Targeting Gut Microbiome and the Recovery of Muscle Loss Associated with Cancer (Cachexia): An Overview of the Possible Effect of Bee Products

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Abstract

Current research emphasizes the contribution of gut microbiome to numerous health conditions including cancer and skeletomuscular disorders. Cachexia is a debilitating condition of progressive loss of body tissues. It occurs in up to 80% of patients with tumors, and it impairs their functioning and quality of life and increases morbidity and premature death. Several randomized trials indicate that interventions, which target gut microbiome can correct and revert tissue loss in aged people. However, less is known about the effect of such strategies in cachectic muscle loss. This report briefly sheds the light on the role of gut-muscle axis in cachexia and demonstrates few examples on interventions addressing gut microbiome and their effect on cachectic muscle. It also speculates the literature for the skeletal muscle-promoting activity of bee products, particularly bee honey and propolis (which are quite handy), within the context of cachexia. Implications for future studies are discussed.

Keywords: *Bee honey, cachexia,cancer,gut microbiome/microbiota, gut-muscle axis, probiotics, propolis, skeletal muscle loss.*

Introduction

Overview of Cachexia: Excessive unintentional weight loss is a common feature of neoplastic disorders. Cachexia is a syndrome characterized by progressive weight loss due to tissue loss that involves fat and non-fat components of the body. It occurs in 50–80% of cancer patients.[1-3] Cachexia increases sense of fatigue, alters patients 'functioning, decreases mobility, threatens their wellbeing and quality of life, and heightens morbidity. Cachexia is a direct cause of mortality in 20% of cancer patients while cachectic patients with neoplasms exhibit a one-year mortality rate of 80%.[2]

There is less agreement on the definition of cachexia, and several models have been proposed (Table 1). The common feature among all the available classifications is unintentional weight loss.[4-6] According to a proposal set by the SCRINIO Working Group,unintentional loss of 10% or more of body weight is sufficient to diagnose cachexia in cancer patients.[5] Fearon and others defined

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cachexia as either weight loss>5% within the last 6 months, weight loss >2% along with a body mass index < 20 kg/m^2, or fat free mass index < 14.6 and 11.4 kg/m^2 for men and women, respectively.[6] According to Evans and colleagues cachexia diagnosis is based on weight loss and low body mass index, in addition to the presence of 3 of 5 criteria: fatigue, anorexia, muscle wasting, muscle weakness, and abnormal biochemistry including high levels of inflammatory markers e.g., c-reactive protein or anemia indicated by low hemoglobin level.[4]

Table 1. Different classifications of cachexia.

Cachexia classifications	Diagnosis criteria	Ref.
SCRINIO Working Group	Unintended weight loss ≥ 10% from habitual weight.	5
Fearon and colleagues	Unintended weight loss>5% within the last 6 months or weight loss >2% along with a BMI < 20 kg/m^2 or fat free mass index < 14.6 and 11.4 kg/m^2 for men and women, respectively.	6
Evans and colleagues	Unintended weight loss ≥ 5% within the last year or BMI < 20 kg/m^2 plus 3 of these criteria: anorexia, muscle weakness, fatigue, fat free mass index < 17.0 and 15.0 kg/m^2 for men and women, respectively, disturbed biochemistry e.g., serum albumin <32 g/L, or c-reactive protein >5.0 mg/L or hemoglobin<12 g/dl.	4

The mechanism of cachexia: The dynamic of cachexia is multifaceted. The immune system of individuals with neoplasms is continuously activated, which is associated with high production of pro-inflammatory cytokines and free radicals. Tumors also induce major alterations in the cellular antioxidant system in host tissues. Disturbed redox and high levels of cytokines accelerate the activity of molecules involved in proteolysis and lipolysis.[3,7] In fact, cumulative evidence attributes the progressive nature of muscle loss occurring in cachexia to excessive accumulation of factors that negatively regulate protein turnover in skeletal muscle. Common molecules that derive muscle atrophy in cachectic muscle include atrogin-1, ubiquitin-proteasome system, muscle ring finger-1, myostatin, etc.[1]

Anorexia and decreased food intake experienced by most cancer patients is a major contributor to cachexia.[2] On one hand, low food intake decreases body supply with nutrients necessary for cellular function, which promotes break down of proteins and lipids in major stores in the body (e.g., skeletal muscle) as an alternative source of energy.[8-13] Thus, inadequate protein intake promotes muscle wasting. Therefore, cachexia is associated the development of malnutrition[12-15] and other wasting disorders e.g., sarcopenia and frailty.[16-18] On the other hand, improper dietary supply evokes major alterations in the structure of the resident intestinal flora.[19, 20]

