severe manifestation of PAD (25).

There are also some other important complications to mention. Diabetic foot ulcers result from an interplay of peripheral symmetric neuropathy, which causes abnormal weight bearing and multiple injuries in the insensate feet; macro- and microvascular disease; the increased susceptibility of diabetic patients for infections; and poor wound healing (3,4). Around 4-10% of diabetics suffer from foot ulceration, with an overall lifetime risk of 25% (26), making diabetes the most common nontraumatic cause of lower limb amputation (27). A higher susceptibility to infection not only precipitates foot ulcers, it also causes candidal and periodontal infections to occur more frequently. Moreover, there are some unusual infections that may be pathognomonic for diabetes, like necrotizing papillitis, mucormycosis of the nasal sinuses, and malignant otitis externa. Infections are more likely and have a more severe course in poorly controlled DM due to defective neutrophil chemotaxis and phagocytosis. Macro- and microvascular disease may lead to vascular insufficiency, impeding inflammatory cells from reaching the infection site (4).

Both Type 1 and Type 2 DM also change the skeletal composition and increase fracture risk. Although Type 2 diabetics typically have a normal or even increased bone mineral density, they possess a higher fracture risk, which may be explained by microarchitectural changes including an increase in cortical porosity and a decrease in bone turnover (4,28).

1.1.4 Screening and Diagnosis

According to the current American Diabetes Association (ADA) guidelines (29), screening for T2DM with either an informal assessment or with an assessment tool, of potential risk factors should be considered in all asymptomatic adults. In general, testing should begin at 45 years, and in case of normal results, repeated at 3-year intervals. Regardless of age, asymptomatic patients, who are obese or overweight, should be further tested if they have one or more additional risk factors, which are summarized in Table 1. Prediabetic patients should be evaluated yearly, and women with a history of gestational diabetes mellitus (GDM) should undergo lifelong testing in 3-year intervals (see Table 1).

In individuals who were diagnosed with diabetes, it is important to determine and treat other cardiovascular risk factors as well.

Fasting plasma glucose (FPG), 2-h plasma glucose (PG) during 75g oral glucose tolerance test (OGTT) and Hemoglobin A1c (HbA1c) levels are equally suitable to test for diabetes and prediabetes (30). In order to confirm the diagnosis of diabetes, one of the following criteria needs to be met: FPG of ≥126 mg/dL (7.0 mmol/L); or 2-h PG of ≥200 mg/dL (11.1 mmol/L) during OGTT; or HbA1c level of ≥6.5%. Fasting in FPG is defined by no caloric intake of at least 8 hours. For OGTT, the WHO guidelines should be followed, using a glucose load containing the equivalent of 75-g anhydrous glucose dissolved in water. For the testing of HbA1c, the performing laboratory is obligated to use a method that is certified according to the National Glycohemoglobin Standardization Program (NGSP) and standardized to the Diabetes control and complications trial (DCCT) assay (30).

Prediabetes is defined by the presence of impaired fasting glucose (IFP) and/or impaired glucose tolerance (IGT), and can be established when there is either FPG between 100 mg/dL (5.6 mmol/L) and 125 mg/dL (6.9 mmol/L), or 2-h PG during 75-g OGTT is 140 mg/dL (7.8 mmol/L) to 199 mg/dL (11.0 mmol/L), or HbA1c level is 5.7–6.4%. Although prediabetes is not regarded as a clinical entity itself, it is important to recognize it, since it increases the risk for diabetes and cardiovascular disease (30). Table 2 summarizes the diagnostic criteria for diabetes and prediabetes.

Table 1. Criteria for testing diabetes in asymptomatic adults

1. Testing should be considered in overweight or obese (BMI ≥25 kg/m^2) adults who have one or more of the following risk factors:

- First-degree relative with diabetes
- High-risk race/ethnicity (e.g., African American, Latino, Native American, Asian American, Pacific Islander)
- History of CVD[a]
- Hypertension (≥140/90 mmHg or on therapy for hypertension)
- HDL[b] cholesterol level <35 mg/dL (0.90 mmol/L) and/or a triglyceride level >250 mg/dL (2.82 mmol/L)
- Women with polycystic ovary syndrome
- Physical inactivity
- Other clinical conditions associated with insulin resistance (e.g., severe obesity, acanthosis nigricans)

2. Persons with prediabetes (HbA1c ≥5.7% [39 mmol/mol], IGT[c], or IFG[d]) should be tested yearly

3. Women who were diagnosed with GDM[e] should have lifelong testing at least every 3 years.

4. For all other patients, testing should begin at age 45 years, and should be repeated in 3-year intervals in case of normal test results

Adapted from ADA: Classification and Diagnosis of Diabetes: Standards of Medical Care in Diabetes - 2020
[a] Cardiovascular disease
[b] High-density lipoprotein cholesterol
[c] Impaired glucose tolerance
[d] Impaired fasting glucose
[e] Gestational diabetes mellitus

Table 2. Diagnostic criteria for diabetes and prediabetes

	Diabetes	Prediabetes
FPG [a]	≥126 mg/dL (7.0 mmol/L)	100 mg/dL (5.6 mmol/L) - 125 mg/dL (6.9 mmol/L)
OR: 2-h PG [b] during 75g OGTT [c]	≥200 mg/dL (11.1 mmol/L)	140 mg/dL (7.8 mmol/L) - 199 mg/dL (11.0 mmol/L)
OR: HbA1c	≥6.5%	5.7–6.4%
OR: Random PG if classic symptoms of hyperglycemia or hyperglycemic crisis	≥200 mg/dL (11.1 mmol/L)	

Adapted from ADA: Classification and Diagnosis of Diabetes: Standards of Medical Care in Diabetes - 2020
[a] Fasting plasma glucose
[b] Plasma Glucose
[c] Oral glucose tolerance test

1.1.5 Prevention and Treatment

Referring to ADA recommendations, individuals with prediabetes are advised to adhere to an intensive behavioral lifestyle intervention program as suggested by the Diabetes Prevention Program (DPP) (30). This program includes the goal of achieving and maintaining 7% weight loss of initial body weight and practicing moderate-intensity physical activity, being equivalent to brisk walking, for at least 150 minutes per week. These lifestyle interventions in individuals with prediabetes are able to reduce the incidence of type 2 diabetes by 58% over 3 years (31). Although reducing fat and absolute calorie intake, as well as engaging in physical activity, are of utmost importance in preventing the progress to type 2 diabetes in at-risk population, it is not always easy to maintain these goals long-term (32). Because of this, it is essential to provide continuing support and, if needed, other additional therapeutic options like pharmacotherapy. Pharmacotherapy with metformin should be considered in prediabetics if certain other criteria are met, such as moderate- and severe-risk

obesity (BMI ≥35 kg/m^2), age less than 60 years, or prior GDM in women. Other measures for diabetes prevention in at-risk population includes the cessation of tobacco consumption, as well as identifying and treating other cardiovascular risk factors such as hypertension (30).

Acute symptoms of diabetes usually cease when the PG is lower than 200 mg/dL (11.1 mmol/L) (3), which is why the main goal of diabetes treatment is the prevention of long-term complications, as they develop at lower levels compared to acute symptoms.

Monitoring of glycemic levels is carried out by both the patient´s at-home measurement of PG, which represents the short-term glycemic status, as well as HbA1c levels measured at the doctor´s office, which indicate the degree of long-term glycemic control over the last 2-3 months. Generally speaking, in patients who are able to establish a successful glycemic control and meet their treatment goals, the HbA1c level should be measured at least two times per year.

In patients whose therapy changed, or with insufficient glycemic control, the testing of HbA1c is recommended quarterly. Self-monitoring of blood glucose (SMBG) in type 2 DM should be done three or more times daily if the patient takes multiple insulin injections, in order to adjust short-acting insulin boluses, as well as long-acting insulin doses. If the patient receives oral medication, it is not needed to measure SMBG as frequently, but rather use it as marker of medication and diet effectiveness. Nowadays there are also continuous glucose monitoring (CGM) devices available, which rather act as a complementary glucose measuring method, without being able to replace SMBG. Particularly patients with multiple hypoglycemic episodes, hypoglycemic unawareness, or patients not reaching their treatment goals despite great effort, could benefit from these CGM devices.

Glycemic target levels should be tailored to the individual patient, taking into consideration the age, comorbidities and compliance to the pharmacotherapy and lifestyle interventions. For most non-pregnant adults, a HbA1c value of <7% is aimed at (34). If desired by the patient and approved by the provider, HbA1c goals even less than 6.5% may be sought. In this case, it is important to assess whether this goal can be achieved without an increased risk of hypoglycemia or other treatment-related complications. However, if the patient has a history of hypoglycemia, extensive micro- and macrovascular complications and comorbidities, or a diminished life span, HbA1c goals of less than 8% are appropriate.

