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FACULTY OF PHARMACEUTICAL SCIENCES
PHARMACY-BIOCHEMISTRY COURSE

Effect of supplementation with resveratrol on biomarkers associated with atherosclerosis in humans.

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Final Paper of the Pharmacy-Biochemistry Course of Faculty of Pharmaceutical Sciences/University of São Paulo.

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São Paulo
2020

Acknowledgments

First, I would like to praise and thank God, for sustaining me and opening doors and opportunities, even when things didn't seem possible and making me meet incredible people during this journey.

I am extremely grateful to my parents, for all their love, never sparing resources and efforts to provide me with quality education and forging my character.

I sincerely wish to express my gratitude towards Professor Inar Castro, for accepting me, inspiring and guiding, always full of grace, joy, patience and wisdom, encouraging me to look further and higher.

The completion of this work would not be possible without the help of Tamires Santana who, with immense dedication and patience, discussing, demanding results, always helped me, regardless of how dreadful the circumstances were. Thank you for being a great example of inspiration and good humor in this academic environment.

For my friends who found some way to encourage me and at the same time, respect the quarantine situation in which we find ourselves. To my beloved nephews who provided me some moments of distraction, relief and affection which comforted my heart.

Last but not least, I would like to extend my appreciation to all those who were not cited but contributed, somehow, to the development of this project and future plans.

"Talent wins games, but only teamwork and intelligence win championships."

Michael Jordan

LIST OF FIGURES

LIST OF TABLES

SUMMARY

Abbreviations

Akt; Protein Kinase B

ALT; Alanineaminotransferase

MAP; Mean arterial pressure

ARE: Antioxidant Response Element

AST; Aspartate aminotransferase

MCP; Monocyte chemoattractant protein

AMPK; Adenosine monophosphate kinase

MDA; Malondialdehyde

MMP; Matrix Metalloproteinase

APO; Apoliprotein

NAFLD; Non-alcoholic fatty liver disease

BMI; Body Mass Index

NF-κB; Nuclear factor kappa B

BUN; Blood Urea Nitrogen

NOS; Nitric oxide synthases

CAD; Coronary artery disease

Nrf2; Nuclear factor erythroid 2-related fator 2

oxLDL; oxidized LDL

COX; Cyclooxygenase

CRP; C-reactive protein

PCA; Principal Component Analysis

PGC-1α; peroxisome proliferator-activated receptor gamma coactivator 1α

PI3K; Phosphatidylinositol 3-kinase

CVD; Cardiovascular disease

PTEN; Phosphatase and tensin homolog

DBP; Diastolic blood pressure

QUICKI; Quantitative Insulin Sensitivity Check Index

FoxOs; forkhead box protein O

FRAP; Ferric reducing ability of plasma

RAGE; Receptor of Advanced Glycation End product

GGT; γ-glutamyltransferase

RelA; nuclear factor NF-kappa-B p65 subunit

ROS; Reactive oxygen species

GSH; Glutathione

SBP; Sistolic blood pressure

GST; Glutathione-S-transferase

SGOT; Serum Glutamate Oxaloacetate Transaminase

GPx; Glutathione peroxidase

SGPT; Serum Glutamate Pyruvate Transaminase

HDL; High-density lipoprotein

SIRT; Sirtuin

HMG-CoA; 3-hydroxy-3-methyl-glutaryl-CoA

SMC; Smooth muscular cell

HO; Heme oxygenase

HOMA-IR; Homeostatic Model Assessment of Insulin Resistance

SOD; Superoxide dismutase

hsCRP; High sensivity CRP

SR; Scavenger receptor

ICAM; Intercellular adhesion molecule

TC; Total cholesterol

IL; Interleukin TG; Triglycerides

Keap-1; Kelch-like ECH-associated protein 1

LDL; Low-density lipoprotein

TMD2; Type 2 diabetes melitus

LOX; Lectin-like oxLDL receptor

TNF-α; Tumor Necrosis Factor

VCAM; Vascular cell adhesion protein

Abstract

Background: Several current trials have investigated the effect of resveratrol supplementation on cardiovascular-related biomarkers. However, there is no consensus regarding its efficacy in humans, mainly due to several factors including dosage, intervention time, and studied population. Our objective was to conduct a review applying a multivariate statistical approach to identify the experimental conditions associated with the results presented in studies in which humans received resveratrol supplementation.

Material and methods: The literature search included Pubmed, Science Direct, and Google Scholar databases. It was included the studies published in the last 10 years that investigated the impact of resveratrol on atherosclerosis-related biomarkers. Biochemical data were collected from 27 studies on the baseline and after the intervention time. From 81 biomarkers evaluated in at least one study, 12 biomarkers were selected for the multivariate analysis. The net change of these 12 biomarkers was calculated. Principal component analysis (PCA) followed by cluster analysis was applied to visualize the association among the biomarkers and also for assays grouping. One-way ANOVA followed by Tukey test was applied to compare the protocol parameters and biomarker's net change among the groups.

Results: Resveratrol reduced total cholesterol, LDL cholesterol, triglycerides, and blood pressure, while increased HDL cholesterol. It was also identified that resveratrol reduced high-sensitive C-reactive protein (hsCRP) and tumor necrosis factor-α (TNF-α), but not interleukin (IL) 6 or leptin. In contrast, resveratrol improved the concentration of IL-10 and adiponectin, which are involved in anti-inflammatory responses. Applying multivariate analysis, we separated the studies into three clusters. The groups showed that the resveratrol effect could be dependent on protocol parameters, such as health conditions of the patients and intervention time.

Conclusions: In general, the supplementation with resveratrol improved the lipoproteins profile, arterial pressure and inflammatory biomarkers. Regarding to the study design, our analysis showed that a longer intervention time can be necessary to achieve HDL increase in the patients.

Key words: resveratrol, atherosclerosis, multivariate analysis, biomarkers

1. INTRODUTION

Cardiovascular disease (CVD) remains the main cause of morbidity and mortality globally (Benjamin *et al.*, 2019). It is a collective term for diseases of the heart and blood vessels, such as coronary artery disease (CAD) and stroke. Atherosclerosis is an inflammatory disease characterized by endothelial dysfunction, accumulation of lipids in the arterial intima, in particular, low-density lipoprotein (LDL), immune system cell recruitment and inflammatory response, and is a central condition underlying the majority of CVD development (Libby *et al.*, 2019).

Some biomarkers have been traditionally used in the clinical trials to assess the risk of atherosclerosis development and cardiovascular events (Kampoli *et al.*, 2009). In general, these biomarkers are associated to dyslipidemia, diabetes and hypertension (Aday and Ridker, 2019). However, some clinical trials have also reported biomarkers associated to the inflammatory response, such as interleukins (IL), tumor necrosis factor (TNF)-α, C-reactive protein (CRP), and adhesion molecules (Kampoli *et al.*, 2009; Dyck *et al.*, 2019)

Current therapeutic approaches in the treatment of atherosclerosis focus on reducing plasma cholesterol levels (Moss, Williams and Ramji, 2018). However, a significant residual risk remains, linked to the role of inflammation in all stages of the disease (Moss and Ramji, 2016; Aday and Ridker, 2019; Libby *et al.*, 2019). Factors related to lifestyle, such as an unhealthy diet, favor the development of CVDs. There is evidence that adherence to the Mediterranean diet is effective in preventing CVDs (Martínez-González, Gea and Ruiz-Canela, 2019). Recently, the study PREDIMED (PREvencion con DIetaMEDterranea) demonstrated that the protective effect of the Mediterranean diet is attributed to the variety of foods rich in monounsaturated fatty acids, vitamins, minerals and polyphenols. This study showed positive results in reducing cardiovascular events and also in inflammatory biomarkers related to atherosclerosis (Medina-Remón *et al.*, 2017; Estruch *et al.*, 2018).

Among the polyphenols present in the Mediterranean diet, resveratrol stands out in grape and red wine (Gambini *et al.*, 2015). The benefits attributed

to resveratrol are mainly its antioxidant, antiplatelet and anti-inflammatory properties (Baur and Sinclair, 2006; Berman *et al.*, 2017; Pannu and Bhatnagar, 2019). In this context this polyphenol has been popularized due to the possibility of its use as a nutritional supplement (Kulkarni and Cantó, 2015). Since then, several studies conducted *in vitro* and *in vivo* have identified the resveratrol as an important agent against diabetes, neurodegenerative diseases, cancer, aging, obesity, and cardiovascular diseases (Pannu and Bhatnagar, 2019). However, clinical trials of nutritional intervention with resveratrol have shown controversial results (Dyck *et al.*, 2019).

The effects of resveratrol for prevention of CVDs and biomarkers associated with atherosclerosis in humans is limited and inconclusive, mainly due to several factors including dosage, intervention time, and studied population (Haghighatdoost and Hariri, 2018). Thus, this study aims to apply a multivariate statistical approach to identify the experimental conditions associated with the results presented in studies in which humans received resveratrol supplementation.

2. LITERATURE REVIEW

2.1 Atherosclerosis

Atherosclerosis is an unresolved chronic inflammatory disease characterized by the formation of fatty plaques in the intima of large and medium-sized arteries, especially in regions where there is blood flow disorder such as curvatures or bifurcations (Tabas, García-Cardeña and Owens, 2015). Clinically, these become more evident in adulthood, but their development has been present since childhood (Ross, 1999).

