ABSTRACT

The growing prevalence of obesity affects millions of people around the world and has gained increased attention over the years because it is associated with the development of other chronic degenerative diseases. Different organizations recommend lifestyle changes to treat obesity; nevertheless, other strategies in addition to lifestyle changes have recently been suggested. One of these strategies is the use of probiotics in fermented dairy products; however, a need exists to review the different studies available related to the potential antiobesity effect of these products. Because probiotic fermented dairy products that support weight management are not available in the market, there is a great opportunity for the development of functional dairy products with new lactic acid bacteria that may present this added health benefit. Thus, the purpose of this overview is to highlight the importance of probiotic fermented dairy products as potential antiobesogenic functional foods and present in vitro and in vivo studies required before this kind of product may be introduced to the market. Overall, most studies attributed the antiobesity effect of fermented dairy foods to the probiotic strains present; however, bioactive peptides released during milk fermentation may also be responsible for this effect.

Key words: probiotic dairy products, obesity, body weight reduction

INTRODUCTION

A large number of people (1.9 billion adults) suffer from overweight or obesity, which is defined by the World Health Organization as an abnormal or excessive fat accumulation that may impair health (WHO, 2016). Body mass index (BMI) is a measurement for overweight and obesity, which are diagnosed when the BMI is above 30 kg/m². The principal cause of this disease is an energy imbalance between calories consumed and calories expended, generally due to an increased high-fat food intake and sedentary lifestyle; however, genetics are also involved (Kim et al., 2011). In fact, obesity is highly associated with a higher risk for the development of metabolic syndrome, which includes cardiovascular diseases, type 2 diabetes, hyperlipidemia, and some types of cancer (Kondo et al., 2010).

Given the serious health consequences associated with obesity and the effect on health costs, it is necessary to look for other weight management strategies. The most commonly used recommendations by different organizations and medical centers are lifestyle changes, such as an increase of physical activity and a restricted calorie diet (WHO, 2016; Yarborough et al., 2018). In addition to this, pharmacological therapy may also be recommended as adjunctive support for obesity treatment; however, this recommendation is based on the preference, tolerance, and adverse effects for each patient (Trigueros et al., 2013).

Recently, a useful additional strategy recommended may be to include functional foods in a restricted calorie diet. Functional foods promote health status, improve well-being, and reduce the risk of disease beyond basic nutritional needs (Flambard and Johansen, 2007). In this context, there is an opportunity for the food industry to develop functional foods that may help reduce obesity and its associated health risks (Picó et al., 2006; Enkhmaa et al., 2018). In this regard, a functional food with potential antiobesity effect may inhibit food intake, decrease bioavailability of nutrients (e.g., lipase inhibition), stimulate energy expenditure, inhibit adipocyte proliferation and differentiation, or modulate gut microbiota (Trigueros et al., 2013; Mohamed et al., 2014).

Fermented dairy foods have been associated with multiple health benefits, particularly linked to probiotic strains and bioactives produced by their metabolic activity of milk components. Indeed, many of these health benefits have been associated with milk-derived
bioactive peptides, released from dairy proteins (Aguilar-Toalá et al., 2017). Also, fermented dairy foods may present potential antiobesity effects, which have been attributed to calcium, medium-chain fatty acids, conjugated linoleic acid, lactose, peptides, and lactic acid bacteria, particularly probiotic strains (Ebringer et al., 2008; Dror et al., 2017). Moreover, probiotics affect gene expression in host tissues, such as colon, small intestine, liver, adipose, and muscles, and could be a promising approach in the management of obesity (Arora et al., 2013; Mulders et al., 2018). Although probiotics are not limited to lactic acid bacteria of dairy origin, the term dairy probiotics refers to the majority of probiotics that can actively ferment milk or be viable in fermented milk at high numbers (Zoumpopoulou et al., 2018). Thus, dairy probiotics present a new field of applications in the development of functional dairy foods for weight management support.