Exogenous insulin, as well as antidiabetic drugs that stimulate endogenous insulin release regardless of glycemic levels, carry the risk of causing hypoglycemia. Hypoglycemia has 3 levels regarding severity, Level 1 being defined by a PG between ≥54 mg/dL (3.0 mmol/L) and <70 mg/dL (3.9 mmol/L); Level 2 with PG <54 mg/dL (3.0 mmol/L); and Level 3 being characterized by a severe event including an altered mental and/or physical status requiring assistance for treatment of hypoglycemia (33). Frequent hypoglycemic episodes call for adjustment of the antidiabetic therapy, behavioral intervention, and sometimes technology, like CGM, to prevent and identify hypoglycemia on time. Early recognition and intervention in hypoglycemia are important, since in patients suffering from Level 2 or 3 hypoglycemia lacking adrenergic or neuroglycopenic symptoms, hypoglycemic unawareness is likely to develop. In the treatment of hypoglycemia, pure glucose is preferred over other carbohydrate sources rich in fat or proteins, since they would delay the glycemic recovery. In patients unable or unwilling to take glucose by mouth, subcutaneous glucagon injections are indicated. Relatives, or people who are in close contact with hypoglycemia-susceptible diabetics, should be instructed properly on location and application of glucagon injections.

Nowadays, there are plenty of drugs on the market for the treatment of T2DM, with various beneficial and side effect profiles. Therefore, only the essentials of pharmacologic therapy, as recommended by the ADA, are discussed in the section below. In general, metformin is recommended as first line therapy in most patients after diagnosis, in addition to lifestyle interventions, including weight reduction and physical activity (34). Metformin is the drug of choice, being effective and safe, as well as having multiple favorable effects on HgA1c concentration, weight and CV mortality (35). It is applied orally, being more pleasant to patients when compared to injection therapy, such as insulin or GLP-1 agonists. Side effects of metformin include gastrointestinal disturbances, like bloating and diarrhea; lactic acidosis in the case of very high circulating drug levels; as well as vitamin B12 deficiency, making periodical vitamin B12 testing advisable. In case of contraindications or intolerance to metformin, other, usually second line, medication is given in accordance to the patient´s clinical condition (see Table 3).

Dual combination therapy becomes necessary when the patient´s HbA1c level is ≥1.5% higher than the glycemic target. When there is severe hyperglycemia with associated catabolic effects, such as weight loss, ketosis, or polyuria and polydipsia, introduction of insulin becomes essential. Insulin or sulfonylureas can also be used as initial therapy in type 2

diabetics with severe hyperglycemia and can be later replaced by oral therapy as acute glucose toxicity is controlled. Despite some studies suggesting that initial dual combination therapy might be beneficial, current standard of pharmacotherapy in T2DM consists of stepwise addition of medication to the initial metformin regimen. According to the recommendations, patients with concomitant atherosclerotic cardiovascular disease (ASCVD), CKD, or HF, should receive either a SGLT-2 inhibitor or a GLP-1 RA as part of treatment regimen, without regard to HbA1c level, since both drug groups are associated with CV benefit. In individuals lacking these comorbidities, choice of combination therapy is guided by the avoidance of adverse effects, especially hypoglycemia and weight gain, cost, and patient preferences. Table 3 summarizes the recommendations for pharmacotherapy in T2DM related to the individual patient´s characteristics. For patients in whom weight loss is an important factor, GLP-1 RA with proven efficacy for promoting weight loss, such as semaglutide or liraglutide, are recommended. Since DPP-4 inhibitors and GLP-1 RAs both belong to the group of incretins, they should not be given together but rather replace each other if the patient experiences significant side effects while taking the other drug.

Assessing the effectiveness and side effects of the treatment regimen regularly, approximately every 3-6 months, for the individual patient is necessary in order to adjust the dose or change to a different medication (34).

Table 3. Pharmacotherapy in T2DM according to patient characteristics

1st line therapy in all	Patient characteristics	add if HbA1c above target; add in ASCVD [a] /HF [b] /CKD [c] independently of HbA1c	add if HbA1c above target	add if intolerance to medication or HgA1c above target despite quadruple therapy
	ASCVD [a]	GLP-1 RA	SGLT2i	DPP-4 if not on GLP-1 RA *or* Basal insulin *or* TZD *or* SU
	HF [b] /CKD [c]	SGLT2i	GLP-1 RA	DPP-4 if not on GLP-1 RA *or* Basal insulin *or* SU (Avoid TZD in HF)
Metformin	Minimizing hypoglycemia	DPP-4i or GLP-1 RA *or* SGLT2i *or* TZD	SGLT2i +/- TZD DPP-4i *or* GLP-1 RA +/- TZD SGLT2i +/- DPP-4i *or* GLP-1 RA	Later generation SU with lower risk for hypoglycemia *or* basal insulin
	Promoting Weight loss	GLP-1 RA efficient for weight loss *or* SGLT2i	SGLT2i GLP-1 RA efficient for weight loss	DPP-4 if not on GLP-1 RA *or* SU *or* TZD *or* Basal insulin
	Reducing costs	SU TZD	TZD SU	Insulin therapy *or* DPP-4i *or* SGLT2i each with lowest acquisition cost

Adapted from ADA: Pharmacologic Approaches to Glycemic Treatment: Standards of Medical Care in Diabetes—2020

[a] Atherosclerotic cardiovascular disease

[b] Heart Failure

[c] Chronic kidney disease

1.2. Arterial Stiffness as Cardiovascular Parameter

Epidemiological studies have concluded that arterial stiffness is a strong and independent predictor of cardiovascular events, as wells as a risk factor for CV and all-cause mortality (36). For better understanding and assessment of arterial stiffness with its various measurement techniques, it is important to comprehend underlying hemodynamic principles. Importantly, blood flow and pressure are not constant over time, meaning that the pressure wave oscillates around the mean BP. The upper and lower limits of this pressure oscillation are known as systolic and diastolic pressures, respectively, and influence to a great part the LV afterload and coronary artery perfusion. At systole, when the SV is accommodated in the proximal aorta, the local pressure rises and generates a pressure gradient towards the peripheral arteries, causing blood flow to the periphery. In this model, one needs to consider aortic input impedance, which combines factors opposing LV ejection such as the peripheral resistance (PR), the viscoelastic properties and dimensions of large arteries, as well as the intensity and timing of pressure wave reflections (37).

One main hemodynamic function of the arterial system is that it works as a conduit, meaning that it delivers an adequate amount of blood from the heart to peripheral tissues. This function is impaired when the arterial lumen is narrowed and the peripheral resistance of arteries is increased, both factors being caused to a great extent by atherosclerosis and hypertension (37). Another important principle is the so-called “windkessel” function, corresponding to cushioning or dampening, which is important in order to provide a constant flow to peripheral tissues (37) . Due to viscoelastic properties, around 50% of SV is temporarily stored in the aorta and large arteries, stretching the arterial walls and increasing the blood pressure. Accordingly, a part of the energy produced by the heart during systole is redirected to the distension of large arteries and thereby stored in the vessel walls. With diastole, this energy is then again released, causing a continuous blood flow to the periphery during the whole cardiac cycle. However, when the arterial system cannot be stretched because it is too stiff, this cushioning does not function properly and the stroke volume generated by the heart will flow exclusively during systole, and therefore blood will reach peripheral tissues only during this time. Stiffness can be reciprocally defined by the term compliance, describing the absolute change in volume for a given change in pressure: $C = \Delta V/\Delta P$ (37).

In this simplified hemodynamic model, it is important to consider that the aorta and large arteries have both conduit and cushioning functions, and that cushioning is progressively reduced towards the more muscular and less elastic peripheral arteries (38). Therefore, the more peripheral arteries predominantly have a conduit function and are stiffer compared to the large elastic arteries. Because of these differences in conduit and cushioning functions of the respective arteries, the pressure wave velocity is not infinite and equal over the whole arterial system, but rather heterogenous, with marked pressure amplification towards the periphery, which can be explained by the increasing stiffness of the peripheral arteries (38).

Pathophysiologically, arterial stiffening is caused by aging and CV risk factors, including DM, obesity, hypercholesterolemia, smoking and hypertension, through several mechanisms such as increasing breaks in elastin fibers, accumulation of collagen, fibrosis, inflammation, medial smooth muscle necrosis, calcifications, and diffusion of macromolecules within the arterial wall (38). Additionally, a rise in the MAP enhances arterial stiffness, a mechanism that influences the entire arterial system passively (37).