The pathogenesis of atherosclerosis can be divided into three stages: initiation, progression and complication (Libby *et al.*, 2019). Initial activation of the endothelium increases permeability, which facilitates deposition and accumulation of lipids, mainly LDL, in the subendothelial space (Glass and Witztum, 2001; Libby *et al.*, 2019). The retained LDL is subject to oxidative and enzymatic modifications in the subendothelial space, giving rise to oxidized LDL (oxLDL) (Glass and Witztum, 2001). These modifications trigger an inflammatory response characterized by increased expression of adhesion

molecules and chemokines by activated endothelial cells, such as vascular cell adhesion protein 1 (VCAM-1), intracellular adhesion molecule-1 (ICAM-1) and monocyte chemoattractant protein (MCP-1) (Chistiakov *et al.*, 2017). The inflammatory signals lead to monocyte recruitment that can bind to the adhesion molecules (Moore and Tabas, 2011). Adherent monocytes migrate into the subendothelial space and differentiate into macrophages (Moore and Tabas, 2011). These cells sense and bind oxLDL with several scavenger receptors (SR) such as SR-A1, CD36, and lectin-like oxLDL receptor-1 (LOX-1), which results in their loss of functionality, becoming foam cells (Moore and Tabas, 2011; Chistiakov *et al.*, 2017).

Foam cells contribute to amplify the inflammation through the release of cytokines. As the stimulus does not stop, this sustains leukocyte recruitment and contributes to plaque growth and instability (Bäck *et al.*, 2019). As a consequence, the smooth muscle cells (SMCs) undergo cell proliferation and migration, increase their production of the extracellular matrix, proteoglycans, leading fibrous cap formation (Tabas, García-Cardeña and Owens, 2015). Moreover, SMCs may switch the phenotype to macrophage-like cells and take up oxidized lipoproteins that contribute to the number of foam cells in advanced lesions (Borén *et al.*, 2020).

At later stages of plaque development, as macrophage lipid metabolism dysregulates, cells become apoptotic showing defective efferocytosis, which results in secondary necrosis and in the release of cellular components and lipids that form the necrotic core (Moore, Sheedy and Fisher, 2013; Bäck *et al.*, 2019), thinning of the fibrous cap (Yurdagul *et al.*, 2018). The necrotic core is the primary feature of atherosclerotic plaque vulnerability, contributing to it rupture and lumenal thrombosis, that underlies myocardial infarction and stroke (Bäck *et al.*, 2019).

Taking into account the persistent expression of inflammatory cytokines and recruitment of immune cells, new evidence suggesting that chronic inflammation plays an important role in plaque progression (Borén *et al.*, 2020). However, lipid-lowering remains the first-line therapy for atherosclerosis, although several patients present a residual risk. The recent study “Canakinumab Antiinflammatory Thrombosis Outcomes Study (CANTOS)”

provided pivotal support for the inflammatory hypothesis of atherosclerosis (Ridker *et al.*, 2017). The results demonstrated that canakinumab (a monoclonal antibody to IL-1β) significantly reduced high-sensitivity CRP (hsCRP) and IL-6 levels and the incidence of recurrent cardiovascular events in patients with previous myocardial infarction. Unfortunately, the anti- IL-1β antibody therapy was associated with a small but significant increase of fatal infections (Ridker *et al.*, 2017).This highlight the urgency for alternative agents in the atherosclerosis prevention and treatment (Moss, Williams and Ramji, 2018). Supplementation or consumption of enriched foods with natural bioactive compounds could be a complementary strategy to ensure both prevention and treatment (Bhullar and Udenigwe, 2016). Nutraceuticals are products derived from food sources that have health benefits beyond their nutritional value. Because nutraceuticals are derived from food sources, they generally are more tolerable and safer than pharmaceuticals, becoming an important strategy in the prevention of atherosclerosis (Moss, Williams and Ramji, 2018).

2.2Resveratrol: bioavailability and metabolism

Resveratrol (3,5,4'-trihydroxystilbene) is a phytoalexin predominantly found in grapes, wine, berries, and related food products (Wenzel and Somoza, 2005; Pannu and Bhatnagar, 2019). This compound was first isolated in 1939 from the roots of the white hellebore (*Veratrum grandiflorum*), which are widely used in Chinese medicine (Weiskirchen and Weiskirchen, 2016).Their synthesis occurs in plants by a mechanism of resistance to stressful factors, such microorganisms infections, UV radiation and chemicals (Gambini *et al.*, 2015). Resveratrol contains two aromatic rings which are attached by a methylene bridge and exists in two isomeric configurations, *cis* and *trans,* being the *trans*-isomer the most stable form, as shown in Figure 1 (Gambini *et al.*, 2015).

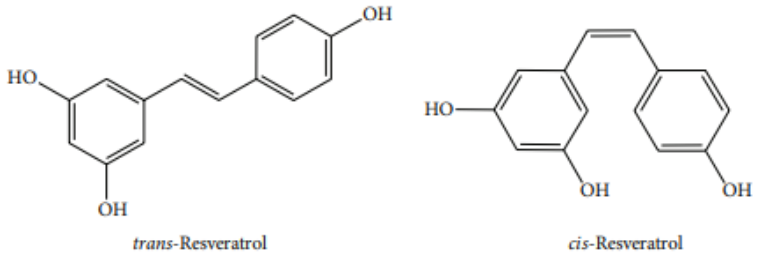

Figure 1: Chemical structures of trans-resveratrol and cis-resveratrol.

Resveratrol is extensively metabolized and rapidly eliminated and therefore it shows a poor bioavailability (unchanged drug concentration on blood) (Walle *et al.*, 2004). After ingestion, ~77–80% is absorbed in the intestine by passive diffusion or by complexes with membrane transporters, such as integrins (Walle *et al.*, 2004; Delmas *et al.*, 2011). After absorption, like other xenobiotics, the *trans*-resveratrol undergoes phase II metabolism in the liver, producing polar metabolites of easy excretion by urine (Springer and Moco, 2019). Extensive metabolization generates glucuronide and sulfate forms of resveratrol, which might retain some biological activity (Delmas *et al.*, 2011; Walle, 2011). A small fraction of resveratrol escapes from conjugation and exits from the enterocyte via basolateral membrane (Springer and Moco, 2019). Thus, in the bloodstream, resveratrol can be found as glucuronide, sulfate or free (Gambini *et al.*, 2015). The presence of resveratrol and their metabolites in the bloodstream can be attributed to the enterohepatic circulation (Wenzel and Somoza, 2005; Delmas *et al.*, 2011), where it can be reabsorbed in the intestine after hydrolysis, going through portal circulation into the liver again for further metabolism, and via systemic circulation reaching peripheral tissues (Springer and Moco, 2019).

In the bloodstream, owing to its low water solubility, resveratrol circulates attached with albumin or lipoproteins, such as LDL (Delmas *et al.*, 2011; Pannu and Bhatnagar, 2019). This binding was already detected in human LDL isolated from blood samples of healthy volunteers bounded in a non-covalent manner (Urpi-Sarda *et al.*, 2007). It may be hypothesized that when these complexes reach specific membrane receptors in the cells, the resveratrol dissociates and enter into the cell under their free form (Delmas *et al.*, 2011; Gambini *et al.*, 2015). Furthermore, these complexes could be considered reservoirs of resveratrol, contributing to their distribution and also scavenging reactive oxygen species (ROS), which are involved in LDL oxidation (Delmas *et al.*, 2011).

2.3 Potential influence of the resveratrol on atherosclerosis biomarkers

Resveratrol gained great attention in the early 1990s for its relationship with the "French Paradox" reported by Renaud & de Lorgeril (1992). A term to

describe the observation that moderate consumption of alcoholic beverages, especially wine having a high content of -resveratrol, could be linked to the protection from cardiovascular disease in the French population despite their high intake of saturated fat (Catalgol *et al.*, 2012). Since then, an extensive research has been carried out to explain the cardioprotective effect (Baur and Sinclair, 2006). Several studies described that the health benefits attributed to resveratrol are mainly its antioxidant, antiplatelet, and anti-inflammatory properties, being these properties largely attributed to their chemical structure (Zhang and Tsao, 2016; Haghighatdoost and Hariri, 2019).

As discussed above, LDL oxidation is a crucial step in atherosclerosis development, that stimulates the recruitment of immune cells and inflammatory response (Zordoky, Robertson and Dyck, 2014). Interestingly, resveratrol may be involved in several pathways directly or indirectly in these processes (Bonnefont-Rousselot, 2016). *In vitro* studies revealed that resveratrol protects LDL against peroxidative degradation by both chelating and free radical scavenging mechanisms (Frankel, Waterhouse and Kinsella, 1993; Belguendouz, Fremont and Linard, 1997). In a clinical trial conducted by Tomé-Carneiro et al. (2012a), a 8 mg resveratrol-rich grape supplement containing grape polyphenols reduced LDL-c, oxidized LDL, and apolipoprotein (Apo) B in statin-treated patients (Tomé-Carneiro *et al.*, 2012a). In addition, some articles indicated lipid-lowering effect of resveratrol (Chen *et al.*, 2015; Simental-Mendía and Guerrero-Romero, 2019). A more recent meta-analysis (Haghighatdoost and Hariri, 2018) suggested that resveratrol has no effect on plasma LDL-cor TG levels. Moreover, *trans*-resveratrol might be able to change total cholesterol and increase HDL-C in cross-over trials (Haghighatdoost and Hariri, 2018). Multiple potential mechanisms, including regulation expression of hepatic 3-hydroxy-3-methyl-glutaryl-CoA (HMG-CoA) reductase, activation of sirtuin 1 (SIRT1), as well modulation of the adenosine monophosphate kinase (AMPK) signaling pathway, could be related of amelioration fatty acid and lipid metabolism (Zordoky, Robertson and Dyck, 2014; Hunter and Hegele, 2017).

