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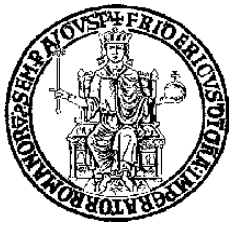

Department of Clinical Medicine and Surgery

PhD Program in

Advanced biomedical-surgical therapies

XXXIII CYCLE

"The effects of a hypocaloric diet and cinchona supplementation on nutritional status and body composition in a population of obese adults"

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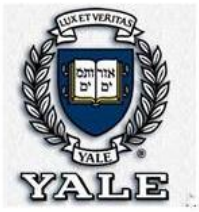

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List of abbreviations

CVD	Cardiovascular disease
B.M.I	Body mass index
C group	Cinchona group
P group	Placebo group
WHO	World Health Organization
Interleukin	IL-6
Tumor necrosis factor-α	TNF-α
Chemoactive protein monocyte-1	MCP- 1
High-fat diet	HFD
Central nervous system	CNS
Arcuate nucleus	ARC
agouti-related protein	AgRP
proopiomelanocortin	POMC
Leptin Receptor	LEPR
Insulin Receptor Substrate	IRS
Phosphatidylinositide-3 Kinase	PI3K
Cholecystokinin	CCK
Neuropeptide Y	NPY

Tyrosine- Tyrosine Polypeptide	PYY
Glucagon-like peptide 1	GLP1
cyclic Adenosine Monophosphate	cAMP
Growth Hormone Receptor	GHSR
Bioelectrical Impedance Analysis	BIA
Resistance	R
Reactance	Xc
Livelli di Assunzione Raccomandata di Nutrienti	LARN
Waist circumference	**WC**
Hip circumference	**HC**
Visceral adipose tissue	**VAT**
Subcutaneous adipose tissue	**SAT**
Glycemia	**Gly**
Total cholesterol	**COL-tot**
HDL cholesterol	**Col-HDL**
LDL cholesterol	**Col-LDL**
Triglycerides	**Try**
Velocity of Eritro-Sedimentation	**VES**
Protein C Reactive	**PCR**
Standard error of the mean	**SEM**

Fat mass	**FM**
Fat-free mass	**FFM**
Gastrointestinal	**GI**
Quinine hydrochloride	**QHCL**
Bitter taste receptors	**T2R**

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To my family,

for their unconditional love and support…

To me,

for my strength and tenacity…

Abstract

Cardiovascular disease (CVD) is the leading cause of mortality and disability in humans worldwide. Obesity is a chronic disease present worldwide, considered as an etiological risk factors that can cause the onset of cardiovascular diseases. Currently, the most effective therapies to reduce the cardiovascular risk associated with obesity is an healthy lifestyle represented by dietary changes and physical activity. The aim of this study was to evaluate the effects of a hypocaloric diet associated with a cinchona supplementation on nutritional status and body composition in a population of obese adults.

A total of forty obese subjects of both sexes with Body Mass Index (BMI) $\geq$ 30 kg/m^2 attending at Outpatients Clinic of the Departmental program "Physiology Nutrition Unit ", School of Medicine, "Federico II" University of Naples, were recruited. Subjects were randomized into 2 groups. The first group was treated with hypocaloric diet for 2 months plus supplementation of Cinchona (C group); the second one was treated with hypocaloric diet for 2 months plus a placebo supplementation (P group). Anthropometric measurements as well as bioimpedance analysis, abdominal fat distribution and basal metabolic rate were evaluated at baseline and after 60 days.

At the end of observations, we observed a significant improvement in nutritional status and body composition in patients belonging to C group compared with subjects belonging to P group.

. Therefore, this study demonstrates that the association of hypocaloric diet with a nutraceutical supplementation is able to induce a more significant weight loss than that obtained with a hypocaloric diet alone.

Chapter 1:

Introduction

1. 1.Cardiovascular disease

Cardiovascular disease (CVD) is the leading cause of mortality and disability in humans worldwide [1]. Currently, cardiovascular disease accounts for 31% of mortality in developed countries, and the rate of CVD around the world is expected to rise. The pathogenesis of CVD is predominantly of atherosclerotic origin leading to the development of coronary artery disease, cerebrovascular disease, venous thromboembolism, and peripheral vascular disease, resulting in myocardial infarction, cardiac arrhythmias or stroke (Figure 1.1).

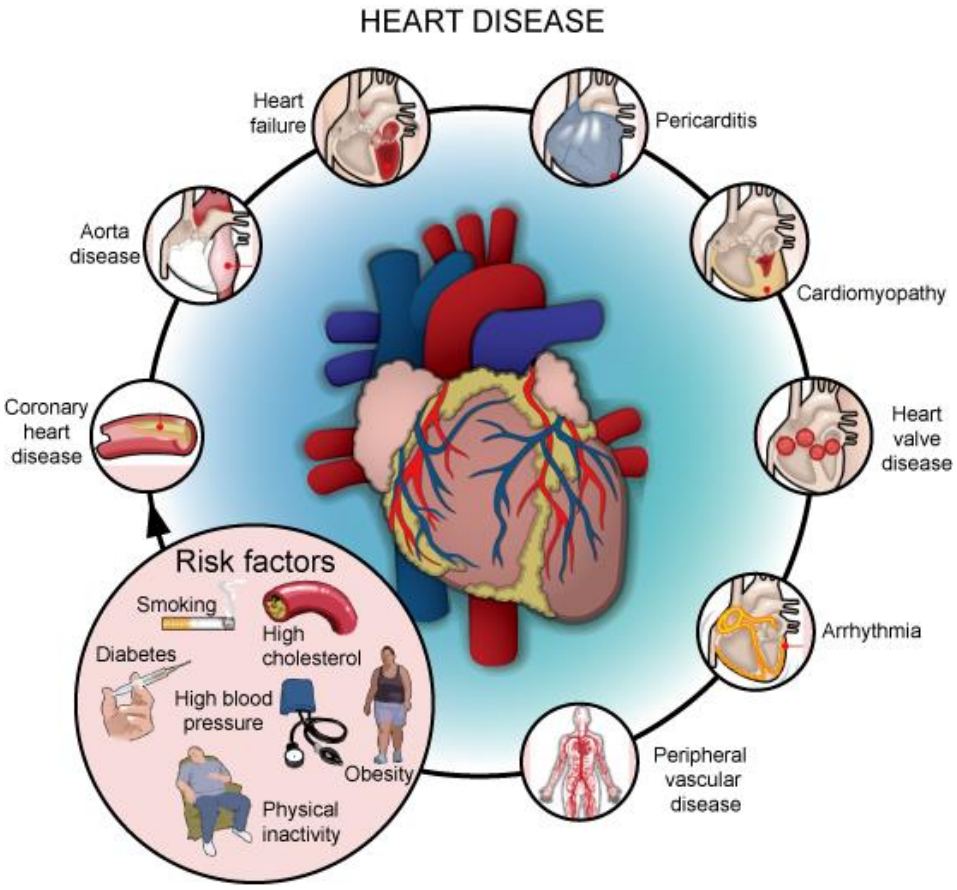

Figure 1.1: Types of heart disease

The World Health Organization (WHO) determined that more than 75% of premature CVDs could be prevented through a reduction of risk factors [2, 3, 4]. The incidence of cardiovascular disease, indeed, can be modified with careful risk factor reduction and primary prevention, leading to a reduction in premature disability and morbidity and prolonging survival and quality of life.

Dyslipidemia, hypertension, diabetes, obesity, smoking and lack of physical activity represent the etiological risk factors that can cause the onset of cardiovascular diseases [1].