The two most commonly used non-invasive methods to assess arterial stiffness in clinical practice are measurement of pulse wave velocity (PWV), which is a direct measure of arterial stiffness, and pulse wave analysis (PWA), which is an indirect measure, determined by other factors as well.

1.2.1 Pulse Wave Velocity

PWV assesses the stiffness of an artery as a hollow structure, integrating the geometry of the artery (thickness, h; and radius, r), the intrinsic elastic properties of the arterial wall as defined by the Young´s modulus (E) and the blood density (ρ), all factors being summarized in the Moens and Korteweg formula: $PWV^2 = Eh/2r\rho$ (37).

PWV is determined clinically by measuring the distance between the two arterial recording sites over the body surface (Δx) and the transit time of the arterial pulse along the respective arterial segment (Δt): $PWV = \Delta x / \Delta t$ (39). The aorta is the principal artery contributing to the cushioning function, and thus is the most affected by the pathophysiological effects of arterial stiffening and associated with negative CV outcomes

(40). Accordingly, the measurement of the carotid-femoral, or aortic, PWV is considered as gold standard for assessing arterial stiffness (38).

As mentioned earlier, the stiffness gradient increases towards the peripheral, more muscular, conduit arteries, reflected by a higher PWV in these arteries and less prognostic value compared to the aortic PWV (40). To summarize, the stiffer the artery, the higher the PWV of the artery, since it is not as easily stretched and the pulse wave is therefore propagated along the rigid segment at a higher speed. However, the stiffness also depends on pressure, meaning that it increases with higher BP. Therefore, a decrease in stiffness together with a decrease in BP does not necessarily indicate improved arterial wall properties. However, an increase in stiffness together with a decreased or constant BP, indicates deterioration (37).

Although there are no comprehensive reference values currently established for PWV, in order to "classify" the CV risk associated with the respective PWV values, many authors of epidemiological studies stratified subjects into tercile groups according to their PWV. The predictive role of PWV is even more pronounced in higher risk disease states, including coronary artery disease, renal disease, hypertension and diabetes (36).

Regarding DM, growing evidence shows that arterial stiffness is associated with the pathogenesis of the disease. In these patients, particularly AGEs and endothelial NO dysregulation are contributing to the increase in arterial stiffness. Moreover, arterial stiffness is suggested to be associated with the development and progression of target organ dysfunction in diabetics, including nephropathy, retinopathy and autonomic neuropathy (41).

1.2.2 PWA: Augmentation Index

Aortic stiffness, together with SV and ejection velocity, determines the arterial pressure wave amplitude, affecting systolic, diastolic and pulse pressures (PP) in the aorta. The arterial pressure waveform represents the interplay between forward wave, created by ventricular contraction, and reflected waves, which arise at peripheral branch points or sites of impedance mismatches (37). In cases where the PWV is low, as in elastic arteries, reflected waves tend to impact on central arteries during diastole. This is beneficial hemodynamically, since the increase in early diastolic pressure improves coronary perfusion without raising

systolic pressure and LV afterload. In stiff arteries and therefore higher PWV, the reflected waves impact the central arteries earlier, during systole, and therefore add to the forward wave and amplify the systolic pressure, while decreasing aortic pressure during diastole (37,38).

The effects of reflected waves on the pressure shape and amplitude can be assessed in absolute terms, meaning the augmented pressure in millimeter of mercury (mmHg), or relatively, by the central augmentation index (cAIx). The AIx is defined as the ratio of the augmentation pressure (AP), which is the difference between the second (reflected) and first (forward) systolic peak (P2-P1), over the pulse pressure (PP), the difference between systolic and diastolic pressure (P1-P3): AIx (%) = AP/ PP. A typical carotid pressure waveform and the belonging peaks, as recorded by applanation tonometry, are shown in Figure 1 (38).

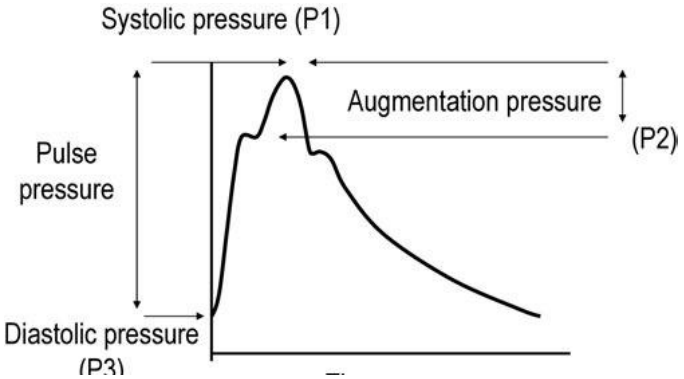


Figure 1. Carotid pressure waveform
Adapted from: Expert consensus document on arterial stiffness: methodological issues and clinical applications.

In individuals with less arterial stiffness and lower PWV, the travelling time of the pressure wave from the aorta to the reflecting sites and back is long, causing the reflected wave to impact the forward travelling wave later during diastole, with P2 appearing after the peak systolic pressure created by the ventricle, P1. Thus, the reflected wave does not add to the forward travelling wave, and the corresponding pressure wave shape is characterized by a negative AIx. Increasing arterial stiffness and PWV, on the other side, are marked by the reflected wave impacting earlier during systole and adding up to systolic pressure, which is represented in the pressure waveform by P2 occurring before P1, causing the cAIx to be positive (37). Peripheral arteries such as the brachial artery, show only slight changes in

arterial stiffness with age, meaning that with increasing central arterial stiffness, the pressure amplification is not as marked as in young and healthy subjects with low central stiffness (38).

Accordingly, cAIx was confirmed to be of significant predictive value of CV events and all-cause mortality, independently of BP and HR (42).

Impedance mismatches, particularly marked in young subjects with a low aortic PWV and pressure amplification towards the peripheral arteries, facilitate reflections of the forward wave. The impedance mismatches and partial reflections of the forward wave at subsequent arterial segments restrict the direct transmission of pulsatile energy to the microcirculation and capillaries (37). With increased stiffness and pressure augmentation, the stiffness gradient towards the periphery is gradually diminished, decreasing the impedance mismatches and reflections, and causing the transmission of pulsatile energy into the microcirculation (37). This process may be dangerous in organs with high perfusion and low resistance such as the brain and kidneys, resulting in an increased risk for dementia and stroke, as well as declining glomerular function (43).

Importantly, pulse wave shape is not only affected by the PWV, which itself is influenced by age and BP, but also by the amplitude of the reflected wave and its point of origin, as well as the HR (38). In tachycardia, the left ventricular ejection time (LVET) is shortened, meaning that the reflected wave is not able to overlap with the forward wave during systole, but impacts during diastole, indicated by negative augmentation pressure and a negative cAIx. In unchanged PWV, but with lower HR, the reflected wave is able to impact and add to the forward travelling wave during systole, resulting in positive augmentation pressure and a positive cAIx (37,38). Conclusively, HR is inversely related to cAIx. Furthermore, the validity of AIx depends on the physiological functioning of the LV, generating normal flow in the presence of afterload. In case of systolic dysfunction of the LV, the ejection depends on the afterload, resulting in a paradoxical in pressure augmentation due to wave reflections not manifesting as pressure augmentation, but as relative decrease of flow during late systole (44). These effects are reflected in the pulse wave shape by an aortic pressure wave with minimal or no systolic augmentation, short ejection duration, and a marked reflected wave during diastole, therefore making PWA unreliable under these

circumstances. Interestingly, the effects of an impaired LV systolic function explain the discrepant increase of central PWV and cAIx in diabetics (44,45).

Additionally, sex, height and ethnicity were shown to be determinants of cAIx as well (46,47). Due to the variability of multiple physiological factors associated with cAIx, it is difficult to establish appropriate normative equations for hemodynamic assessment. One equation proposed by Janner *et al* (48), incorporates the individual´s age, body height, sex, and HR, and has shown promising results in calculating reference values for cAIx, that may be applicable in populations living in different environmental conditions and with different lifestyles, within the same ethnicity (49). In order for equations, including the one mentioned above, to be more sensitive in detecting CV risk and facilitate comparability between different populations, it is suggested to design equations for narrower age groups, as well as incorporate other determinants of cAIx such as DBP and MAP (49). Unfortunately, recording of pulse waves and calculation of cAIx are strictly device specific, due to the application of different measuring principles, which cannot be used interchangeably (50).