A wealth of studies reports a strong causal relation between gut microbiome and pathologies contributing to many serious disorders such as cancer,[21] obesity, insulin resistance[22,23] depression,[24] major neurodegenerative disorders such as Alzheimer's disease and Parkinson's disease[25, 26] and even the current coronavirus disease 2019.[27] What is common between all these conditions is that ingestion of pathogens, toxins, and unhealthy food such as food high in fat and low in fiber promote the growth of toxic intestinal microbes and limit the growth of beneficial bacteria.[9,28] Metabolites of toxic bacteria cause local insults to the gut (permeability or dysbiosis) and pass to the systemic circulation where they reach many organs and trigger gene mutations that support pathogenesis.[28, 29] Recent studies confirm the presence of a strong association between gut microflora and skeletal muscle wasting, which is mediated by numerous physiological alterations that promote muscle protein degradation such as insulin resistance as well as increased production of free radicals and inflammatory mediators in skeletal muscle (Figure 1). These studies stronglysuggest the presence of a gut-muscle axis.[10, 14, 15, 26, 27, 30, 31]

Generalized wasting and muscle weakness associated with cachexia lead to serious functional decline, general weakness, exhaustion, and promote inactivity.[13] Limited physical activity promotes extra muscle loss via multiple mechanisms involving gut microbiome alteration, oxidative stress, and metabolic

dysfunction.[12] In addition, refeeding attempts through the use of amino acids fail to counteract anabolic resistance (i.e., increase protein synthesis) under conditions of low physical activity.[12, 26, 32]

Targeting cachexia through gut promoting interventions: The literature shows that dietary proteins and amino acids promote muscle protein synthesis during early stages of muscle atrophy, which is associated with preservation of body tissues.[33-41] However, cachectic individuals respond poorly to refeeding. In other words, cachectic muscle mass does not increase with increasing dietary intake of proteins.[12] In the meantime, treatment options for people with cancer-induced cachexia are quite limited, and treatment effects are rather discouraging, which entails poor quality of life in this group of patients.[42]

Gut bacteria represent a major factor in anabolic resistance since pathogenic ones degrade amino acids and impede the delivery of amino acids to skeletal muscle in order to trigger muscle protein synthesis.[29] Experimental evidence shows that the development of cancer alters the composition of microbiome of the gut by promoting flagellated pathogens, which boost the production of inflammatory cytokines and reactive oxygen species resulting in cachexia.[42] The classical phenotype of cachexia associated with tumors involves reduced levels of *Lactobacillales* and propagation of *Enterobacteriaceae* and *Parabacteroides*.[43] Experimentally, blocking activin receptors, which regulate the continuously occurring turnover of the epithelial membrane of the gut, could not correct cancer-induced alterations in gut microbiota.[42] However, cumulative knowledge denotes that manipulation of the gut microbiota through fecal microbiota transplant as well as prebiotics and probiotics may treat cachexia in laboratory animals.[43]

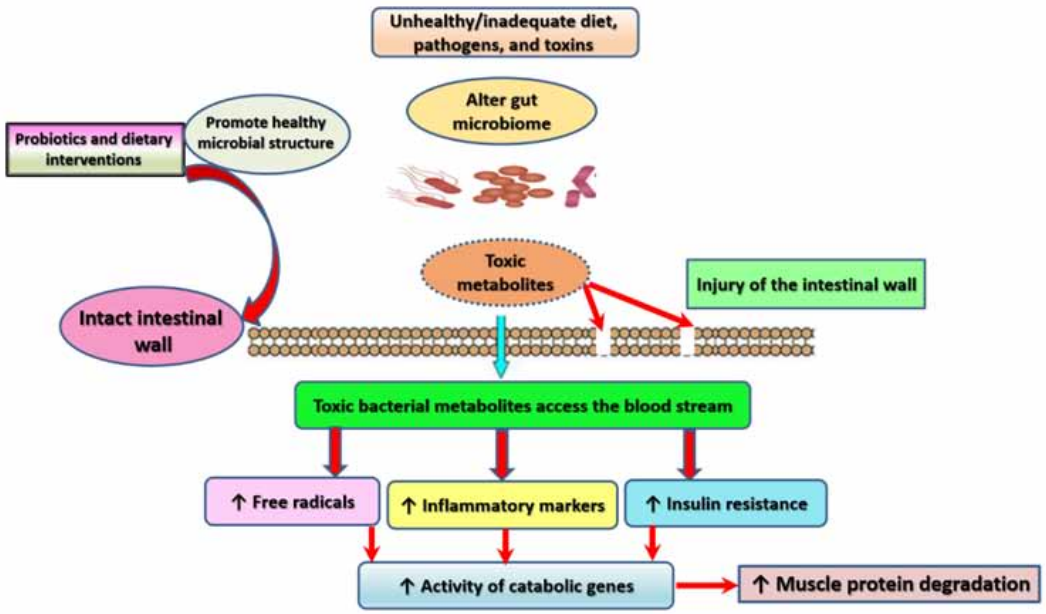

Figure 1. Schematic illustration of the contribution of gut alterations to muscle loss along with the protective role of interventions that target microbial community in the gut.

