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Review

Modification of the microbiota in obese individuals following a Very Low-Calorie Ketogenic Diet

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Abstract. *Background and aim:* Recent studies have shed light on the efficacy of Very Low Carbohydrate Ketogenic Diets (VLCKDs) in fighting obesity. This review aims to explore the mechanisms by which VLCKDs influence weight loss, particularly through their interaction with the gut microbiota and modulation of the Enteric Nervous System (ENS). The symbiotic relationship between the gut microbiota and its host is essential for maintaining physiological homeostasis, and disturbances in this relationship can lead to various health issues, including obesity. *Methods:* This review synthesizes findings from various studies that examine the impact of VLCKDs on the gut microbiota and ENS, and their subsequent effects on obesity. It delves into the dynamics of gut microbiota-host interactions and how VLCKDs can lead to a beneficial remodeling of the gut microbial ecosystem. *Results:* Evidence suggests that VLCKDs can significantly alter the composition of the gut microbiota, leading to a state that favors weight loss and metabolic health. The diet ability to modify the gut microbiota and ENS interaction plays a critical role in its effectiveness in reducing obesity and potentially rectifying gut dysbiosis. *Conclusions:* VLCKDs have been confirmed to be effective in reducing obesity through their significant therapeutic potential, which includes gut microbiota and ENS modulation. The restoration of a balanced gut microbiota is crucial for fighting obesity and its associated pathologies. VLCKDs emerge as a promising therapeutic approach, suggesting a paradigm shift in the treatment of obesity and related conditions by leveraging diet interventions to manipulate the gut ecosystem.

Key words: VLCKD, gut microbiota, enteric nervous system, obesity, diet

Introduction

Obesity is a chronic and multifactorial disease whose diffusion is rapidly increasing all around the world (1). According to the World Health Organization (WHO), the prevalence of overweight and obesity cases has tripled from 1975 to 2016 (2). In Europe, obesity and overweight are responsible for over 1.2 million deaths annually (3). Obesity is associated with an increased risk of various diseases, such as cancer, cardiovascular disease, type 2 diabetes mellitus (T2DM), chronic respiratory disease, dyslipidaemia, ovarian polycystic syndrome, and sleep apnoea syndrome (4–6). Additionally, obesity has been linked

to negative effects on psychological well-being (7–9) and cognitive function (10–14) and may exacerbate dementia (15–17). Lifestyle modifications are widely considered the most effective strategy for preventing and treating obesity, overweight and related pathologies (18–20). The main goal is weight loss, and research shows that the most effective diet is one that obese patients can stick to consistently over both the short and long term (21,22). In recent years, several dietary patterns have been proposed. However, it has not been conclusively proven that the Mediterranean diet is more effective than other diets in achieving long-term weight loss, despite some clinical studies. This may be due to the difficulty of adhering to a diet that includes complex carbohydrates and reduces fat intake (23). Individuals with obesity may struggle to adhere to diets due to their preference for high-calorie foods and drinks with a high glycaemic index that stimulate serotonin and "carb-craving" (24). However, it is well-established that a high consumption of carbohydrates, particularly refined sugars, contributes to the development of chronic diseases (25). Scientific evidence has shown significant interest in low-carbohydrate diets, particularly ketogenic diets (KDs), for their preventive and therapeutic roles in weight loss and various diseases (26,27). Changes in diet can affect the Enteric Nervous System (ENS), also known as the second brain, due to the symbiotic association created by the intestinal microbiota with its host (28–32). This balance is linked to certain diseases, including obesity (33). However, it is important to note that the composition of gut microbiota can also be influenced by certain diseases, such as obesity (34). The aim of this review is to investigate how ketogenic diets can be used as a tool against obesity and their impact on the intestinal microbiota in obese individuals.