1.2.3 Systolic and Diastolic Blood Pressure, Pulse Pressure

Although in the beginning of brachial blood pressure measurement special attention was given to the importance of mean and diastolic pressures, later research, including the Framingham study, demonstrated that systolic BP is more closely related to CV risk at any stage of hypertension (51). SBP is of great value in assessing most hemodynamic mechanisms in hypertension. The progressive increase of SBP and PP with age can be explained in part by the increase of PWV associated with arterial stiffening and its hemodynamic consequences on central pressure relations as mentioned earlier. Correspondingly, SBP rises constantly over the lifespan, whereas DBP rises until middle age but thereafter declines (52). This explains why SBP is more appropriate than DBP for risk prediction. Additionally, high SBP can be seen as a risk indicator of increased aortic stiffness in subjects over 55-60 years. PP was shown to predict adverse cardiovascular and cerebrovascular events as well (53,54) and was suggested to be a slightly better indicator than SBP regarding carotid stenosis (54) and stroke mortality (55), emphasizing the importance of central BP measurement.

DBP and MAP are relatively uniform over the whole arterial tree and between individuals (56). SBP and PP on the other side, are highly variable between persons and

within the individual´s circulation itself. Due to pulse pressure amplification (PPA) towards the periphery, particularly expressed in young individuals, brachial SBP and PP cannot be used as surrogates for central SBP and PP, as they would overestimate central values (38).

Regarding the clinical significance of central BP values, central SBP was suggested to be the best indicator to define hypertension (57), and to be a slightly better predictor for CV events, compared to brachial BP (58). Additionally, central SBP is more closely associated with target-organ damage, including carotid intima-media thickness and left ventricular mass (59). Central PP was also shown to be marginally superior to brachial PP in predicting clinical events (42). Moreover, retinal artery disease is more closely associated with central PP than with brachial BP (60). However, it should be kept in mind that an acute reduction in PP does not necessarily correlate with a reduction in arterial stiffness, as acute vasodilation decreases PP proportional to baseline values, independently of changes in aortic stiffness (61).

Finally, aortic PWA, recorded either directly from the carotid artery or derived by transfer function from the radial artery waveform, should include central PP, central SBP and cAIx as major parameters (38). Although providing useful additional information on wave reflections, it should be kept in mind that these parameters represent arterial stiffness only indirectly, being influenced by various other factors, including the amplitude and the reflectance point of the reflected wave, as well as HR and ventricular contractility. Aortic PWV is the gold standard in assessing arterial stiffness, being directly associated with arterial stiffness and having the highest predictive value of CV risk.

1.3. Red Wine as Cardiovascular Lifestyle Intervention

Epidemiological studies have shown that light to moderate red wine (RW) consumption is associated with protection against cardiovascular diseases (CVD) (62). Since CVD is the leading cause of mortality and morbidity in type 2 diabetics, the effect of RW on the CV system in these individuals is of high interest. The CASCADE study (63), designed as 2-year RCT, revealed that chronic, low to moderate RW consumption in well-controlled type 2 diabetics with low risk of alcohol abuse is safe and may modestly reduce cardiometabolic risk. RW was shown to be superior to white wine and water in improving lipid profiles, including an increase in HDL cholesterol and apolipoprotein(a)1, as well as a decrease in total cholesterol-HDL-C ratio, which are important contributing factors in atherosclerosis and

CVD. Moreover, RW was associated with a reduction in the number of components comprising the metabolic syndrome. Both red and white wine, with the later showing a modest advantage that might be a coincident, were associated with improved glycemic control as reflected by changes in FPG levels and the homeostasis model assessment of insulin resistance (HOMA-IR) score (63).

Regarding hypertension, a major cardiovascular risk factor in T2DM, RW was shown to produce a biphasic effect on 24-h ambulatory BP in diabetic individuals (64). This means, that moderate RW consumption results in a decrease of diastolic blood pressure (DBP) at night, while both diastolic and systolic BP are elevated during the next day. However, regarding heart rate (HR), moderate RW intake was associated with increase of both asleep and awake HR, which is an unfavorable outcome on CV risk. Arterial stiffness parameters, including PWV and AIx, were shown to improve with acute intake of RW in healthy as well as in coronary artery disease participants (65).

However, there is some lack considering the effect of RW on arterial stiffness parameters such as aortic PWV, although it is a known independent and strong predictor of all-cause and cardiovascular mortality (36). This is the reason why the effect of RW on arterial stiffness is of high interest, since it may reflect new clinical consequences of RW intake on Type 2 diabetic patients. There have been some studies indicating a favorable effect of RW on arterial stiffness, but only in healthy volunteers, postmenopausal women and patients with coronary artery disease (65), while there is lacking information concerning diabetic patients. With CVD being one of the most significant complications in T2DM (13), more comprehensive lifestyle recommendations on improvement of cardiovascular health in these patients are desirable. By studying the effects on arterial stiffness in this particular group of patients, another step will be made in order to complement the ongoing discussion about recommendations for moderate red wine consumption in the growing population of diabetic patients.

The cardiovascular effects of RW are likely to arise from an interplay between its different compounds, particularly ethanol and the polyphenols, with their respective properties being discussed in the sections below.

1.3.1 The Effect of Ethanol on Cardiovascular Protection and T2DM Development

Although initially thought that cardiovascular (CV) protective effects arise solely from polyphenols contained in RW, studies have concluded that some beneficial effects result from the ethanol component itself, and that these beneficial effects are associated with other alcoholic beverages as well, such as white wine, beer and spirits (66). Above all, the health effects of ethanol depend strongly on both the amount of alcohol consumed, as well as the pattern of consumption (67). Light to moderate drinking is defined as 1-2 standard drinks per day, one drink being equivalent to 15-20 g of ethanol, corresponding to approximately 150 ml of wine, 350 ml of beer, or 40 ml of an average spirit. In general, the health benefits follow a J-shaped curve, meaning that light to moderate alcohol intake is associated with a decreased risk of CV events and overall mortality compared to strict abstaining. While women benefit from 1 standard drink per day, in males this benefit is associated with 1-2 standard drinks per day. Heavy drinking on the other side, defined as 3 or more standard drinks per day, correlates to an increased risk of CV events and mortality. The adverse effects of heavy drinking mainly include the increased morbidity and mortality associated with hemorrhagic stroke, induction of liver injury, precipitation of high-fat diet-induced steatohepatitis, neural toxicity, and the increase in risk of various neoplasms, such as breast, oral and gastrointestinal cancer. Furthermore, even light to moderate drinking causes psychosocial consequences, including impaired driving, and is contraindicated in persons susceptible to the development of alcoholism, especially when there is familial alcoholism (67).

In both males and females, regardless of age in adults, low to moderate drinking is associated with a significant decrease in incidence of myocardial infarction (MI) (68). In addition, this association is also proven in risk groups, including persons with diabetes, hypertension, metabolic syndrome, known CVD, overweight, and in smokers. Even in men complying with a healthy lifestyle, including regular exercise, healthy dieting, and abstaining from smoking, a 40-50% reduction in risk for MI was demonstrated (67).

Cardioprotective mechanisms of ethanol consist of direct effects on cardiomyocytes as well as indirect extra-cardiac effects (67). Indirect effects of moderate alcohol intake include beneficial changes in blood lipid profiles, such as an increase in high-density lipoprotein (HDL) and apolipoprotein(a)1, platelet aggregation and fibrinolytic activity, explained by a decrease in fibrinogen (66), thereby decreasing the risk of adverse cardiovascular events.

With special regard to diabetes, moderate alcohol intake is also associated with improved insulin sensitivity after MI (69), and reduction in risk of new-onset T2DM (70).

Direct effects of ethanol include the stimulation of myocardial blood flow. This is explained by an ethanol-induced increase in expression of nitric oxide synthase (NOS), thereby leading to an increase in nitric oxide (NO) generation, as well as transient receptor potential vanilloid 1 (TRPV1) channel activation on perivascular sensory nerve terminals. These then stimulate the release of CGRP, which in turn increases coronary blood flow (67).

Another beneficial effect of alcohol intake is the increased tolerance of cardiomyocytes to ischemia/reperfusion (I/R). This effect resembles ischemic preconditioning (IPC), a cardioprotective intervention where tissues are exposed to short cycles of I/R, after which a prolonged period of coronary artery occlusion and reperfusion are induced. In the early phase of IPC, arising within minutes and lasting for about 2-4 hours, pre-existing mediators are thought to protect against subsequent ischemic damage. These early-phase, or first window, mediators include adenosine, bradykinin, opioids, protein kinase C, ROS and the mitochondrial K_{ATP} channel.