To date, the beneficial health effects of fermented dairy foods have been extensively studied (Tapsell, 2015) and the antiobesity effect of probiotics has been reviewed (Dahiya et al., 2017; Ejtahed et al., 2019; Elshaghabee et al., 2019). Also, the effect of dairy peptides on satiety (Kondrashina et al., 2020) and body weight via lipid metabolism (Ricci-Cabello et al., 2012; Torres-Fuentes et al., 2015) have been discussed. However, a review dealing with the evaluation of probiotic fermented dairy products with potential antiobesity effect is needed. Therefore, the objective of this review was to present available information on the evaluation of the potential antiobesity effect of probiotic fermented dairy products, by in vitro and in vivo studies. Particularly, mechanisms associated with the potential antiobesity effect, such as those related to lipase inhibition, inhibitors of adipogenesis and adipogenic factors, the regulatory effect on lipid metabolism, and appetite suppressants are addressed.

**IN VITRO STUDIES**

The general approach for the production of fermented dairy products with antiobesity effect is to screen lactic acid bacteria (LAB) strains to determine their potential lipase inhibitory activity (LIA) and test the inhibition of adipocyte proliferation and differentiation in cell lines. The search for bioactive compounds that inhibit pancreatic lipase (PL) enzyme in vitro is one of the strategies that determine the potential efficacy in the management of obesity, due to the fact that lipid metabolism is very important to maintain body homeostasis (Sternby et al., 2002). Pancreatic lipase is a triacylglycerol acyl hydrolase (EC 3.1.1.3) that is synthesized and secreted by the pancreas and plays a key role in the efficient digestion of triglycerides.

Pancreatic lipase acts in the interface with specific fat emulsion properties (droplet size) and is responsible for the hydrolysis of 50 to 70% of total dietary fats into diacylglycerols, monoacylglycerols, glycerol, and fatty acids (Liang et al., 2014). Lipase inhibitory activity is mainly associated with 2 mechanisms: direct enzymatic inhibition by an inhibitor such as Orlistat, which is a pharmaceutical agent used as a potent PL inhibitor, and the modification of fat emulsion properties (Ogawa et al., 2015). Changes in fat emulsion droplet size and activity inhibition influence the effects of lipase hydrolysis by reducing dietary fat absorption, thus helping in weight reduction (Ogawa et al., 2015). Generally, spectrophotometric methods are the most widely used techniques for the evaluation of PL inhibition, because they are simple and time saving. One of these methods measures the release of p-nitrophenol at 405 nm using p-nitrophenyl palmitate as substrate, and the results may be expressed as percentage of LIA (Gupta et al., 2003).

Park et al. (2014a) studied 188 Lactobacillus strains for LIA. Of all evaluated strains, only 3 strains presented LIA (>60%). Among these 3, Lactobacillus plantarum Q180 exhibited the highest (P < 0.05) inhibitory activity (83.61%) compared with the negative control (supernatant without L. plantarum Q180). Then, this strain was also evaluated for its physiological characteristics, and results demonstrated that L. plantarum Q180 showed potential as a probiotic with antiobesity effect (Park et al., 2014a). Additionally, in another study, LIA was determined in different Lactobacillus strains. Overall, L. plantarum FH185 presented the highest (P < 0.05) LIA (70%; Park et al., 2015). Interestingly, both studies also evaluated the inhibition of 3T3-L1 adipogenesis that will be discussed in the next section (Park et al., 2014a, 2015).

Furthermore, Park et al. (2014b) investigated the optimal conditions for the production of a functional milk fermented by L. plantarum Q180 with LIA. The optimal conditions for the fermentation of skim milk (9.5% vol/vol) with the highest LIA (52.86%) were 37°C for 28 h (Park et al., 2014b). In another study, LIA from fermented milk with L. plantarum Q180 was attributed to peptides released through milk proteolysis. In this regard, a purified peptide obtained from milk fermented by L. plantarum Q180 with LIA was characterized. After evaluation, the peptide composed of Asp, Thr, Ile, Ser, Ala, and Gln presented an IC50 of 2.817 μg/mL for LIA (Kim and Lim, 2020). Lipase inhibitory peptides were isolated from fermented milk by UF, reversed phase column chromatography, reverse phase HPLC and gel permeation HPCL to increase LIA and peptide yield (Kim and Lim, 2020). Although a purified peptide with high LIA was obtained, the
sequence of this peptide was not reported. Nevertheless, the peptide sequences of lipase inhibitory peptides from other sources have been reported (Jakubczyk et al., 2019).