Due to the oxidative and inflammatory characteristics of atherosclerosis, the increase the defense mechanism must be a target for prevention or treatment (Ungvari *et al.*, 2007). Several studies demonstrated that resveratrol

has effective scavenger properties by reducing a variety of oxidants, including hydroxyl radical ($HO^{\bullet}$), superoxide anion radical ($O_2^{\bullet -}$), hydrogen peroxide (H_2O_2) and peroxynitrite (Leonard *et al.*, 2003; Xia *et al.*, 2017).

A recent study (Meng, 2020) organized resveratrol antioxidant molecular mechanisms: resveratrol unanchors Nrf2 in cytoplasm, disrupting Keap-1-dependent ubiquitination and degradation. The built-up Nrf2 translocates into the nucleus, binds to ARE, and initiates the transcription of many antioxidative genes such as SOD and catalase and also HO-1 via FoxOs. Resveratrol prevents Akt activation by upregulating PTEN, a major antagonist of PI3K. The existing activated Akt will reduce, leading to decreased FoxOs phosphorylation, preventing the translocation to cytoplasm, remaining more FoxOs in the nucleus to act as transcriptional factors. Resveratrol activates AMPK that maintain the stability of cytoplasm FoxOs, facilitating the translocation to nucleus, and activated AMPK phosphorylates PGC-1α which translocates to the nucleus where it is deacetylated by SIRT1 and promote Nrf2 gene expression and reduced oxidative stress (Malaguarnera, 2019, Pannu and Bhatnagar, 2019).

Activated AMPK activates SIRT1, which inhibit MAPK signaling pathways and results in autophagy. Activated SIRT1 will inhibit RelA acetylation (SIRT1 substrate), decreasing NF-kB-induced expression of inflammatory factors such as TNF- α, IL-1β, IL-6, metalloproteases (MMP)-1, 3 and Cox-2 (Malaguarnera, 2019). Nrf2 interaction leads to decreased expression of ICAM-1, consequently, inhibiting monocyte adhesion (Meng *et al*., 2020; Seo et al., 2019).

Resveratrol reduce oxidative stress by increasing the activity and expression of several anti-oxidant enzymes including SOD, catalase, glutathione reductase, GPx, GST and heme oxygenase-1 (Ungvari *et al.*, 2007; Ramprasath and Jones, 2010; Bonnefont-Rousselot, 2016), where SOD catalyzes the dismutation of superoxide ion to form H_2O_2, which is converted into water and molecular oxygen by catalase and GPx, oxidizing glutathione that is recovered by glutathione reductase. Resveratrol AMPK-α activation restore intracellular GSH, inhibiting monocyte differentiation and reducing pro-inflammatory cytokine production (Meng *et al*., 2020).

Clinical trials have found a decrease in plasma levels of inflammatory cytokines following resveratrol supplementation, including IL-6, TNF-α, hsCRP

or CRP (Timmers *et al.*, 2011). These suppressive effects on the inflammatory mechanism suggest that resveratrol may delay the onset of atherosclerosis (Seo *et al.*, 2019). However, given that these studies are performed using highly variable protocols, there is still not a consensus about the cardio protective action from resveratrol supplementation.

3. OBJECTIVE

This study aimed to apply a multivariate statistical approach to identify the experimental conditions associated with different results presented in studies in which humans received resveratrol supplementation.

4. MATERIAL AND METHODS

4.1 Criteria for studies selection and data insertion

It was applied either one of the following keywords or multiple of them at the same time: "resveratrol", "*cardiovascular diseases*", "*obesity*", "*dyslipidemia*", "*supplementation"*, "*clinical trial", "biomarker", "atherosclerosis"* e "*risk factors",* on Pubmed, Science Direct and Google Scholar databases. To be included in our analysis, the studies published in the last 10 years had to meet the following criteria: (1) original articles with randomized double/triple blind controlled trial design; (2) studies conducted on humans; (3) use of resveratrol for intervention; (4) be related to cardiovascular disease. From this analysis, only essays published in English and that met the inclusion criteria were selected. Eligible studies were reviewed and the following data were inserted in the datasheets: first author, year of publication, study design, number of participants, age, sex, resveratrol dose and intervention duration. Also, the biochemical data were collected on the baseline and after the intervention time. The changes of biomarkers from each study were calculated as the % difference between the values observed after and before (baseline) the intervention, discounted the placebo effect. It was considered in each study, the treated group and its respective placebo group. In the studies where more than one intervention was evaluated, the same placebo was applied to the other interventions. About 81 biomarkers evaluated in at least one study were included, considering the initial and final values. However, the multivariate

analysis and discussion were carried out with only 12 biomarkers that were physiologically more interesting among the most present biomarkers.

4.2. Statistical Analysis

Data were initially summarized and presented as mean ± SD, median, mode and range (minimum and maximum values). After, from 81 biomarkers, 12 were selected to the multivariate analysis. The net change of these 12 biomarkers was applied as active variables and the 32 assays obtained from 27 studies were taken as cases (rows). First of all, the Principal Component Analysis was used to plot the 32 assays according to the plane generated by the two first principal factors. In this analysis, mean substitution was applied when data was not present, and the analysis was based on correlation. After, Cluster Analysis was carried out on standardized data applying Ward's method and Euclidean distance. Joining (Tree Clustering) analysis was used to visualize the association among the biomarkers and also for assays grouping. One-way ANOVA followed by Tukey test was applied to compare the protocol parameters and biomarkers net change among the groups. Calculations and graphs were performed using the Statistica v. 13.4 (TIBCO Software Inc, Round Rode, Texas, USA).

5. RESULTS

From the studies published in the last 10 years found in the databases using the keywords, 27 that followed the inclusion criteria were selected to our analysis: Timmers *et al.*, 2011; Fujitaka *et al.*, 2011; Tomé-Carneiro *et al.*, 2012b; Magyar *et al.*, 2012; Yoshino *et al.*, 2012; Bhatt, Thomas and Nanjan, 2012; Crandall *et al.*, 2012; Gliemann *et al.*, 2013; Militaru *et al.*, 2013; Poulsen *et al.*, 2013a; Movahed *et al.*, 2013; Tomé-Carneiro *et al.*, 2013; Méndez-Del Villar *et al.*, 2014; Chachay *et al.*, 2014; Made, Plat and Mensink, 2015; Chen *et al.*, 2015; Faghihzadeh, Adibi and Hekmatdoost, 2015; Agarwala *et al.*, 2016; Zortea *et al.*, 2016; Imamura *et al.*, 2017; Kjær *et al.*, 2017; Mansur *et al.*, 2017; Khodabandehloo *et al.*, 2018; Seyyedebrahimi *et al.*, 2018; Fodor *et al.*, 2018; Abdollahi *et al.*, 2019; Hoseini *et al.*, 2019; Simental-Mendía and Guerrero-

Romero, 2019; Walker *et al.*, 2019; Batista-Jorge *et al.*, 2020. The major reasons to exclude the studies were: absence of control group, open label or uniqueness of design (only one article with that type of study design). Some articles were decomposed in assays, excluding the part of the trial that tested a compound that was not resveratrol.

In general, studies showed variation in sample size from 9 to 92 individuals with an average of 26 individuals, despite the wide variation, the median and mode were 25 individuals. The mean ages of participants ranged from 32 to 67 years. Their treatment duration ranged between 28 and 360 days, and the resveratrol dose ranged from 8 to 3000 mg/day, but most of trials used a dose of 500mg/day. The detailed characteristics of the studies are presented in **Table1**.

Table 1: General characteristics presented in the selected studies protocols.

	Mean ± SD	**Median**	**Mode**	**Range**
Individuals (n)	26 ± 17	25	25	9 - 92
Female/Male (%)	43/57	-	-	-
Age (y)	55 ± 9	58	60	32 - 67
Time (days)	105 ± 104	60	56	28 - 360
Dose (mg)	497.59 ± 658.36	225.00	500.00	8.00 – 3,000.00

General characteristics and routine drugs used by the patients according to the two groups (Treated or Control) were shown in **Table 2**. A similar proportion or value was observed to the number of individuals, gender and age was observed. The studies included participants with a large range of different health conditions and under pharmacological treatment.

Table 2: General characteristics and routine drugs used by the patients according to the two groups. Values expressed as mean ± SD (n).

	Treated	**Control**	**Groups (n)**
Individuals (n)	25 ±14 (32)	26 ± 19 (32)	64
Female/Male (%)	38/62 (30)	38/62 (30)	60
Age (y)	55 ± 8 (32)	55 ± 10 (32)	62
Diabetes	16 (9)	17 (9)	18
Dyslipidemia	44 (2)	61 (2)	4
Obesity	26 (2)	39 (2)	4

Hypertension	29 (9)	22 (9)	18
Stable Angina	2 (2)	3 (2)	4
Smokers	6 (9)	7 (9)	18
Aspirin	12 (4)	11(4)	8
Clopidogrel	1 (4)	2 (4)	8
Statins	19 (10)	19 (10)	20
β-Blockers	19 (7)	18 (7)	14
Calcium Channel Blockers	6 (4)	7 (4)	8
ECA/ARB Inhibitors	21 (7)	20 (7)	14

Table 3 presents the values of all 81 biomarkers determined in the patients at the beginning (T_0) and at the end of the trial (T_F) according to the group (Treated or Control).

