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Title

The Effects of a Cinchona Supplementation on Satiety, Weight Loss and Body Composition in a Population of Overweight/ Obese Adults: A Controlled Randomized Study

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Abstract:

Obesity is a risk factor for several diseases present worldwide. Currently, dietary changes and physical activity are considered the most effective treatment to reduce obesity and its associated comorbidities. To promote weight loss, hypocaloric diets can be supported by nutraceuticals. The aim of this study was to evaluate the effects of a hypocaloric diet associated with *cinchona succirubra* supplementation on satiety, body weight and body composition in obese subjects. Fifty-nine overweight/obese adults were recruited, randomized into two groups and treated for 2 months. The first group (32 adults) was treated with a hypocaloric diet plus cinchona supplementation (the T-group); the second one (27 adults) was treated with a hypocaloric diet plus a placebo supplementation (the P-group). Anthropometric measurements as well as bioimpedance analysis, a Zung test and biochemical parameters were evaluated at baseline and after 60 days. T-group adults showed significant improvement in nutritional status and body composition compared to those at the baseline and in the P-group. Moreover, T-group adults did not show a reduction in Cholecystokinin serum levels compared to those of P-group adults. In conclusion, our data demonstrate that a hypocaloric diet associated with cinchona supplementation is effective in inducing more significant weight loss and the re-establishment of metabolic parameters than those obtained with a hypocaloric diet.

Keywords: obesity; cinchona; nutraceutical treatment; body composition analysis; gut hormone; bitter tast.

A tutte quelle donne che,

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1 Introduction

1.1 Obesity

Obesity is a chronic disease with a multifactorial etiology that is prevalent worldwide, currently affecting over a third of the world's population [1]. Globally, it is estimated that around 605 million people are obese, and more than 1.9 billion adults are overweight [2,3].

The obesity rate has increased dramatically in both males and females across all age groups, with a proportionally higher prevalence in older individuals and women. Obesity increases the risk factors for many pathological conditions, such as cardiovascular diseases, hypertension, infertility, diabetes mellitus and dyslipidemia [4, 5].

The World Health Organization (WHO) has defined obesity as an abnormal or excessive accumulation of fat that represents a health risk, as indicated by a body mass index (BMI) ≥ 30 kg/m^2, while a body mass index (BMI) over 25 kg/m^2 is considered overweight [6].

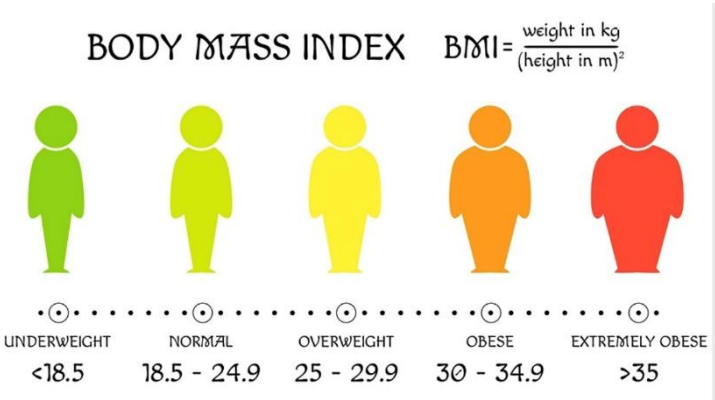


Fig.1: Body Mass Index

Previous studies have shown that a weight loss of 5–10% versus baseline in subjects, with obesity associated with other comorbidities, can significantly improve health and counteract mortality [4]. The etiology of obesity is multifactorial, involving, indeed, factors both hereditary and genetic, family history, socioeconomic and sociocultural as well as social and environmental factors.

These are primarily characterized by a sedentary lifestyle and unhealthy eating habits, but also psychological and behavioral factors [7-12].

Moreover, obesity is defined as a condition of chronic inflammation that can have a significant impact on mental health and overall well-being [13].
Currently, obesity has reached global epidemic proportions in both adults and children, resulting in a significant financial burden on healthcare.
This is an exponentially increasing condition, with estimates projected to rise in the coming decades if preventive strategies are not implemented. It has indeed been estimated that 1.5 billion people worldwide are obese and, if this trend is not reversed, an additional 167 million people will be obese by 2025.

1.2 The appetite regulation mechanism

The appetite regulation mechanism is a complex process that involves the interaction of various signals and circuits in the body, including the central nervous system, hormones and the gastrointestinal system. Metabolic homeostasis proceeds through the regulation of the stimuli for satiety or appetite. Our body has developed mechanisms to regulate the sense of satiety or appetite based on the organism's energy conditions. This regulation involves the central nervous system as well as a series of peripheral stimuli, originating, for example, from the gastrointestinal tract or metabolic organs such as adipose tissue. These stimuli release factors, such as Leptin or other intestinal peptides, informing the brain about the metabolic state of our organism. Therefore, the control of food intake involves signals collected from the periphery (adipose tissue, stomach, intestine) processed at the hypothalamic level and integrated at the cortical level, determining eating behavior and, consequently, the intake or avoidance of food

1.3 The Role of Hormones in Appetite and Weight Regulation

The central nervous system (CNS), particularly the hypothalamus, plays a pivotal role in the regulation of appetite and energetic homeostasis in response to changes in peripheral circulating signals, such as hormones and nutrients. The reception and integration of these signals primarily occur in the arcuate nucleus (ARC), housing two distinct populations of neurons: one releasing agouti-related protein (AgRP), and the other releasing proopiomelanocortin

(POMC). These neurons participate in the intricate regulation of energy homeostasis, perceiving and integrating a myriad of metabolic signals [14].
Observations in the hypothalamus indicate that leptin binds to its receptor (LepR-b), initiating a signaling pathway that induces STAT3 stimulation, subsequently activating POMC neurons and inhibiting NPY/AgRP/GABA neurons, resulting in an anorexigenic effect. Leptin stands out as a key component in the physiological system governing body weight regulation [15]. Moreover, substantial evidence suggests that insulin binding to the insulin receptor substrate (IRS) produces a similar anorexigenic effect as leptin, activating a phosphorylation cascade through phosphatidylinositide-3-kinase (PI3K) in POMC neurons [16].
Tyrosine–tyrosine polypeptide (PYY) and glucagon-like peptide 1 (GLP-1), produced by L cells in the small intestine, contribute to an anorexigenic effect. Following food ingestion, PYY plasma concentration increases, signaling food intake from the intestine to the appetite-regulating system in the brain. PYY exerts its effects by binding to its Y2 receptor [37]. Conversely, GLP-1 binds to a membrane receptor, GLP-1-R, whose activation stimulates cyclic adenosine monophosphate (cAMP) production by adenylyl cyclase. This mechanism initiates the transmission of intracellular signaling [17].

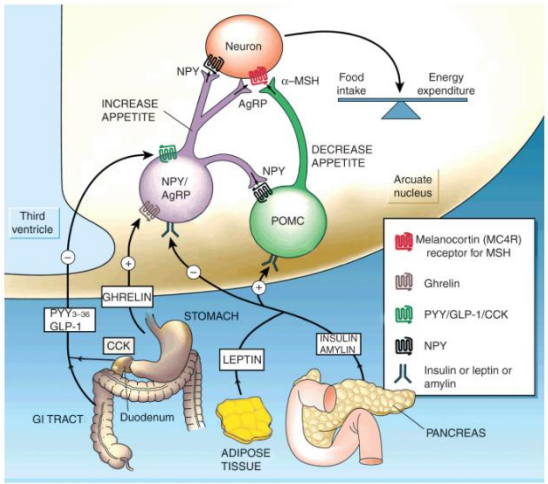


Fig.2: Neuroendocrine Regulation of Appetite and Body Weight.

1.3.1 Gastrointestinal System in Appetite Regulation:

Gut hormones play an important role in the regulation of energy balance; they, indeed, modulate feeding behavior as well as energy expenditure and nutrient partitioning [18]. Our body is capable of rapidly adapting to the sensation of satiety through synaptic plasticity, signifying the neurons' ability to promptly adjust to changes in environmental conditions and external stimuli. While the regulatory center for appetite is localized in the hypothalamus, molecules released in the intestine can act as mediators for chemoreceptors, mechanoreceptors and osmoreceptors, generating both long-term reflexes mediated by the central nervous system (CNS) and autonomic nervous system (ANS) and shorter reflexes acting through the enteric nervous system, whose regulation is nonetheless influenced at the central level.
In the context of short-term regulation of the gastrointestinal system, various phases are distinguished:

- ***Cephalic control phase***: represents the initial contact with food. Sight, smell and touch induce the CNS to send preparatory signals for food intake, mediated by both the central nervous system and the autonomic nervous system.

- ***Gastric control phase*****:** depends on stimuli from the stomach, such as wall distension, which increases gastrin secretion and acidity.

- ***Intestinal control phase*****:** depends on stimuli from molecules in the diet, stimulating intestinal osmoreceptors and mechanoreceptors. Pancreatic juice is released in response to intestinal stimuli, while acidity in the duodenum activates intestinal chemoreceptors. Both long and short-term reflexes induce the release of secretin, which, in turn, stimulates pancreatic duct cells to release bicarbonate-rich pancreatic juice, reducing duodenal acidity.