Nutrition is one of the most studied factors in the pathogenesis of CVD as it affects several cardiometabolic risk factors such as obesity, lipid profile, blood pressure, glucose homeostasis, endothelial function, cardiac function, metabolic expenditure, visceral adiposity and microbiome [5,6].

Previous studies have shown that the Mediterranean diet based on a daily intake of whole cereals, vegetables, legumes, fruit and olive oil, a moderate intake of fish and poultry and a lower intake of dairy products, red and processed meats, is associated with lower incidence and mortality of CVD (Figure 1.2).

Figure 1.2: The Mediterranean Diet Pyramid.

1.1.1.CVD risk factors

CVD risk factors can be classified into modifiable and non-modifiable risk factors (Table 1.1). Non-modifiable risk factors such as age, sex, ethnicity and family history cannot be changed directly, but their effect can be mitigated by adopting a healthy lifestyle and preventive treatments.

- Age: older people show a higher risk of developing cardiovascular disease than younger people; although the aging process cannot be changed, a healthy lifestyle is recommended to counteract and prevent the onset of cardiovascular diseases.
- Sex: in the past, cardiovascular disease was considered a male disease. Studies are currently underway to investigate this issue. Some studies, indeed, have shown that women can develop cardiovascular disease at an older age than men and this onset seems to be linked to the hormonal changes following menopause.
- Ethnicity: population studies have shown that people from South Asia, Africa or Caribbean, have a higher risk to develop cardiovascular disease; in addition, these people also show an increased risk to develop type 2 diabetes, considered a risk factor for cardiovascular disease. Currently the causes of this predisposition are not yet clear, but it is recommended to have a healthy lifestyle to prevent the development of heart and circulatory diseases.
- Family History: A family history of cardiovascular disease, as well as of hypertension, hypercholesterolemia, and type 2 diabetes is considered a risk factor. A healthy lifestyle is generally recommended to counteract and reduce the risk of cardiovascular disease in those with a genetic predisposition to the condition [7, 8, 9, 10, 11, 12].

Modifiable risk factors such as excess weight and obesity, as well as hypertension, smoking, diabetes, and a sedentary lifestyle are among the most important risk factors for cardiovascular diseases. Currently, positive results in mortality reduction were obtained through hypertension, hypercholesterolemia, and

smoking prevention and treatment. Exceptions to these positive trends are represented by obesity and diabetes, which, on the other hand, are constantly growing [13, 14, 15]. Furthermore, the mechanisms linking obesity to the development of CVD, are currently not clear. Some studies suggest that in an obese subject, the expansion of adipose tissue and the production of pro-inflammatory cytokines are able to directly compromise systolic and diastolic heart function and induce the formation of atherosclerotic plaques. Furthermore, the change in body composition appears to affect hemodynamics and alter the structure of the heart. Currently, the most effective therapies for reversing cardiovascular risk factors associated with obesity have been dietary changes associated with increased physical activity [16, 17].

RISK FACTORS FOR CVD

NON-MODIFIABLE	MODIFIABLE
• Age	• Overweight or Obesity
• Sex	• Diabetes
• Ethnicity	• Smoking
• Family History	• Hypertension
	• Hypercholesterolemia
	• Excessive alcohol
	• Stress

Table 1.1: Risk factors for CVD

1.2. Obesity

Obesity, a complex and multifactorial disease associated with excessive adiposity or body fat, currently affects over a third of the world's population [13, 18]. Obesity is a major risk factor for associated comorbidities, such as cardiovascular diseases, type 2 diabetes mellitus, cancer, and mortality. Currently, obesity has reached global epidemic proportions in both adults and children, resulting in a substantial financial burden on healthcare. The World Health Organization (WHO) defines obesity as an excessive accumulation of fat diagnosed with a body mass index (BMI) ≥ 30 kg / m^2, which could

compromise health [16, 19, 20] (Figure 1.3). Obesity is a consequence of an energy imbalance between caloric intake and energy expenditure, leading to a positive energy balance with a consequent increase in body weight [21, 22]. Factors both hereditary or genetic, family history, socioeconomic and sociocultural conditions are considered risk factors for obesity [13]. Moreover, obesity is defined as a condition of chronic inflammation and presents a multifactorial etiology, such as genetic factors and hormone imbalance, as well as diet and the environment [23]. Inflammation is a protective response of the body to maintain the homeostasis of tissues and organs and can be of two types: short-lived acute inflammation, characterized by edema and migration of leukocytes, or long-lasting chronic inflammation, characterized by the presence of lymphocytes and macrophages and by the proliferation of blood vessels and connective tissue. Chronic inflammation induces the secretion of inflammatory adipokines from adipose tissue, such as interleukin (IL-6), tumor necrosis factor-α (TNF-α), chemoactive protein monocyte-1 (MCP- 1), and resistin [24, 25]. The consumption of an unbalanced diet, characterized by an excess of fats and carbohydrates, can cause inflammation in the peripheral organs, in particular in the adipose tissues [25, 26, 27]. The consequences of dysfunctional adipose tissue closely linked to obesity and metabolic disorders are inadequate angiogenesis, hypoxia, inflammation, as well as fibrosis. In a pathological obesity condition, adipocyte hypertrophy and hypoxia are able to cause inflammation, inducing the production of many cytokines and chemokines, involved in the beginning and development of the inflammatory response associated with obesity in adipose tissue and obesity-induced insulin resistance [28, 29]. Furthermore, this condition induces a reduction of the production of adiponectin, important for its anti-inflammatory function and for its ability to improve sensitivity to insulin and regulate energy expenditure, predisposing to a pro-inflammatory state and oxidative stress, thus contributing to the pathogenesis of obesity [25, 30, 31]. Many studies have shown that obesity induced by a high-fat diet (HFD) causes chronic hypothalamic inflammation [32, 33, 34].

Figure 1.3: Body Mass Index.

1.2.1. The Role of Hormones in Appetite and Weight Regulation

The central nervous system (CNS), in particular the hypothalamus, plays a fundamental role in regulating appetite and energetic homeostasis in response to changes in peripheral circulating signals, such as hormones and nutrients. The reception and integration of these signals occur mainly in the arcuate nucleus (ARC), which contains two distinct populations of neurons, one releasing agouti-related protein (AgRP) and the other releasing proopiomelanocortin (POMC). These neurons are involved in the regulation of energy homeostasis, sensing and integrating numerous metabolic signals [31]. It has been observed that in the hypothalamus, leptin binds its receptor (LepR-b) and triggers a signaling pathway, which induces STAT3 stimulation with subsequent activation of POMC neurons and NPY/AgRP/GABA neurons inhibition, exerting an anorexigenic effect. Leptin is one of the main components of the physiological system involved in the regulation of body weight [35]. Furthermore, several data have shown that insulin binding to the insulin receptor substrate (IRS) exerts the same anorexigenic effect of leptin by activating a phosphorylation cascade through phosphatidylinositide-3-kinase (PI3K) in POMC neurons [36]. Experimental and human studies have shown that cholecystokinin (CCK) reduces food intake dose-dependently in response to meal initiation. CCK is synthesized both in the gastrointestinal tract and in the hypothalamus, representing the most abundant neuropeptide in the CNS. At the hypothalamic level, indeed, CCK, binding to CCK-B receptor and

interacting with NPY, induces satiety. CCK has been studied as a potential therapeutic substance for the management of obesity [31]. Tyrosine–tyrosine polypeptide (PYY) and glucagon-like peptide 1 (GLP-1) are produced by L cells in the small intestine, leading to an anorexigenic action. In response to food ingestion, PYY plasma concentration increases, signaling food ingestion from the intestine to the appetite-regulating system in the brain: PYY mediates its effects by binding to its Y2 receptor [37]. GLP-1, on the other hand, acts by binding to a membrane receptor, GLP-1-R, whose activation stimulates the production of cyclic adenosine monophosphate (cAMP) by the enzyme adenylyl cyclase. This mechanism triggers the transmission of intracellular signaling [38]. Conversely, ghrelin binds its receptor, the growth hormone receptor (GHSR), inducing the activation of AgRP neurons and the inhibition of POMC neurons, exerting orexigenic effects [31, 39] (Figure 1.4).