Recently, Gil-Rodríguez and Beresford (2019) evaluated the LIA of fermented milks with 31 different LAB strains including different genera (Lactobacillus, Lactococcus, and Pediococcus). The results showed that fermented milks with Lactobacillus helveticus SC8, SC44 and SC45 presented LIA > 49%. In this study the activity of the selected samples was preserved after removal of casein and microbial cells during the filtration and fractionation process. Furthermore, the activity was also preserved in the <3 kDa fraction in all 3 samples tested. This suggested that lipase was inhibited by small size peptides released during milk fermentation. Thus, authors suggested that LIA was attributed to fractions (<3 kDa) containing 2 peptides (2 kDa), and that the mechanism of action was by enzyme inhibition at its active site (Gil-Rodríguez and Beresford, 2019). Furthermore, this evidence supported the fact that different LAB strains and its fermented dairy products, may present PL inhibition and may be used as functional foods for weight loss (Gil-Rodríguez and Beresford, 2019).

In contrast to that study, Ogawa et al. (2015) investigated the mechanism associated with the suppression of lipid absorption by the probiotic strain Lactobacillus gasseri SBT2055. Data obtained by these authors in an in vitro study indicated that the activity of this strain was not due to a direct inhibition of the enzyme, but due to an increase in fat droplet size. Interestingly, authors suggested that L. gasseri LG2055 may potentially interact with bile acids and destabilize the fat emulsion, which may result in its coalescence. This results in a decrease in the oil-water interface surface, which subsequently hinders lipase activity. It was observed that L. gasseri SBT2055 suppressed enzyme activity in a dose-dependent manner (1–100 μg/mL) and when compared with 4 other strains, it showed the highest (P < 0.05) LIA (70%). Lactobacillus gasseri SBT2055 increased fat emulsion droplet size, resulting in the suppression of lipase-mediated fat hydrolysis. Indeed, L. gasseri SBT2055 displayed antiobesity properties by suppressing fatty acid release using a simple oil-in-water emulsion as a physiological model (Ogawa et al., 2015). Therefore, authors concluded that the influence of L. gasseri SBT2055 on the physicochemical properties of fat emulsion provides a mechanism for the probiotic-mediated suppression of lipid absorption (Ogawa et al., 2015). Moreover, the antiobesity effect of fermented milk with L. gasseri SBT2055 was also assessed in in vivo and clinical trials and are further discussed in next sections.

Zhou et al. (2013) reported that the probiotic strain Lactobacillus pentosus S-PT84 isolated from shibazuke (fermented Japanese Kyoto pickle) presented PL activity in a dose-dependent manner. The authors indicated that some probiotic strains or its fermented products decreased lipid absorption due to its lipid binding capacity in the intestine (Matsumura, 2010). Furthermore, the heat killed L. pentosus S-PT84 strain also exhibited LIA suggesting that cells lysates (postbiotics) rather than viable cells in fermented products may present LIA. Moreover, LIA was attributed to the cell wall components of Lactobacillus strains, such as the S-layer proteins and lipoteichoic acids that bring hydrophobicity to the cell surface, facilitating adhesion to the oil-water interface of the enzyme by changing its properties (Zhou et al., 2013).

In this last regard, authors found no difference in LIA between L. pentosus S-PT84 and its intact cell wall; it is possible that these cell surface proteins were also involved in the inhibitory effect, because L. pentosus S-PT84 was thought to have an extra-thick cell wall. Because PL is a surface-active enzyme, it is notable that the activity depends directly on the substrate lipid surface. Therefore, a significant level of hydrophobicity around the cell wall surface of S-PT84 may enhance the adhesion to the substrate interface; consequently, impeding PL from acting on its lipid substrate. Also, L. pentosus S-PT84 cells may bind to PL by hydrophobic interaction and block the enzyme active site, behaving as a true lipase inhibitor (Zhou et al., 2013). Thus, this study suggested that postbiotics from specific strains of Lactobacillus may also present LIA. Therefore, the evaluation of this probiotic strain in fermented milk may be of interest because it could present LIA from metabolites resulting from fermentation as well as from postbiotics.

