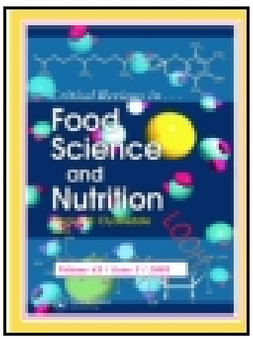

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Hop-derived Bitter Compounds, Glucose Homeostasis, GLP-1.

COULD HOP-DERIVED BITTER COMPOUNDS IMPROVE GLUCOSE HOMEOSTASIS BY STIMULATING THE SECRETION OF GLP-1?

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ABSTRACT

Hops (*Humulus lupulus* L.) is by far the greatest contributors to the bitter property of beer. Over the past years, a large body of evidence demonstrated the presence of taste receptors in different locations of the oral cavity. In addition to the taste buds of the tongue, cells expressing these receptors have been identified in olfactory bulbs, respiratory and gastrointestinal tract. In the gut, the attention was mainly directed to sweet Taste Receptor (T1R) and bitter Taste Receptor (T2R) receptors. In particular, T2R has shown to modulate secretion of different gut hormones, mainly Glucagon-like Peptide 1 (GLP-1), which are involved in the regulation of glucose homeostasis and the control of gut motility, thereby increasing the sense of satiety. Scientific interest in the activity of bitter taste receptors emerges because of their wide distribution in the human species and the large range of natural substances that interact with them. Beer, whose alcohol content is lower than in other common alcoholic beverages, contains a considerable amount of bitter compounds and current scientific evidence shows a direct effect of beer compounds on glucose homeostasis. The purpose of this paper is to review the available literature data in order to substantiate the novel hypothesis of a possible direct effect of hop-derived bitter compounds on secretion of GLP-1, through the activation of T2R, with consequent improvement of glucose homeostasis.

KEYWORDS

Hop-derived Bitter Compounds; Glucose Homeostasis; GLP-1.

INTRODUCTION

Hops (*Humulus lupulus* L.), a widely used agent in the brewing industry, is the main contributor to the bitter property of beer (**Liu et al.,2015**). Therefore, beer is the most important dietary source of a number of hop-derived substances. The effects of beer on glucose homeostasis seem to be still controversial (**Huang et al.,2017; Sluik et al.,2017**). Since numerous years, Scientific Community agrees to state that a moderate alcohol consumption of wine and beer shows the strongest positive effects on health, contributing to reduce, in particular, cardiovascular risk (**de Gaetano et al.,2016**). In this context, the known "French Paradox" demonstrated how abstemious and high-alcohol consumers have a cardiovascular risk higher than moderate alcohol consumers (**Renaud et al.,1992**). This association, defined "U relation" and confirmed by other studies, underlines the beneficial effects of low alcohol doses on insulin resistance and, at the same time, the cardioprotective effect of non-alcoholic compounds in drinks, in particular, polyphenols (**Chiva-Blanch et al.,2013**). However, for many years, the interest was focused on wine, specifically due to its content in polyphenolic compounds. Recently, scientific research is moving towards the focus also on the effects of a moderate consumption of beer, which contains sufficient amounts of polyphenols as well, such as flavonoids and phenolic acids (**Arranz et al.,2012**). This consideration could explain the mechanism of action that makes beer comparable to wine about the effects on health. Some clinical trials demonstrated the role of beer consumption in improving glucose metabolism (**Imhof et al.,2009**; **Shai et al.,2007; Beulens et al.,2008**), and suggested that a further mechanism by which moderate alcohol consumption associated with beer consumption protects against cardio-vascular disease could be mediated by the effects of beer-derived polyphenols on glucose homeostasis (**Arranz et al.,2012**, **Chiva-Blanch et al.,2013**). In a very recent meta-analysis, beer consumption of 20--30 g/day was associated with a significant reduction of the risk of Type 2 Diabetes Mellitus (T2DM) (**Huang et al.,2017**).

However, the presence in the beer of considerable amounts of hop-derived bitter compounds (**Kao and Wu,2013**) might provide an alternative explanation for the favourable effects of moderate beer consumption on glucose metabolism. Indeed, many studies in literature pointed out the co-localization of bitter taste receptors with endocrine cells of gastrointestinal secreting gut hormones linked with glucose metabolism (**Avau et al.,2015**, **Andreozzi et al.,2015**). Therefore, our attention has focused on the possible influence of bittering acids contained in the beer on the release of the gut hormones involved in the control of glucose homeostasis. The purpose of this paper is to summarize the available literature and to provide a scientific plausibility to the novel hypothesis that effects of beer on glucose homeostasis regulation could be also derived from the interaction between hop-derived bitter compounds and bitter receptors in gastrointestinal tract.