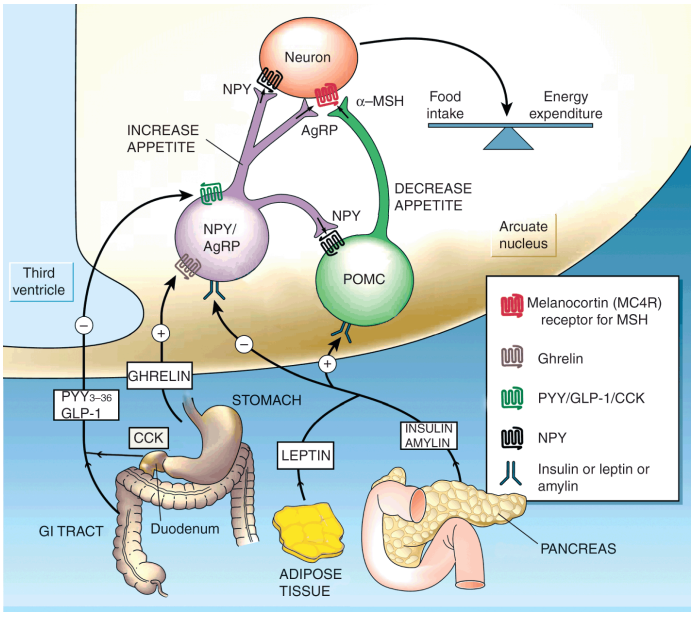

Figure 1.4: Neuroendocrine Regulation of Appetite and Body Weight.

1.2.2. Body composition as risk factor for CVD

Obesity is characterized also by an alteration of the body composition; numerous studies have shown that body composition is directly related to health. A good percentage of fat mass, indeed, is associated with good health; on the contrary, an altered body composition with excess fat mass can greatly increase the risks of numerous diseases such as cardiovascular disease. The percentage of fat mass, therefore, can be considered an important predictor of the risk of cardiovascular disease, in all individuals [40]. Bioelectrical Impedance Analysis or Bioimpedance Analysis (BIA) is a non-invasive, safe and economical technique that can monitor body composition over time. The BIA, based on the assumption that the body is a cylindrical conductor, indeed, allows to detect changes in body weight, favoring early intervention and prevention. BIA provides the measurement of body mass and fluids which represent a fundamental tool for evaluating health.

This analysis involves the placement of two electrodes on the person's right hand and foot and determines the impedance, a combination of resistance, R, i.e. the opposition to the flow of an injected alternating current, at any current frequency, through intra- and extracellular ionic solutions, and reactance, Xc, i.e. the dielectric component of cell membranes and organelles, and tissue interfaces. BIA can be assessed through single frequency or multi-frequency electrical currents. The single frequency BIA is performed at 50 KHz (Figure 1.5) [41].

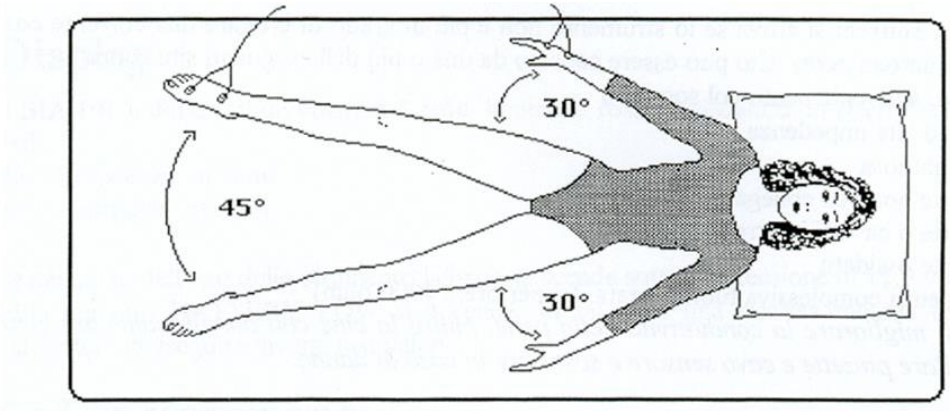

Figure 1.5: Bioimpedance Analysis (BIA)

1.3. Obesity and nutraceuticals

To date, the common interest is to find a treatment that can fight or prevent obesity and promote and protect individual health. Currently, nutraceuticals compounds, natural products derived from food sources, are under investigation for their helpful activities in many diseases, becoming a safer alternative / supplement to conventional therapy, which, on the contrary, provides a combination of pharmaceutical agents often ineffective and accompanied by several side effects. [42, 43].

The word “Nutraceutical” was born from the fusion of two terms: “nutrition” and “pharmaceutical”, coined by Stephen De Felice in 1989. Nutraceutical are foods or part of food with beneficial health skills; these compounds, indeed, seem to have a beneficial effect on health in terms of the prevention or treatment of one or more pathologies [44].

Based on different characteristics such as chemical composition, origin or pharmacological form of administration, nutraceuticals can be classified into nutrients, herbals or dietary supplements [45]. These compounds showed an anti-inflammatory activity, as well as antioxidant, immunoregulatory and modulation of the intracellular signaling pathway activities [46].

In particular, some studies have shown that the use of nutraceuticals could regulate food intake (intestine-brain axis), energy expenditure (energy homeostasis-thermogenesis), lipids metabolism (lipase inhibition) and life cycle of adipocytes (adipocytokines from abnormal adipocytes) by an epigenetic or genetic mechanism [47].

Further studies are needed to clarify the effects of nutraceuticals in the prevention of obesity and its complications as well as the safest dosage, treatment time and its bioavailability.

1.4. Cinchona

Cinchona belongs to the Rubiaceae family, originally from South America. The main part of the plant which is mainly used for medicinal purposes is the bark which it can be up to 30 cm long and 2-6 cm thick. The most important property of cinchona is due to the presence of several types of alkaloids; chichonine, with higher activity compared to other compounds, as well as quinine, quinidine and cinchonidine are the main alkaloids present in this plant. In the past, these alkaloids were used in the treatment of malaria and other diseases [48, 49].

Numerous studies have shown further beneficial activities of these alkaloids such as antiobesity as well as antitumor, antioxidant, anti-inflammatory, antimicrobial activity [49].

- **Antiobesity property:** Cinchonine has been widely used for its antimalarial effects, but some studies have shown that cinchonine has also an anti-obesity activity that seems to be more effective than that exerted by other phytochemicals, specific to counteract obesity (Figure 1.6). Furthermore, cinchonine has also been shown to reduce plasma lipid levels in mice fed HFD (high fat diet), modulating hyperlipidemia and hyperglycemia [50, 51].
- **Antitumor property:** Studies have shown that quinine is able to inhibit cell proliferation and induce apoptic cell death in cancer, suggesting that quinine may be used as an anticancer agent in the future [52].
- **Antioxidant property:** The cinchona bark also contains compounds such as phenols, flavonoids and phytosterols able to exert antioxidant activity [53].
- **Antiinflammatory activity:** In 1984, quinine and cinchonine were isolated to treat lupus. To date, quinine is also used as an anti-inflammatory to treat nocturnal leg cramps and arthritis [54].
- **Antimicrobial activity:** Some studies have shown that cinchona was effective in the treatment against many microorganisms harmful to humans [55].