On the other hand, LIA can indirectly influence the inhibition of the inflammatory process that is associated with obesity (Toita et al., 2016). Thus, the correlation between the amino acid composition and the sequence in peptides with anti-inflammatory properties may contribute in the designing of peptides with high in vivo anti-inflammatory (Jakubczyk et al., 2019) and antiobesity effects. The composition of peptides inhibiting the inflammatory process has indicated that the main role is played by the dominance of leucine, serine, tyrosine, and arginine residues, compared with non-anti-inflammatory epitopes (Jakubczyk et al., 2019). Furthermore, hydrophobic and polar residues of peptides were determined as specific motifs of anti-inflammatory epitopes (Gupta et al., 2017). Although it is well-known that the antiobesity agent Orlistat induces inhibition of lipase activity by interaction with the catalytic sites of the enzyme, little is known about
the interaction of antiobesogenic dairy derived peptides with lipase inhibition.

Another strategy for the evaluation of the efficacy of probiotics and fermented dairy products in the management of obesity consists in the inhibition of adipocyte proliferation and differentiation (Torres-Fuentes et al., 2015). Adipose tissue is composed of adipocytes cells, which plays a central role in the maintenance of lipid homeostasis and energy balance by storing triglycerides as fuel for energy reserves. Besides, it is a major endocrine and secretory organ that releases a wide range of adipokines, such as adiponectin and leptin that regulate fat storage (Zuk et al., 2002). Thus, the inhibition of adipocyte proliferation and differentiation may promote weight loss by reducing hyperplasia and hypertrophy that is characteristic in obese subjects (Torres-Fuentes et al., 2015).

The most commonly used cell line for the study of antiobesity effect in terms of adipocyte inhibition is the fibroblastic 3T3-L1 preadipocyte line. These cells undergo pre-adipocyte to adipocyte conversion and accumulate triglycerides upon differentiating in culture, due to the expression of \textit{PPARG} and \textit{CEBPA} genes. These are adipocyte-specific genes that cause the transcriptional activation of some adipocyte-specific mRNA with encoding enzymes such as fatty acid synthase (\textit{FAS}), lipoprotein lipase (\textit{LPL}), acetyl-CoA carboxylase, stearoyl-CoA desaturase-1 and PPAR co-activator-1 (Kim et al., 2015). These enzymes are involved in lipogenesis and adipogenesis processes that induce the synthesis of fat globules, modulating lipid metabolism (Giri et al., 2006; Furukawa et al., 2017).

Several studies have reported that some probiotic strains or even their cell components were found to be effective in inhibiting adipogenesis in the 3T3-L1 cell line (Table 1). However, there were only few studies that reported the use of 3T3-L1 cells in the evaluation of probiotic strains in dairy fermented foods (Ho et al., 2013).

Park et al. (2011) reported that the cell extract from a probiotic strain, \textit{L. plantarum} KY1032 (KY1032-CE), significantly decreased (\textit{P} < 0.05) lipid accumulation by 30\% in differentiated 3T3-L1 cells at 0.1\% of concentration. Besides, this cell extract showed an increase in lipolysis of 7\%. The anti-adipogenic effect of KY1032-CE was attributed to a decrease in the expression levels of adipocyte-specific genes, 28\% for \textit{PPARG} and 23\% for \textit{CEBPA}. Thus, KY1032-CE decreased mRNA and protein expression of these genes, suppressing the differentiation of 3T3-L1 cells into adipocytes. However, because the increase of lipolysis was not significant (\textit{P} > 0.05), the authors concluded that the extract inhibited adipogenesis, but it may not likely break down pre-existing fat droplets in adipocytes (Park et al., 2011).