HOP-DERIVED BITTER COMPOUNDS IN BEER

Beer is a beverage obtained from the fermentation of a must of water and cereal, to which hops are added. This complex production process makes this drink a harmonious container of nutrients and bioactive compounds. A traditional approach to chemical composition would describe beer as a drink composed almost exclusively of water and ethyl alcohol with a modest amount of carbohydrates, poor in protein and no fat. Alcohol content in beer is lower than in other common alcoholic beverages (**de Gaetano et al.,2016**). It could represent an advantage, because daily consumption of standard drink of beer (330 ml, according to U.S. Department of Agriculture, USDA) can be considerate as moderate alcoholic intake. This moderate consumption has positive effects on health, in particular reducing T2DM risk. Indeed, epidemiological studies showed how moderate alcohol consumption decrease this risk in man (47000 subjects, 36% lower risk) and women (110000 subjects, 58% lower risk) (**Kondo,2004**).

Beer nutritional composition has been widely described. USDA provided a detailed description taking in account all micro- and macronutrient (**USDA,2017**) (***Table 1***). For many years, public opinion supported the hypothesis that beer was more caloric than other alcoholic beverages; because of this

reason, it was thought to increase body weight. Several studies showed that this idea was incorrect (**Hätönen et al., 2012**). Cross-sectional and prospective studies, indeed, demonstrated that beer consumption does not cause significant increase in body mass index and/or waist-to-hip ratio (**Kondo,2004**).

The addition of hops enriches the beverage of compounds such as bitter compounds, aldehydes, ketones, higher alcohols, polyphenols that give all those typical organoleptic characteristics and, above all, a potential bioactivity. Amount of bitter compounds present in beer is reported in ***Table 2*** and bitter compounds have identified and quantified by an High-Performance Liquid Chromatography with Diode-Array Detection (HPLC-DAD) and Mass Spectrometry protocol (**Kao and Wu,2013***)*. It must be considered that, although mammals have an adversity to bitter compounds due to the association of bitter taste to toxins, beer bitter compounds are believed responsible for imparting to beer the refreshing bitter taste well recognized by the beer consumer in all well brewed brands of beer.

Individually, these nutrients and other compounds are also present in other foods and beverages, but considering the complex production technology, it could be hypothesized that beer is not a mere mix of different ingredients but that all components undergo changes during the production process and that they expose their function to human in a synergistic manner. Hops (*Humulus lupulus* L.), by far the greatest contributor to the bitter property of beer, is a plant belonging to the *Cannabinaceae* botanic family. In most Countries, hops producers grow non-fertilized female plants, because only female, conical, flowers can be used in brewery. Each female flower has a powdered and yellow substance at the base, called lupulin. The lupulin contains bitter resins, essential oils, tannins and terpenes. The brewing and commercial value of hops is due to its amaricating and flavouring properties (**Liu et al.,2015**). Total resins represent the soluble fraction of hops both in methyl alcohol and in diethyl ether. Based on their solubility in organic solvents, they are distinguished by “soft resins” and “hard resins”. Soft resins, soluble in hexane and in paraffinic hydrocarbons, include α- and β-acids; hard resins, which are non-

soluble in paraffinic hydrocarbons, include, instead, oxidation products of α- and β-acids. Different chemical structures have been identified for both α- acids and β-acids: humulone, co-humulone, ad-humulone, lupulon, co-lupulon, ad-lupulon with their isomers **(Zhang et al.,2004; Taniguchi et al.,2015)**.

The bitter characteristics of beer, both for the variety of substances and for their quantity, depend on the diversity, quantity and hops adding time. Hops varieties are distinguished by aromatic and bittering, depending on the relation between aromatic and bitter substances. From the amount of resin added, it will have beers of different International Bittering Units (IBU) value. In addition, the α- and β-acids are also isomerized during the baking of the must, so if the resin is added at the start of cooking, the percentage of iso-acids increases. This is one of the most important considerations to be taken into account in our hypothesis that beer may be related to regulation of glucose homeostasis.

Hops have characteristic secondary metabolites (unique hops compounds: α-acids, β-acids and xanthohumol; highly characteristic hops compounds: α-humulene, β-caryophyllene) which are transformed during the brewing process into bittering and flavoring components (**Steenackers et al.,2015**). Among essential oils, monoterpene β-myrcene and the sesquiterpenes α-humulene and β-caryophyllene are the major compounds responsible for aroma, while only trace levels of oxygenated and sulfur compounds are also found (**Almaguer et al.,2014**). Mono- and sesquiterpenoid-related alcohols, such as linalool, geraniol, nerolidol, nerol, and terpineol contribute to the hop aroma. A complex mixture of phenolics, such as flavonols (e.g. quercetin), flavan-3-ols (e.g. catechin), phenolic acids (e.g. ferulic acid), and corresponding glycosides and polymers, are also present in the beer (**Champagne and Boutry,2017**). Whilst α and β-acids are the main responsible of the bitterness of the beer, essential oils from the hops are responsible for the bulk of the aroma and flavour. Bittering is applied by boiling hop α-acids, converting them to iso-α acids, which are bitter. In particular, throughout the traditional wort-boiling process, the specialized metabolites are converted into valuable compounds. Therefore, in the beer