Very low-calorie ketogenic diet and obesity

In recent years, scientific research has shown interest in the ketogenic diet (KD) as an alternative weight loss method. The diet reduces appetite and produces ketone bodies (KBs), such as acetoacetate, beta-hydroxybutyric acid (BHB), and acetone. The KD is a normal-protein diet with a significant reduction in carbohydrate content. In 1921, a diet was proposed to treat epilepsy by mimicking fasting through ketonemia (35). In physiological conditions, when the body's glucose reserves are insufficient for producing oxaloacetate for fat oxidation during the Krebs Cycle, ketone bodies (KBs) are produced as an alternative energy source. This leads to a shift towards fatty-acid oxidation, and the overproduction of acetyl-CoA results in the production of KBs in the hepatic mitochondrial matrix (36). During cases of KD or fasting, the body enters a metabolic state known as "ketosis" due to the low or absent intake of dietary carbohydrates and the use of KBs by the Central Nervous System (CNS) (37,38). The scientific interest in ketogenic diets has increased in recent years due to their therapeutic effects against various pathologies, including epilepsy (39), cardiovascular diseases (CVD), T2DM, infertility, endometriosis in women, overweight, and obesity (40). A diet is considered ketogenic if it contains no more than 30-50g of carbohydrates per day and a normal protein content. Various types of KD are available, differing in calorie content and macronutrient ratio (41). One of these variants is the Very Low-Calorie Ketogenic Diet (VLCKD), which is commonly used for managing obesity. In a study conducted by Bueno and colleagues (42), the conventional low-fat diet was compared to the VLCKD over the long term, and it was concluded that the VLCKD resulted in a more significant weight loss (42). VLCKD is characterised by a carbohydrate intake of less than 50g per day, a normo-protein intake of 1–1.5g of protein per kg of ideal body weight, a low-fat intake of 15–30g per day, and a very low-calorie intake of approximately 500-800 kcal per day (26). The VLCKD protocol consists of three stages: active, re-education, and maintenance (26). The protocol includes the use of high-biological-value protein, replacement artificial meals, and natural foods. In 2015, the European Food Safety Authority (EFSA) established a scientific panel to determine the composition of artificial meal for weight control. The panel recommended a daily protein content of 75g, a minimum carbohydrate content of 30g for brain activity, a minimum fat content of 20g, and a minimum energy intake of 600 kcal (EFSA Panel on Dietetic Products, Nutrition and Allergies (NDA) (43). Moscogiuri and colleagues '2021

meta-analysis confirmed these findings, demonstrating significant reductions in BMI, body weight, waist circumference (WC) as an expression of visceral adipose tissue (VAT), Fat Mass (FM), glycemia, glycated haemoglobin (HbA1c), total cholesterol, LDL-cholesterol and triglycerides. The results showed a greater reduction in all parameters compared to other dietary protocols, except for blood sugar, HbA1c, total cholesterol, and LDL cholesterol (26). Therefore, the VLCKD may be a viable treatment option for individuals with obesity and related metabolic complications, but caution should be exercised and possible contraindications should be considered (44).