Particularly PKCε was shown to play a major role in both ethanol-induced cardioprotection and early phase IPC, this was proven by studies in which PKC was pharmacologically inhibited, leading to an absence of protective effects. The second window of protection, or late-phase IPC, is characterized by signaling molecules such as ROS and NO, protein kinases including PKC, Akt, MAPK and transcription factors such as NFκB or STAT1/3, which were induced by the preconditioning stimulus. These molecules then lead to the transcription and de novo synthesis of protective proteins including COX-2, heat shock protein, iNOS and aldehyde dehydrogenase, which result in protection against ischemic damage. These late-phase effects appear at around 24 hours after induction and persist for approximately 48-72 hours. These infarct-sparing mechanisms mediated by IPC, can also explain the mode of action of chronic low-dose alcohol consumption on cardioprotection. It was shown that chronic low-dose alcohol intake, similar as in IPC, increased active levels of PKCε and Akt, NO generation, mitochondrial KATP channel, as well as α1-adrenoreceptors, adenosine A1-receptors and phospholipase C (67).

Additionally, moderate alcohol intake is associated with beneficial effects on vascular endothelial cells, in part by inhibiting leukocyte-platelet/endothelial cell interactions, thereby maintaining endothelial barrier function (67).

Furthermore, moderate alcohol consumption was shown to promote anti-inflammatory effects in adipose tissue by promoting the expression of the adipocyte hormone adiponectin, which is also associated with a reduction in risk of diabetes (71), as well as CHD (72). Ethanol also reduces the release of inflammatory cytokines and pro-inflammatory mediators from adipose tissue (66).

Light to moderate alcohol intake was associated with a risk reduction for acquiring T2DM, supposedly by reducing low-grade systemic inflammatory processes linked to diabetes development (67). This anti-inflammatory ability is reflected by the reduction in inflammatory markers associated with moderate alcohol intake, including CRP, fibrinogen, soluble tumor necrosis factor receptor-2 and soluble vascular adhesion molecule-1 in type 2 diabetes (73). On the other side, moderate alcohol intake in type 2 diabetics was not associated with an increase in specialized pro-resolving mediators of inflammation (SPMs), which are molecules contributing to mitigate chronic inflammation (74). Hence, the exact mechanisms and influence of moderate alcohol intake on inflammatory processes in Type 2 diabetics are uncertain.

Since the improvement in glycemic control observed in the CASCADE study (63) was primarily pronounced in slow ethanol metabolizers, and the benefit was associated with both red and white wine with similar ethanol concentrations, this favorable glycemic outcome may be attributed to the ethanol component in wine.

1.3.2 Cardiovascular and Antioxidant Effects of Polyphenolic Compounds in Red Wine

A great proportion of the favorable effects of RW on cardiovascular outcome is thought to be associated with its contained polyphenols, which were shown to be inversely related to CHD (75). Polyphenols in RW can be divided into various subgroups, a simplified distinction can be made between the flavonoids including quercetin, anthocyanin, proanthocyanidin and catechin, and on the other side the non-flavonoids such as the phenolic acids and the stilbene resveratrol.

Although some of the beneficial effects of RW can be designated to ethanol, as described in the section above, some of the cardioprotective effects are attributed to the high polyphenolic content of RW. This superior effect of RW is also indicated by the lower mortality of cardiovascular and other causes in low to moderate RW intake as compared to the similar intake of other alcoholic beverages (76). In multiple studies, RW was associated with inhibition of LDL oxidation, reduction in susceptibility to lipid peroxidation, as well as enhancement of the antioxidant capacity in humans. The extent of antioxidative capacity of individual polyphenols depends on their chemical structure, namely the number of their hydroxyl groups in the aromatic ring (77,78). Polyphenols have the ability of direct radical scavenging, meaning they are oxidized by radicals, making the reactive oxygen species more stable and less reactive (79). Some of the polyphenols, such as quercetin, are able to reduce I/R injury by interfering with inducible NO synthase activity (79). Although at normal concentration, NO produced by constitutive NO synthase is beneficial in maintaining vasodilation, the excessive NO concentrations produced by inducible NO synthase are associated with oxidative damage. In this situation, NO may react with free radicals, thereby producing highly damaging peroxynitrite, which directly oxidizes LDLs, leading to irreversible cell membrane damage. However, through antioxidant action of polyphenols, free radicals are scavenged and cannot react with NO, thus preventing damage (79). Additionally, a proportion of polyphenols, including quercetin and silibin, have the ability to inhibit xanthine oxidase, which is important considering that this enzyme is a source of oxygen free radicals, particularly in ischemic conditions (80).

While the amount of non-flavonoid phenols in red and white wines is similar, there is a much higher quantity of flavonoids in red wine as compared to white wine, suggesting the overall higher quantity of polyphenolic compounds is the reason why red wines show a higher antioxidant effectiveness than white wines (81). The increase in antioxidant capacity after RW consumption was also shown in studies using dealcoholized red wine, emphasizing that this effect can be attributed to the polyphenols (82). However, later evidence suggested that both polyphenols and plasma urates contribute to the antioxidant capacity of RW, with urates being present in smaller quantities in dealcoholized RW (83). Accordingly, the total antioxidative capacity is higher in alcohol-containing RW as compared to dealcoholized RW.

Other antioxidant-independent beneficial effects of polyphenols include the improvement of endothelial function, by stimulating the production of vasodilating factors

such as NO, endothelium-derived hyperpolarizing factor (EDHF) and prostacyclin, and inhibition of the vasoconstrictor endothelin-1 in endothelial cells (75). Additionally, polyphenols inhibit pro-angiogenic factors including vascular endothelial growth factor (VEGF) and matrix metalloproteinase-2 (MMP-2) in smooth muscle cells (75), as well as inhibit platelet aggregation (79). However, in vivo studies have shown that for the absorption of vasoactive polyphenols, some presence of ethanol would be necessary (84), as an increase of plasma NO production can be observed after RW intake but not after consumption of an ethanol-free polyphenolic beverage (85).

The antioxidant and vasoactive effects of polyphenols are most probably achieved by the synergistic action of the individual compounds together, as there was shown a negative correlation between the antioxidant and vasodilatory activity of individual polyphenols (86).

There are some controversies regarding the bioavailability and actual in vivo effects of polyphenols, since the plasma concentrations of polyphenols vary greatly between the different compounds and are in general lower than the concentrations used in cell culture models and animal studies. Most polyphenols are extensively metabolized during absorption, leading to glucuronidated, sulphated and methylated forms of the original polyphenolic compound, with more research being needed regarding the in vivo effects of these conjugated forms (87).

2. OBJECTIVES

The objective of this study was to examine the effects of moderate daily red wine intake on arterial stiffness and hemodynamic parameters in participants with well-controlled T2DM.

The hypothesis to be tested: subacute, moderate daily red wine intake, defined as 300 ml per day over three weeks, decreases arterial stiffness, measured by aortic PWV and cAIx, in well-controlled Type 2 diabetic patients.

3. MATERIALS AND METHODS

3.1. Study Participants

The study included 21 patients with Type 2 diabetes mellitus which were recruited from the Department of Endocrinology, University Hospital Split and by family doctors from family medicine practices in Split. They were informed about the opportunity to participate in this study. We included men with history of well-controlled Type 2 diabetes mellitus (HbA1c 5,0-7,5%), in the age range between 40 to 70 years.

Study participants were treated with antidiabetic therapy prescribed by an endocrinologist: all were treated with metformin at a dose of 500-2000 mg daily; four subjects were on dual therapy with metformin and DDP-4 inhibitors (vildagliptin, alogliptin, or sitagliptin) or acarbose. Finally, one participant was additionally treated with the third drug, SGLT2 inhibitor, empagliflozin.

Exclusion criteria were previous cardiovascular events such as myocardial infarction, stroke, as well as arterial or venous thromboembolism; acute infections; other acute or chronic diseases with exception of well-controlled arterial hypertension; severe obesity (BMI >35 kg/m^2); and smoking. Among the participants mentioned, three were excluded: one due to inability to schedule an appointment during morning hours because of his overnight work, one due to refusal of drinking wine because he did not tolerate it well, and one due to uncontrolled arterial hypertension detected at the first visit.

3.2. Study Design

The study was organized as a cross-over interventional study with the first two weeks being the drive-in period. Participants were instructed to abstain from any alcoholic beverage during this time and to fill out a diet-questionnaire for three consecutive days. The first measurement was conducted after this drive-in, no-alcohol period.

The second period lasted for three weeks, during which the participants were instructed to drink 300 ml of red wine per day with meals, split between lunch and dinner. The participants were again asked to fill out the diet-questionnaire for three consecutive days. The second measurement was conducted directly after this three-week red wine intervention.

The red wine was produced from the grape variety *Plavac mali*, 2015, Volarević winery, Croatia, with ethanolic content of 14.7 vol% and polyphenolic content of 3210 mg gallic acid equivalents/L. The wine was provided by our research group after performing the first measurement, at the beginning of the second period of the study. The participants were asked to return the empty bottles at the time of the second measurement.