there are bitter compounds that are not present in the hops (**Champagne and Boutry,2017**). Isomerized α-acid can also be added after fermentation. During fermentation, prenyl flavonoids are lost due to incomplete hop extraction in must (13-25%), absorption of insoluble must (18-26%) and adsorption of yeast cells. After brewing beer, xanthohumol are largely in the form of isoxanthohumol (about 22--30% of hop xanthohumol); about 10% of desmethylxanthohumol is completely transformed into prenyl naringenin; geranyl naringenin behaves in a very similar way to the desmethylxanthohumol. In addition, malt carbohydrates form soluble complexes with xanthohumol and isoxanthohumol (**Stevens et al.,1999a***)*. The isoxanthohumol is the most abundant flavonoid in beer, ranging from 0.04 to 3.44 mg/l *(***Stevens et al.,1999b***)*.

HOP-DERIVED BITTER COMPOUNDS AND GLUCOSE METABOLISM: CURRENT EVIDENCE

Current scientific evidence shows a direct effect of beer compounds on glucose homeostasis (**Huang et al.,2017**). In particular, it was found that in healthy men the moderate daily consumption of beer (330 mL; 16 g alcohol), even over a short period of use (30 d), induced significant changes in glucose homeostasis and lipid profile, thereby reducing insulin resistance (**Nogueira et al.,2017**). An *in vivo* study on murine model showed that in type 2 diabetic KK-A(y) mice, the administration of xanthohumol decreased plasma levels of glucose, triglyceride and weight of white adipose tissue, while increasing levels of adiponectin, with an interesting effect in attenuating diabetes (**Nozawa,2005**). This effect of xanthohumol on reduction of plasma glucose levels and weight was confirmed in other studies on rodent model of obesity, such as Zucker fa/fa rats **(Legette et al.,2013)** or T2DM mice (**Miranda et al.,2016**), with a clear dose-dependent effect. In addition, Miranda et al. reported also a significant decrease in triglycerides, total cholesterol, Low Density Lipoprotein (LDL)-cholesterol, Interleukin (IL)-6, Monocyte Chemoattractant Protein (MCP)-1, insulin and leptin levels induced by xanthohumol administration, with improvement of markers of systemic inflammation and metabolic syndrome **(Miranda et al.,2016**). More recently, the effects of xanthohumol were analyzed in association of 8-

prenylnaringenin, another beer-derived polyphenol, on liver and skeletal muscle lipid and glycolytic metabolism in T2DM mice model (**Costa et al.,2017**). The Authors found that these polyphenols promoted hepatic and skeletal muscle AMP-Activated Protein Kinase (AMPK) activation, and reduced the expression of target lipogenic enzymes (sterol regulatory element binding protein-1c and fatty acid synthase) and acetyl-coenzyme A carboxylase activity, thus preventing body weight gain and improving plasma lipid profile, insulin resistance and glucose tolerance.

In vitro studies showed that these compounds are able to activate Peroxisome Proliferator-Activated Receptors (PPAR) α and PPARγ, which modulate the transcription of genes involved in regulation of glucose metabolism. PPARγ agonists, such as pioglitazone, indeed, are used to treat hyperglycemia or T2DM. Several studies showed that isohumulones improved glycemic and lipidemic metabolism, acting with a mechanism similar to those of pioglitazone. Interestingly, unlike pioglitazone, isohumulone does not cause increased body weight. In type 2 diabetic mice, isohumulones reduced blood levels of glucose, triglyceride and free fatty acids. In subjects with mild T2DM, isohumulones reduced not only plasma glucose and Hemoglobin A1c (HbA1c), but also systolic blood pressure, supporting its role as a natural agent for treatment of obesity, diabetes and associated complications, such as hypertension (**Kondo,2004**). A study in humans investigated the hypoglycemic effect of isohumulones in subjects with prediabetes. Isomerized hop extract, containing note amounts of isohumulones, was tested using soft capsule by Obara et al. (**Obara et al.,2009**) In their study, 95 subjects with prediabetes, were randomly assigned to each of the four study group: A (placebo), B (16 mg of isohumulones), C (32 mg of isohumulones) and D (48 mg of isohumulones); each treatment was followed for 12 weeks. Results showed that 48 mg isohumulones for 12 weeks were effective in decreasing fasting blood glucose, HbA1c, body weight and abdominal fat, suggesting a possible role for isohumulones a novel natural treatment for the management of obesity and T2DM (**Obara et al.,2009**). In addition, Costa et al. focused on the effects exerted by beer-derived polyphenols on diabetic wound healing, reporting that antioxidant

and anti-inflammatory effects of xanthohumol supplemented in beer were effective in modulating oxidative stress, inflammation and angiogenesis, three intermingled processes implicated in the pathogenesis of diabetes mellitus and diabetic vascular complications (**Costa et al.,2013**).