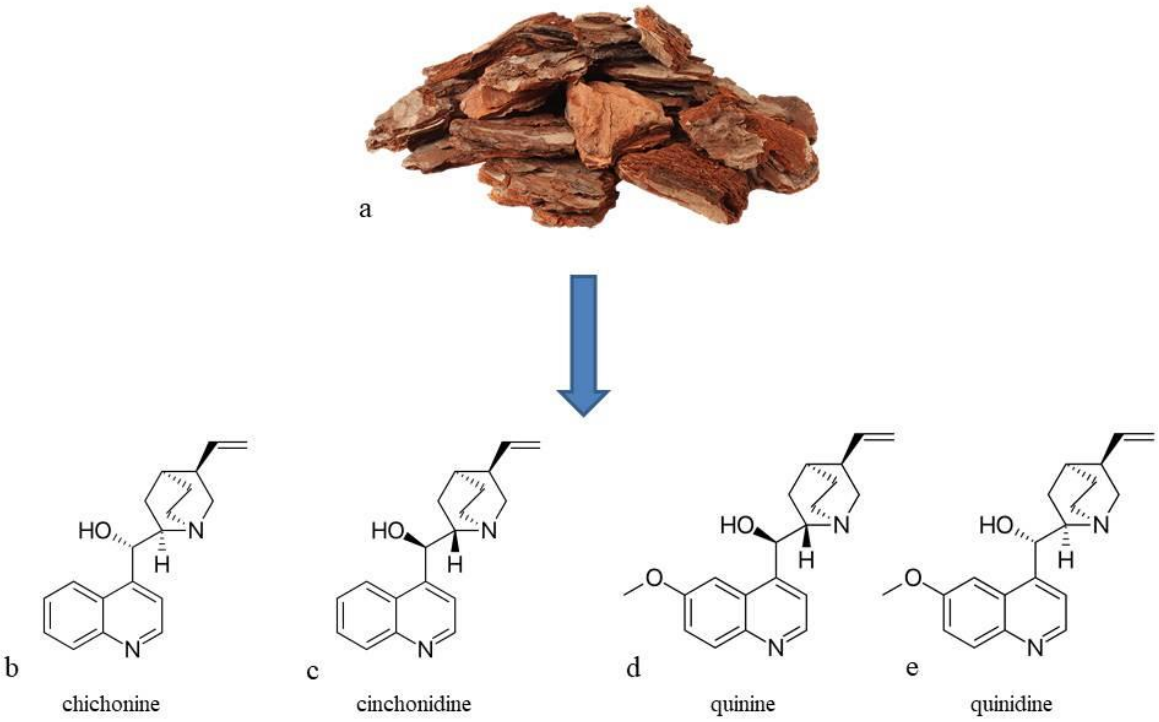

Figure 1.6: a) Cinchona bark; b) Chemical formula of Cinchonine; c) Chemical formula of Cinchonidine; d) Chemical formula of Quinine; e) Chemical formula of Quinidine.

Chapter 2:

Aim

The aim of the present study was to assess the effects of nutraceutical supplementation with cinchona on a population of obese adults. In particular, our purpose was to evaluate nutraceutical effects on body weight, changes in body composition and nutritional status in obese subjects.

Chapter 3:

Matherials and Methods

3.1.Clinical study

3.1.1.Study design

The study protocol was approved by the Ethical Committee of the Federico II University Medical School of Naples (EC approval code: 8615) and all patients gave written informed consent.

Forty obese subjects of both sexes with Body Mass Index (BMI) ≥ 30 kg/m^2 attending at Outpatients Clinic of the Departmental program "Physiology Nutrition Unit ", School of Medicine, "Federico II" University of Naples, were recruited.

After baseline evaluations, subjects were randomized (R) into 2 groups: The first group (20 patients: M: n=9; F: n=11) was treated with hypocaloric diet for 2 months plus supplementation of Cinchona (C group); the second one (20 patients: M: n=10; F: n=10) was treated with hypocaloric diet for 2 months plus a placebo supplementation (P group). Patients were excluded when affected by diseases, such as cancer, acute and chronic metabolic and inflammatory diseases, chronic renal disease type 1 and type 2 diabetes treated with insulin and/or hypoglycemic drugs or treated with drugs to lose weight or treated with hormone therapy.

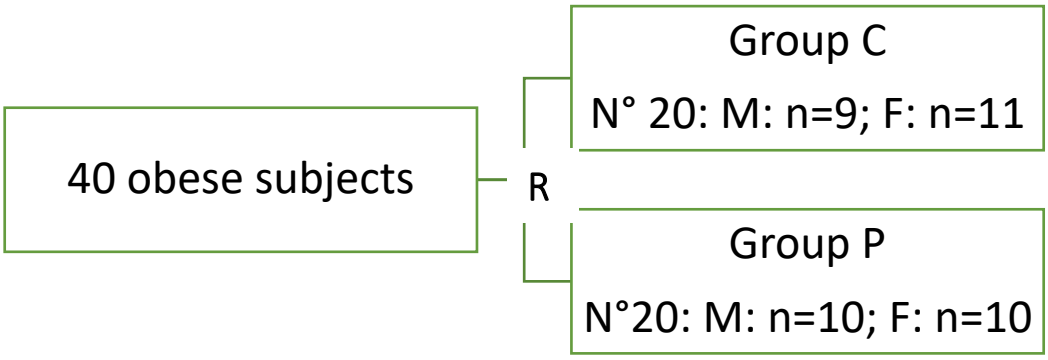

Figure 3.1.: Clinical study design. C= obese subjects administered with hypocaloric diet plus cinchona; P= control group - obese subjects administered with hypocaloric diet;

3.1.2. Dietary treatment:

A personalized diet was recommended for each patient of all 2 groups, according to the LARN (Livelli di Assunzione Raccomandata di Nutrienti) guidelines [56]. A hypocaloric diet (the calorie restriction was 40% of the total energy needs), with 55-60% of the total caloric intake in carbohydrates, 10-15% in proteins and 20-25% in fatty acids (<7% from saturated fat), has been recommended for all obese patients.

3.1.3. Supplementation:

The subjects belonging to group C were treated with 800 mg of Cinchona per day (two Cinchona capsules per day, one hour before main meals).

The consumption of Cinchona is not associated with health risks.

Participants who did not take pills for two or more days were excluded from the study. Each patient received by the Department of Pharmacy, "Federico II"University of Naples, the overall amount of Cinchona pills for the whole treatment.

3.1.4. Study protocol

The subjects were evaluated at the moment of recruitment (time T0), after 30 (time T1) and 60 (time T2) days of treatment, using standardized protocols.