This study was conducted at the Department of Pharmacology of the University of Split School of Medicine, in accordance with the statements of the Nuremberg code and ethical principles for medical research as defined by the Declaration of Helsinki and its amendments, and was approved by the Ethics Committee of the University of Split School of Medicine (class: 003-08/13-03/0003, number: 2181-198-03-04-13-0042). All participants took part in this research voluntarily, after all methods and procedures have been presented and explained. Their identities are protected, representatives of the Ethics Committee of the Faculty of Medicine can have access to the documentation.

All measurements were performed by the research team of the Department of Pharmacology, University of Split, as a part of the project financed by the Croatian Science Foundation, under the title: Biological effects of wine: the influence of vinification technology, dealcoholization and aging of wine, project leader Prof. Mladen Boban.

3.3. Anthropometric, hemodynamic and arterial stiffness measurements

The measurements were taken at the research laboratory of the Pharmacology Department of the University of Split School of Medicine, two times: on the last day of the first, drive-in, no alcohol period and accordingly, at the end of the second, wine consumption-period of the study. They were scheduled in the morning, from 7 to 10am, with each participant being scheduled individually for the same time of day at the first and second measurement, respectively. Participants were instructed to continue their usual dietary habits and physical activities the day before the measurement, and to come to the appointment after an overnight fast, before breakfast. The study was organized in two parts: the first ten participants were included in July, and additional eleven ones in October, 2019.

At the first measurement, the participant´s medical history was taken, as well as anthropometric data including height, weight, and upper arm circumference. A calibrated medical scale with an altitude meter (Seca, Birmingham, UK) was used to measure body mass

and height. Body mass index (BMI) was calculated by dividing body mass value (kg) by the squared height value (m^2). After the wine consumption-period, the measurements of weight and upper arm circumference were repeated. The second step at both measurement appointments was for the participants to lie down on a bed in supine position, for 15 minutes. Thereafter, a blood sample of 50 ml was drawn from each participant at each visit. Several parameters were measured, for the purpose of this diploma thesis we have focused on glucose and HbA1c. HbA1c measurement was conducted with a laboratory assay certified by the NGSP as traceable to the DCCT reference, according to the American Diabetes Association (ADA) guidelines (29,88). All samples were analyzed in the same laboratory by a competent biochemist according to the standard protocol.

Five minutes after phlebotomy, several cardiovascular parameters were determined by the oscillometry-based Arteriograph™ device (TensioMed®, Budapest, Hungary). Parameters included peripheral systolic and diastolic blood pressure, so this device was actually used for measurement of peripheral arterial blood pressures. The device originally functions by the principle of oscillometry, which is based on the pressure changes in the occluded artery under an inflated cuff connected to a piezoelectric sensor that registers the measured pressure signals. The measurement begins by placing a cuff on the right upper arm, namely the brachial artery. The Arteriograph first measures systolic and diastolic blood pressure, and then the cuff is inflated to a suprasystolic value of 35 mmHg above the systolic pressure. In this way, the inflated cuff serves as a sensor to register pulsatile changes in the occluded brachial artery (89)(see 1.2.3).

Thereafter, we used the applanation tonometry-based SphygmoCor® (Version 8.1; AtCor Medical Inc., Sydney, Australia) device. Measurements were taken while participants resting in supine position. First, applanation tonometry of the right radial artery was performed, with the integrated transfer function generating central pressure wave forms. Secondly, we performed measurement of the carotid-femoral PWV with the SphygmoCor® device. For this, the distance between the right carotid and right femoral artery was measured as a direct line between the points of the strongest pulsation of the right carotid/femoral artery and supra-sternal notch, by a simple flexible measurement scale device and their respective pressure waveforms were recorded consecutively. The intersecting tangent algorithm has been used in the SphygmoCor Software and the direct carotid femoral distance has been corrected by 0.8 to accomplish appropriate PWV values (90).

The pulse transit time was derived from the relation of the respective pulses to the R-waves of the simultaneously recorded ECG (see 1.2.1).

In summary, parameters obtained by the SphygmoCor® were peripheral blood pressure parameters including systolic, diastolic, pulse pressure and the peripheral augmentation index (pAIx); heart rate (HR); central (aortic) pressure parameters including systolic, diastolic, pulse pressure and central augmentation pressure; central augmentation index (cAIx) and central augmentation index at frequency 75/min (cAIx@HR75); and PWV.

In order to maintain a constant environment during measurements and thereby prevent measurement bias, the temperature in the research laboratory was constantly regulated between 22°C and 24°C and talking was prohibited during measurements. Repeated measurements were performed to attain a minimum of two valid readings according to the built-in quality control demand. Quality score ranged from zero to 100, yet, measurements with quality control of less than 90 were repeated, in order to achieve adequate results. All measurements with the tonometry-based SphygmoCor® were performed by the same operator, trained and experienced with the operation of the device, in order to prevent inaccuracy from operator-dependent variability.

3.4. Statistical Analysis

GraphPad Prism version 6 for Windows (GraphPad Software, San Diego, CA, USA) was used for statistical data analysis and graph design. Normality of data distribution was estimated by the Kolmogorov-Smirnov test. Correspondingly, data were expressed as mean±standard deviation (SD). Statistical analysis was performed by using Student's t test. The level of significance was set at $P<0.05$.

4. RESULTS

4.1. Basic anthropometric parameters, glucose levels and glycemic indicator HbA1c

As presented in Table 4, there was no significant difference in basic anthropometric parameters, glucose levels and glycemic control indicator HbA1c before and after wine consumption.

Table 4. Anthropometric parameters, glucose levels and glycemic control indicator HbA1c before and after wine consumption

Parameters	Drive-In (N=18)	Wine (N=18)	*P**
Age	55	55	
Weight (kg)	102.6±21.0	102.2±21.3	0.184
BMI (kg/m^2)[a]	30.12±4.35	29.96±4.32	0.204
HbA1c (%)[b]	6.60±0.96	6.66±0.96	0.692
Fasting plasma glucose (mmol/l)	7.91±2.25	7.86±2.59	0.899

Data are presented as mean±standard deviation
[a] Body Mass Index
[b] Hemoglobin A1c
* Student´s t test

4.2. Peripheral and central hemodynamic parameters

Moderate drinking of wine affected almost all the measured hemodynamic parameters in diabetic participants with significant decrease of peripheral diastolic pressure (79.72±11.47 mmHg in control, no alcohol period vs. 76.39±11.15 mmHg for intervention, wine consumption period, *P*=0.034).

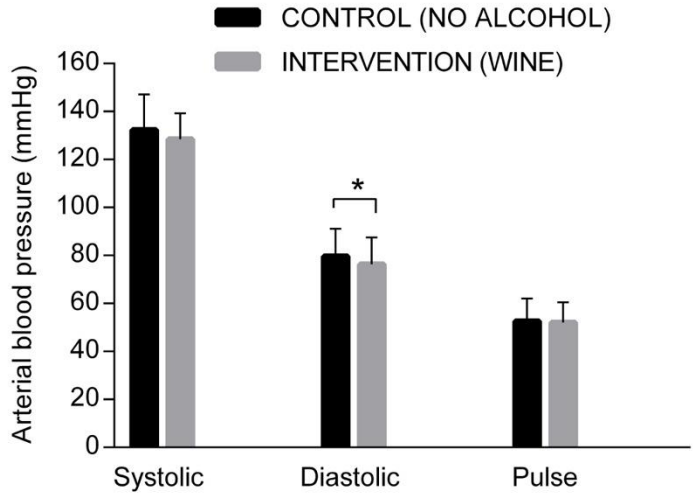
Although wine consumption induced reduction of peripheral systolic pressure (132.30±14.88 mmHg in control, no alcohol period vs. 128.50±10.70 mmHg for intervention, wine consumption period), as well as of central systolic (120.30±14.53 mmHg in control, no alcohol period vs. 115.90±10.98 mmHg for intervention, wine consumption period), and central diastolic pressure (80.11±12.46 mmHg in control, no alcohol period vs. 77.28±10.98 mmHg for intervention, wine consumption period), these changes were not statistically

significant (P=0.206, P=0.122, and P=0.105, for peripheral systolic, central systolic and central diastolic pressure, respectively).

Peripheral and central pulse pressures were not affected by wine consumption (52.56±9.52 mmHg in control, no alcohol period vs. 52.11±8.32 mmHg for intervention, wine consumption period (P=0.826) for peripheral, and 39.44±8.64 mmHg in control, no alcohol period vs. 38.86±8.07 mmHg for intervention, wine consumption period (P=0.756), for central pulse pressure, respectively).

These data are presented in Figure 2 A for peripheral and B for central hemodynamic parameters.