The hop-derived bitter acids also contribute to the overall microbial stability of beer *via* favoring the brewer's yeast over other microorganisms (**Champagne and Boutry,2017**). Recent evidence suggests the anti-inflammatory potential of hops-derived compounds, which are exerted *via* the inhibition of plasma lipopolysaccharide (LPS)–stimulated prostaglandin E_2 production, nitric oxide formation, cyclooxygenase 2 abundance, and the proinflammatory transcription factors Nuclear Factor kappa B (NF-kB) pathway in macrophages **(Desai et al.2009)**. In particular, tetrahydroisoalpha acids, a well-defined hop extract (termed META060) derived from hops, has been found to exert anti-inflammatory activity on LPS-stimulated tumor necrosis factor alpha (TNF-a) and interleukin-6 (IL-6) production in isolated peripheral blood mononuclear cells **(Konda et al.,2010)**. Everard et al. (**Everard et al., 2012**) analyzed the effect of META060 on body weight gain, fat mass development, glucose homeostasis and gut barrier function markers in diet-induced obese and type 2 diabetic mice treated with META060 for 8 weeks. In this study, the Authors reported that META060 was effective in reducing body weight gain, adipogenesis, glucose intolerance, and fasted hyperinsulinemia, and normalizing insulin sensitivity markers. Of interest, these effects were associated with the improvement of gut barrier function, with consequent reduced metabolic endotoxemia and inflammation.

Besides the effects of specific hop-flavored flavonoids of the beer, only few studies directly investigated the effect of the beer on glucose metabolism. As reported by Hätönen KA et al. (**Hätönen et al.,2012**) beer produced a high Glycemic Index (GI) value, the numerical index that ranks carbohydrates according to how they affect blood glucose levels (**IOS,2010**), due to its content of complex maltooligosaccharides (**Hätönen et al.,2012**). However, Hosaka et al **(Hosaka et al.,2008**) evaluated the effect of alcohol on blood glucose levels in T2DM patients treated by diet alone, within 2h after the

respective intake of three different alcoholic beverage: beer, sake (a fermented alcoholic beverage) and shochu (a distilled alcoholic beverage). Beverage used in this study, indeed, had a different nutritional composition, in particular, relative to alcohol (beer: 3.7%; sake: 12.3%; shochu: 20.5%) and glucose (beer: 3.1%; sake: 4.9%; shochu: 0%), while the amount of each beverage was calculated to obtain an almost equal intake of ethanol. The Authors found that, although a limited intake of alcoholic drinks was required to improve blood glucose levels, beer consumption caused the greatest increase in blood glucose levels compared to other beverages due its higher carbohydrate contents. Nevertheless, it should be considered that that the amount of beer consumed in the study (800 ml) was higher than the standard drink (330 ml), as described by USDA (**USDA,2017**). Additionally, it has reported that the consumption of non-alcoholic beer tended to lower the GI compared to beer (**Hätönen et al.,2012**). Taking in account these considerations, it is not possible to conclude that beer consumption *per se* worsens glucose homeostasis, but only that glucose content of this beverage could be involved in this negative metabolic mechanism. Among further possible mechanisms that improve glucose metabolism through beer consumption, bitter taste receptors stimulation by beer bitter compounds could play a fundamental role.

BITTER TASTE RECEPTORS

Bitter taste is considered to be a toxicity detector, which provides a final analytical detector just before ingestion, in a warning and defensive modality (**Yan et al.,2001**). Bitter is sensed by 25 subtypes of the Taste Receptor Type 2 (TAS2R or T2R) family of with seven-transmembrane G protein-coupled receptors expressed in the oral cavity (**Lu et al.,2017**). Recently, taste receptors have been identified in extra-oral tissues, suggesting additional functions for these receptors besides taste perception (**Chandrashekar et al.,2006**). α-gustducin, a taste-specific G-protein that detects sweetness, umami and bitterness, is a heterotrimeric protein composed of the products of the GNAT3 (α-subunit), GNB1 (β-subunit) and GNG13 (γ-subunit) (**Wong et al.,1996**). After the binding of bitter taste compounds to T2Rs on taste receptor-expressed cells, T2R signal transduction starts with the dissociation of α-gustducin into