The nutritional status was assessed by anthropometric measurements: weight (Seca GmbH & Co KG, Hamburg, Germany), height (wall-mounted stadiometer to the nearest 0.1 cm), body mass index (BMI), waist circumference (WC), hip circumference (HC) [57]. To evaluate the body composition, bioelectrical impedance analysis (BIA) was undertaken by tetrapolar BI (RJL 101; Akern SRL, Florence, Italy). BIA was performed with a single-frequency measurement (50 kHz) [58]. Visceral adipose tissue (VAT) and subcutaneous adipose tissue (SAT) were evaluated on fasting subjects by the same operator with Bodymetrix BX2000 instrument. In particular, VAT and SAT were measured 1 cm above the umbilicus at the end-expiration and applying the same probe pressure for all subjects. Each measurement was performed 3 times and the mean of the 3 measurements was used for analysis [59]. The hand muscle strength (kgm) was measured on the dominant and nondominant hands to the nearest kilogram using a hand dynamometer (78010; Lafayette Instrument Company, Lafayette, IN, USA). During measurement, the participant was in an upright position and the arm of the measured hand was unsupported and parallel to the body [60]. Indirect calorimetry was performed in the measurement of energy expenditure to assess energy needs by VMAX 2900 calorimeter. In particular, the calorimeter uses the amount of inspired and expired gas exchanges to calculate energy expenditure. The canopy system was used during the test with no compression mask in order to utilize a light comfortable device with no pressure or contact with the subject's face. The participant was placed in the supine position, free of physical and psychological stress, fasting and awake [61].

Blood glucose (Gly), total cholesterol (COL-tot), HDL cholesterol (Col-HDL), LDL cholesterol (Col-LDL), triglycerides (Try) were measured and monitored during the treatment.

3.1.5. Compliance

Compliance with the dietary intervention was assessed by monitoring the dietary intake at baseline and every month until the end of the study by Food Frequency Questionnaires [62].

Assessment of compliance with the physical activity was verified by asking subjects to complete a physical activity questionnaire [63]. The questionnaires confirmed that patients belonging to both groups carried out regular physical activity for the entire duration of the treatment.

Compliance with the nutraceutical supplementations were evaluated by the completion of a daily questionnaire asking each volunteer to record the time of consumption of the supplement. In addition, the number of capsules at the end of the study was recorded.

3.1.6. Sample size and statistical power

The sample size calculation was performed using MedCalc. The outcome for the calculation of sample size was the reduction in body weight in cinchona treated groups. A difference of 20% between C group and P group was estimated. Power and significance levels were set at 0.80 and 0.05, respectively. Using these parameters the estimated sample size was of 20 participants per group.

3.1.7. Statistical analysis

All data were expressed as mean ± standard error of the mean (SEM). Data were tested for normal distribution with the Kolmogorov-Smirnov test. Parametric tests (Student t test, ANOVA and Bonferroni post hoc test) or nonparametric tests (Wilcoxon, Mann-Whitney and Kruskal-Wallis tests) were used. Multivariate analysis of variance was not performed because of the small sample number. The statistical significance was set at $p < 0.05$.

Chapter 4:

Results

4.1 Nutritional status evaluation

All patients were accurately evaluated at baseline and were reconsidered after 30 and 60 days. At the end of the observations, the subjects belonging to C group presented significant changes in body composition compared to T0 and P groups (Table 4.1). Biochemical parameters were monitored and remained stable throughout the study. Patients recruited for this study did not show significant difference in biochemical parameters compared their T0 (Table 4.2) .

N°40	C group N°20 Male: n=9; Famale: n=11		P group N°20 Male: n=10; Famale: n=11	
	T0	T2	T0	T2
Age	51.5±1.5		48.7±1.5	
BW	94.8±1.1	85.8±1.1*	97.8±1.2	93.0±0.9
BMI	38.2±1.0	34,5±1.0*	36.7±0.4	34.9±0.3
WC	112.8±0.6	104.5±0.5*	100.2±1.2	97.8±1.1
HC	118.7±1.1	110.3±1.3*	122.7±0.4	118.3±0.4
FM (%)	37.9±0.9	31.7±1.4*	41.5±1.0	39.7±0.4
FFM (%)	62.1±0.7	68.2±1.0*	58.5±0.9	60.3±0.3
VAT	17.2±1.8	10.8±0.6*	14.6±0.5	13.4±0.8
SAT	30.7±1.1	24.3±0.8*	26.1±0.5	23.8±0.5*

Table 4.1: Anthropometric, body composition and abdominal fat distribution measurements at baseline and after 60 days of treatment. Data are reported as mean ± SEM. *p<0.05 T1 vs T0 values; § C group and P group did not show significant differences at T0.

Abbreviations: SEM, standard error of mean; T0, basal conditions; T2, after 60 days of treatment; BMI, body mass index (Kg/m2); WC, waist circumference (cm); HC, hip circumference; FM, fat mass (%); FFM, fat-free mass (%); VAT, visceral adipose tissue (mm); SAT, subcutaneous adipose tissue (mm).

	C group T0	P group T0
Glycemia (Gly)	92.0±0.8	88.0±0.6
Total cholesterol (COL-tot)	187.5±2.5	196.0±1.6
HDL cholesterol (COL-HDL)	67.5±2.4	77.6±2.5
LDL cholesterol (COL-tot)	102.4±4.6	112.3±1.7
triglycerides (Try)	87.2±0.8	58.0±0.7

Table 4.2: Biochemical parameters at T0. Data are reported as mean ± SEM.

Abbreviations: SEM, standard error of mean; T0, basal conditions; Gly, glycemia (mg/dL); COL-tot, Total cholesterol (mg/dL); COL-HDL, HDL cholesterol (mg/dL); COL-LDL, LDL cholesterol (mg/dL); Try, Triglycerides (mg/dL).

4.2 Cinchona group (C group)

Cinchona supplementation improved nutritional status of patients, inducing an average reduction of 9.6%±0.3 of Body Weight (94.8±1.1 vs 85.8±1.1 Kg, P=0.02, Figure 4.1 and 4.2) as well as an average reduction in BMI of 9.6%±0.3 (38.2±1.0 vs 34.5±1.0 Kg/m^2, P=0.04 Figure 4.3 and 4.4). Furthermore, waist circumference significantly decreased by 7.7%±0.4 after 60 days of treatment (112.8±0.6 vs 104.5±0.5 cm, P=0.02) as well as there was a decrease by 7.2%±1.2 of hip circumference (118.7±1.1 vs 110.3±1.3 cm, P=0.02) (Figure 4.5 and 4.6). Finally, bioimpedance analysis showed a significant reduction of 17.6%±2.3 in fat mass (37.9±0.9 vs 31.7±1.4 %; P=0.02) in C group after 60 days of cinchona supplementation. In contrast, FFM (62.1±0.7 vs 68.2±1.0 % P=0.02) appeared to be improved by 9.7%±1.2 (Figure 4.7 and 4.8).

It is interesting to observe the abdominal fat distribution; in particular, a significant reduction in VAT and SAT by 31.3%±2.8 and 19.2%±3.2, on the average, respectively, was observed (17.2±1.8 vs 10.8±0.6 mm and 30.7±1.1 vs 24.3±0.8 mm respectively P=0.001) (Figure 4.9 and 4.11).

After 60 days of treatment, C group showed no significant changes in basal metabolic rate as well as no significant alterations were observed in hand muscle strength.

4.3 Placebo group (P group)

After 60 days treatment, no significant changes in body weight (- 4.7%±1.3) (Figure 4.1 and 4.2) as well as in BMI (- 4.7%±1.3) (Figure 4.3 and 4.4), waist circumference (-2.4%±0.5) and hip circumference (-3.8%±0.9) were observed in patients belonging to P group compared with T0 subjects (Figure 4.6). Similarly, no significant variations in fat mass (-4.2%±8.2) and fat free mass (+ 3.9%±4.8) were detected after 60 days of hypocaloric diet (Figure 4.8). Furthermore, no significant change was observed in VAT (-8.6%±1.3); conversely, SAT showed a slight significant reduction by 8.6%±0.7 (26.1 ± 0.5 vs 23.8 ± 0.5 mm, P=0.007) (Figure 4.10 and 4.11).