Since regulation involves both central and intestinal stimuli, the role of intestinal hormones such as cholecystokinin, ghrelin and secretin is crucial for regulating pancreatic juice production, necessary to neutralize acidity from the stomach. The synthesis and release of these hormones are regulated by the amount of protein and fat digestion products present in the duodenum.

Stomach distension during food intake sends signals to the brain indicating satiety. This, in turn, can be inhibited or stimulated by hormonal signals.

1.3.2 Secretion of Gastrointestinal Hormones

Hormones such as CCK and peptide YY (PYY) are released during digestion to signal satiety. These hormones produced by the intestine communicate with tissues both internally and externally and play a fundamental role in the regulation of metabolic homeostasis. Of particular importance are: Ghrelin, released by enteroendocrine cells called Gr in the stomach; Cholecystokinin (CCK) and Peptide YY (PYY), released by cells mainly located in the upper small intestine; Glucose-Dependent Insulinotropic Polypeptide (GIP), released by K cells (mostly in the upper small intestine); Glucagon-Like Peptide-1 (GLP-1); hormones released by the pancreas, such as Insulin and Abscisic Acid; hormones released by adipose tissue, such as Leptin. The release of these hormones into the blood determines their arrival to the central nervous system, modulating the metabolic conditions of the organism.
Experimental and human studies have demonstrated that cholecystokinin (CCK) reduces food intake dose-dependently upon meal initiation. CCK, synthesized in both the gastrointestinal tract and hypothalamus, emerges as the most abundant neuropeptide in the CNS. At the hypothalamic level, CCK binding to the CCK-B receptor and interacting with NPY induce satiety. CCK has been explored as a potential therapeutic substance for managing obesity [19].

- ***Cholecystokinin (CCK)*** consists of various forms, with the most common being CCK-8, released by I cells in the intestinal mucosa of the duodenum and part of the jejunum. CCK acts through two main types of receptors: CCK1 (formerly known as CCK-A), predominantly found in the periphery, and CCK2 (formerly known as CCK-B), active in the central nervous system. Therefore, CCK acts on two types of receptors, located both in the intestine and in the nervous system. Its release occurs primarily in the presence of free amino acids or after a meal rich in fats or proteins.

The main role of CCK-A is to activate specific receptors, located on gastric parietal cells, to initiate the secretion of hydrochloric acid in the stomach. Additionally, it improves the digestion and absorption of nutrients by stimulating the contraction of the gallbladder, leading to the release of bile, essential for the digestion and absorption of fats and fat-soluble vitamins. Cholecystokinin also acts on the pancreas, stimulating the release of digestive enzymes for fats, proteins and carbohydrates. Elevated blood concentrations of cholecystokinin can be observed 15 minutes after the start of a meal and persist for up to three hours.
The effect of the tetrapeptide CCK-B is to regulate appetite by slowing gastric emptying, contributing to the feeling of satiety and by activating receptors mainly located centrally: in the cortex, basal ganglia, striatum, mammillary bodies and nucleus accumbens.
A study in rats demonstrated that following the administration of CCK-B antagonists or a genetic defect in its transcription, obesity developed in rats due to excessive food consumption (Covasa et al. 2001).

- ***Ghrelin***: On the contrary, ghrelin binds to its receptor, the growth hormone secretagogue receptor (GHSR), inducing the activation of AgRP neurons and inhibiting POMC neurons, leading to orexigenic effects [20].
  Secreted by the stomach, ghrelin is known as the hunger hormone and stimulates appetite. It is a hormone produced at the gastric level by P/D1 cells and epsilon cells of the pancreas, which acts centrally by activating orexigenic neurons for the production of NPY. Ghrelin is secreted in fasting conditions, with higher plasma concentrations observed before a meal (fasting), and decreasing after food intake. It plays a crucial role in regulating appetite and energy balance. Its main function is to stimulate hunger, regulate food intake, and accelerate gastric emptying. When the stomach is empty, ghrelin is released into the bloodstream and acts on the hypothalamus, triggering a series of stimuli that lead to increased appetite and promote food intake.
  In addition to its roles in increasing appetite, ghrelin also influences other physiological processes. Indeed, ghrelin can increase gastric motility and acid secretion, preparing the stomach for the entry of food.

Furthermore, ghrelin can affect blood glucose levels by influencing insulin secretion, glycolysis, and gluconeogenesis.

Moreover, at the rewarding circuit level, ghrelin increases the release of dopamine, a neurotransmitter that has a strong link for food, encouraging an individual to seek it out.

Levels of this hormone fluctuate throughout the day. Eating clearly reduces ghrelin concentration, but the decrease varies depending on what is consumed: carbohydrates and proteins act to limit ghrelin concentrations to a greater extent than fats, resulting in a sense of satiety in less time. Ghrelin levels, on the other hand, increase following a low-calorie diet, explaining why it can be challenging to maintain weight loss results

Ghrelin, CCK, GLP-1 and PYY: they contribute to three presumed motivational processes that provide unconditional basic control over the initiation and size of meals:

- Hunger, which refers to the process of acquiring energy for obtaining food and initiating a meal;
- Satiety, which leads to meal termination;
- Postprandial satiety, which inhibits food intake after meals and extends the interval among meals [21].

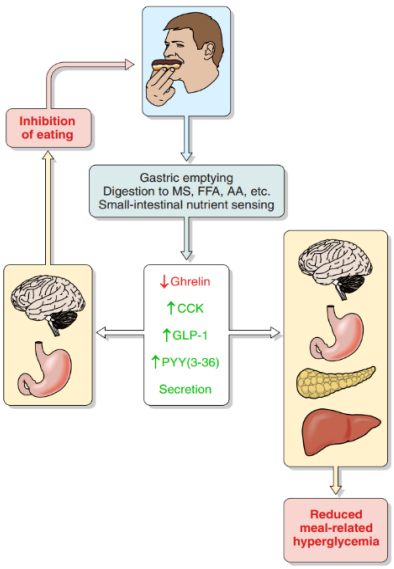


Fig.3: Overview of the ghrelin, CCK in the control of eating and meal-related glycemia

1.4 The eating behavior: Psychological and Environmental Influences

The interaction between the central and peripheral systems of our organism is fundamental for the regulation of food intake. Appetite and satiety are not solely regulated by stimuli from the Central Nervous System (CNS) but also by stimuli originating from the gastrointestinal tract, external and sensory stimuli, which are grouped into homeostatic and hedonistic regulatory stimuli, whose regulation underlies eating behavior. Through complex mechanisms, these two regulatory systems guide energy intake and expenditure.

The regulation of food intake involves the integration of signals from the periphery of our organism, such as adipose tissue, stomach, and intestine. These signals, processed at the hypothalamic level and integrated at the cortical level, determine a behavior of food-seeking (resulting in intake) or food avoidance. The hypothalamus is a brain area involved in various functions, including the regulation of metabolism, secretion of peptide hormones, etc., acting on three systems: the endocrine system, the autonomic nervous system and the system of motivations and emotions, better known as the rewarding circuit.

Its central location, between the two cerebral hemispheres, is strategic, as each hypothalamic nucleus is closely correlated with important brain areas. This interconnected system is fundamental for the regulation of eating behavior through anorexigenic and orexigenic circuits (Morton et al., 2006).

Psychological and environmental factors can influence appetite. Stress, negative emotions or eating habits can lead to altered appetite regulation.

The appetite regulation mechanism is a dynamic and interconnected system that adapts to physiological, metabolic and environmental conditions. Dysregulations in this system can contribute to conditions such as obesity or eating disorders.

Metabolic regulation is not as straightforward; if it were, when one eats enough or in abundance, the signals that transmit the sense of satiety to the brain should immediately interrupt the appetite stimulus. This phenomenon does not always occur because the system is more complex, involving not only homeostatic signals but also hedonistic and rewarding impulses. This is often associated with weight accumulation and the development of conditions such as overweight and obesity.