Gα and Gβγ subunits. The Gα subunit stimulates Phosphodiesterase (PDE) and then a decrease of the intracellular cAMP level, while Gβγ stimulates phospholipaseβ2 (PLCβ2) for activation of the IP3/DAG pathway, thereby leading to the release of calcium ions into the cytosol due to the activation of the inositol trisphosphate receptor (InsP3R) in the endoplasmic reticulum. The activation of the IP3/DAG pathway is followed by the Na^+ influx through the membrane cation channel Transient Receptor Potential M5 (TRPM5), which depolarizes the cells and causes the release of gut hormones from vesicles gastrointestinal tract and neurotransmitter ATP through gap junction hemichannels (**Park et al.,2015; Avau et al.,2015; Lu et al.,2017**). The subsequent cell depolarization and neurotransmitter release activate sensory nerves cranial nerves VII, IX, and X that project to the nucleus of the solitary tract in the medulla (**Katz et al.,2000**). The discovery of taste receptors and their downstream signaling pathways in the endocrine cells of gastrointestinal tract suggests that they might sense nutrients, similarly to the taste receptor cells on the tongue, thereby contributing to the regulation of gut motility and gut hormones release, which modulate hunger signalling (**Avau et al.,2015**; **Depoortere et al.,2015**; **Andreozzi et al.,2015; Avau and Depoortere,2016**). Therefore, although a complex set of signaling cascades modulates the release of gut hormones, a therapeutic potential in the treatment of obesity and T2DM of bitter agonists might be currently suggested (**Avau et al.,2015**).

EFFECT OF BITTER COMPOUNDS ON GLUCAGON-LIKE PEPTIDE 1

Taste receptors expressed on endocrine cells of gastrointestinal tract could be involved in the modulation of the secretion of gut hormones, mainly Glucagon-like Peptide 1 (GLP-1) (**Rozengurt et al.,2006**). The endocrine cells of the gastrointestinal tract represent <1% of the intestinal epithelium, but, as a whole, they constitute the largest endocrine organ of the body, producing and releasing >20 identified hormones (**Rehfeld, 1998**). GLP-1 is a peptide-derived hormone belonging to the incretin family, which is primarily produced by the cleavage of the proglucagon in endocrine intestinal L cells (**Campbell and Drucker, 2013**). During fasting, in humans, this hormone is present in a range of 5 to 10 pmol/L, while

reaching two to three times higher levels after meals. Its half-life is very short, about 2 minutes, due to degradation by Dipeptidyl Peptidase (DPP)-4. GLP-1 exists in two biologically active form: GLP-1-(7-37) and GLP-1-(7-36)NH_2 (**Campbell and Drucker, 2013**). This hormone is secreted in response to a variety of nutrients, such as carbohydrates, fibers, proteins, amino acids, Monounsaturated Fatty Acids (MUFA), Polyunsaturated Fatty Acid (PUFA) and non-nutritional sweeteners. GLP-1 increases the glucose-dependent insulin release by pancreatic β-cells and suppresses the glucagon secretion. Specifically, when plasma glucose levels are normal, GLP-1 is not able to stimulate insulin secretion; in this way it does not cause hypoglycemia (**Pham et al.,2016)**. GLP-1 also confers glucose sensitivity to glucose-resistant β cells, stimulates β cell proliferation, and inhibits β cell apoptosis. Additionally, GLP-1 slows gastric emptying, promotes satiety and suppresses energy intake (**Steinert et al.,2016; Zanchi et al.,2017**). As a representative incretin, GLP-1 is currently one of the most important target for the treatment of T2DM (**Troke et al.,2014**). Nevertheless, increasing endogenous GLP-1 secretion as a therapeutic strategy has led to heightened interest in endocrine cells of gastrointestinal tract and the gut-pancreatic axis (**Ezcurra et al.,2013**). In particular, the activation of T2R has been studied as a potential therapeutic target of T2DM because of its stimulation of GLP-1 resulting in hypoglycemic effect (**Depoortere,2014**).

The effects of T1R on GLP-1 secretion have been previously reported, and L cells of the gut are able to "taste" glucose through the same mechanisms used by taste cells of the tongue (**Jang et al.,2007**). Interestingly, it was also observed that T2R haplotype is associated with impaired glucose homeostasis, as a Single Nucleotide Polymorphisms (SNPs) in this haplotype alter the normal T2R9 receptor response, resulting in an impairment of glucose homeostasis (**Dotson et al.,2008; Shin et al.,2008)**. In the recent years, remarkable progress has been made in demonstrating the expression of molecular transducers in bitter taste signaling of cells in the gastrointestinal tract involved in the secretion of gut hormones (**Li et al.,2017**). In particular, induced increase in GLP-1 by bitter compounds has been considered to have an