Table 3: Initial (baseline T0) and final values (TF) of biomarkers according to the group. Values are expressed as mean ± SD.

Biomarker		**Treated**	**Control**	**Groups (n)**
Bodyweight (Kg)	T_0	87.17 ± 14.52	87.07 ± 16.59	40
Bodyweight	T_F	85.47 ± 13.79	84.34 ± 15.53	32
BMI (Kg/m^2)	T_0	29.66 ± 3.29	29.62 ± 3.39	56
Body Mass Index	T_F	28.82 ± 3.31	29.03 ± 2.82	44
Waist circunference (cm)	T_0	102.92 ± 9.72	102.44 ± 9.25	26
Waist circumference	T_F	99.55 ± 7.93	98.98 ± 7.60	22
Hips circunference (cm)	T_0	103.00 ± 5.17	102.22 ± 3.08	10
Hips circumference	T_F	103.35 ± 4.61	101.46 ±2.41	8
Waist:Hip ratio	T_0	ND	ND	0
Waist:Hip ratio	T_F	0.88 ± 0.16	0.88 ± 0.16	12
Energy intake (kcal/day)	T_0	1978.29 ± 333.11	2014.72 ± 302.23	6
Energy intake	T_F	1896.34 ± 305.37	1884.22 ± 234.04	6
Lean mass (kg)	T_0	71.63 ± 2,59	75.62 ± 1.92	6
Lean mass	T_F	75.20 ± 1.12	74.33 ± 0.00	4
Fat mass (kg)	T_0	35.58 ± 4.07	35.99 ± 0.74	8
Fat mass	T_F	42.60 ± 0.00	40.80 ± 0	2
Visceral fat volume (cm^3)	T_0	2934.50 ± 1621.49	2558.75 ± 1413.78	8
Visceral fat volume	T_F	1077 ± 0	811.00 ± 0	2
Subcutaneous abdominal fat volume (cm^3)	T_0	5576.50 ± 2437.91	6447.25 ± 3534.49	8
Subcutaneous abdominal fat	T_F	2287.00 ± 0	2065.00 ± 0	2

volume				
SBP (mmHg)	T_0	129.79 ± 9.53	130.44 ± 10.68	46
Sistolic Blood Pressure	T_F	126.55 ± 9.53	128.87 ± 8.67	40
DBP (mmHg)	T_0	79.51 ± 6.17	79.56 ± 6.73	46
Diastolic Blood Pressure	T_F	80.71 ± 12.80	81.36 ± 12.54	40
MAP (mmHg)	T_0	96.30 ± 0	95.30 ± 0	2
Mean Arterial Pressure	T_F	96.87 ± 4.49	95.90 ± 4.45	6
Blood glucose (mg/dL)	T_0	119.68 ± 30.90	115.97 ± 28.00	58
Blood glucose	T_F	114.46 ± 26.77	114.82 ± 28.31	58
Insulin (µIU/mL)	T_0	20.15 ± 22.90	19.96 ± 21.32	34
Insulin	T_F	11.78 ± 3.68	11.98 ± 3.52	34
HbA1c (%)	T_0	8.53 ± 2.40	6.56 ± 0.93	6
GlycatedHemoglobin	T_F	8.29 ± 2.96	6.77 ± 0.49	6
HOMA-IR	T_0	3.80 ± 1.51	3.52 ± 1.25	28
Homeostatic Model Assessment of Insulin Resistance	T_F	3.40 ± 1.11	3.40 ± 0.94	30
C-Peptide (ng/mL)	T_0	4.27 ± 0	4.35 ± 0	2
C-Peptide	T_F	4.14 ± 0	4.24 ± 0	2
QUICKI	T_0	0.31 ± 0.01	0.31 ± 0.01	6
Quantitative Insulin Sensitivity Check Index	T_F	0.32 ± 0.01	0.32 ± 0.01	6
TC (mg/dL)	T_0	197.16 ± 28.76	191.95 ± 32.33	58
Total Cholesterol	T_F	186.01 ± 24.09	185.16 ± 26.29	52
TG (mg/mL)	T_0	160.54 ± 43.85	151.52 ± 32.59	60
Triglycerides	T_F	153.06 ± 27.89	151.02 ± 36.19	57
LDL (mg/mL)	T_0	118.30 ± 18.76	113.69 ± 21.55	57
Low-DensityLipoproteinCholesterol	T_F	112.55 ± 20.26	109.30 ± 20.49	52
HDL (mg/mL)	T_0	45.81 ± 6.10	45.67 ± 6.92	60
High-DensityLipoproteinCholesterol	T_F	46.35 ± 6.54	46.02 ± 6.53	54
Non-HDL C (mg/dL)	T_0	146.65 ± 18.24	153.04 ± 13.01	10
Non-HDL Cholesterol	T_F	138.91 ± 21.73	145.33 ± 17.92	10
VLDL (mg/dL)	T_0	49.25 ± 0	24.10 ± 0	2
VeryLowDensityLipoprotein	T_F	39.10 ± 0	27.40 ± 0	2
ApoA (g/L)	T_0	28.42 ± 46.90	29.11 ± 47.82	6
Apolipoprotein A	T_F	13.33 ± 23.92	12.53 ± 22.23	8
ApoB (g/L)	T_0	1.11 ± 0.18	1.11 ± 0.15	4
Apolipoprotein B	T_F	1.14 ± 0.09	1.08 ± 0.12	6

Free fatty acids (mmol/L)	T_0	13.82 ± 18.59	11.40 ± 15.25	4
Freefattyacids	T_F	8.67 ± 11.43	11.84 ± 15.92	4
PAI-1 (ng/mL)	T_0	16.95 ± 0.29	18.10 ± 0.69	8
PlasminogenActivatorInhibitortype 1	T_F	14.48 ± 1.29	21.53 ± 3.81	8
Fibrinogen (g/L)	T_0	3.52 ± 0.18	3.31 ± 0.28	12
Fibrinogen	T_F	3.63 ± 0.05	3.81 ± 0.66	12
D-Dimer(mg/mL)	T_0	0.13 ± 0	0.12 ± 0.01	8
D-Dimer	T_F	0.11 ± 0.01	0.13 ± 0.01	8
hsCRP (mg/L)	T_0	3.87 ± 1.97	3.96 ± 1.94	22
High-sensitivity CRP	T_F	3.89 ± 0.91	4.17 ± 1.26	24
CRP (mg/mL)	T_0	2.10 ± 1.54	1.68 ± 1.58	8
C-reactiveprotein	T_F	1.70 ± 1.74	1.36 ± 1.63	10
TNF-α (pg/mL)	T_0	10.16 ± 5.36	9.52 ± 4.73	20
Tumor Necrosis Factor α	T_F	9.86 ± 4.82	9.87 ± 5.03	24
IL-6 (pg/mL)	T_0	2.88 ± 2.15	2.82 ± 2.38	22
Interleukin-6	T_F	2.54 ± 1.57	2.57 ± 1.47	26
IL-8 (pg/mL)	T_0	1.47 ± 0	1.84 ± 0	2
Interleukin-8	T_F	2.10 ± 1.67	3.46 ± 0.69	4
IL-10 (pg/mL)	T_0	13.88 ± 8.67	12.72 ± 6.60	10
Interleukin-10	T_F	15.00 ± 7.88	12.24 ± 5.79	10
IL-18 (pg/mL)	T_0	184.00 ± 0	216.00 ± 0	4
Interleukin-18	T_F	177.50 ± 13.44	247.00 ± 4.24	4
IL-1β(pg/mL)	T_0	2.02 ± 3.14	2.33 ± 3.54	6
Interleukin-1β	T_F	1.77 ± 2.66	2.06 ± 2.83	8
IFN-γ(pg/mL)	T_0	1.23 ± 0	0.73 ± 0	2
Interferon-γ	T_F	0.99 ± 0	0.73 ± 0	2
VCAM-1 (ng/mL)	T_0	653.78 ± 489.56	661.63 ± 509.34	8
Vascular celladhesionprotein 1	T_F	617.55 ± 435.79	646.44 ± 468.98	10
ICAM (ng/mL)	T_0	228.44 ± 196.69	243.22 ± 209.19	6
Intercellular adhesion molecules	T_F	244.68 ± 168.73	265.69 ±177.26	8
MCP-1 (pg/mL)	T_0	107.04 ± 74.19	94.51 ± 64.47	4
Monocyte Chemoattractant Protein-1	T_F	112.75 ± 92.85	88.99 ± 63.94	4
sCD-40 (ng/mL)	T_0	6.10 ± 0	7.50 ± 0	4
Soluble CD-40 ligand	T_F	6.35 ± 0.21	7.80 ± 0.42	4
Leptin (ng/mL)	T_0	20.01 ± 8.21	20.28 ± 8.96	12
Leptin	T_F	18.73 ± 5.57	17.95 ± 3.60	14
Adiponectin (μg/mL)	T_0	9.74 ± 4.37	9.23 ± 4.00	20