Gut microbiota, diet and obesity

Microbial communities are found in various parts of the human body, with the gastrointestinal tract being the most populated and well-studied. The intestinal microbiota consists of around 500 different species of bacteria and weighs approximately 1-2 kg (45). The colon is the area with highest concentration of microorganisms, with approximately 10^9-10^{12} CFU/mL present (46,47). Each bacterium possesses its own gene pool, known as the "microbiome". The weight of bacterial genes is significant with the microbiota consisting approximately 100 times more genes than the human genome (2-4 million genes) (48). Recent scientific research focused on the impact of the microbiota on human health (49). The Human Microbiome Project has identified both healthy and harmful bacteria in relation to their host. It has been estimated that there are approximately 2,500 species of bacteria with a large inter-individual variability in terms of composition. The predominant healthy gut flora is made up of *Firmicutes* and *Bacteroidetes*, while other *phyla*, such as *Actinomycetes, Proteus, Fusobacteria, and Verrucomicrobia* are less common (50). In 2015, the concept of the "superorganism" was introduced (51). Scientific research has focused on the role of the microbiota in human health. There is a link between the composition of the microbiota and the host, resulting in a symbiotic relationship (52). The health of the host influences the health of the microbiota and vice versa (48). The gut microbiota depends on host for its nutritional and hydric requirements, as intestinal bacteria use the food that remains undigested by the host as an energy source. The microbiota uses its digestive enzymes to produce metabolites and compounds that can affect the health of its host. The intestinal ecosystem remains healthy if it finds a favorable environment for its growth. However, if microbiota growth is unhealthy or harmful metabolites are produced, the health of the host will be negatively affected (53). In this sense, a symbiotic mutualistic relationship is created between the two, where the health of one depends on that of the other. The gut microbiota plays a crucial role in maintaining the host's health (48,54). Bacteria have a defined "metabolic" function as they produce Short Chain Fatty Acids (SCFAs) and vitamins B through the fermentation of non-digestible dietary residues and endogenous mucus. They also have an immune function as they stimulate the maturation of Gut-Associated Lymphoid Tissue (GALT) and the systemic and local immune response through links and communication with ENS. The gut microbiota plays a protective role through the "barrier effect" by inhibiting the growth of pathogenic bacteria. This is achieved by competing for attachment sites and available nutrients (54,55). Scientific research has recently focused on the concept that the environment can modify our genes, and it has recently emerged that the microbiota also has an important epigenetic role. Gut bacteria produce metabolites, including butyrate (56), which regulate gene expression inhibiting histone deacetylase (54). Each individual has a unique bacterial fingerprint (57). In 2010, Turnbaugh and colleagues analyzed the fecal microbiota and found that homozygous twins had over 50% diversity of microbial species (58). The microbiota of the intestine is sterile at birth and remains surprisingly stable over time (59). It is only from the second year of life that the microbiota begins to be colonized and stabilized, forming the composition that will persist into adulthood (60). Throughout the life cycle, the composition of the gut microbiota can be influenced by some external factors, such as the mode of birth (31), type of breastfeeding (28), age (61), diet (30), medication use (32), and environment (62). This creates a state of balance between external environmental factors, host and the microbiota, known as "*eubiosis*". If this balance is disrupted, a condition called "*dysbiosis*" can

occur, in which pathogenic microorganisms contribute to the pathogenesis of diseases (52). The identification of dysbiosis is primarily based on the characterization of the microbial population, particularly the relationship between the two dominant *phyla*, expressed as the *Firmicutes/Bacteroidetes* ratio. This ratio has been associated with several diseases. The gut microbiota is considered an emerging target for the nutritional or therapeutic prevention and management of several diseases, including diarrhea, irritable bowel syndrome, allergy, multiple sclerosis, type 1 and type 2 diabetes, inflammatory bowel diseases, rheumatoid arthritis, Alzheimer's and Parkinson's diseases, autism, obesity, and atherosclerosis (63). In 2006, Turnbaugh and colleagues showed that obese mice have an increased *Firmicutes/Bacteroidetes* ratio (64). Several animal model studies have shown that obese mice have a lower abundance of *Bacteroidetes* and a higher abundance of *Firmicutes* compared to normal-weight control mice. Turnbaugh discovered that obesity and changes in gut microbiota, which are determined by obesity status, increase the ability to obtain energy from the diet (65). Regarding the impact of diet, gut microbiota uses dietary nutrients to direct fundamental biological processes. Therefore, a balanced and varied diet not only helps prevent pathologies but also affects overall health and the sense of physical and mental well-being (66–70). Several studies have shown that various types of diets can influence the composition of gut microbiota (71,72). For instance, the Western Diet is characterized by an excess of saturated fat (73). This not only increases the risk of cardiovascular disease linked to increased triglycerides, but also alters the composition of the microbiota. This leads to an increase in *Escherichia Coli,* which in turn increases the catabolism of choline. Choline is a molecule produced by the liver that acts as a coenzyme in numerous metabolic reactions. The catabolism of choline converts it into trimethylamine (TMA) which is a potentially dangerous and toxic metabolite for the body. Fats affect the gut microbiota both as substrates for bacterial metabolic processes and by exerting a toxic influence that inhibits bacterial growth (74). Patients with dyslipidemia, such as non-alcoholic steatohepatitis (NASH) or atherosclerosis, have shown an alteration of the gut microbiota (75). Furthermore, certain diseases may alter the composition of the gut microbiota itself. For instance, individuals with T2DM have a distinct gut microbiota composition compared to healthy individuals (33). Additionally, an increase in body mass index (BMI) due to obesity and overweight, can also alter the composition of the intestinal microbiota, resulting in a change in the *Firmicutes/ Bacteroides* ratio compared to the healthy microbial composition (34,76,77). However, the scientific data regarding the relationship between BMI and microbiota is controversial due to variations in study populations and their lifestyles. While the link between obesity and microbiota is clear, it is important to analyse different co-variables. Therefore, it would be more convincing to examine the correlation between different types of diets and gut microbiota composition (63).