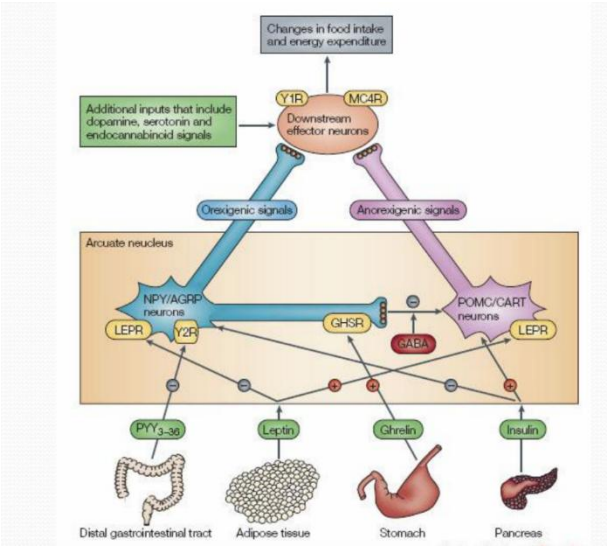
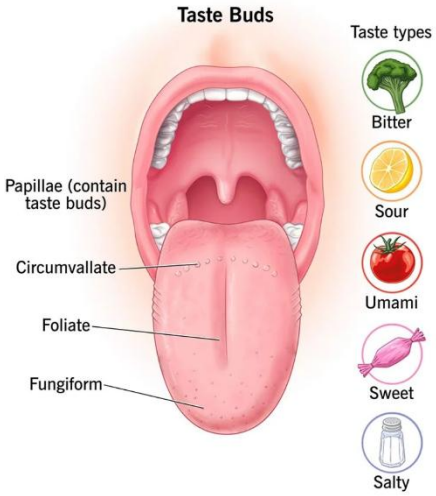


Fig.4: Peripheral signals that reach the hypothalamic nuclei and modulate food intake regulation

1.5 The Taste

Taste is one of the five senses, allowing us to detect everything we eat and drink. Cells programmed to detect specific tastes have highly specialized sensory receptors. The collection of these cells forms taste buds located within the taste papillae, situated on the tongue and soft palate. Their task is to integrate primary tastes through specific receptors positioned in distinct areas of the tongue, eventually developing more complex taste sensations. On the tongue, distinct regions can be discerned, each dedicated to the perception of specific taste modalities, including bitterness, saltiness, sweetness, umami (pertaining to the detection of savory substances such as L-amino acids and ribonucleotides), and acidity. The taste receptors are a class of receptors associated with G proteins (G-protein coupled receptors), not confined solely to the oral cavity, but also found in other epithelial tissues. This is why agonist molecules of these receptors can activate them in other epithelial tissues where they are



expressed. The ability of gastrointestinal cells to detect molecules plays a crucial role in various stages of digestion. It has been found that this capability is involved in hormonal activation pathways that regulate food intake, as well as pancreatic secretion and gastrointestinal motility, influencing the amounts of nutrients absorbed [22].

Taste perception provides information about the chemical characteristics of substances that come into contact with the taste cells of the oropharyngeal mucosa. Taste cells, indeed, serve as the gustatory sensory receptors capable of interacting with chemical molecules dissolved in saliva and transducing the informative content of the chemical stimulus into nerve signals. The majority of taste stimuli consist of hydrophilic, non-volatile molecules, including salts, acids, sugars, amino acids, and proteins. The taste of a substance is a characteristic dependent on its chemical composition. It is believed that the various hundreds of perceptible flavors arise from the combination of five primary qualities: sweet, salty, bitter, sour, and umami.

1.5.1 Diverse Roles of Taste Receptors: From Chemical Sensation to Gut Hormone Regulation

Primary tastes differ not only in the chemical nature of the compounds that evoke them but also in the reception and signal transduction mechanisms that occur at the level of taste receptors. Taste receptors or taste cells are specialized epithelial cells organized into structures called taste buds. Taste buds contain several dozen sensory receptors, in addition to immature cellular elements (basal cells). The reception and transduction mechanisms occur in different ways depending on the chemical nature of the substance [23-25].

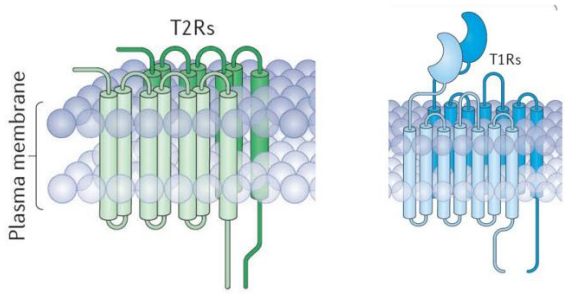


Fig.5: GPCR receptors for bitter and sweet taste

The reception of chemical stimuli occurs through the interaction of these stimuli with transmembrane proteins with seven alpha-helical domains that are coupled to a heterotrimeric G protein called gustducin. These proteins belong to two main classes or families: the TAS1R (T1R) family and the TAS2R (T2R) family. The receptors interact with various organic molecules and signal transduction mechanisms are mediated by different receptors depending on the nature of the chemical substance.

The existence of T1R receptors in enteroendocrine cells in the stomach is responsible for regulating digestive functions. Their activation leads to the formation of incretins, such as GLP-1, which, in turn, promotes the release of insulin from pancreatic beta cells. Additionally, T1R receptor activation increases the transcription and expression of the glucose transporter GLUT2 and the Na+/glucose cotransporter SGLT-1 in the apical portion of intestinal epithelial cells, facilitating glucose absorption (Mace et al., 2007; Margolskee et al., 2007).

While the function of bitter taste receptors (T2R) in the gastrointestinal tract is less clear, recent studies using classic bitter substances, such as phenylthiocarbamide, have demonstrated that their stimulation results in an increase in intracellular calcium and the release of the hormone cholecystokinin (CCK). CCK is involved in the digestive process, capable of reducing gastric motility and increasing the sense of satiety through action on CCK1 receptors located on the sensitive fibers of the vagal nerve controlling food intake. This supports the crucial role of the TASR2 receptor in increasing satiety through the release of CCK. Recently, it has been shown that gut hormone secretion can be regulated by G-protein-coupled taste receptor (TAS2R) family 2, a family of taste receptors activated by bitter molecules.

For example, basic toxic substances activate T2R, which, through the release of cholecystokinin, slows the passage of the toxic substance from the stomach, reduces hunger stimulation and protects the body from the toxic effects of the substance in question (Glendinning et al., 2008). Furthermore, in the colon, activation of T2R by toxins results in increased water and anion secretion, leading to liquid release in the intestine and subsequent diarrhea (Kaji et al., 2009).

Evolutionally, TAS2R has played important roles allowing organisms to detect potentially harmful substances and to avoid their ingestion [8]. Being allocated in the gastrointestinal tract, it plays important roles in intestinal chemoreception

coupling bitter molecules binding to gut hormone secretion (Rozengurt et al., 2007).

The expression of TAS2Rs varies among people and there exist interindividual variations associated with gut hormone secretion upon taste receptor stimulation [80, 81]. Previous in vivo studies indicate that the stimulation of TAS2R family members expressed by enteroendocrine cells (ECCs) is effective in inducing the release of gastrointestinal peptides, such as CCK and GLP-1, which are involved in the control of the sense of hunger and satiety, and sequentially of food intake.

CCK hormone, released by the duodenal I cell after the consumption of a meal rich in lipids, is the most studied peptide up to date that is able to inhibit the sense of hunger, mediated by CCK1 receptors on vagal afferents [26, 27].

1.6 Genetic Variability and Individual Sensitivity in Bitter Taste Perception

The bitter taste is mediated by G protein-coupled receptors belonging to the TAS2R subfamily of the taste cell membrane [28, 29]. These receptors present 7 transmembrane domains with alpha-helical structure and a conserved amino acid residue. Humans possess approximately 25 TAS2R receptors for bitterness, encoded by gene clusters located on chromosomes 5p, 7q, 12p, and so far, about 550 molecules, capable of binding these receptors, have been identified [30, 31]. However, this number represents only a small fraction of the thousands of bitter-tasting compounds of plant origin that exist in nature. Since the number of compounds significantly exceeds the number of receptors, it appears likely that individual receptors respond to more than one type of bitter chemical [32]. It is known that some receptors respond to a limited number of compounds, while others respond to a wide range of chemicals [33]. There are genetically inherited individual differences in the perception of bitter taste reflecting the rich allelic diversity of TAS2R receptors [34]. Variations in the sequence of the TAS2R19 gene have been associated with differences in the ability to perceive the bitter taste of quinine [39]. Individuals can be classified as tasters or nontasters based on their sensitivity to PROP (6-n-propylthiouracil (PROP) test, which is used to assess individual sensitivity to bitter taste) and to PTC (Population Cross-Sectional Tasting, a term that might be used in studies or research on taste preferences within a population).

1.7 Regulation of Gastrointestinal Hormones and Energy Intake: Implications for Obesity Management

Food intake and/or the ingestion of bitter substances can result in an increase in the release of CCK and a reduction in hormones, such as ghrelin and motilin, causing a decrease in gastrointestinal motility, in the hunger sense and, finally, in food intake [35].

The existence of T1R receptors in enteroendocrine cells in the stomach is responsible for regulating digestive functions. Their activation leads to the formation of incretins, such as GLP-1, which, in turn, promotes the release of insulin from pancreatic beta cells. Additionally, T1R receptor activation increases the transcription and expression of the glucose transporter GLUT2 and the Na+/glucose cotransporter SGLT-1 in the apical portion of intestinal epithelial cells, facilitating glucose absorption (Mace et al., 2007; Margolskee et al., 2007).

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The effects on the secretion of gastrointestinal (GI) hormones and consequently on the slowing of gastric emptying play an important role in regulating energy intake, representing a new approach to the management or prevention of obesity and its comorbidity [36-38].

1.8 Non-pharmacological approaches to obesity: Nutraceuticals

Considering the clinical framework, behavioral aspects and associated risks, obesity and its treatment pose a significant challenge for modern medicine. In accordance with the guidelines of international societies committed to combating obesity, it is crucial to carefully consider all individual approaches for patients, taking into account factors such as age, gender, comorbidities and socioeconomic status (Lingvay I. et al., 2022). Appropriate behavioral methods, based on dietary modifications and increased physical activity, form the foundation of therapeutic options for obesity.