Adiponectin	T_F	10.22 ± 4.75	9.24 ± 4.02	20
Estradiol (pg/mL)	T_0	37.47 ± 0	34.61 ± 0	2
Estradiol	T_F	36.86± 0	35.59 ± 0	2
Estrone (pg/mL)	T_0	14.97 ± 0	4.06 ± 0	2
Estrone	T_F	14.07 ± 0	5.87 ± 0	2
Total antioxidant capacity (mM)	T_0	320.97 ± 554.20	308.79 ± 532.96	6
Total antioxidant capacity	T_F	480.58 ± 679.28	459.86 ± 649.75	4
Sirtuin (ng/mL)	T_0	0.93 ± 0.18	1.25 ± 0.57	4
Sirtuin	T_F	3.36 ± 3.39	3.36 ± 3.45	4
MDA (µmol/L)	T_0	3.66 ± 0	3.63 ± 0	2
Malondialdehyde	T_F	3.36 ± 0	3.55 ± 0	2
Total tiol(µmol/L)	T_0	321.30 ± 0	307.55 ± 0	2
Total tiol	T_F	368.00± 0	290.00 ± 0	2
Carbonyl protein (nmol/mg protein)	T_0	5.50 ± 0	4.75 ± 0	2
Carbonyl protein	T_F	4.48 ± 0	5.36 ± 0	2
FRAP (µmol/L)	T_0	928.50 ± 330.22	584.00 ± 739.63	4
Ferric reducing ability of plasma	T_F	975.00 ± 291.33	870.00 ± 222.03	4
SOD	T_0	4.95 ± 3.19	4.54 ± 2.49	4
Superoxide dismutase	T_F	2.83 ± 0	3.08 ± 0	2
Catalase	T_0	3.66 ± 3.66	3.55 ± 3.76	4
Catalase	T_F	4.21 ± 4.48	3.87 ± 4.23	4
HO-1	T_0	10.38 ± 0	9.85 ± 0	2
Heme oxygenase 1	T_F	10.22 ± 0	9.69 ± 0	2
NOS	T_0	11.74 ± 0	11.94 ± 0	2
Nitric oxide synthases	T_F	12.09 ± 0	12.04 ± 0	2
Nrf2	T_0	6.32 ± 0	6.11 ± 0	2
Nuclear factor erythroid 2-related factor 2	T_F	5.62 ± 0	6.10 ± 0	2
RAGE	T_0	9.73 ± 0	9.97 ± 0	2
Receptor of Advanced Glycation End product	T_F	10.80 ± 0	11.21 ± 0	2
ALT (U/L)	T_0	39.86 ± 10.07	35.34 ± 8.39	22
Alanine aminotransferase	T_F	40.06 ± 11.49	37.56 ± 9.70	18
AST (U/L)	T_0	31.52 ± 3.34	28.30 ± 3.56	16
Aspartate aminotransferase	T_F	31.22 ± 8.76	29.22 ± 6.26	12
GGT (U/L)	T_0	33.75 ± 3.77	36.02 ± 7.35	14
γ-glutamyltransferase	T_F	33.74 ± 4.51	36.50 ± 5.38	10
SGOT (U/L)	T_0	19.52 ± 0	23.00 ± 0	2

Serum Glutamate Oxaloacetate Transaminase	T_F	20.21 ± 0	19.72 ± 0	2
SGPT (U/L)	T_0	24.26 ± 0	28.22 ± 0	2
Serum Glutamate Pyruvate Transaminase	T_F	26.13 ± 0	23.83 ± 0	2
Lactate dehydrogenase (U/L)	T_0	348.5 ± 14.43	319.00 ± 16.17	8
Lactate dehydrogenase	T_F	345.50 ± 3.54	319.50 ± 14.85	4
Alkaline phosphatase (U/L)	T_0	191.06 ± 12.91	186.27 ± 11.47	10
Alkaline phosphatase	T_F	181.21 ± 17.52	181.80 ± 17.30	6
Direct bilirubin (mg/dL)	T_0	0 ± 0	0 ± 0	0
Direct bilirubin	T_F	0.24 ± 0	0.23 ± 0	2
Total bilirubin (mg/dL)	T_0	0.24 ± 0	0.21 ± 0	2
Total bilirubin	T_F	0.64 ± 0.17	0.61 ± 0.10	6
Creatinine (mg/dl)	T_0	0.89 ± 0.12	0.90 ± 0.08	18
Creatinine	T_F	0.89 ± 0.09	1.01 ± 0.27	14
Albumin (g/L)	T_0	36.40 ± 17.66	36.57 ± 17.96	10
Albumin	T_F	31.37 ± 23.70	32.00 ± 21.04	6
Uricacid (mg/dL)	T_0	6.28 ± 1.67	6.41 ± 1.92	14
Uricacid	T_F	5.58 ± 0.30	5.59 ± 0.74	10
T4 (ng/dL)	T_0	1.15 ± 0.06	1.20 ± 0.12	8
Thyroxine	T_F	1.20 ± 0	1.15 ± 0.07	4
TSH (mU/dL)	T_0	1.95 ± 0.29	2.25 ± 0.17	8
TyroidStimulatingHormone	T_F	2.05 ± 0.49	1.95 ± 0.07	4
RBC ($x10^{12}$/L)	T_0	4.85 ± 0	4.89 ± 0	2
Red Blood Cell	T_F	4.73 ± 0	4.82 ± 0	2
HGB (g/L)	T_0	148.20 ± 0	146.60 ± 0	2
Haemoglobin	T_F	147.30 ± 0	144.40 ± 0	2
WBC ($x10^9$/L)	T_0	6.36 ± 0.41	6.16 ± 0.12	8
White Blood Cell	T_F	5.86 ± 0.86	6.13 ± 0.29	6
Platelets ($x10^9$/L)	T_0	183.41 ± 20.38	199.71 ± 6.07	4
Platelets	T_F	169.00 ± 0	200.50 ± 0	2
BUN (mmol/L)	T_0	5.05 ± 0	5.25 ± 0	2
Blood Urea Nitrogen	T_F	5.15 ± 0	5.40 ± 0	2
FGF21 (pg/mL)	T_0	224.70 ± 0	226.90 ± 0	2
Fibroblast Growth Factor 21	T_F	201.40 ± 0	223.95 ± 0	2
CK-18 (IU/L)	T_0	265.30 ± 0	284.80 ± 0	2
Cytokeratin-18	T_F	238.40 ± 0	283.85 ± 0	2

ND, not determined

From these 81 biomarkers, 12 were selected to the multivariate analysis based on the clinical relevance and the higher variation. The % difference observed on these 12 selected biomarkers after the treatment and also discounting the alteration observed in the control group (Net change) is shown in **Table 4**, and summarized in **Figure 2**.

Table 4: Change (%) observed after treatment and Net Change (%) of biomarkers selected to the multivariate analysis. Values are expressed as mean ± SD.

Biomarker	Change after treatment[1]	Net change[2]	N (studies)
SBP (%)	-3.34 ± 4.62	-2.76 ± 4.43	17
DBP (%)	2.00 ± 16.10	-1.84 ± 4.21	17
TC (%)	-4.61 ± 6.00	-2.69 ± 5.99	23
TG (%)	-2.85 ± 14.96	-1.33 ± 18.90	24
LDL (%)	-5.15 ± 8.68	-2.07 ±10.25	22
HDL (%)	1.70 ± 7.15	0.92 ± 7.07	24
hsCRP (%)	12.34 ± 56.80	-4.23 ± 24.35	11
TNF-α (%)	1.76 ± 23.16	-2.58 ± 13.34	10
IL-6 (%)	1.05 ± 27.67	1.63 ± 32.05	11
IL-10 (%)	15.24 ± 22.31	14.82 ± 12.32	5
Leptin (%)	1.94 ± 15.59	2.46 ± 18.71	6
Adiponectin (%)	-16.61 ± 47.93	6.64 ± 9.85	9

[1] $((TF\text{-}T_0)/T_0)*100$ for Treated group
[2] $[((TF\text{-}T_0)/T_0)*100$ for Treated group] – $[((TF\text{-}T_0)/T_0)*100$ for Control group]

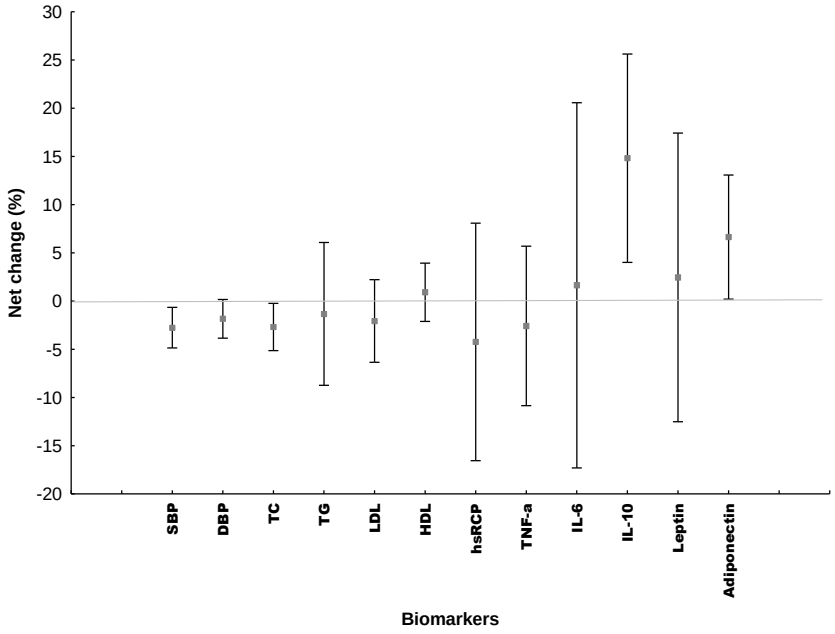

Figure 2: Net changes (%) observed in the assays expressed as mean ± 1.96 SE.