important role in host defence through reduction of toxic food intake (**Kim et al.,2014**). Several *in vitro* studies showed that the interaction of various bitter compounds with T2R on human and murine endocrine cells of gastrointestinal tract determines the secretion of gut hormones. The scientific evidence is summarized in ***Table 3***. In details, Rozengurt et al. reported that the stimulation of both human colon and intestinal endocrine cell lines (HuTu-80 and NCI-H716 cells expressing GLP-1) with the bitter-tasting compound phenylthiocarbamide, which binds T2R38 induced a rapid increase in the intracellular Ca^{2+} concentration in these cells (**Rozengurt et al.,2006**). Additionally, the administration of denatonium benzoate, a ligand of bitter taste receptor cells, in diabetic mice (Lepr−/− (db/db) mice) resulted in decreased blood glucose levels and increased GLP-1 levels compared with the control group (**Kim et al.,2014**). On the other hands, as previously mentioned, Avau et al. demonstrated that the administration of denatonium benzoate resulted in a reduced gastric emptying on mice and increased satiation in humans (**Avau et al.,2015**). More recently, Park J et al. found that GLP-1 hormone is co-localized with T2R5 in the human duodenum and ileum tissue and that 1,10-phenanthroline, which is known agonist of T2R5, is able to secrete GLP-1 hormone through stimulation of T2R5 in human endocrine cells of gastrointestinal tract (**Park et al.,2015**). Berberine, the main component of a number of particularly bitter medicinal plants, including Coptis Rhizome, has been successfully used for T2DM (**Yu et al.,2015; Liu et al.,2015; Chang et al.,2015**). In previous studies, berberine-induced GLP-1 secretion has reported as a possible mechanism for berberine hypoglycemic effect **(Yu et al.,2010; Lu et al.,2009; Yu et al.,2015)**. Yu Y et al. demonstrated that berberine stimulated the GLP-1 secretion *via* activation of gut-expressed bitter taste receptors through the PLCβ2 signaling cascades in human enteroendocrine NCI-H716 cells, but not TRPM5 (**Yu et al.,2015**). Recent studies revealed that also Qing-Hua Granule, a compound originated from Gegen-Qinlian-Decoction, one of the well-known traditional Chinese medicines clinically employed also to treat T2 diabetic patients, regulated glucose homeostasis by inducing GLP-1 secretion *via* activation of T2R5 expressed in gastrointestinal tract in db/db diabetic mice (**Li et al.,2017**). Additionally,

agonists of T2R38, such as the bitter compound 6-n-propylthiouracil, are able to increased GLP-1 levels and have suggested as novel and effective agents in diabetes management (**Pham et al.,2016**).

Taking in account the nutrition composition of beer and, in particular, its amount of bitter compounds, it is tempting to speculate that just these nutrients would be involved in the activation of GLP-1 pathway, thereby contributing to beer hypoglycemic effects. Nevertheless, other beer compounds might offer a further explanation for the favorable effects of beer consumption on glucose homeostasis. Indeed, besides the possible effects on GLP-1 secretion of beer carbohydrates, mainly malt oligosaccharides and starch, interestingly polyphenols from various sources have proven to stimulate L-cells to secrete GLP-1 (**Domínguez Avila et al.,2017**). However, in the previous literature it has been hypothesized that the main effects of polyphenols on GLP-1 secretion is due to different mechanisms, including the increase in its half-life by inhibiting DPP4, the stimulation of β-cells to secrete insulin and stimulate the peripheral response to insulin, increasing the overall effects of the GLP1-insulin axis, but it was not yet hypothesized that this happens through the stimulation of the bitter receptors. Additionally, alcohol *per se* has also been shown to influence a number of hormones linked to satiety, including GLP-1 (**Traversy and Chaput,2015**).

CONCLUSION AND FUTURE PROSPECTIVES

The discovery of a new pathway for the secretion by L-intestinal cells of the GLP-1 following the presence of macronutrients in the intestine, has led to the research for incretins as a new class of antidiabetic drugs. Increasing endogenous GLP-1 secretion as a therapeutic strategy has led to heightened interest in nutrient-sensing mechanisms and endocrine cells of gastrointestinal tract. Recent evidence indicated that a number of bitter compounds interact with receptors belonging to the T2R family expressed on human and murine enteroendocrine cell membrane and are able to stimulate the release of different gut hormones, mainly GLP-1, which is strictly involved in glucose metabolism. Therefore, it tempting to speculate that also bitter compounds in the beer, when introduced in small amounts with

carbohydrates (which instead stimulate the sweet taste), could favor the metabolism of carbohydrates by stimulating insulin secretion through gut hormone pathways, with a possible novel mechanism showed in ***Figure 1***.

The main limitation in the use of hop-derived bitter compounds in clinical practice is that they are contained in beer whose excessive consumption could result in several adverse health effects due to both alcohol and the high content of glucose. Thus, in view of the current evidence we hypothesize that could be a novel possible mechanism through which the beer may regulate glucose metabolism, due to its bitter components, thereby suggesting a nutraceutical approach using bitter molecules from beer in a pharmaceutical formulation, such as acid-resistant capsules in order to avoid the side effects related to excessive consumption of beer. Nevertheless, it should be considered that beer is a mix of different ingredients, such as polyphenols, which exert their function in a synergistic manner. Future studies are mandatory to investigate whether the possible contribution of different beer bitter compounds to the favorable effects exerted by beer on glucose homeostasis is also mediated by the stimulation of the secretion gut hormones. In the same way, it could be possible investigate if consumption of dealcoholized beer could improve glucose homeostasis making the bitter compounds of beers a promising strategy for management diabetic patients.