<table>
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<th>Evaluation</th>
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<th>Concentration</th>
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<tr>
<td>Pancreatic lipase (PL) inhibition</td>
<td>\textit{Lactobacillus plantarum} Q80</td>
<td>100 \mu g/mL</td>
<td>Inhibited PL activity by 83%</td>
<td>Park et al., 2014a</td>
</tr>
<tr>
<td>Pancreatic lipase (PL) inhibition</td>
<td>\textit{Lactobacillus plantarum} Q80</td>
<td>10 mg/mL</td>
<td>Inhibited PL activity by 85%</td>
<td>Park et al., 2014b</td>
</tr>
<tr>
<td>Pancreatic lipase (PL) inhibition</td>
<td>\textit{Lactobacillus pentosus} S-PT84</td>
<td>30 × 10^8 cfu/2.7 mL</td>
<td>Inhibited PL activity by 50%</td>
<td>Zhou et al., 2013, Gil-Rodriguez and Beresford, 2011, Park et al., 2011</td>
</tr>
<tr>
<td>Pancreatic lipase (PL) inhibition</td>
<td>\textit{Lactobacillus gasseri} SBT2055</td>
<td>100 \mu g/mL</td>
<td>Inhibited PL activity by 50%</td>
<td>Ogawa et al., 2015</td>
</tr>
<tr>
<td>Pancreatic lipase (PL) inhibition</td>
<td>Milks fermented by different lactic acid bacteria</td>
<td>10 mg/mL</td>
<td>Inhibited PL activity by 50%</td>
<td>Gil-Rodríguez and Beresford, 2011, Park et al., 2013, Ho et al., 2013, Kim and Lim, 2000</td>
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Table 1. In vitro studies of the antiobesity effect of probiotics strains and fermented dairy foods

Peptides from milk fermented by \textit{L. plantarum} Q80

Kim and Lim, 2000
However, it would be interesting to evaluate if extracts from milk fermented by this probiotic strain may be able to also decrease lipid accumulation.

On the other hand, several metabolites produced by LAB, may also present an antiobesity effect. In this sense, it has been reported that γ-aminobutyric acid (GABA), besides having an antihypertensive effect, may also have antiobesity effects (Park et al., 2013). Therefore, the antiobesity effect of *L. plantarum* LG42, a GABA-producing strain was determined. The supernatant obtained by the freeze-dried *L. plantarum* LG42 cells, exhibited a reduction of lipid accumulation of 58% at a concentration of 40 μg/mL and caused an inhibition on intracellular triglyceride accumulation when compared with untreated cells. Furthermore, the activity of the enzyme GPDH decreased in 3T3-L1 cells treated with 40 μg/mL of the supernatant, which is an enzyme that is important for triacylglycerol synthesis. Also, PPARG and CEBPA levels were downregulated and, thus, decreased the expression level of adipogenic markers, such as adipocyte fatty acid binding protein (aP2), leptin, and fatty acid translocase (CD36). However, authors were not able to conclude that the antiobesity effect of *L. plantarum* LG42 was attributed to GABA (Park et al., 2013).

As previously mentioned, *L. plantarum* Q180 strain not only exhibited LIA (Park et al., 2014a) but was also able to inhibit adipogenesis in 3T3-L1. After an 8-d treatment with different concentrations of *L. plantarum* Q180 throughout the differentiation period, results evidenced a significant inhibition (*P* < 0.05) of 3T3-L1 adipogenesis in a dose-dependent manner. Lastly, a concentration of 100 μg/mL of *L. plantarum* Q180 was the most effective at reducing lipid content by 14.63% (Park et al., 2014a). Similarly, the inhibition of 3T3-L1 adipogenesis by *L. plantarum* FH185 was also assessed (Park et al., 2015). Results showed that after 8-d treatment, 3T3-L1 adipogenesis was inhibited also in a dose-dependent manner, being 100 μg/mL the most effective to decrease lipid content by 18.63% (Park et al., 2015).

On the other hand, to the best of our knowledge, only one study has evaluated adipogenesis in 3T3-L1 cells with dairy products, in particular kefir. This is a fermented milk drink with a creamy texture, sour taste and subtle effervescence that is produced by adding a starter culture termed kefir grains to milk. Kefir grains contain lactose-fermenting yeasts and non-lactose-fermenting yeasts, lactic and acetic acid-producing bacteria, entangled within a polysaccharide and protein matrix called kefiran (Dimidi et al., 2019).