A)



B)

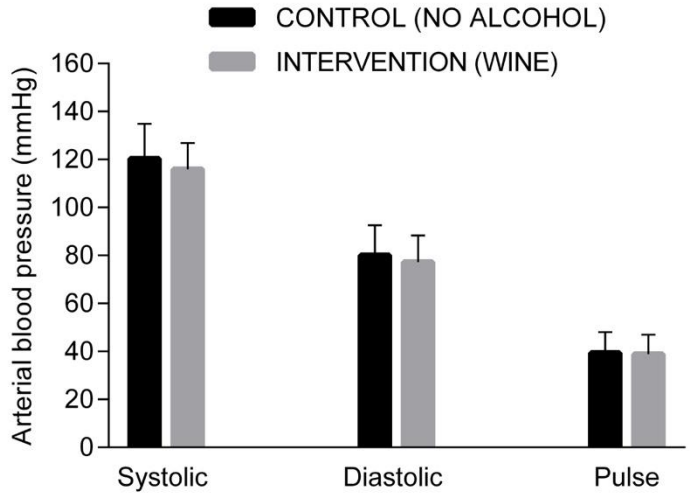


Figure 2 A and B. Peripheral (A) and central (B) systolic, diastolic and pulse pressure in diabetic participants before and after consumption of wine
Data are presented as mean±standard deviation. N=18
* Student´s t test, *P*=0.034

4.3. Arterial stiffness parameters

As shown in Figure 3, wine consumption induced significant decrease of PWV in our diabetic participants (7.42±1.44 m/s for control, no alcohol period vs. 6.98±1.44 m/s for intervention, wine consumption period, *P*=0.013). Although there was also a trend of decline of other arterial stiffness parameters following wine consumption, these differences were not statistically significant (Table 5).

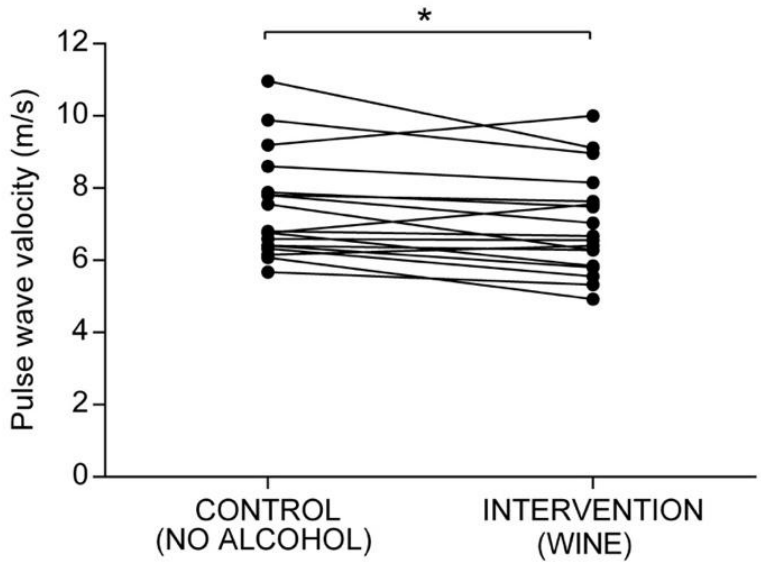


Figure 3. Pulse wave velocity in diabetic participants before and after consumption of wine
Data are presented as mean±standard deviation. N=18
* Student´s t test, *P*=0.013

Table 5. Peripheral and central augmentation indices in diabetic participants before and after wine consumption

Augmentation indices (%)	**Control (drive-in, no alcohol period) (N=18)**	**Intervention (following 3 weeks of moderate wine consumption) (N=18)**	***P****
pAIx[a]	-26.42±17.27	-28.72±16.07	0.440
cAIx[b]	22.81±13.35	19.67±11.30	0.195
cAIx@HR[c]	19.97±12.33	17.44±11.15	0.256

Data are presented as mean±standard deviation (N=18)
* Student´s t test
[a] Peripheral Augmentation Index
[b] Central Augmentation Index
[c] Central Augmentation Index adjusted for heart rate of 75/min

5. DISCUSSION

This study has shown that three weeks of moderate red wine consumption significantly improved PWV in participants with well-controlled Type 2 diabetes mellitus. Furthermore, wine consumption induced decrease of peripheral arterial diastolic pressure. On the other side, this intervention had no effect on glycemic control, at least not on the level of fasting plasma glucose and HbA1c.It is the expected result as this parameter represents a measurement of blood sugar control over the previous two to three months. However, some other experimental studies with a longer duration of wine consumption intervention showed similar lack of significant improvement of this specific glycemic control indicator. The study of Gepner *et al* compared the effects of moderate red and white wine consumption over a period of 2 years in Type 2 diabetics, with primary outcomes being cholesterol and blood glucose levels. Red wine was shown to have a beneficial effect on cholesterol levels, by significantly increasing HDL-cholesterol (HDL-C) and decreasing the total cholesterol to HDL-C ratio; while white wine was associated with a significant decrease in fasting plasma glucose (FPG) levels. Neither of both wine interventions improved HbA1c levels, and no changes were observed in terms of blood pressure, body weight, or waist circumference compared to the control group of mineral water drinkers (63). Similar results were presented in a recently published meta-analysis by Ye *et al* (91).

In our study with diabetic participants subjected to a lifestyle intervention with relatively short, subacute moderate red wine consumption, measurement of fructosamine, as an indicator of total glycated serum protein and a biomarker with a half-life of 14–21 days, which measures glucose control over a two-to-three week period (92), would have been a better choice and is planned for future research.

Regardless of our results, the question of possible correlation between HbA1c levels and arterial stiffness in the assessment of cardiometabolic risk is intriguing. Liang *et al* performed a large non-diabetic sample study of Chinese population and showed strong association between HbA1c and PWV that was amplified in older and hypertensive population (93).

Another parameter that is often under the scope of cardiovascular risk investigators is body weight. In our study, expectedly, there were no changes in participants' body weight and BMI following wine consumption. As shown in Table 4, the BMI of our participants is

higher than 30 kg/m^2, which corresponds to obesity class I. Interventional studies in which modest weight loss has been achieved by lifestyle interventions in overweight subjects with impaired glucose tolerance show that weight loss is associated with improved insulin-glucose homoeostasis (31,94). Several studies have suggested a link between moderate wine consumption and body weight. Recently, Poudel *et al* reported that among non-smoking adolescents from Denmark, consumption of alcohol, and in particular wine, seems to be associated with less weight gain until midlife (95).

Comparison of differences in hemodynamic parameters before and after wine consumption indicate the following: participants' peripheral and central systolic and diastolic pressures were reduced for approximately 5 and 3 mmHg, respectively. It is worth mentioning that with a 2 mmHg lower systolic blood pressure the risk of stroke mortality can be reduced by 10 % and other cardiovascular mortality by 7% (96).

Our results are, however, in contrast with the results of previously mentioned CASCADE randomized control trial (63) where consumption of wine by diabetic participants lasted for two years and induced no changes in blood pressure. Although it is hard to compare these two studies, it is important to point out that in CASCADE study, participants drank twice as much wine as our participants.

In contrast to that, the decrease of pulse pressures at central and peripheral level was less pronounced. In general, peripheral pressures were more affected by wine consumption than central ones and only peripheral diastolic pressure decrease was statistically significant.

However, it is important to be aware of some technical limitations of the devices used for both hemodynamic and arterial stiffness measurement. When measuring central blood pressure non-invasively, for example by radial artery tonometry, the by transfer function generated central pressure wave form needs to be calibrated to brachial cuff systolic and diastolic blood pressure values. Since the measured cuff blood pressure values tend to be lower than the actual brachial artery pressure as measured by invasive means, the derived central pressures tend to be erroneously low (97). Further complicating the analysis of radial waveforms, the pressure amplification from the brachial artery, used for calibration, to the radial artery needs to be considered. Although carotid artery pressures are only needed to be scaled to brachial diastolic blood pressure and mean arterial pressure, which are less variable

throughout the arterial tree when compared to systolic blood pressure, obtaining carotid waveforms of sufficient quality can be difficult in some, particularly in obese, patients. In theory, brachial systolic blood pressure should be more variable than central systolic blood pressure, because of the inherent population variability in pulse pressure amplification towards peripheral arteries. This theoretical superiority of central systolic blood pressure in providing more accurate values is however diminished in practice by possible measurement and model errors encountered in central systolic blood pressure calculation (98). Further studies are needed in order to convincingly outline the superiority of central pressure measurement over brachial pressure measurement, especially when considering the high correlation (r = 0.6–0.9) between the two (97).