Table 5 presents the contribution of the net change of these 12 biomarkers to the Components Analysis. Based on eigenvalues, only 42.84% of the variation was explained by the two Principal Components.

Table 5: Factor coordinates of the variables (Biomakers), based on correlations until factor 6.

Biomarker	Factor 1	Factor 2	Factor 3	Factor 4	Factor 5	Factor 6
SBP	**0,51**	-0,30	-0,15	-0,42	0,11	-0,46
DBP	**0,57**	-0,30	-0,12	-0,39	-0,44	-0,27
TC	**0,79**	0,23	-0,31	0,17	-0,32	0,07
TG	**0,55**	0,05	-0,35	0,49	0,20	-0,29
LDL	**0,64**	0,26	0,03	0,41	-0,30	0,34
HDL	-0,03	**-0,80**	0,04	-0,01	-0,26	0,32
hsCRP	**-0,63**	0,16	-0,34	-0,10	-0,54	-0,11
TNF-α	-0,02	**0,87**	-0,21	-0,23	0,01	0,11
IL-6	**-0,69**	-0,03	-0,51	0,09	-0,22	-0,03
IL-10	0,36	-0,20	-0,20	-0,47	0,14	**0,59**
Leptin	0,14	**0,65**	0,41	-0,44	-0,13	-0,04
Adiponectin	0,02	-0,08	**0,79**	0,21	-0,33	-0,12

It was observed the positive contribution of the pressure and non-HDL lipoproteins and negative contribution of the hsCRP and IL-6 to the Factor 1, while HDL contribute negatively and TNF-α and leptin positively to the Factor 2. Adiponectin contributed to Factor 3 and IL-10 to Factor 6. **Figure 3** shows the distribution of the assays according to the plane generated by the two PCs.

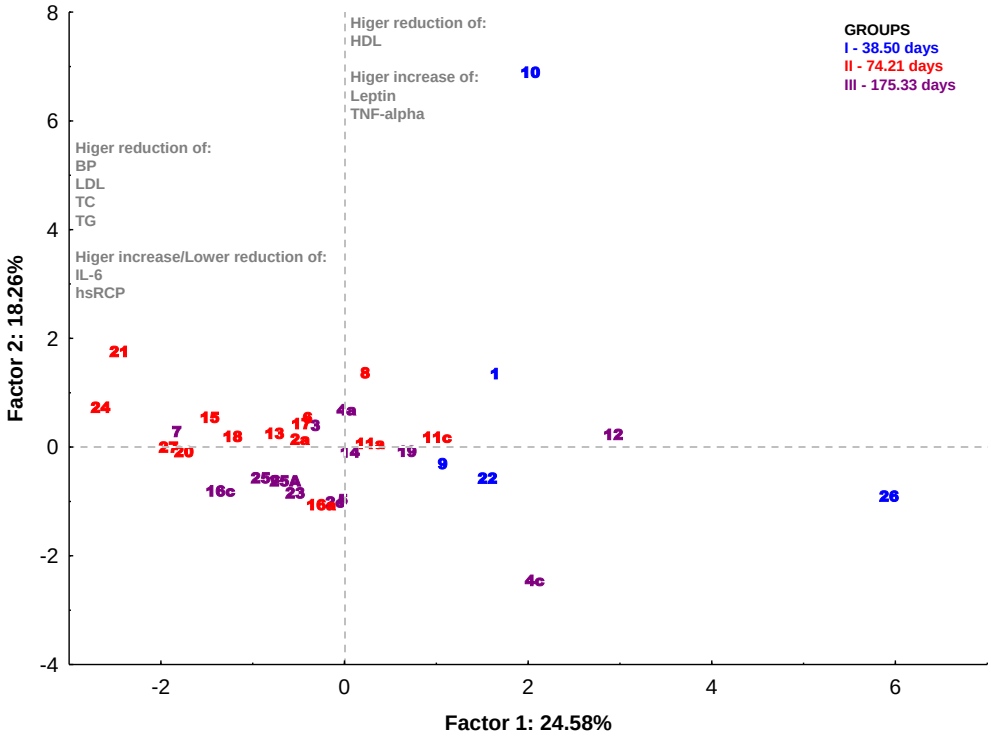

Figure 3: Projection of the assays on the factor-plane (1 x 2).

Except for the assays 21, 24, 10, 26, and 4c, all others showed high similarity. In the studies 21 (Seyyedebrahimi *et al.*, 2018) and 24 (Khodabandehloo *et al.*, 2018) type 2 diabetes mellitus (T2DM) patients were supplemented with 800 mg/d resveratrol for 8 weeks. Both studies were characterized by a significant reduction in systolic and diastolic blood pressure compared to the placebo group. There were no significant differences in TG levels, total cholesterol, LDL-c, HDL-c between the resveratrol and placebo groups. However, when compared with their baseline, significant decrease in LDL-c level was observed in the resveratrol group. In study 26 (Chachay *et al.*, 2014) was conducted with overweight or obese men diagnosed with non-alcoholic fatty liver disease (NAFLD), who were supplemented with a high dose of resveratrol (3,000 mg/day) for 8 weeks. A significant increase in LDL after resveratrol treatment was observed, a small increase in TG, and no changes regarding blood pressure and TC and HDL-c were observed. In addition, a decrease in IL-6 and hsCRP were observed in the treatment group, while other biomarkers didn't show any changes.

The study 10 (Poulsen et al., 2013a) was conducted only with obese men supplemented with 1,500 mg/day of trans-resveratrol for 4 weeks. There was a decrease in HDL, and an increase of leptin in the resveratrol treatment group. Concerning to the study 4c (Tomé-Carneiro *et al.*, 2012b), patients in primary prevention for CVD were supplemented for 12 months with a resveratrol-rich grape supplement containing 8 mg of resveratrol in the first 6 months and after this period subjects receive the double dose (16 mg of resveratrol) for more 6 months. After treatment, it was observed that the grape supplement rich in resveratrol decreased hsCRP and TNF-α levels while IL-6 values remained unchanged.

Cluster Analysis was applied to group the biomarkers and assays according to their similarities. **Figure 4** and **Figure 5** present the dendrogram obtained from padronized values of biomarkers and assays, respectively. According to the dendrogram obtained from cluster analysis, the studies could be divided into three clusters. **Table 6** shows the characteristics of each group

of assays according to the selected biomarkers and protocol parameters that were summarized in **Figure 6** and **Figure 7**.

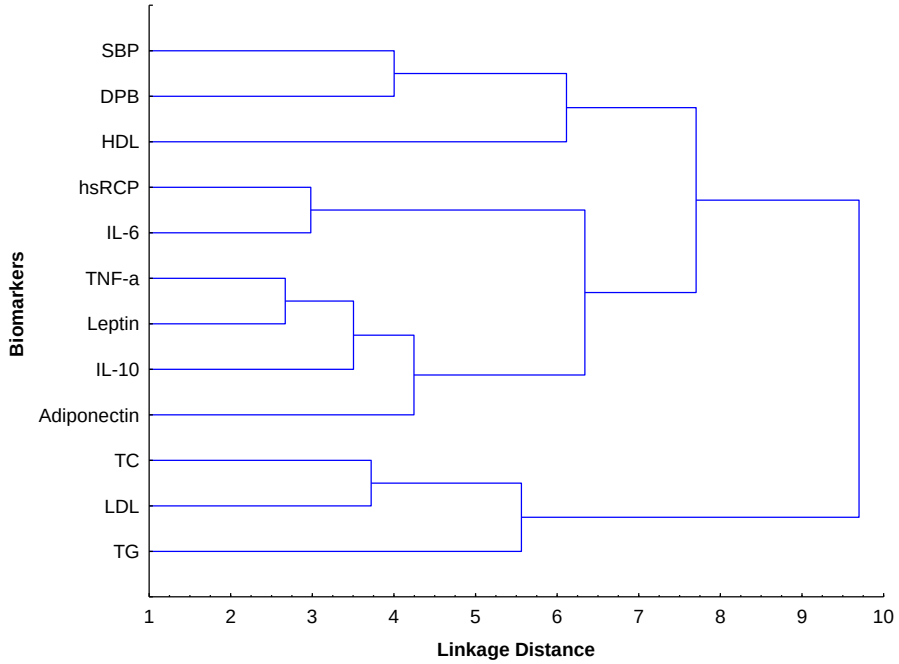

Figure 4: Tree Clustering applied to the biomarkers.