No significant variations in basal metabolic rate and in hand muscle strength were detected in patients treated with placebo plus hypocaloric diet.

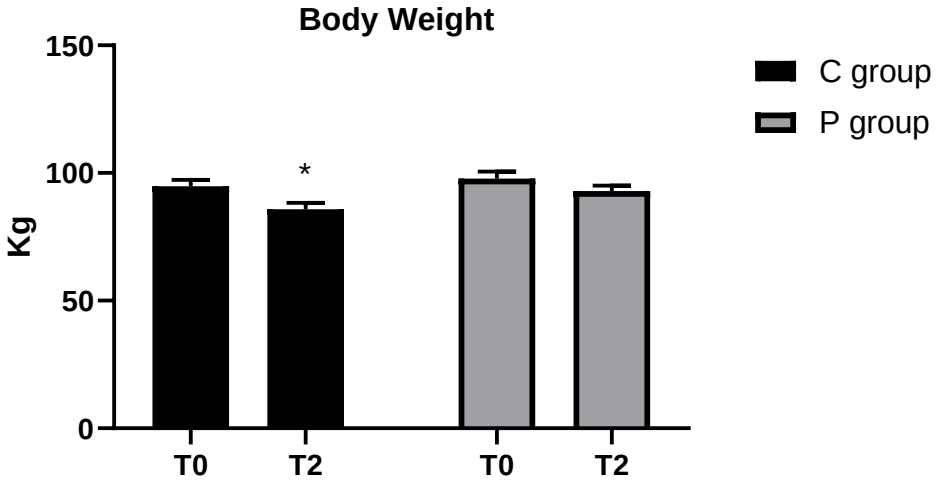

Figure 4.1: Variations in body weight (BW) in C group and P group after 60 days of treatment. Data are reported as mean ± SEM. *P<0.05 T2 vs T0.

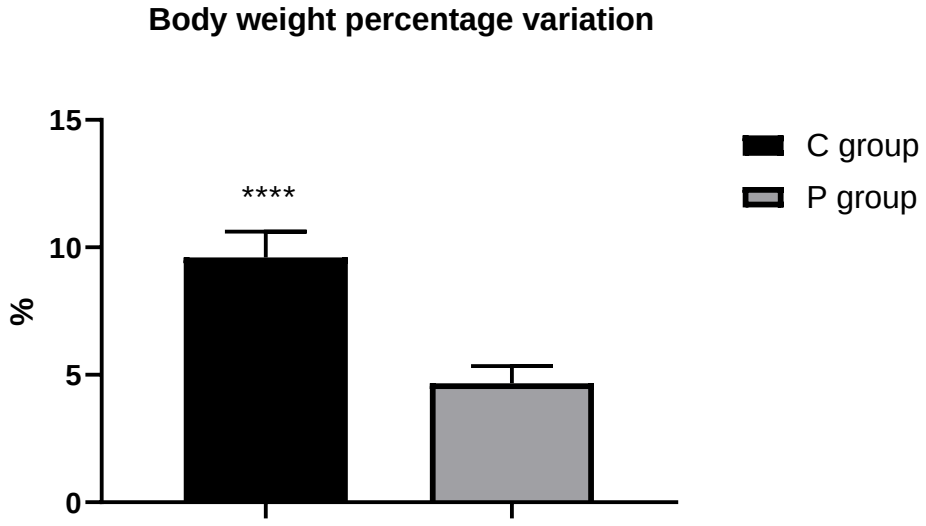

Figure 4.2: Body weight (BW) percentage variation in C group and P group after 60 days of treatment. Data are reported as mean ± SEM. ****P<0.0001 C group vs P group.

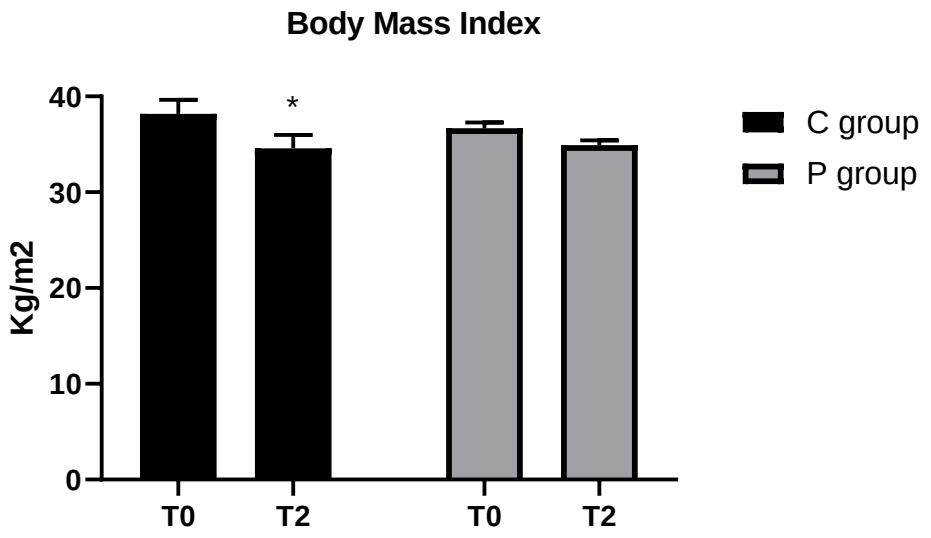

Figure 4.3: Variations in body mass index (BMI) in C group and P group after 60 days of treatment. Data are reported as mean ± SEM. *P<0.05 T2 vs T0.

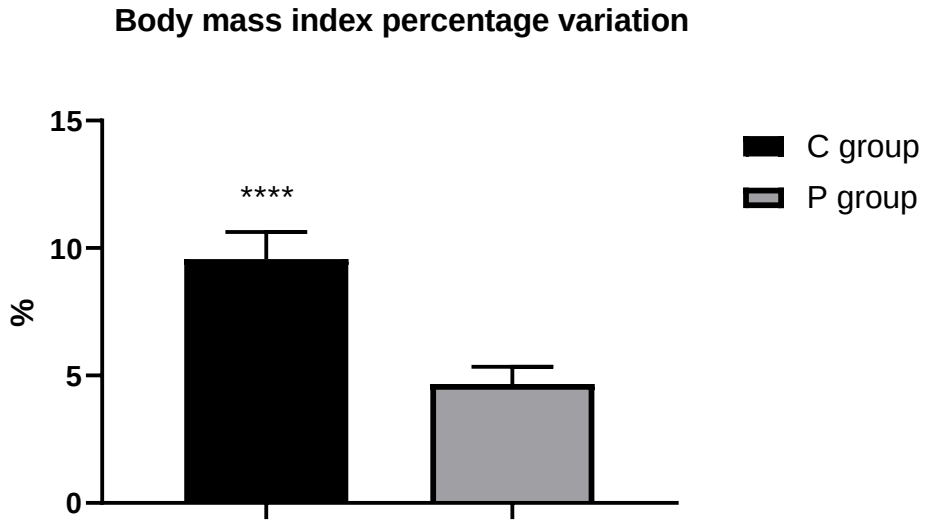

Figure 4.4: Body Mass Index (BMI) percentage variation in C group and P group after 60 days of treatment. Data are reported as mean ± SEM. ****P<0.0001 C group vs P group.