Newer cuff-based devices such as the Arteriograph™ device (TensioMed®), which was used in our measurements but exclusively for blood pressure measurement, may produce more accurate central blood pressure measurements. As they scale brachial waveforms obtained with pulse volume plethysmography to the measured brachial cuff pressure, they prevent any influence of brachial-radial amplification. Moreover, they are supposedly less operator-dependent than tonometry-based devices. However, as brachial cuff pressure measurements tend to be lower than the true value, this may still obscure central pressure values to some extent in these devices as well (95).

Regarding the PWV, this is the most important result of the study. In general, baseline values of arterial stiffness parameters of our study participants might suggest that their endothelial function and arterial elasticity is rather preserved regardless of their diabetic disease. Only one participant exhibited PWV higher than 10 m/s which is according to the guidelines (99) rather robust but significant marker of end-organ damage (measured value of this participant was 10.96 m/s).

On the other side, potential existence of initial vascular damage that is not yet clinically evident might be suggested by the fact that consumption of wine induced significant improvement of carotid-femoral PWV.

There are some advantages and limitations of our study. The design of the study as a cross-over interventional trial in which each participant acts as a control to himself is ideal for testing of the hypothesis set. However, the fact that we conducted the study at two time points

is limiting, although there is no statistical difference between the measured parameters of the July participants in comparison to those of October. This will be avoided in future studies.

An additional limitation is that we were not able to perform a pilot study to test either intra- or inter-operator variability. However, as already mentioned, our measurements of arterial stiffness parameters were performed by experienced and trained professional.

More studies are needed to improve the understanding of the mechanisms linking PWV as a mediator of arterial stiffness cardiovascular risk of T2DM patients. and to direct strategies for individuals with diabetes to benefit arterial function (100). The robust prediction of arterial stiffness over many years of life is observed even when diabetes mellitus and hypertension have been well controlled over time (101). Thus, prevention and early treatment of T2DM is critically important for the prevention or attenuation of arterial stiffness and attendant CVD mortality and morbidity. In regard to that, it seems that moderate wine consumption could be valuable.

6. CONCLUSION

Three weeks of moderate red wine consumption significantly improved PWV in participants with well-controlled Type 2 diabetes mellitus. This indicates that relatively short period of moderate wine consumption could beneficially affect compromised arterial function. Furthermore, wine consumption induced decrease of peripheral arterial diastolic pressure. The underling mechanisms are complex and, in this case, probably not directly correlated with HbA1c level.

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8. SUMMARY

Introduction: Type 2 Diabetes Mellitus (T2DM) is a prevalent cause of morbidity and mortality, particularly due to chronic complications development, most commonly cardiovascular diseases. Among markers for assessment of clinical and subclinical signs of cardiovascular disease, the presence of arteriosclerosis, and stiffening of arterial walls as indicated by the arterial stiffness, was proven to be an important predictor of adverse cardiovascular events. Carotid-femoral pulse wave velocity (PWV) is considered as gold standard for arterial stiffness measurement. On the other side, moderate red wine (RW) consumption was shown to have cardioprotective effects by multiple epidemiologic and experimental studies.

Objective: The objective of this study was to examine the effects of moderate daily red wine intake on arterial stiffness and hemodynamic parameters in participants with well controlled T2DM.

Subjects and Methods: 18 well controlled-T2DM participants (taking metformin alone or with oral antidiabetic drugs as chronic therapy) were included in the cross-over interventional study during five weeks; the first two weeks being the drive-in period with no alcohol consumption (control), followed by three weeks of moderate, 300 ml per day, consumption of red wine *Plavac mali* with meals (intervention). At the end of each experimental period we assessed anthropometric data (body mass index), performed biochemical blood analysis (fasting plasma glucose, and HbA1c), while oscillometric and tonometric measurements of arterial stiffness (pulse wave velocity, peripheral and central augmentation indices) and hemodynamic parameters (heart rate, peripheral and central systolic, diastolic and pulse pressure) were performed by Arteriograph and SphygmoCor devices, respectively.

Results: Moderate RW consumption in well-controlled T2DM participants induced significant decrease of PWV (7.42±1.44 m/s for control, no alcohol period vs. 6.98±1.44 m/s for intervention, wine consumption period, P=0.013) and peripheral arterial diastolic pressure (79.72±11.47 mmHg in control, no alcohol period vs. 76.39±11.15 mmHg for intervention, wine consumption period, P=0.034), while other arterial stiffness and hemodynamic parameters were not significantly changed although showing a trend of decline.

Conclusion: In a cohort of subjects with well controlled T2DM, in whom clinical symptoms of cardiovascular disease were not yet expressed, there was a decrease in PWV. This indicates an improvement in arterial stiffness even after moderate consumption of red wine of relatively short duration.

9. CROATIAN SUMMARY

Naslov: Učinci umjerene konzumacije crnog vina na hemodinamske parametre i parametre arterijske elastičnosti u ispitanika oboljelih od šećerne bolesti tipa 2.

Uvod: Šećerna bolest tipa 2 važan je uzrok obolijevanja i smrtnosti posebice zbog razvoja kroničnih komplikacija od kojih su najčešće kardiovaskularne bolesti. Među markerima za procjenu kliničkih i subkliničkih znakova kardiovaskularnih bolesti, smanjenje arterijske elastičnosti, arterioskleroza, i pojava ukrućivanja stijenke arterija, engl. arterial stiffness, pokazalo se pouzdanim prediktorom kardiovaskularnog rizika. Karotidno-femoralna brzina pulsnog vala smatra se zlatnim standardom procjene arterijske elastičnosti/krutosti. S druge strane, kardioprotektivni učinci umjerene konzumacije crnog vina pokazani su u epidemiološkim i eksperimentalnim istraživanjima.

Cilj: Istražiti učinke umjerene konzumacije crnog vina na hemodinamske parametre i parametre arterijske elastičnosti u ispitanika oboljelih od šećerne bolesti tipa 2, kod kojih je bolest dobro nadzirana.

Ispitanici i metode: U ukriženo intervencijsko istraživanje uključeno je 18 ispitanika s dobro nadziranom šećernom bolesti tipa 2 (uz kroničnu terapiju metforminom) tijekom pet tjedana: dva tjedna bez konzumacije ikakvih alkoholnih pića, drive-in faza pokusa (kontrolna), nakon kojega je slijedilo tri tjedna umjerene konzumacije crnog vina sorte Plavac mali od 300 ml dnevno uz obrok (intervencija). Na kraju svake faze pokusa ispitanicima su izmjereni antropometrijski parametri (indeks tjelesne mase), učinjena biokemijska analiza krvi (plazmatska koncentracija glukoze natašte i koncentracija HbA1c), a hemodinamski parametri (srčana frekvencija, periferni i središnji sistolički, dijastolički i pulsni tlak) i parametri arterijske elastičnosti (brzina pulsnog vala, periferni i središnji augmentacijski indeksi) određeni su oscilometrijskom (Arteriograph) i tonometrijskom (SphygmoCor) metodom.

Rezultati: Nakon umjerene konzumacije vina došlo je do smanjenja brzine pulsnog vala (7,42±1,44 m/s u kontrolnoj fazi pokusa bez konzumacije alkoholnih pića vs. 6,98±1,44 m/s nakon intervencije, P=0,013) i perifernog dijastoličkog tlaka (79,72±11,47 mmHg u kontrolnoj fazi pokusa bez konzumacije alkoholnih pića vs. 76,39±11,15 mmHg nakon intervencije, P=0,034), dok su promjene ostalih hemodinamskih parametara i parametara arterijske elastičnosti bili statistički neznačajne, unatoč ostvarenom trendu smanjena.

Zaključci: U kohorti ispitanika s dobro nadziranom šećernom bolesti tipa 2, kod kojih još nisu izraženi klinički simptomi kardiovaskularne bolesti, došlo je do smanjenja brzine pulsnog vala. To ukazuje na poboljšanje arterijske elastičnosti čak i nakon umjerene konzumacije crnog vina relativno kratkog trajanja.

10. CURRICULUM VITAE

Personal Information:

Name and Surname:	Anika Katarina Januš
Date of birth:	27th of March 1996, in Leonberg, Germany
Nationality:	Croatian
Address:	Jörgstraße 9, 70499 Stuttgart, Germany
E-Mail:	janus.anika@gmail.com

Education:

2014 - 2020:	University of Split School of Medicine, Split, Croatia
2006 - 2014:	Gymnasium in der Glemsaue, Ditzingen, Germany

Activities:

1/2020 - 3/2020:	Clinical Rotations as part of the Erasmus+ Program in the Departments of Internal Medicine and General Surgery, St.-Agnes Hospital Bocholt, Germany
8/2019:	Internship in the Department of Orthopedic- and Trauma Surgery, St.-Agnes Hospital Bocholt, Germany
8/2018 - 9/2018:	Internship in the Department of Gynecology and Obstetrics, Triemlispital Zürich, Switzerland