Studies have been conducted to assess the effectiveness of various types of diets, including vegan, low-calorie ketogenic and Mediterranean diets. The adoption of a low-calorie diet with balanced proportions of carbohydrates, proteins and fats has proven effective in the dietary treatment of obesity (Wiechert M et al., 2022). However, the change in habits and lifestyle, though crucial and decisive, is a lengthy and challenging process that requires considerable dedication from patients (Chao A.M et al., 2021). Often, even after achieving goals, it can be difficult to maintain them over time due not only to the recurrence of old habits but also to altered hormonal mechanisms, particularly following particularly restrictive diets.

In this regard, there are studies on nutraceutical products that suggest how the integrated approach of diet, physical activity and the use of nutraceuticals can represent a potential strategy against obesity.

As of now, there is a collective focus on identifying treatments that can combat or prevent obesity while promoting and safeguarding individual health. Nutraceutical compounds, derived from natural products found in food sources, are currently under investigation for their beneficial activities in various diseases. They represent a safer alternative or supplement to conventional therapy, which often involves a combination of pharmaceutical agents that can be ineffective and accompanied by numerous side effects [40, 41].

The term "Nutraceutical" was coined by by Stephen De Felice in 1989, combining the words "nutrition" and "pharmaceutical". Nutraceuticals encompass foods or components of food that possess beneficial health properties. These compounds appear to have a positive impact on health, either in preventing or treating one or more pathologies [42]. Classified based on characteristics such as chemical composition, origin, or pharmacological form

of administration, nutraceuticals can be categorized into nutrients, herbals, or dietary supplements [43].

These compounds have demonstrated anti-inflammatory, antioxidant, immunoregulatory and modulation of intracellular signaling pathway activities [44]. Notably, certain studies have indicated that the use of nutraceuticals may regulate food intake (intestinal-brain axis), energy expenditure (energy homeostasis-thermogenesis), lipid metabolism (lipase inhibition), and the life cycle of adipocytes (adipocytokines from abnormal adipocytes) through epigenetic or genetic mechanisms [45].

Further research is essential to elucidate the effects of nutraceuticals in preventing obesity and its complications, as well as determining the safest dosage, treatment duration and bioavailability. However, relatively recent studies are focusing on the association between taste and energy intake

1.9 Cinchona

Cinchona, a genus of flowering plants from South America and a member of the Rubiaceae family, has a history of widespread use in pharmacology due to its anti-malarial, analgesic and anti-flu properties [54-59]. The bark of certain species, particularly Cinchona officinalis, is valued as a natural source of quinine. The bark, up to 30 cm long and 2-6 cm thick, is the primary medicinal component of the plant. Cinchona is renowned for its alkaloid content, with chichonine exhibiting higher activity compared to other compounds. Notable alkaloids include quinine, quinidine and cinchonidine. Historically, these alkaloids were utilized in the treatment of malaria and other ailments [46]. Recent studies have unveiled additional beneficial activities of these alkaloids, including antiobesity, along with antitumor, antioxidant, anti-inflammatory and antimicrobial properties [47].

- *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 [48,49].

- Antitumor property: Studies have shown that quinine is able to inhibit cell proliferation and induce apoptotic cell death in cancer, suggesting that quinine may be used as an anticancer agent in the future [50].
- *Antioxidant property*: The cinchona bark also contains compounds such as phenols, flavonoids and phytosterols able to exert antioxidant activity [51].
- *Anti-inflammatory 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 [52].
- *Antimicrobial activity*: Some studies have shown that cinchona was effective in the treatment against many microorganisms harmful to humans[53]

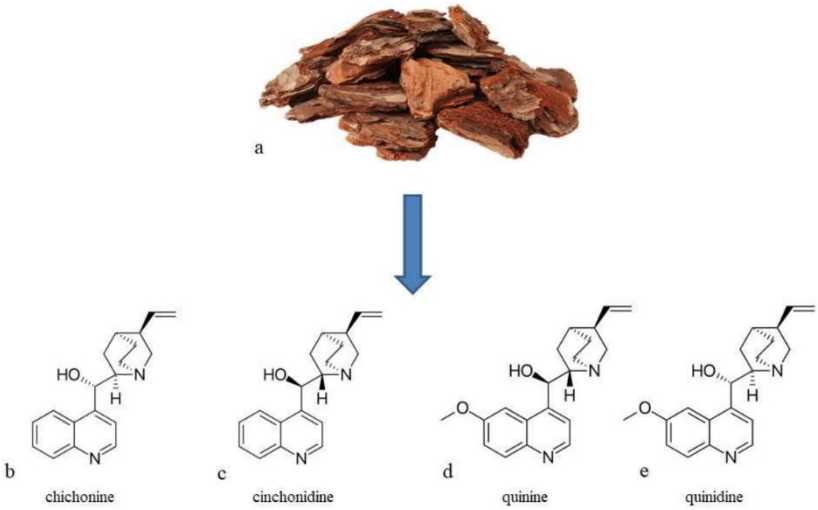

Fig.6: a) Cinchona bark; b) Chemical Formula of Chinconine; c) Chemical formula of Cinchonidine; d) Chemical formula of Quinine e) Chemical formula of Quinidine

1.10 Exploring the Role of Cinchona, between Pharmacophoric Similarity and Regulation of Satiety and Obesity

The choice of quinine is associated with its pharmacophoric similarity to a substance already widely used, melatonin. Melatonin is a derivative of the amino acid tryptophan and is the primary neuroendocrine product of the pineal gland, present both in the CNS and widely distributed in the gastrointestinal system. Until a few years ago, melatonin was exclusively considered a hormone that regulates circadian rhythms and seasonal biorhythms. However, today we know that this represents only one of its characteristics, as an increasing number of studies highlight its anti-inflammatory, antioxidant, immunomodulatory, thermoregulatory and antitumor properties (Ortega-Peña et al., 2021).

Melatonin is one of the substances capable of activating bitter receptors in the gastrointestinal tract, which, through hormonal stimulation, modulate and influence digestive processes and food intake. In vitro observations on gastric and duodenal tissue have shown the existence of endogenous mediators capable of controlling the metabolic cycle of two TAS2R receptor subtypes. This has led to the hypothesis that the ligand of duodenal TAS2R receptors could be represented by melatonin.

Melatonin, along with its derivatives, is structurally very similar to some bitter compounds, such as quinine. The binding of melatonin to TAS2R receptors stimulates the release of CCK and clarifies the mechanism by which hunger stimuli are inhibited during sleep and in the presence of elevated circulating melatonin levels. Given the characteristics of melatonin on food intake, it was possible to highlight through a similarity search screening how the alkaloids present in Cinchona bark (quinine, quinidine, cinchonine and cinchonidine) share the pharmacophoric portion with melatonin.

These compounds all have a suitably functionalized ring portion that overlaps with the pharmacophore of melatonin, suggesting that they could act as duodenal TAS2R receptor agonists. Several studies on quinine and melatonin have also shown that quinine administration increases CCK levels in CCK-secreting cells and that when quinine administration is integrated with melatonin administration, the released CCK levels are even higher (Tenore et al., 2018). This synergy between quinine and melatonin, well-demonstrated in

vitro, is thought to be due to a crosstalk interaction between MNTR and TAS2R receptors.

Historically used for malaria treatment, quinine has also found its way into food products over time as a bitter substance. In the pursuit of effective strategies to manage and combat obesity, researchers have delved into the intricate mechanisms governing satiety, the feeling of fullness or satisfaction after eating. Understanding these mechanisms is crucial, as they play a pivotal role in regulating food intake and, consequently, body weight. One intriguing avenue of exploration in the context of satiety and obesity is the potential influence of cinchona for its possible role in modulating appetite and satiety.

The initial investigation associating quinine with metabolism was documented in 1973. This study revealed that the inclusion of quinine in the diet resulted in a reduction in body weight of male rats with lateral hypothalamic lesions versus intact male rats (keesey et al., 1973). Following this, numerous subsequent studies corroborated a notable decrease in both food intake and body weight among rats that consumed a diet supplemented with quinine.
As the scientific community delves deeper into the intricate connections between satiety and cinchona, there is a promising prospect for novel approaches to address the complex challenges posed by obesity.
To date, the common interest is to find a treatment that can counteract or prevent obesity, promoting and protecting individual health. A large amount of data indicate that a healthy diet and an active lifestyle play a key role in the prevention and treatment of obesity [61]. In particular, several dietary supplements, commonly called nutraceuticals, have been shown to have health benefits [62, 63]. Furthermore, nutraceutical use associated with an appropriate diet and daily physical activity can represent an effective treatment for body weight loss and metabolic syndrome [54, 64].

2 Aim of the study

The aim of the present study was to assess the effects of a nutraceutical supplementation with *cinchona succirubra* (cinchona), a bitter taste agonist, on a population of overweight/obese adults.
Previous studies demonstrate the beneficial effects of dietary cinchonine in preventing obesity [25]; in particular, our purpose was to evaluate nutraceutical effects on satiety, weight loss, changes in body composition and nutritional status in a group of overweight/obese adults.

This investigation seeks to contribute to the evolving landscape of obesity research, providing potential insights that could inform innovative strategies for weight management and health promotion.