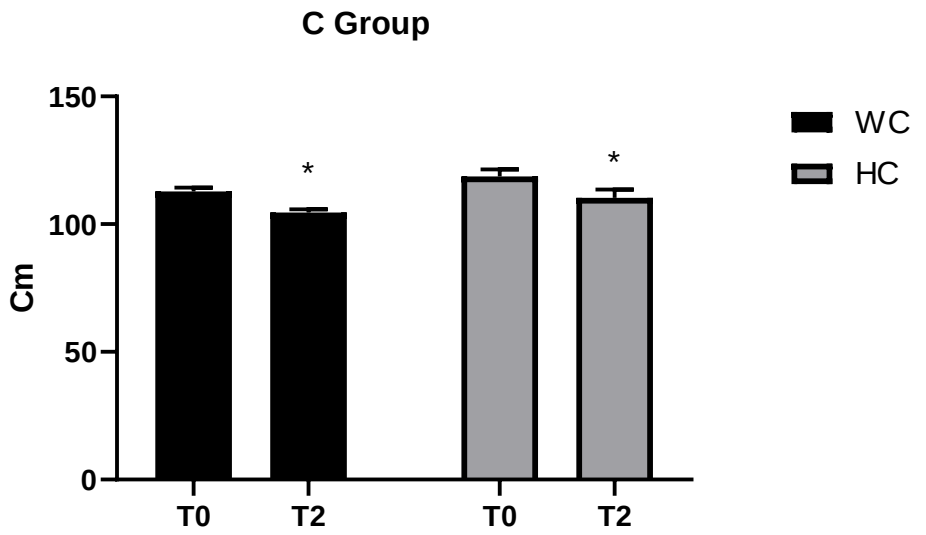

Figure 4.5: Variations in waist circumference (WC) and hip circumference (HC) in C group after 60 days of treatment. Data are reported as mean ± SEM. *P<0.05 T2 vs T0.

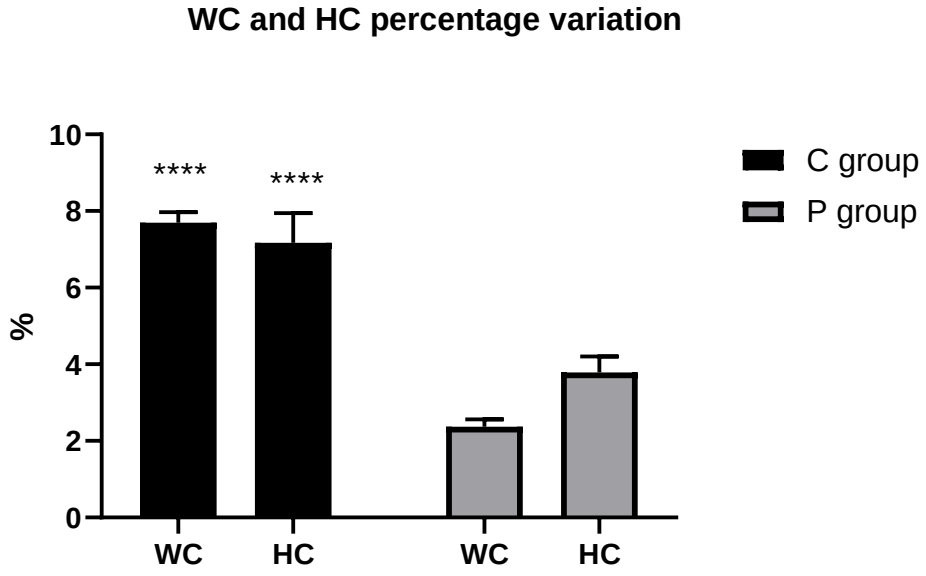

Figure 4.6: WC and HC percentage variations in C group and P group after 60 days of treatment. Data are reported as mean ± SEM. ****P<0.0001 C group vs P group.

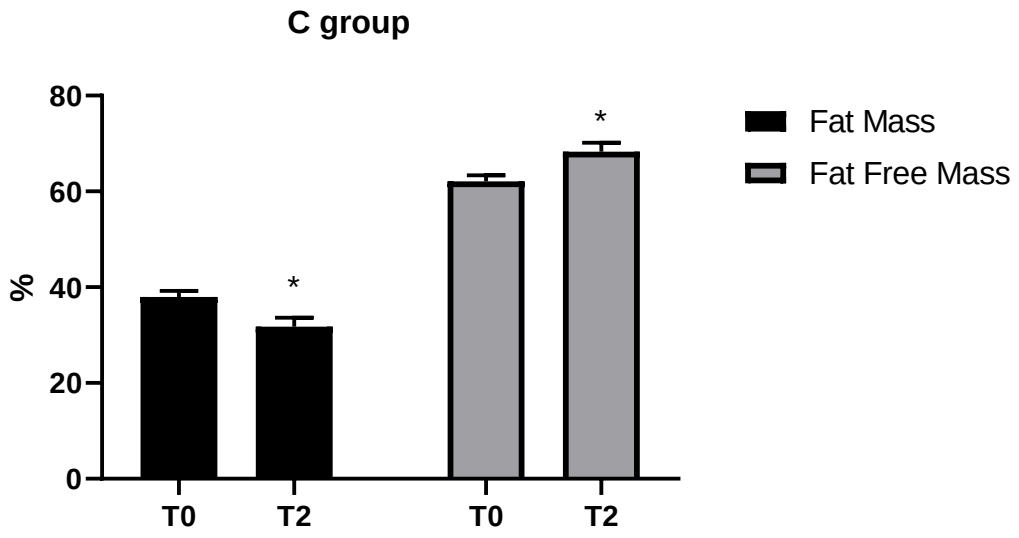

Figure 4.7: Variations in body composition in C group after 60 days of treatment. Data are reported as mean ± SEM. *P<0.05 T2 vs T0.

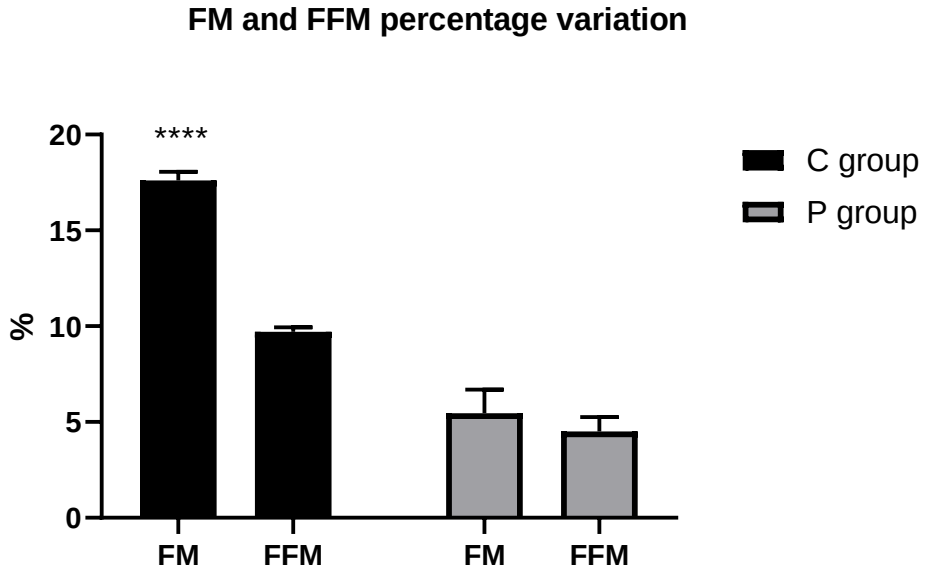

Figure 4.8: Body composition percentage variations in C group and P group after 60 days of treatment. Data are reported as mean ± SEM. ****p<0.0001 C group vs P group.