Abbreviations

T1R **,** Taste Receptor 1

T2R, Taste Receptor 2

GLP-1, Glucagon-like Peptide 1

T2DM, Type 2 Diabetes Mellitus

USDA, U.S. Department of Agriculture

HPLC-DAD, High-Performance Liquid Chromatography with Diode-Array Detection

IBU **,** International Bittering Units

LDL **,** Low Density Lipoprotein

IL-6, Interleukin 6

MCP-1, Monocyte Chemoattractant Protein-1

AMPK, AMP-Activated Protein Kinase

PPAR, Peroxisome Proliferator-Activated Receptor

HbA1c, Hemoglobin A1c

GI, Glycemic Index

DPP IV, Dipeptidyl Peptidase IV

MUFA **,** Monounsaturated Fatty Acids

PUFA, Polyunsaturated Fatty Acid

SNPs, Single Nucleotide Polymorphisms.

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AUTHOR CONTRIBUTIONS

The Authors' responsibilities were as follows: LB and GA: were responsible for the concept of this paper and drafted the manuscript; GM, AA, GCT, AC and SS: provided a critical review of the paper. All Authors contributed to and agreed on the final version of the manuscript.

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Table 1. Nutritional composition of beer (data from USDA,2017)

Nutrient	Units	Mean content	
		Per 100 g	Per standard drink,333.3 ml
Water	g	91.96	306.4
Energy	kcal	43	143.2
Protein	g	0.46	1.53
Total lipid (fat)	g	0	0
Carbohydrate, by difference	g	3.55	11.8
Fiber, total dietary	g	0	0
Sugars, total	g	0	0
Minerals			
Calcium, Ca	mg	4	13.3
Iron, Fe	mg	0.02	0.06
Magnesium, Mg	mg	6	20
Phosphorus, P	mg	14	46.6
Potassium, K	mg	27	89.9
Sodium, Na	mg	4	13.3
Zinc, Zn	mg	0.01	0.03
Copper, Cu	mg	0.005	0.016
Manganese, Mn	mg	0.008	0.026
Selenium, Se	µg	0.6	2
Fluoride, F	µg	44.2	147.2
Vitamins			
Vitamin C, total ascorbic acid	mg	0	0
Thiamin	mg	0.005	0.016
Riboflavin	mg	0.025	0.08
Niacin	mg	0.513	1.7
Pantothenic acid	mg	0.041	0.14
Vitamin B-6	mg	0.046	0.14
Folate, DFE	µg	0.6	2
Choline, total	mg	10.1	33.6
Vitamin B-12	µg	0.02	0.06
Vitamin A, RAE	µg	0	0
Vitamin E (alpha-tocopherol)	mg	0	0
Vitamin D	IU	0	0
Vitamin K (phylloquinone)	µg	0	0
Amino acids			
Alanine	g	0.012	0.04
Aspartic acid	g	0.016	0.05
Glutamic acid	g	0.047	0.15
Glycine	g	0.013	0.04

Proline	g	0.035	0.12
Other			
Alcohol, ethyl	g	3.9	13

Table 2. Bitter compounds present in beer (*Kao and Wu,2013*)

Bitter compound	Mean content (µg/g)
Isoxanthohumol	36.2
Xanthohumol	29.6
8-prenylnaringenin	7.84
6-prenylnaringenin	19.2
Cohumulone	44.7
Humulone	123
Adhumulone	21.8
Colupulone	44.2
Lupulone	33.2
Adlupulone	5.76

Table 3. Main studies demonstrating effect of bitter substances on glucagon-like peptide-1 secretion

Authors	*Type of study*	*Bitter substance*	*Cell line/experimental model*	*Receptor*	*Main experimental method*
Rozengurt et al.,2006	In vitro	Phenylthiocarbamide	HuTu80NCIH716	T2R38	RT-PCR
Kim et al.,2014	In vitro and in vivo	Denatonium benzoate	NCIH716Lepr−/− (db/db) mice	T2R	RT-PCRSmall interfering (si)RNA preparation
Parket al.,2015	In vitro	1,10-phenanthroline	Human L cells	T2R5	RT-PCR
Yuet al.,2015	In vitro	Berberine	NCIH716	T2R38	Western blot chemiluminescence assay
Phamet al.,2016	In vitro and in vivo	Propylthiouracil	Knockout HuTu-80 old male BALB/c mice	T2R38	Small interfering (si)RNA preparation IHC
Li et al.,2017	In vitro and in vivo	Qing-Hua Granule	db/db diabetic mice	TS2R /TS2R5α-gustducin PLCβ2TRPM5	RT-PCR,Western blotIHC

T2R, Taste Receptor Type 2; ***GLP-1,*** Glucagon-like Peptide-1; ***RT-PCR,*** Real-time Polymerase Chain Reaction; ***T2R5,*** Taste Receptor Type 2 Member 5; ***T2R38,*** Taste Receptor Type 2 Member 38; ***IHC,*** Immunohistochemistry*;* ***PLCβ2,*** 1-phosphatidylinositol-4, 5-bisphosphate phosphodiesterase β-2; ***TRPM5,*** Transient Receptor Potential Cation Channel Subfamily Member 5.