3 Materials and Methods

3.1 Study Design

The study protocol was approved by the Ethical Committee of the Federico II University Medical School of Naples—A.O.R.N. Cardarelli (EC approval code: 204/2023); all adults gave written informed consent. The clinical trial was registered at www.isrctn.com, accessed on 25th July 2023 (ISRCTN 13055163 number).
Overweight/obese adults attending Outpatients Clinic of the Departmental program "Diet therapy in transplantation and chronic renal failure", School of Medicine, "Federico II" University of Naples, were recruited. A low-calorie diet was recommended for all overweight/obese adults.

3.2 Inclusion and Exclusion Criteria

The adults eligible for the study were over 18 years of age with overweight/obesity, attending Outpatients Clinic of the Departmental program "Diet therapy in transplantation and chronic renal failure", School of Medicine, "Federico II" University of Naples. Overweight/obesity was defined by a BMI >25 and <45 kg/m^2.
Adults were ineligible if the following were observed:

- ✓ They participated in other clinical trials;
- ✓ They declined to consent;
- ✓ They showed a weight change of more than 3 kg in the last 2 months;
- ✓ They were treated with weight loss medications and/or had a history of bariatric surgery;
- ✓ They were treated with hormonal therapies (estrogens, thyroxine or progesterone);
- ✓ They currently have cancer or received a cancer diagnosis within the last 5 years;
- ✓ They suffered with acute or chronic metabolic and inflammatory diseases (Crohn's disease, rheumatoid arthritis, etc.);

- ✓ They suffered from type 1 or type 2 diabetes or had been treated with insulin and oral hypoglycemic drugs (changes in the regulation of intermediary metabolism).

Patients with type 2 diabetes, treated with diet alone, could be included in the study.
After baseline evaluations, subjects were randomized into 2 groups: the first group (32 adults) was treated with a hypocaloric diet for 2 months plus the supplementation of cinchona (T group); the second one (27 adults) was treated with a hypocaloric diet for 2 months plus a placebo supplementation (P-group). Adults affected by diseases, such as those reported above, cancer, acute and chronic metabolic and inflammatory diseases, 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 were excluded.

3.3 Dietary Treatment

A personalized diet was recommended for each patient of both groups, om accordance with LARN (Livelli di Assunzione Raccomandata di Nutrienti) guidelines [28,29] [65, 66]. A hypocaloric diet (calorie restriction was 40% of the total energy needs), with 55–60% of the total caloric intake comprising carbohydrates, 10–15% of that comprising proteins and 20–25% of that being composed of fatty acids (<7% from saturated fat), was recommended for all obese adults.

3.4 Supplementation

The consumption of cinchona is not associated with health risks [67-69].
The *Cinchona succirubra* bark powder used for the nutraceutical formulation was provided by NGN Healthcare New Generation Nutraceuticals srl. To allow the nutraceuticals to exclusively address duodenal EECs, the nutraceuticals were encapsulated in gastro-resistant capsules containing 400 mg of *cinchona succirubra* bark powder microencapsulated with maltodextrins (two cinchona capsules per day, one hour before main meals). The placebo capsules used were produced using only maltodextrins and administered in accordance with the same methods. The subjects belonging to the T-group were treated with 800 mg

of cinchona per day (two cinchona capsules per day, one hour before main meals). Participants who did not take pills for two or more days were excluded from the study.

3.5 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.
Nutritional status was assessed via anthropometric measurements: weight (Seca GmbH & Co KG, Hamburg, Germany), height (measured using a wall-mounted stadiometer to the nearest 0.1 cm), body mass index (BMI), waist circumference (WC) and hip circumference (HC) [70]. In particular, total body water (TBW), fat mass (FM) and fat-free mass (FFM) percentages were detected to evaluate the body composition, via bioelectrical impedance analysis (BIA) undertaken using a tetrapolar BIA (RJL 101; Akern SRL, Florence, Italy). BIA was performed with a single-frequency measurement (50 kHz) [71]. The Zung test for assessing well-being was performed at baseline and at the end of the study. The Zung test represents a 20-item Likert scale, with scores ranging from 20, ‘‘no depression’’, to 80, ‘‘major depression’’, which are converted into index scores by dividing the sum of the raw scores by 80 and multiplying the result by 100 [72]. Blood glucose (Gly), total cholesterol (TC), HDL cholesterol (HDL-c), LDL cholesterol (LDL-c), triglycerides (TG), uric acid, alanine amino transferase (ALT) and aspartate aminotransferase (AST) were measured and monitored during the treatment. All subjects, at T0 and at the end of the treatment, were tested for bitter taste to classify the subjects as tasters or non-tasters [73].

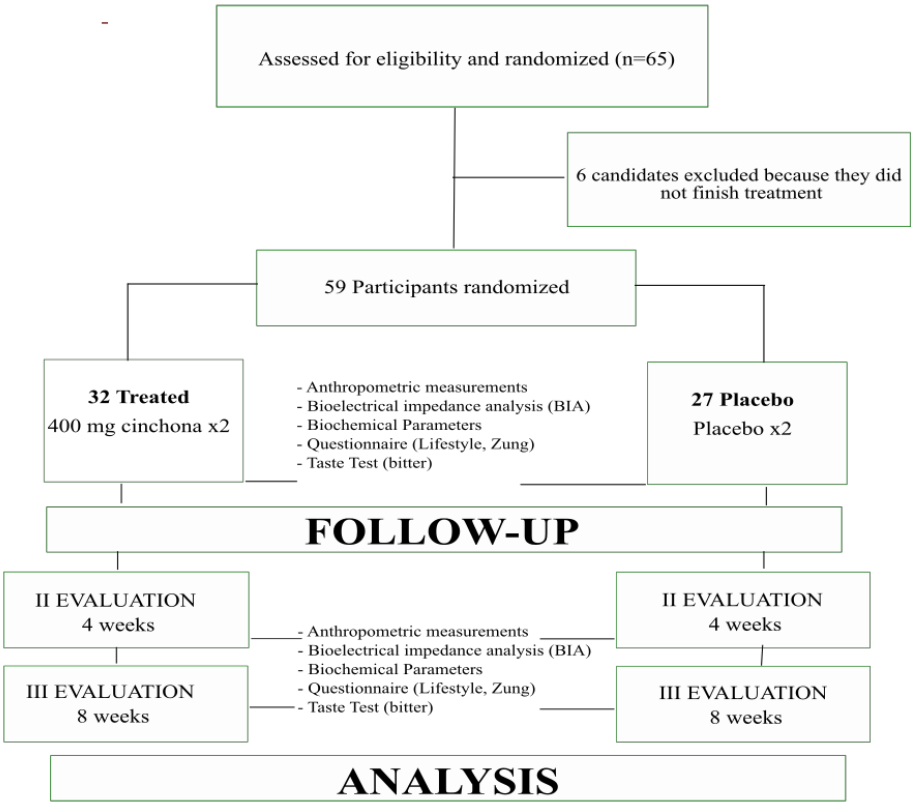

Fig.7: Flow chart illustrating study population.

3.6 Cinchona Alkaloid Status Assessment

To determine cinchona tasting status, all subjects consecutively placed five paper strips on their tongue; the first one was a control strip, whereas the others were impregnated with 0.4, 0.9, 2.4 and 6.0 mg of quinine extract, respectively. "Tasters" were defined as subjects who perceived bitter taste from any of the quinine impregnated blotting paper strips.

3.7 Dot Blot Protocol for CCK and Ghrelin Analyses

Serum CCK and ghrelin levels were measured via Dot-blot. Briefly, five microliters of serum samples was spotted on a nitrocellulose membrane to then be processed via Western blotting. CCK and ghrelin levels were immunodetected using anti-ghrelin (Santa Cruz, CA, USA, dilution 1:250) and anti-cholecystokinin (Santa Cruz, CA, USA, dilution 1:250) anti-bodies.

3.8 Compliance

Compliance with the dietary intervention was assessed by monitoring the dietary intake at baseline and every month until the end of the study using food frequency questionnaires [74].
The assessment of compliance with physical activity was verified by asking subjects to complete a physical activity questionnaire [75]. The questionnaires confirmed that adults belonging to both groups carried out regular physical activity for the entire duration of the treatment.
Satiety was assessed by means of visual analog scales (VAS), a previously validated questionnaire [76]
Compliance with the nutraceutical supplementations was evaluated using a daily questionnaire asking each volunteer about the time of the consumption of the supplement as well as evaluating the presence of adverse events.
In addition, the number of capsules at the end of the study was recorded.

3.9 Sample Size and Statistical Power

Sample size calculation was performed using MedCalc. The outcome for the calculation of sample size was the reduction in body weight in the T-group. A difference of 25% between the T-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 30 participants per group.

3.10 Statistical Analysis

All data were expressed as the mean ± standard error of the mean (SEM). Data were analyzed with the IBM SPSS 20.0 program (Statistical Package for Social Science, SPSS, Chicago, IL, USA). A paired-sample t-test, independent-sample *t*-test and chi-squared test were performed. Hormone levels were compared among groups using ordinary two-way ANOVA, correcting for multiple comparisons, and controlling the false discovery rate using the original FDR method of Benjamini and Hochberg [77, 78]. Statistical significance was set at $p < 0.05$.