Ho et al. (2013) evaluated the adipocyte differentiation and lipid accumulation in 3T3-L1 cells from 3 different fractions (kefir liquid culture broth fraction Fr-1, soluble fraction Fr-2 and insoluble fraction Fr-3) from kefir. Results showed that all 3 fractions presented adipocyte differentiation by displaying an inhibitory effect on lipid accumulation. Yet, Fr-3 (0.1 μg/mL) exhibited the highest decrease (*P* < 0.05) in lipid accumulation by 60% in 3T3-L1 cells. Additionally, F-3 treatment significantly decreased the expression of adipogenic transcription factors, such as sterol regulatory element binding protein (SREBF1), PPARG, and CEBPA during adipocyte differentiation. Interestingly, by suppressing the expression of SREBF1, the expression of aP2 and FAS was reduced. Moreover, F-3 significantly decreased the expression of proinflammatory cytokine TNF-α. The inhibitory effect of GPDH of kefir fractions was also determined during adipocyte differentiation; because this enzyme is important for triglyceride synthesis. Results demonstrated that all 3 kefir fractions presented a decrease in GPDH activity, thus suggesting that this effect may suppress adipocyte differentiation (Ho et al., 2013). Nevertheless, there was no discussion on the possible bioactive compounds associated with the effect. Knowing the specific LAB and yeasts, as well as the bioactive compounds involved in the effect, may be important to gain some understanding on the mechanisms involved in the anti-adipogenic effects of the kefir F-3 insoluble fraction.

A wide range of microbial species have been identified in kefir grains, commonly including *Lactobacillus* spp, *Lactococcus* spp, *Streptococcus* spp, *Acetobacter* spp, *Saccharomyces* spp, *Candida* spp, *Kluyveromyces* spp and *Leuconostoc* spp. Nevertheless, the microbial composition of kefir may change by the end of fermentation and varies between commercial and artisanal kefir products. Indeed, *Lactobacillus kefiri* can represent 80% of all *Lactobacillus* species in the final fermented milk (Dimidi et al., 2019). Thus, studies on the anti-adipogenic effect of *Lactobacillus kefiri*, rather than the consortium of bacteria and yeast, may help to elucidate the bioactives and mechanisms involved.

In general, it is apparent that the reduction of lipid accumulation in 3T3-L1 cells exerted by the different LAB is due to the downregulation of the main transcription factors involved in adipogenesis such as PPARG, and CEBPA. Those transcription factors regulate expression of target genes such as FABP4, CD36, and LEP, that are central to the pathway that links insulin resistance to obesity, promote lipid accumulation, and produce leptin that regulate energy expenditure and food intake, respectively (Kim et al., 2015). Nevertheless, the regulation in 3T3-L1 cells by probiotics was strain dependent rather than determined by species.

All these in vitro studies demonstrated the potential of viable probiotic strains or their lysates in the treatment of obesity-related problems. However, to the best of our knowledge, only 4 studies evaluated the potential
antiobesity effect of fermented dairy products in vitro (Ho et al., 2013; Park et al., 2014b; Gil-Rodríguez and Beresford, 2019; Kim and Lim, 2020). Thus, the use of several probiotic strains or their metabolites in fermented dairy products for the management of obesity remains to be fully explored. A general approach may be to start by screening dairy probiotics and postbiotics for LIA and inhibition of adipocyte differentiation of 3T3-L1 cells by fermented dairy products. Furthermore, it is also necessary to carry out in vivo studies and clinical trials to demonstrate the biological effect in living organisms.

**IN VIVO STUDIES**

Animal model studies work as good indicators to evaluate the antiobesity effect of dairy fermented foods, because animals may show an underlying genetic predisposition to be obesity prone or resistant, like humans (Hariri and Thibault, 2010). Rats and mice are known as the standard models for studying dietary obesity, due to their sensitivity to develop obesity with ad libitum access to high-fat diets. Sprague-Dawley rats, Wistar rats, and C57BL/6C mice are some examples; however, there are also strains genetically modified, such as Zucker fa/fa rats and ob/ob mice. In this sense, several studies demonstrated the antiobesity effect of fermented dairy products using different models (Table 2).