Probiotics have been increasingly used in the last few decades as a treatment approach that involves implanting live microorganisms in specific doses e.g., *Lactobacilli* and *Bifidobacteria*. "Prebiotics" is a closely related approach, which involves nutritional modifications that nurture beneficial gut microflora.[44, 45] Health-promoting species of gut microbiome contribute to health of the host by activating xenobiotic metabolism system, stimulating mucosal immunity (secretory IgA), inhibiting the growth of endotoxic bacteria, enhancing the expression of mucin, which promotes stability of the mucosal barrier, and synthesizing beneficial substances e.g., short-chain fatty acids, amino acids, antioxidants, and vitamin K.[24, 29, 44]

Evolving knowledge denotes effectiveness of probiotics in the treatment of muscle wasting in cachectic animal models. Oral consumption of probiotics containing *lactobacillus* (*L.) reuteri, L. gasseri, and L. plantarum*by cachectic mice was associated with restoration of the normal structure and balance of bacterial phyla in the gut as well as with less intestinal permeability. Accordingly, circulating levels of toxic bacterial metabolites dropped resulting in significant reduction in the production of inflammatory mediators and negative regulators of muscle protein turnover such as Atrogin-1, MuRF1, LC3, Cathepsin L in the gastrocnemius and tibialis muscles.[19,20] It is noteworthy that not all lactobacillus species can affect microbiota of the gut in a fashion that allows suppression of the activity of molecules involved in muscle atrophy. For instance, treating cachectic rats with *L. acidophilus* could not inhibit inflammatory responses in skeletal muscle or prevent muscle atrophy.[20] In addition, successful administration of probiotics is rather challenging since secretions of the upper gastrointestinal tract e.g., gastric and pancreatic secretions contribute to the damage of a considerable portion of externally administered probiotic bacteria, especially when administered in a free form.[46]

Research reports limited diversity of gut microbiome in people with musculoskeletal dysfunction such as frailty and cachexia.People with altered gut-microbiome profile express nutritional deficiencies and higher production of inflammatory markers.[11, 31] Healthy foods represent a major source of amino acids and prebiotic dietary elements, which can modify the structure of gut microbiome and promote its diversity.[24, 31] Evidence denotes that around 35% of lactic acid bacteria in fresh fruits and vegetables can successfully survive gastric conditions and reach the intestine.[24, 44]

Bee honey contains a variety of health-promoting lactic acid bacteria e.g., *Bifidobacterium, Fructobacillus, and Lactobacillaceae*. These bacterial species demonstrate strong antimicrobial activity even against the most antibiotic-resistant pathogens.[47] Moreover, honey's high contents of phenolic acids and internal hydrogen peroxide inhibit the growth of endotoxic bacteria in the gut.[24, 48] In fact, honey is considered a full food, which is consumed by bee workers during winter where foraging decreases or stops.[49] It is rich in carbohydrates, proteins, amino acids, vitamins, flavonoids, and oligosaccharides. The latter function as prebiotics, which promote the growth of healthy intestinal microflora such as *Bifidobacterium*.[50-52]

Propolis is a multifunctional bee product that bee workers produce by mixing their saliva and bee pollen with plant exudates they collect from various plants. Propolis is rich in more than 400 bioactive compounds, including phenols, flavonoids, amino acids, vitamins, and trace elements.[53] It expresses several pharmacological activities such as being an antioxidant, anti-inflammatory, anti-aging, anti-microbial, anti-cancer, anti-lipidemic, immunomodulatory, etc.[25,54] Owing to its countless bioactivities, propolis is widely used as a dietary supplement to promote health and well-being.[53,54]