3. Results

3.1. Nutritional Status Evaluation

All adults were accurately evaluated at baseline and were reconsidered after 30 and 60 days. No significant differences in all parameters were observed at the baseline among the two groups with the exception of hip circumference (P-group vs. T-group: $p = 0.019$) and the percentage of total body water (P-group vs. T-group: $p = 0.49$) (Table 1). On the basis of the dietary questionnaire, in the whole cohort, all adults had high adherence to the Med-Diet. At the end of the observations, the adults belonging to T-group presented significant changes in nutritional status and body composition compared to those at T0 (Table 2) and a higher improvement compared to those in the P-groups (Table 2), in particular in body weight ($p < 0.0001$ Table 2, Figure 8) as well as in BMI ($p < 0.0001$, Table 2, Figure 8), waist circumference ($p = 0.047$, Table 2, Figure 9), hip circumference ($p = 0.016$, Table 2, Figure 8), FM% ($p = 0.013$, Table 2, Figure 9), FFM% ($p = 0.006$, Table 2, Figure 9) and TBW% ($p = 0.004$, Table 2, Figure 9). Zung scores for adults belonging to both groups were less than 45 and therefore did not indicate a depression, at the end of the treatment. However, a significant reduction was observed in both the treated ($p < 0.0001$) and the placebo group ($p = 0.008$) (Table 2). Biochemical parameters were monitored throughout the study; parameters, such as HDL-c, and both transaminases remained stable throughout the study in both groups. Moreover, adults perceived the bitter taste of quinine-impregnated blotting paper and were thus all classified as "Tasters in both groups".

Furthermore, satiety questionnaires administered in both groups revealed that the T-group showed greater satiety throughout the duration of treatment compared with that in the P-group confirming the data obtained via hormonal measurements.

VAS analysis revealed that, compared to those in the P-group, adults belonging to the T-group felt more satiated before (3.5 + −0.9 and 4.2 + −0.6 for the P- and T-group, respectively; p value = 0.0059) and during the meals (3.3 + −0.7 and 4.3 + −0.7 for the P- and T-group, respectively; p value < 0.0001), suggesting that cinchona prolonged satiety throughout the day.

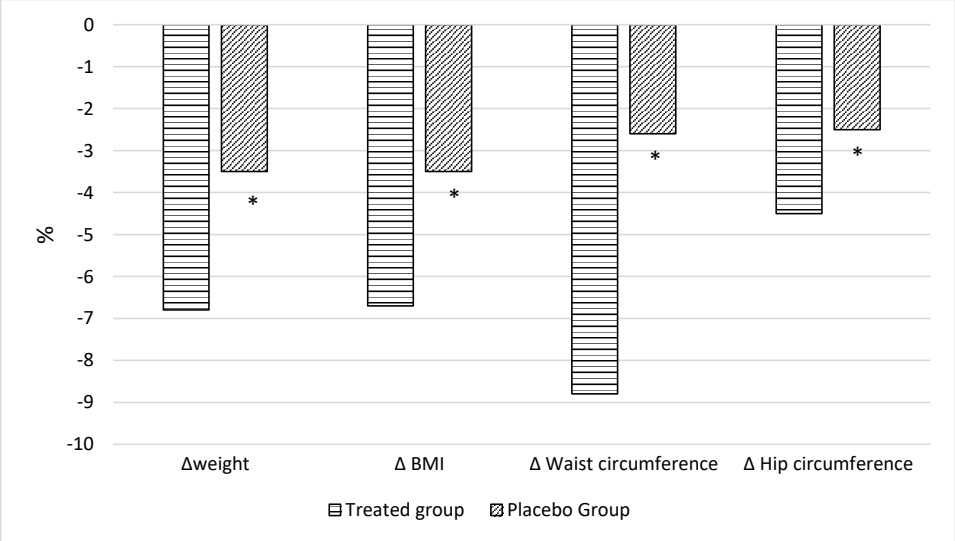

Fig.8: Percentage variations in anthropometric measurements in treated and placebo group compared with baseline. Data are reported as mean. * $p < 0.05$; T0, placebo group vs. treated group.

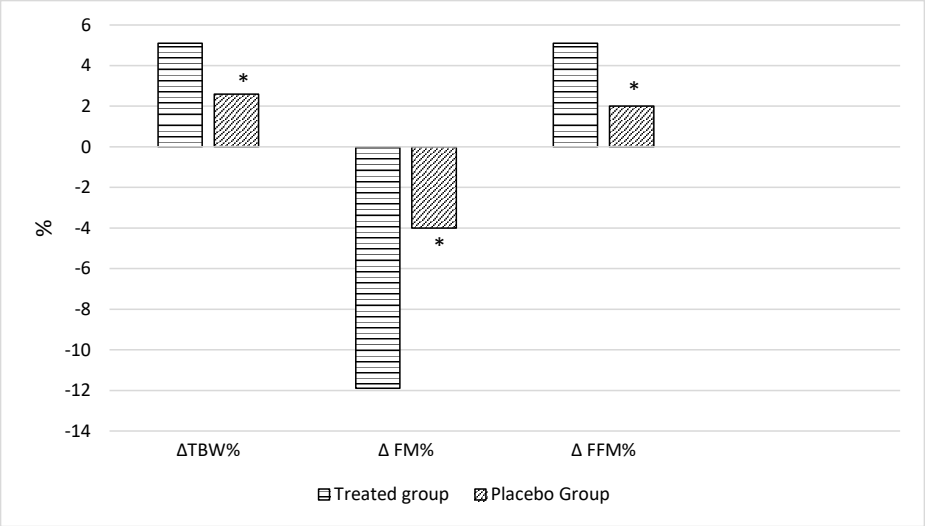

Fig.9**:** Total body water (TBW), fat mass (FM) and fat-free mass (FFM) percentage variations in treated and placebo group compared with those at baseline. Data are reported as mean. * $p < 0.05$; placebo group vs. treated group.

	Nutritional Assessment (T0)	
	Treated Group (n = 32)	**Placebo Group (n = 27)**
Male, n° (%)	9 (28.1)	11 (44.7)
Female, n° (%)	23 (71.9)	16 (55.3)
Age, years	36.7 ± 2.1	40.4 ± 1.9
Body Weight, kg	96.9 ± 1.6	90.7 ± 2.0
BMI, kg/m²	34.6 ± 1.0	33.0 ± 0.8
Waist circumference, cm	102.7 ± 1.1	100.7 ± 1.5
Hip circumference, cm	120.7 ± 1.1	113.7 ± 0.9 *
% Taste	100%	100%
	Comorbidities	
	Treated Group (n = 32)	**Placebo Group (n = 27)**
ZUNG	39.2 ± 1.2	37.3 ± 1.3
DIABETES n (%)		
No prediabetes or diabetes	30 (93.8)	22 (81.5)
Prediabetes	2 (6.2)	4 (14.8)
Diabetes	0 (0)	1 (3.7)
	Plasma Metabolic Parameters (T0)	
	Treated Group (n = 32)	**Placebo Group (n = 27)**
Glucose, mg/dL	92.6 ± 1.4	96.0 ± 1.6
TC, mg/dL	189.2 ± 3.0	185.9 ± 3.2
HDL-c, mg/dL	46.5 ± 1.8	46.6 ± 1.4
LDL-c, mg/dL	118.9 ± 3.5	122.3 ± 3.5
TG/HDL-c ratio	2.5 ± 0.7	2.5 ± 1.3
TG, mg/dL	108.0 ± 3.7	109.5 ± 6.8
AST	22.3 ± 2.3	22.4 ± 1.5
ALT	24.5 ± 3.3	24.7 ± 2.3
Uric Acid	3.9 ± 0.6	4.1 ± 0.8
TBW %	48.0 ± 1.1	51.6 ± 0.8 *
FM %	34.6 ± 1.7	29.8 ± 1.6
FFM %	65.4 ± 1.2	70.2 ± 1.0
PA	7.0 ± 0.3	7.3 ± 0.5
SM	35.7 ± 1.1	36.5 ± 1.5
SMI	12.6 ± 0.4	13.3 ± 0.6

Table 1**.** Baseline clinical characteristics of participants. Data are reported as mean ± SEM. * $p < 0.05$, T0 placebo group vs. T0 treated group. Abbreviations: total cholesterol (TC), high-density lipoprotein (HDL-c), low-density lipoprotein (LDL-c), triglycerides (TG), aspartate aminotransferase (AST), alanine aminotransferase (ALT), total body water (TBW), fat mass (FM), fat-free mass (FFM), phase angle (PA), skeletal mass (SM) and skeletal mass index (SMI).