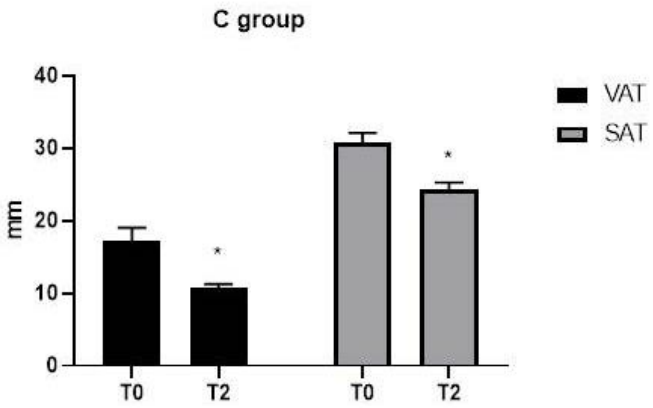

Figure 4.9: Variations in fat abdominal distribution in C group after 60 days of treatment. Data are reported as mean ± SEM. *P<0.05 T2 vs T0.

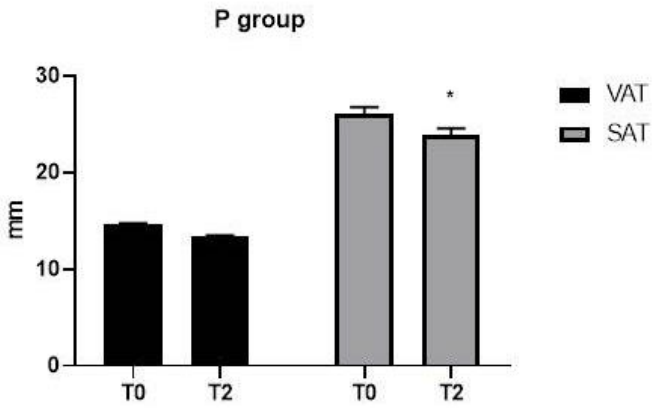

Figure 4.10: Variations in fat abdominal distribution in P group after 60 days of treatment. Data are reported as mean ± SEM. *P<0.05 T2 vs T0.

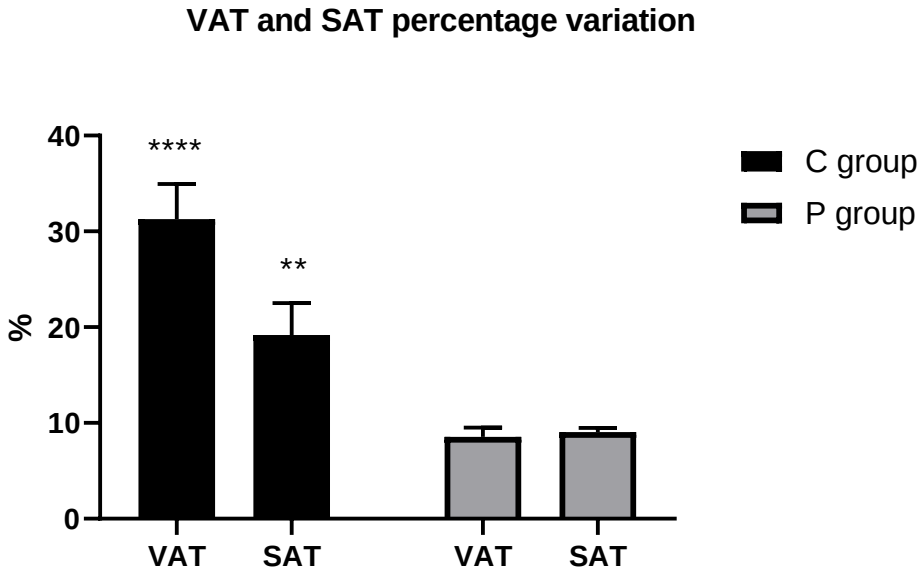

Figure 4.11: Fat abdominal distribution percentage variation in C group and P group after 60 days of treatment. Data are reported as mean ± SEM. ****p<0.0001 VAT C group vs VAT P group; **p<0.009 SAT C group vs SAT P group.

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Chapter 5:

Discussion and conclusions

The present results indicate that a supplementation of cinchona, a natural compound, was able to facilitate weight loss, improving the nutritional status and body composition in a population of obese subjects after 60 days of treatment. Interestingly, patients treated with cinchona, showed an improvement in nutritional status and body composition compared to subjects receiving placebo. In particular, this study demonstrates that the association of hypocaloric diet with a nutraceutical supplementation is able to induce weight loss higher than that obtained with a hypocaloric diet. These results are in agreement with a previous study by Jung et al., who investigated the effects of dietary cinchonine in a mouse model that exhibited adipogenesis and inflammation induced by the high-fat diet (HFD). In particular, their results showed that mice treated with 0.05% dietary cinchonine for 10 weeks presented a reduction in body weight as well as an improvement in blood parameters, such as triglycerides, cholesterol and glycemia, accompanied by an attenuation of proinflammatory cytokines production. It was suggested that cinchona could prevent obesity due to its effects on adipogenesis and inflammation [50]. Furthermore, the group C, treated with cinchona, showed a surprising reduction in fat mass by 17.6%±2.3, compared with placebo. This result is in agreement with a previous study by Cettour-Rose et al., who tested the effect of 0.1% quinine on mouse body weight and body composition. They showed that quinine, a cinchona alkaloid, belonging to the aryl-amino alcohol group of drugs, contributes to the control of body weight and fat mass without affecting food intake in male mice fed a balanced diet, representing a novel tool to counteract the obesity [64].

Furthermore, patients receiving cinchona revealed greater satiety throughout the duration of treatment, suggesting that nutraceutical treatment with cinchona may be able to modulate gastrointestinal (GI) functions, including intestinal hormones, leading to an increased satiety, followed by a reduction in energy intake. Our hypothesis is in agreement with previous

studies conducted on animal and human models which have shown that quinine is able to strongly reduce food intake [65, 66, 67]. In particular, in 2015, Andreozzi et al., in a double-blind crossover study, investigated whether bitter agonists affect food intake and intestinal hormone release in healthy subjects. Twenty healthy volunteers, indeed, were treated with an acid-resistant capsule containing placebo or 18 mg quinine hydrochloride (HCl), 60 minutes before an ad libitum meal, until to reach the satiety. Their data demonstrate that intra-duodenal release of quinine, a bitter taste receptor agonist, significantly reduces caloric intake, with a mechanism likely to involve cholecystokinin (CCK) release and its inhibitory actions on appetite [67]. In 2019, Iven J. et al., using magnetic resonance imaging and examining sensations related to appetite, plasma levels of gastrointestinal hormones, and hedonic food intake, evaluated brain responses at 10 µmol / kg quinine hydrochloride (QHCL) intragastrically administered in 15 healthy women. Their data demonstrated that intragastric QHCl reduces prospective and effective food intake in healthy women by interfering with homeostatic and hedonic brain circuitry in a ghrelin and motilin-mediated group, suggesting that bitter flavors are capable of reducing appetite and food intake, via the gut-brain axis [68]. However, further studies are needed to confirm our hypothesis, to clarify the involvement of cinchona in the control of food intake through the activation of bitter taste receptors (T2R). Moreover, it is important to define the modulation of the intestinal hormones release, such as CCK, and to assess the mechanism of action of this compound.

In conclusion, this study suggests that a hypocaloric diet combined with cinchona supplementation represents a valuable nutritional approach to induce weight loss and body composition change in obesity subjects.

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