VLCKD, microbiota and obesity

Recent scientific evidence demonstrates the efficacy of a KD in targeting obesity (78). The production of KBs leads to appetite suppression (79), although the underlying mechanisms require further investigation. In 2022, Polito and colleagues, demonstrated that in 26 obese subjects, VLCKD lead to a reduction in Heart Rate Variability (HRV), an indicator of Sympathetic Nervous System (SNS), which plays a key role in regulating the body's energy balance (80). The SNS acts centrally to regulate the sense of hunger and satiety, as well as peripherally to regulate energy expenditure in tissues (81–83). However, the microbiota also play an important role in the VLCKD as it involves a significant modulation of the gut microbiota, like all diets (84–87). Although obesity has not been directly linked to a specific bacteria or pathogen, it is associated with a dysbiosis status. Weight loss alone causes a modification in the gut microbiota, with an increase in *Bacteroides* and a decrease in *Firmicutes*, regardless of the diet type (88). However, the type of food chosen in the diet can affect the microbial composition. Several studies have elucidated the effect of VLCKD on the gut microbiota composition in the regulation of various neurological diseases, such as epilepsy (89), autism spectrum disorder (90), multiple sclerosis (86), and neurodegenerative diseases in general (85). More

recently, scientific evidence has also correlated the effects of a VLCKD on the intestinal microbiota in the management of obesity in human models. In 2019, Gutiérrez-Repiso and colleagues, conducted a randomized controlled pilot study to investigate the effect of VLCKD and symbiotics on gut microbiota. The study found that after 4 months of VLCKD, there was an increase in the diversity of gut microbiota, specifically in the healthy bacteria *Butyricimonas* and *Oscillospira.* Notably, this improvement was independent from symbiotic use (91). In 2020, Basciani and colleagues conducted a study on 48 obese subjects who were divided into three groups: VLCKD with whey protein, VLCKD with vegetable protein, and VLCKD with animal protein. The authors observed the effect of 45 days VLCKD on anthropometric parameters, body composition, metabolic parameters, and gut microbiota. The study found that *Firmicutes* and *Actinobacteria* decreased, while *Bacteroides* and *Proteobacteria* increased. This highlights a significant association between the reduction of obesity and inflammation (92). In a 2021 study, Gutiérrez-Repiso and colleagues compared the effects of three different weight loss interventions on the gut microbiota composition: VLCKD, Mediterranean Diet (MD), and bariatric surgery. The study found that the VLCKD led to an increase in *Alistipes* and *Parabacteroides*, which are inversely related to obesity and metabolic syndrome. However, there was also a decrease in *Lactobacillus*, despite previous studies showing that its correlation with obesity is strain-dependent (93). A more recent study by Deledda and colleagues, compared the effects of VLCKD and MD on gut microbiota composition. Both diets resulted in weight loss, reduced BMI and WC. However, only VLCKD showed a statistically significant alteration in the composition of the intestinal microbiota. The study found an increase in the families *Verrucomicrobiota*, *Akkermansiaceae* and *Christensecellacea*, which are bacteria inversely correlated with obesity (94).

Conclusions

In a context where overweight and obesity are increasing, the very low-calorie ketogenic diet could be a valid strategy for weight loss and long-term maintenance of results. Additionally, the intestinal microbiota may play a crucial role in managing and preventing of various diseases, including obesity, which could establish an important therapeutic connection in its management. Recent data shows that after VLCKD, the microbiota in obese subjects returns to a state of "*eubiosis*", which is healthy for the organism. This review suggests that the potential malleability of gut microbiota could be used as a therapeutic target to reduce the prevalence of obesity and overweight with VLCKD. However, due to the limited evidence in human models, further research is needed.

Conflict of Interest: Each author declares that he or she has no commercial associations (e.g. consultancies, stock ownership, equity interest, patent/licensing arrangement etc.) that might pose a conflict of interest in connection with the submitted article.

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