	Nutritional Assessment			
	Treated Group (n = 32)		Placebo Group (n = 27)	
	T0	T2	T0	T2
Body Weight, kg	96.9 ± 1.6	90.4 ± 1.7 *	90.7 ± 2.0	87.5 ± 2.0
BMI, kg/m²	34.6 ± 1.0	32.4 ± 1.1 *	33.0 ± 0.8	31.8 ± 0.8
Waist circumference, cm	102.7 ± 1.1	96.1 ± 1.0 *	100.7 ± 1.5	98.9 ± 1.4
Hip circumference, cm	120.7 ± 1.1	115.2 ± 1.1 *	113.7 ± 0.9	111.7 ± 0.7
ZUNG	39.2 ± 1.2	34.6 ± 1.3 *	37.3 ± 1.3	35 ± 1.4
	Plasma Metabolic Parameteres			
	Treated Group (n = 32)		**Placebo Group (n = 27)**	
	T0	**T2**	**T0**	**T2**
Glucose,mg/dL	92.6 ± 1.4	87.4 ± 1.0 *	96.0 ± 1.6	91.1 ± 1.3
TC, mg/dL	189.2 ± 3.0	173.4 ± 2.9 *	185.9 ± 3.2	171.1 ± 2.8
HDL-c, mg/dL	46.5 ± 1.8	45.3 ± 1.6	46.6 ± 1.4	47.5 ± 1.7
LDL-c, mg/dL	118.9 ± 3.5	106.7 ± 3.5 *	122.3 ± 3.5	106.7 ± 3.0
TG/HDL ratio	2.5 ± 0.7	2.4 ± 0.8	2.5 ± 1.3	2.0 ± 0.8
TG, mg/dL	108.0 ± 3.7	103.8 ± 4.5	109.5 ± 6.8	84.6 ± 3.8
AST	22.3 ± 2.3	21.4 ± 1.4	22.4 ± 1.5	21.1 ± 1.6
ALT	24.5 ± 3.3	21.6 ± 2.0	24.7 ± 2.3	22.5 ± 2.4
Uric Acid	3.9 ± 0.6	3.3 ± 0.7 *	4.1 ± 0.8	3.0 ± 0.6
TBW %	48.0 ± 1.1	50.6 ± 1.2 *	51.6 ± 0.8	52.4 ± 0.8
FM %	34.6 ± 1.7	31.1 ± 2.1 *	29.8 ± 1.6	28.4 ± 1.6
FFM %	65.4 ± 1.2	68.9 ± 1.4 *	70.2 ± 1.0	71.5 ± 1.0
PA	7.0 ± 0.3	6.9 ± 0.3	7.3 ± 0.5	7.0 ± 0.4
SM	35.7 ± 1.1	35.5 ± 1.1	36.5 ± 1.5	36.0 ± 1.4
SMI	12.6 ± 0.4	12.5 ± 0.4	13.3 ± 0.6	13.1 ± 0.5

Table 2. Anthropometric measurements as well as biochemical parameters and body composition at baseline and after 2 months of treatment. Data are reported as mean ± SEM. * $p < 0.05$, T2 vs. T0; ° < 0.05, placebo group vs. treated group.

3.2. Treated Group (T-Group)

Nutraceutical supplementation improved the nutritional status of adults belonging to the T-group, inducing a significant reduction in Body weight (90.4 ± 1.7 vs. 96.9 ± 1.6 kg, $p < 0.0001$; T2 vs. T0) as well as in BMI (32.4 ± 1.1 vs. 34.6 ± 1.0 kg/m^2, $p < 0.0001$; T2 vs. T0), waist circumference (96.1 ± 1.0 vs. 102.7 ± 1.1 cm, $p < 0.0001$; T2 vs. T0) and hip circumference (115.2 ± 1.1 vs. 120.7 ± 1.1 cm, $p < 0.0001$; T2 vs. T0) when compared with those at baseline. Finally, bioimpedance analysis showed a significant reduction in FM % (31.1 ± 2.1% vs. 34.6 ± 1.7%; $p < 0.0001$, T2 vs. T0) in the T-group after 2 months of nutraceutical supplementation. There was, moreover, a significant increase in FFM% (68.9 ± 1.4% vs. 65.4 ± 1.2%, $p < 0.0001$; T2 vs. T0) and TBW % (50.6 ± 1.2% vs. 48.0 ± 1.1%, $p < 0.0001$; T2 vs. T0) when compared with those at baseline.

It is interesting to observe the bioumoral parameter improvements; in particular, significant reductions in glucose (87.4 ± 1.0 vs. 92.6 ± 1.4mg/dL, $p = 0.025$; T2 vs. T0), TC (173.4 ± 2.9 vs. 189.2 ± 3.0 mg/dL, $p = 0.04$; T2 vs. T0), LDL-c (106.7 ± 3.5 vs. 118.9 ± 3.5 mg/dL, $p = 0.029$; T2 vs. T0) and uric acid (3.3 ± 0.7 vs. 3.9 ± 0.6 mg/dL, $p = 0.07$; T2 vs. T0) were detected when compared with those at baseline. Conversely, HDL-c as well as Try, both transaminases, PA, SM and SMI remained stable throughout the study (Table 2).

3.3. Placebo Group (P-Group)

Adults belonging to the P-group showed an improvement in body weight (87.5 ± 2.0 vs. 90.7 ± 2.0 kg, $p < 0.0001$; T2 vs. T0) as well as in BMI (31.8 ± 0.8 vs. 33.0 ± 0.8 kg/m^2, $p < 0.0001$; T2 vs. T0). Furthermore, a significant reduction in waist circumference (98.9 ± 1.4 vs. 100.7 ± 1.5 cm, $p < 0.0001$; T2 vs. T0) as well as in hip circumference (111.7 ± 0.7 vs. 113.7 ± 0.9 cm, $p = 0.001$; T2 vs. T0) was detected after 60 days of treatment. Finally, bioimpedance analysis showed a significant reduction in FM% (28.4 ± 1.6 vs. 29.8 ± 1.6%; $p = 0.02$, T2 vs. T0) in the P-group after 60 days of placebo supplementation and an increase in FFM% (71.5 ± 1.0 vs. 70.2 ± 1.0%, $p = 0.01$; T2 vs. T0). Conversely, no significant differences were detected in TBW%, PA, SM and SMI compared with those at baseline. A significant improvement was detected in bioumoral parameters after 60 days; in particular, significant reductions in glucose (91.1 ± 1.3 vs. 96.0 ± 1.6 mg/dL, $p = 0.001$; T2 vs. T0), TC (171.1 ±

2.8 vs. 185.9 ± 3.2 mg/dL, $p = 0.05$ T2; vs. T0), LDL-c (106.7 ± 3.0 vs. 122.3 ± 3.5 mg/dL, $p = 0.035$ T2; vs. T0), Try (84.6 ± 3.8 vs. 109.5 ± 6.8 mg/dL, $p = 0.023$; T2 vs. T0) and uric acid (3.0 ± 0.6 vs. 4.1 ± 0.8 mg/dL, $p = 0.010$; T2 vs. T0) were observed compared with those at baseline. Conversely, HDL-c and both transaminases remained stable throughout the study (Table 2).

3.4. Serum CCK and Ghrelin Level

To confirm nutraceutical supplementation components addressing enteroendocrine cells (EECs), CCK and ghrelin serum levels were measured in treated and placebo groups, both at T0 and T2. Previously, we showed that bitter taste receptors are expressed in gastric EECs as well as in intestinal EECs. Gastric EECs secrete ghrelin while intestinal EECs secrete CCK [42,43]. Considering that nutraceutical supplementation was administered with gastro-resistant pills, we expected the stimulation of intestinal EECs but not of gastric EECs. As shown in Table 3 at T0 and T2, placebo and treated adult serum samples were processed via Dot-blot to detect circulating CCK and ghrelin. It is worth noting that the hypocaloric diet decreased CCK levels at T2 in placebo adults (delta −40% ± 10, $p = 0.046$); this reduction has been linked to increased hunger in adults undergoing hypocaloric diets. On the contrary, in adults receiving the nutraceutical supplementation, we did not measure a statistically significant reduction in CCK levels, indicating that nutraceuticals were effective in stimulating intestinal EECs to release CCK. Moreover, we measured ghrelin levels, which were unchanged in both placebo and treated group at the end of observations, indicating that there was no stimulation of gastric EECs.

	Plasma hormonal parameters			
	Treated Group (n = 32)		**Placebo group (n = 27)**	
	T0	**T2**	**T0**	**T2**
CCK pg/ml (± SEM)	14.21 ± 3.4	13.26 ± 1.3	14.11 ± 2.21	5.66 ± 1.10*
Ghrelin ng/ml (± SEM)	7.84 ± 2.6	7.63 ± 2.5	7.53 ± 1.21	7.66 ±1.40

Table 3: Hormonal variations in both groups at baseline and after 2 months of treatment. Data are reported as mean ± SEM. * $p < 0.05$ T2 vs. T0. Abbreviations: cholecystokinin (CCK).