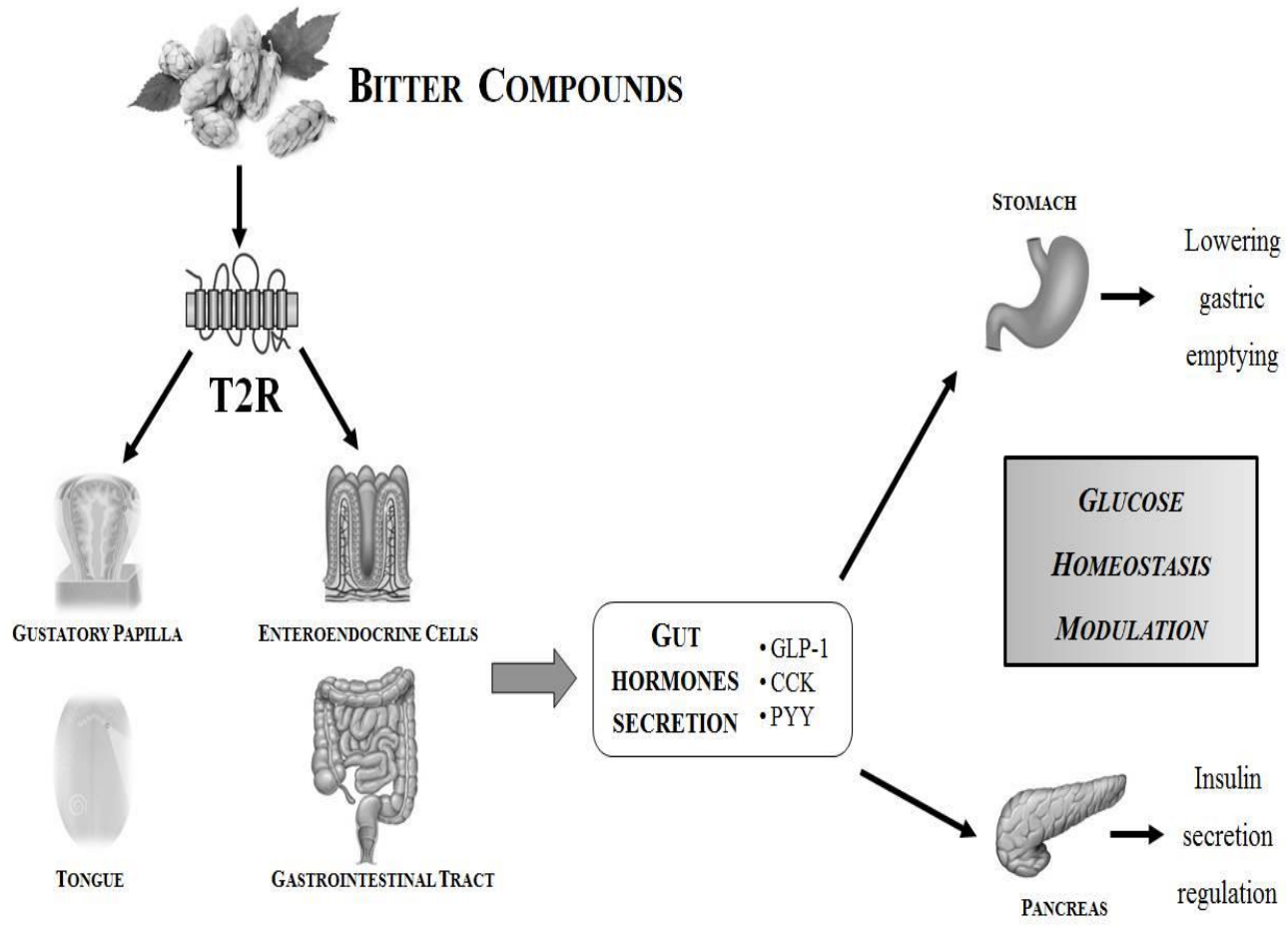

Figure 1: Suggested mechanism of action of beer bitter compounds on glucose homeostasis. The possible mechanism of action by which bitter compounds contained in beer could regulate glucose metabolism involves the stimulation of bitter Taste Receptors (T2R) activity. Briefly, similarly to other bitter compounds, beer bitter compounds could interact with T2R expressed in gastrointestinal tract, resulting in the stimulation of gut hormone secretion, in particular, Glucagon-like peptide 1 (GLP-1), Cholecystokinin (CCK), Peptide YY (PYY), involved in glucose homeostasis.