Bee products such as honey were reported to hinder skeletal muscle wasting and promote weight gain, body fat, and nitrogen contents in cachectic rodents with Walker 256 carcinoma, which mimics the human neoplastic syndrome.[3] These effects were achieved via a multidimensional mechanism that involves attenuation of chronic inflammation, oxidative damage, and catabolism. A mixture of honey and *Aloe vera (L.) Burm. f. (Xanthorrhoeacea*e) modulated the antioxidant system in rats injected with tumors. Antioxidant enzymes such as super oxide dismutase (SOD) and catalase represent the first defense against oxidative stress where SOD catalyzes the dismutation of the superoxide anion (O_2) into oxygen and hydrogen peroxide (H_2O_2) while catalase subsequently converts them into water and oxygen.[3] Honey also downregulated calcium-dependent protein degradation pathway, a key contributor to cell death in cachectic muscle, by modulating the activity of its upstream effector, cysteine protease calpain.[3] It also decreased the chymotrypsin-like activity in the gastrocnemius muscle. The chymotrypsin-like activity corresponds to the catalytic core of the 20S subunit of the ubiquitin–proteasome pathway, which is considered the most important pathway for intracellular protein degradation under catabolic conditions.[3] Likewise, a combination of grape seed polyphenols and propolis, also known as bee glue, attenuated the production of proinflammatory cytokines and corrected muscle wasting in cachectic rats with chronic adjuvant-induced arthritis. Positive effects occurred in animals on continuous low doses compared with animals receiving five different high doses—signifying that correction of cachexia requires chronic treatment.[55] Although the effect of honey and propolis on gut microflora was not examined in these studies, the contribution of these bee products to the resident microflora could not be excluded. This is because honey and propolis were orally consumed,

and there's a probability that they expressed their strong antimicrobial activity in the gut, at least in part, leading to gut healing and repair.

Honey is reported to modify gut microbiome in constipated mice,[56] mice with ulcerative colitis,[57] and human intestinal microbe culture.[58] Experimental evidence shows that honey promotes the growth of health promoting species such as indigenous *Bifidobacterium* in cultures from human colon[58] while co-administration of bee honey with probiotic *Bifidobacterium* prevents the destruction of these bacteria by gastric secretions allowing a large number of cells to reach the intestine to produce their health-inducing activity.[46] In the same way, propolis, which expresses bioactivities similar to those of honey, can positively alter the structure of gut microbiome. For instance, diabetic rats receiving propolis extracts exhibited increased growth of beneficial bacterial species in the gut. This effect was associated with increased production of health-promoting bacterial metabolites (e.g., short chain fatty acids) and levels of tight junction proteins in the ileum denoting correction of gut dysbiosis. Accordingly, levels of fasting blood glucose and glycosylated hemoglobin significantly dropped while glucose tolerance and insulin sensitivity index remarkably increased.[59] It is well-known that glucose-metabolism dysregulation is a key contributor to skeletal muscle loss in diabetes.[26] The mechanism involved entails impairment of protein anabolism in muscle fibers and alteration of the structure of blood vessels resulting in less blood supply to skeletal muscle.[60, 61] Moreover, propolis is reported to correct gut microbiome in rodents on high-fat-diet (HFD). HFD creates pathogenic patterns of gut microbiome involving colonization of bacteria that promote intestinal aberration and promote inflammation and multiple dysfunctions in various remote organs including skeletal muscle, a condition known as sarcopenic obesity—muscle loss along with high fat mass. Interestingly, modifications in gut microbiome induced by propolis were associated increased muscle mass in treated animals.[62]

Bee products, although distinct in their composition and pharmacological activities, share some of their composition and bioactivities. The effect of bee products on gut microbiome has been examined in many studies including cancer samples. In this regard, trans-10-hydroxy-2-decenoic acid (10-HDA, also known as queen bee acid or royal jelly acid) expressed strong antimicrobial activity against numerous pathogenic bacterial species in human cancer colon cells.[63] In addition, 10- HDA is considered a strong anti-inflammatory and anti-oxidant agent,[64, 65] which demonstrates protective effects against skeletal muscle wasting by modulating muscle metabolic functioning and glucose uptake.[53]

To our knowledge, natural honey and propolis are the only bee products known to restore cachectic muscle. In the light of the presented literature, the current article sets a number of questions that worth investigation in future studies: do honey/propolis improve muscle loss in cachexia through modulation of gut microbiome? If so, what is the exact nature of change e.g., in terms of microbial phyla affected, change in bacterial metabolites, repair of gut dysbiosis, and signaling cascades involved in oxidative and inflammatory reactions both locally in the gut and in skeletal muscle? What constituents of honey/propolis are likely to therapeutically affect gut microbiome? Are other bee products (e.g., bee venom, bee pollen, royal jelly, and their key constituents) capable of improving metabolic gut microbiome composition and correcting metabolic and inflammatory dysregulations in cachexia? If so, what are the most muscle promoting agents among all bee products? Accordingly, further investigations of the role of such natural products in cachectic models would promote the development of successful randomized trials in the future.

Conclusion

Use of probiotics and multifunctional foods such as bee honey/propolis is likely to decrease muscle atrophy in cachectic models. Probiotics improve muscle condition by altering gut microbiome structure. Although honey/propolis decreased systemic inflammation and improved skeletal muscle mass in cachectic rodents, their effects on the composition and functionality of gut microbiome were not examined yet, which worth investigation in future studies. Evaluation of the effect of other bee compounds (e.g., bee venom, royal jelly, and bee pollen) on cachectic muscle is also necessary for identification of the most effective substances.

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