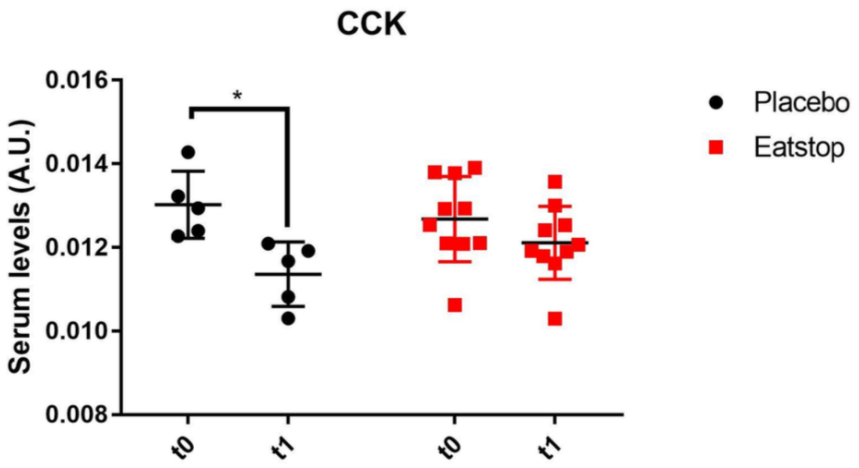

Fig.10: Cck variation in both groups at baseline and after 2 months of treatment

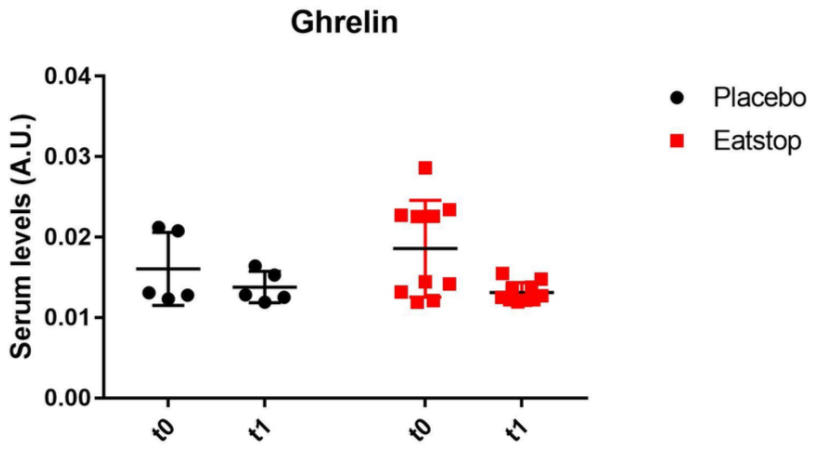

Fig.11: Ghrelin variation in both groups at baseline and after 2 months of treatment

3.11 Questionnaires:

Each patient was asked to complete a VAS scale questionnaire at baseline, 30 days, and 60 days to assess dietary habits, levels of physical activity, and feelings of hunger or satiety. Additionally, at the beginning and end of the treatment, recruited subjects underwent a test to evaluate psychophysical well-being (Zung test).

The collected responses indicated a greater sense of satiety for the group treated with Cinchona succirubra extract, both during and at the end of the intervention, compared to subjects treated with diet alone and a placebo.

4 Discussion

The results of the present study indicate that overweight/obese adults attending Outpatients Clinic of the Departmental program "Diet therapy in transplantation and chronic renal failure", School of Medicine, "Federico II" University of Naples, treated with the supplementation of *cinchona succirubra*, showed a significant improvement in nutritional status and body composition after 60 days of treatment compared with baseline. These adults were compared with a group of overweight/obese adults treated with a hypocaloric diet without supplementation. Interestingly, the cinchona-treated adults showed a reduction in body weight, BMI, waist circumference, hip circumference and FM% that was higher than that detected in the subjects receiving the placebo. Furthermore, a higher improvement in the percentage of FFM and TBW was observed in the T-group, when compared with baseline and adults belonging to the P-group. This study, indeed, demonstrates that the association of hypocaloric diet with cinchona nutraceutical supplementation is effective in inducing higher weight loss than that obtained with a hypocaloric diet.
The study justified the choice of *cinchona succirubra* as a dietary supplement in the broader context of the use of natural substances or nutraceuticals for the treatment of obesity and weight control. The selection of cinchona was influenced by its role as a bitter taste agonist. This property was considered potentially beneficial for modulating food intake, therefore leading to improved weight management. In this context, previous preclinical studies strongly supported our selection.

Our results are in agreement with those of a previous study where Jung and coworkers investigated the effects of dietary cinchonine on the reduction in high-fat-diet- (HFD) induced adipogenesis and inflammation in a mouse model. In particular, the authors showed that HFD-fed 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 in proinflammatory cytokine production. Therefore, the authors suggested that cinchona is able to prevent obesity due to its effects on adipogenesis and inflammation [57].

Furthermore, adults belonging to the treated group showed a surprisi ng reduction in the percentage of FM compared with that in the placebo group, confirming previous data presented by Cettour-Rose and coworkers, who studied the effect of 0.1% quinine on body weight and body composition in male mice fed a balanced diet. In particular, they showed that quinine, a cinchona alkaloid belonging to the aryl-amino alcohol group of drugs, is effective in the management and control of body weight and fat mass without affecting food intake. Therefore, the authors indicated that this could represent a novel tool to counteract obesity [79]. Our present data support the previous suggestions, indicating that the stimulation of taste receptors by nutraceuticals based on cinchona affect the complex interplay of molecules involved in the regulation of food intake, body metabolism and total energy introduction and expenditure, facilitating real control of individual body weight.

It is interesting that adults belonging to the treated group revealed greater satiety throughout the duration of treatment, suggesting that nutraceutical treatment with cinchona is able to modulate gastrointestinal (GI) functions, including intestinal hormones, leading to an increased satiety and the consequent fine modulation of energy intake.

The results of this study indicate that the treatment with cinchona plus hypocaloric diet was accompanied by an interesting resetting of hormonal secretion by intestinal endocrine cells. The hypocaloric diet in the P-group, indeed, induced a decrease in CCK secretion and blood levels, as previously observed in the induction of weight loss via hypocaloric diet treatment. However, it is worth noting that the chinchona supplementation plus the hypocaloric diet did not modify the CCK secretion and blood levels, suggesting that cinchona was effective in facilitating CCK secretion, which was sequentially accompanied by higher satiety in the adults, as detected in our T-group. Therefore, CCK secretion and unmodified blood levels trigger the

adaptation to weight loss induced by cinchona, as characterized by a decrease in hunger stimulation and better compliance to the hypocaloric diet. This likely suggests that this adaptation facilitates weight loss with a real improvement in all the morphometric and functional parameters under all time periods of treatment in our treated cases compared to those in the placebo group. On the other hand, in the P-group, the decrease in CCK secretion and blood levels indicate that the risk of hunger stimulation may play a key role in major alterations or failure in weight loss management.

In conclusion, weight loss management triggers the adaptation of circulating hormones, which is characterized by a decrease in CCK secretion with the consequent risk of failure of weight loss maintenance. Supplementation with cinchona in a hypocaloric diet facilitates higher circulating levels of CCK, which are effective in promoting patient satiety and reducing the risk of hunger stimulation and failure in weight loss attempts. These data call for further studies to clarify the complex interplay of different gut-derived hormones in the modulation of food and energy intake.

5 Limitation

Although this study presents promising results regarding the efficacy of *cinchona succirubra* supplementation in conjunction with a hypocaloric diet for weight loss in overweight/obese patients, there are several limitations that must be acknowledged. First, the sample size and demographic diversity of the study were limited, calling for further research to confirm these findings across a broader population. Additionally, the study duration was relatively short (60 days), indicating the necessity for longer-term studies to evaluate the sustainability and prolonged effects of such treatment. While the study offers promising initial results for morphometric and functional parameters, an in-depth exploration of the long-term implications and potential side effects would be really useful. Furthermore, the biochemical mechanisms contributing to the observed weight loss and hormonal regulation also require further detailed investigation. Finally, while the study suggests an interesting resetting of hormonal secretion and appetite control, these results need to be verified with larger and diverse study populations over longer periods of time to validate the

efficacy and safety of cinchona supplementation and the complex interplay of different gastro-intestinal hormones in weight management.
In this manuscript, we only measure fasting levels of CCK and ghrelin in adults receiving *cinchona succirubra*. In the future, it will be interesting to measure postprandial levels of these appetite hormones upon *cinchona succirubra* supplementation. Their levels will confirm the stimulatory mechanism of action of cinchona on intestinal bitter receptors and/or eventually highlight an unprecedented mechanism of action involving long-term transcriptional modulatory activity on enteroendocrine cells.

6 Conclusions and Future Perspective

The results obtained from this study pave the way for a range of future research opportunities, which are crucial to deepening our understanding and applications in clinical settings. One immediate area to explore is the longitudinal evaluation of the effects of cinchona on weight and body metabolism. Studies extending over longer periods will be critical to assess the long-term safety and efficacy of this dietary supplement. The diversification of the study population in future trials is also essential. Including participants from various demographic backgrounds, different age groups and ethnicities will help determine the effectiveness of cinchona supplementation across a broader spectrum of the population. Furthermore, the mechanisms triggered by cinchona, affecting weight loss and metabolic parameters, need to be clarified. Such studies would provide valuable insights into its role in appetite regulation and energy balance, contributing to an understanding of its therapeutic potential. Significantly, comparative studies will also be interesting in terms of assessing the efficacy of cinchona, when combined with other plant matrices, as sources of alkaloids able to activate bitter taste receptors. Such combinations could potentially enhance the beneficial potential or mitigate any adverse effects, offering a new approach to weight management.

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