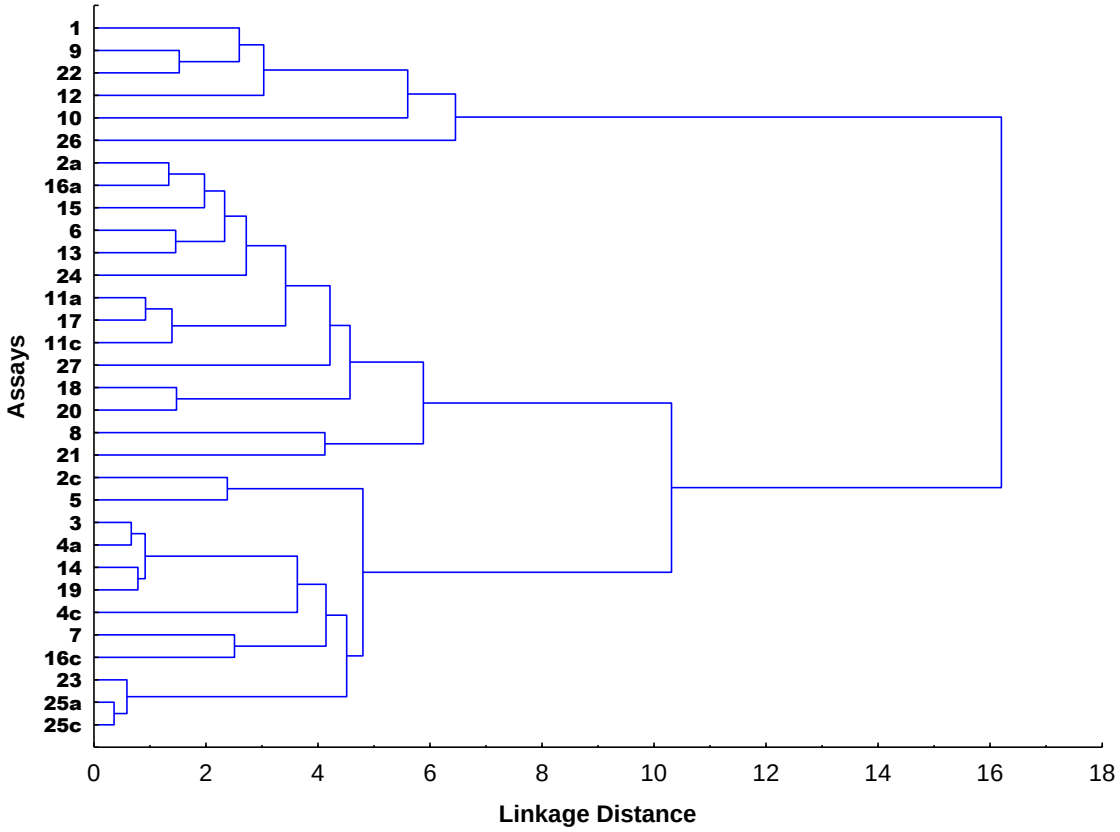

Figure 5: Tree Clustering applied to the assays.

Table 6: Protocol parameters and net changes of biomarkers according to the groups obtained by the Cluster Analysis.

	Group I (n=6)	Group II (n =14)	Group III (n=12)	P value
Individuals (n)	19.83 ± 5.41	21.86 ± 2.10	32.08 ± 5.30	*0.107*
Female (%)	15.74 ± 9.98	35.38 ± 7.96	45.47 ± 9.06	*0.140*
Age (y)	54.16 ± 3.30	53.63 ± 2.46	57.06 ± 1.93	*0.541*
Time (days)	38.50 ± 5.64[a]	74.21 ± 10.38[a]	175.33 ± 40.96[b]	*0.007*
Dose (mg)	1,066.67 ± 474.63	454.14 ± 129.00	263.75 ± 88.07	*0.099*
Impact Factor	5.292 ± 0.929	4.650 ± 1.331	5.212 ± 1.430	*0.938*
Year	2015 ± 0.86	2015 ± 0.86	2015 ± 0.86	*0.949*
Diabetes (n)[2]	-	18.00 ± 7.77	15.33 ± 3.99	-
Dyslipidemia (n)[2]	-	-	43.50 ± 6.50	-
Obesity (n)[2]	-	-	26.00 ± 5.00	-
Hypertension (n)[2]	-	19.50 ± 0.5	31.43 ± 9.57	-
Stable Angina (n)[2]	-	2.00	2.00	-
Smokers (n)[2]	-	4.00 ± 0.84	8.25 ± 2.18	-
Aspirin (n)[2]	-	13.00	11.67 ± 0.67	-
Clopidogrel (n)[2]	-	1	1	-
Statins (n)[2]	-	18.00 ± 1.48	19.50 ± 5.50	-
β-Blockers (n)[2]	-	23.25 ± 2.06	14.00 ± 4.00	-
Calcium Channel Blockers (n)[2]	-	9.00 ± 1.00	3.00	-
ECA/ARB Inhibitors (n)[2]	-	21.50 ± 2.18	20.67 ± 1.68	-
SBP (%)	1.54 ± 1.23[a]	-6.76 ± 1.06[b]	-0.95 ± 1.19[a]	*p<0.001*
DBP (%)	2.17 ± 1.09[a]	-4.51 ± 1.77[b]	-1.39 ± 0.87 [ab]	*0.026*
TC (%)	4.95 ± 1.87[a]	-6.04 ± 1.51[b]	-3.28 ± 1.39[b]	*p<0.001*
TG (%)	23.26 ± 7.11[a]	-10.13 ± 5.01[b]	-3.51 ± 2.12[b]	*p<0.001*
LDL (%)	9.45 ± 2.07[a]	-4.81 ± 3.40[b]	-4.35 ± 2.38[ab]	*0.036*
HDL (%)	-5.04 ± 5.37[a]	-1.16 ± 1.42[a]	6.23 ± 1.35[b]	*p<0.001*
hsCRP (%)	-18.00 ± 16.99	-2.92 ± 7.63	7.23 ± 9.55	*0.361*
TNF-α (%)	9.51 ± 20.08	-4.33 ± 3.14	-6.36 ± 4.26	*0.395*
IL-6 (%)	-45.52 ± 24.66	5.36 ± 10.83	14.82 ± 10.31	*0.061*
IL-10 (%)	22.05	-0.32	17.45 ± 7.13	-
Leptin (%)	29.73	-2.24 ± 18.00	-3.51 ± 5.87	-
Adiponectin (%)	2.41	1.82 ± 1.81	8.95 ± 4.74	-

[1]Values are expressed as mean ± SE.

[2] Mean considering only the studies in which this information was available.

[3]P value obtained by One-way ANOVA. Values followed by the same letter are not different

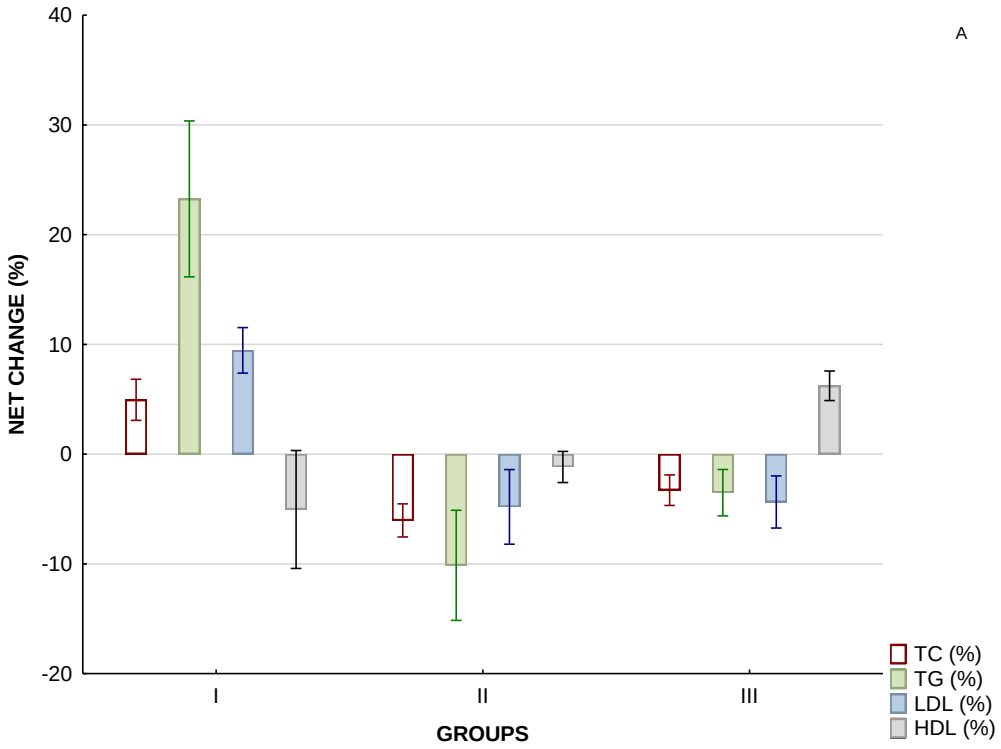

Figure 6: Lipoproteins profile net change values according to the groups

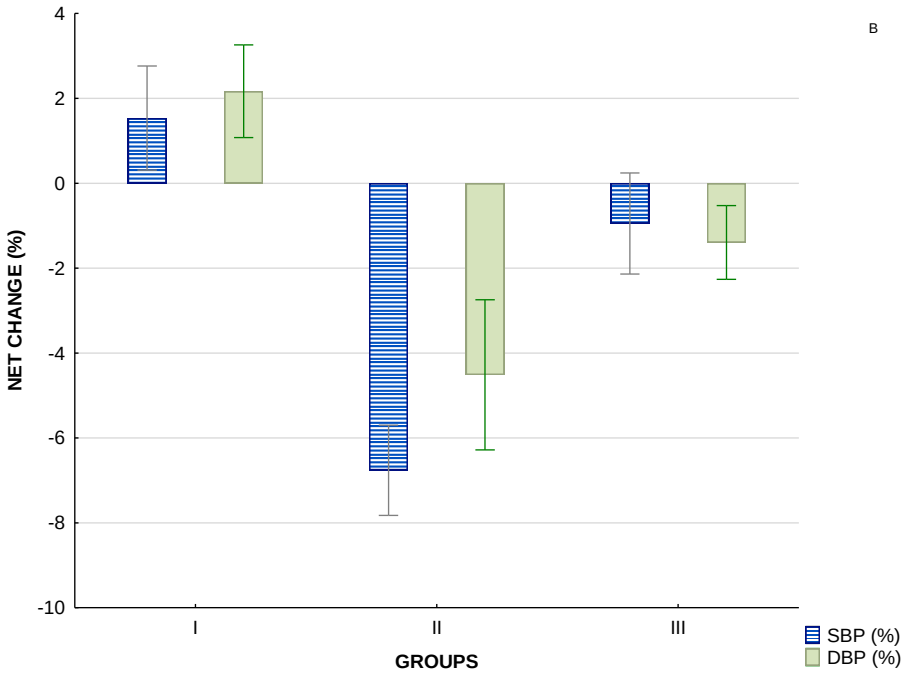

Figure 7: Arterial pressure profile net change values according to the groups.

The groups did not differ in mean terms of the number of participants, sex, and age. There was also no difference between the year of publication or impact factor between the studies. The only difference in protocol parameters between the groups was the intervention time that was longer in group III.

Group I was the cluster with the shortest intervention time and high doses of supplementation. The group II and III showed reduction in SBP and DBP when compared with group I. Concerning to the plasma lipids, groups II and III showed a decrease in TC, TG, and LDL whereas in group I these parameters were increased. HDL increased only in group III, while in group I and II the values were reduced. It is also possible to observe that about inflammatory markers, the group I showed a trend (*p=0.061*) of IL-6 reduction (Table 6). The variables used in the software were standardized.

6. DISCUSSION

In this review, we investigated the association of experimental conditions from clinical studies that evaluated the effects of resveratrol supplementation on biomarkers related to cardiovascular diseases, applying a multivariate approach. It was observed that the effects of supplementation with resveratrol can be influenced by the study designs.

In general, the clinical studies were carried out with subjects over fifty-five (55) years old with a considerable range of different health conditions, risk factors, and taking some medicine. This makes sense, considering that the development of atherosclerosis and other chronic diseases is inherent to aging and is usually evident in people who are obese or have dyslipidemia, diabetes, or hypertension. However, its progression also depends on the lifestyle (Koopman and Kuipers, 2017). Clinical trials have shown that resveratrol supplementation is safe and well-tolerated at different doses, but doses between 2.5 and 5 g/day can cause mild to moderate gastrointestinal symptoms (Patel *et al.*, 2011; Chachay *et al.*, 2014). In addition, some treatments included resveratrol associated with additional compounds (Fujitaka *et al.*, 2011; Militaru *et al.*, 2013). These points highlight the difficulty of interpreting the different results in the clinical studies.

Resveratrol is commonly used as a nutritional supplement; however, due to its potential to modulate different pathways, its multiple mechanisms of action are still unclear (Gambini *et al.*, 2015). Preclinical studies have shown that resveratrol can act on diabetes, neurodegenerative diseases, cancer, aging, obesity, and cardiovascular diseases (Baur and Sinclair, 2006). As observed in

other reviews (Dyck *et al.*, 2019), our findings (Table 3) also indicated that the biomarkers evaluated in the studies with resveratrol supplementation frequently are related to lipid profile (TC, LDL, HDL, and TG); glucose metabolism (fasting glucose, insulin, and HOMA); blood pressure (SBP and DBP); inflammatory (hsCRP, IL-6, TNF-α, leptin) and anti-inflammatory response (adiponectin and IL-10). Despite all potential benefits attributed to resveratrol, we observed that the evaluation of the effects on markers related to antioxidant, endothelial function (NO activity), or oxidative stress is limited. A better assessment of these biomarkers could provide new information on the therapeutic potential of resveratrol as alternative co-therapy.

To investigate the impact of resveratrol on the main parameters among these studies, net changes were calculated for 12 biomarkers (Table 4 and Figure 2). It was possible to observe that the interventions with resveratrol promoted reduction in cholesterol, LDL cholesterol and TG, and increase in HDL cholesterol. In a meta-analysis, Haghighatdoost et al. (2018) investigated the effects of resveratrol supplementation on lipid profile. They observed that the hypolipidemic effect of resveratrol was limited to total cholesterol and no effect on LDL or TG concentrations was observed; The HDL increased significantly only in cross-over trials. The authors suggest the reason for this result could be the selection of participants based on their low HDL cholesterol concentrations (Haghighatdoost and Hariri, 2018). As observed in our results, others authors reported that resveratrol consumption decreased SBP values in trials using a higher-dose, while low-dose of resveratrol did not show a significant favorable effect on SBP; and not affect the physiological regulation of DBP (Matthias B. Schulze *et al.*, 2005; Fogacci *et al.*, 2019). The mechanism underlying the beneficial effect of resveratrol on SBP may involve mainly enhancing eNOS activity and NO bioactivity. The enhanced production of endothelial NO by resveratrol protects the vasculature through its vasodilating action (Li, Xia and Förstermann, 2012). Several meta-analyses have quantified the effects of resveratrol on inflammatory mediators and adipokines (Mohammadi-Sartang *et al.*, 2017; Tabrizi *et al.*, 2018; Haghighatdoost and Hariri, 2019). Similarly, we identified that resveratrol promoted net reduction in hsCRP and TNF-α, but not in IL-6 or leptin. In contrast, resveratrol improved the

concentration of IL-10 and adiponectin, which are involved in anti-inflammatory responses (Tilg and Moschen, 2006)

The application of multivariate analysis allows the joint visualization of a large number of associations and results at the same time according to the variables of interest (Castro, 2005). The similarities between TC, LDL, and TG were negatively correlated with HDL and closer to changes of the SBP and DBP. It has been suggested that the high TG/low HDL cholesterol ratio may influence the obesity-related hypertension and it is a risk factor for hypertension, especially in women, subjects < 60 years old, and those with prehypertension (Jeppesen *et al.*, 2000; Liu *et al.*, 2020). Adipose tissue secretes several adipokines and cytokines that may contribute to vascular complications, such as hsCRP (or CRP), IL-6, TNF-α, and leptin (Tilg and Moschen, 2006). Leptin shows pro-inflammatory actions, including the induce of CRP in the vascular endothelium and the secretion of inflammatory cytokines like TNF-α and IL-6, which are negatively correlated with adiponectin expression (Tilg and Moschen, 2006). Prior investigations have demonstrated that hypoadiponectinemia is associated with the development of atherosclerosis (Ouchi, Kihara and Funahashi, 2003; Matthias B Schulze *et al.*, 2005). Adiponectin induces the production of the important anti-inflammatory cytokines, such as IL-10, an anti-inflammatory cytokine with antiatherogenic properties (Tilg and Moschen, 2006; Han and Boisvert, 2015). Consistent with this, we observed that these markers show a correlation between themselves (Figure 3).

It was also identified that the studies could be classified into 3 groups according to the biomarkers selected in our analysis. Data presented in Table 6 suggested that clinical trials classified in group II and III had a better response to resveratrol supplementation when compared to group I, characterized by shorter period of the intervention. It has already been suggested in some publications, that future studies should assess longer-term resveratrol supplementation (Akbari *et al.*, 2019; Fogacci *et al.*, 2019). Group II had a better effect in reducing blood pressure and also a hypocholesterolemic effect, but included a reduction in HDL levels. In terms of improvement of lipid profile, group III resulted in a reduction in TC, LDL and TG levels, while HDL levels increased, suggesting that a long-term resveratrol intervention may improve

HDL levels. The use of bioactive compounds associated with drug therapy has already shown positive results (Scolaro *et al*., 2018). Thus, resveratrol supplementation administrated accordingly to the protocol followed in group III, could be a complementary therapy for drugs applied to improve the lipoproteins profile.

It is also possible to observe that the participants with some health condition or under drug treatment belong mainly to groups III and II. Altered circulating levels of inflammatory cytokines, such as TNF-α, IL-6, or hsCRP (or CRP), have been reported in metabolic comorbidities (De Heredia, Gómez-Martínez and Marcos, 2012; Taube *et al.*, 2012). It is important to note that there was a great reduction in the levels of hsCRP and IL-6 in the group I, but no reduction in the levels of TNF-α. In contrast, no reduction in IL-6 was observed in groups II and III. Group II showed a slight reduction in hsCRP and TNF-α levels while group III had a reduction only in TNF-α. Taking these data into account, it can be suggested that the choice of study design depend on the target biomarker.

An interesting point was that body composition related studies (10 – Poulsen, 16- Kjær), both with obese danish men, obtained negative results with 500 mg/4 weeks and 1g/16 weeks of resveratrol used in the intervention, but Kjær, with 150 mg of resveratrol, concluded that higher doses of resveratrol have a detrimental effect on glucose and cholesterol metabolism and the optimal dose is related to target tissue. Along with Group 3 data, this findings suggest that maintenance of intake for a longer period and dosage closer to that obtained from diet are related to a better treatment as it is to the Mediterranean diet pattern, highlighting the importance of adhering to good eating habits.

7. CONCLUSION

In general, the supplementation with resveratrol improved the lipoproteins profile, arterial pressure and inflammatory biomarkers. The use of multivariate analysis to assess associations between protocol parameters and effects of resveratrol supplementation on biomarkers related to cardiovascular diseases showed that HDL concentration is directly related to a longer

intervention time. The changes in relation to blood pressure and lipid profile indicated that future studies may be based on protocol parameters similar to those of groups II, III or even a combination between themselves. In addition, the use of resveratrol in combination with drug therapy is an important matter to be better investigated. Finally, the results showed in an interesting way that the best treatment may be related to adhering to a healthy and balanced diet.

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Data e assinatura do aluno(a)

Data e assinatura do orientador(a)