In early studies, it was reported that unfermented milk with *Lactobacillus gasseri* SBT0270 significantly lowered blood serum triglycerides, total cholesterol and LDL; however, it did not present an effect on weight loss (Usman and Hosono, 2000). Unfermented milks of *L. gasseri* were prepared by adding a cell concentrate to 10% skim milk to reach about 2 × 10⁹ cfu/mL. Nevertheless, the effect of *L. gasseri* on the metabolism of triglycerides on serum and liver was not assessed. Therefore, the effect of the administration of milk fermented by *L. gasseri* SBT2055 on serum lipids and leptin, adipocytokine concentration and adipocyte size in adipose tissue of Sprague-Dawley rats were evaluated (Sato et al., 2008). After 4 weeks of intervention, results showed a significant reduction (*P* < 0.05) in the average adipocyte size in mesenteric white adipose tissue and displayed a greater number of small adipocytes from mesenteric and retroperitoneal adipose tissues than the control group. Although serum glucose, adiponectin, and lipids were not significantly different (*P* > 0.05) between groups; serum leptin concentrations decreased 32% in the fermented milk group. It has been reported that the release of leptin depends on the adipocytes size; hence, fermented milk was responsible for the lower concentration of leptin. Overall, these

<table>
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<tr>
<td>Lean Sprague-Dawley rat and obese Zucker rats</td>
<td>Fermented milk by <em>Lactobacillus gasseri</em> SBT2055</td>
<td>Adipocyte size and leptin levels reduction</td>
<td>Sato et al., 2008</td>
</tr>
<tr>
<td>Wistar rat</td>
<td>Different fermented milk by <em>Lactobacillus acidophilus</em>, <em>Lactobacillus bulgaricus</em>, <em>Lactobacillus salivarius</em>, <em>Lactobacillus rhamnosus</em></td>
<td>No effect in obese rats</td>
<td>Forssten et al., 2013</td>
</tr>
<tr>
<td>Male C57BL/6J mice</td>
<td>Kefir powder</td>
<td>BW, epididymal fat, and adipocyte size reduction; decreased expression of genes related to adipogenesis and lipogenesis and proinflammatory cytokines and lipoprotein cholesterol levels; adipocyte size reduction</td>
<td>Choi et al., 2017</td>
</tr>
<tr>
<td>Sprague-Dawley rat</td>
<td>Fermented milk by <em>Lactobacillus fermentum</em> TSI2, <em>L. fermentum</em> S2 or <em>L. fermentum</em> TSI2 and <em>L. fermentum</em> S2</td>
<td>Low-density lipoprotein and high-density lipoprotein cholesterol levels, adipocyte size</td>
<td>Cho et al., 2020</td>
</tr>
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</table>
results suggested a potential role of this fermented milk in the regulation of adipose tissue growth and thus, a potential antiobesity effect (Sato et al., 2008). Nevertheless, because this study was evaluated in lean Sprague-Dawley rats, further studies are needed with animal models fed with a high-fat diet or obese animal models to determine the antiobesity effect.

In this regard, the effect of fermented milk with L. gasseri SBT2055 on adiposity markers in lean and obese Zucker rats and Sprague-Dawley rats were determined in 2 experiments (Hamad et al., 2009). First, fermented milk with L. gasseri SBT2055 and unfermented milk were administered to lean and obese Zucker rats. After 4 weeks of intervention, lean rats treated with fermented milk presented a significantly lower (P < 0.05) mesenteric adipose tissue weight, adipocytes sizes and serum leptin concentration compared with the unfermented milk group. Although these effects were not observed in the obese Zucker rats, the fermented milk group presented smaller adipocytes in the subcutaneous adipose tissue. Moreover, serum leptin was significantly reduced (P < 0.05) in lean rats with fermented milk, though in the obese group no change was observed. In fact, rats administered with fermented milk presented lower serum and hepatic cholesterol and increased the excretion of fecal fatty acids and neutral fecal sterols. Furthermore, in the second experiment, after permanent cannulation with fermented milk or unfermented milk to Sprague-Dawley rats and lymph collection, results indicated that the fermented milk group presented a lower maximum transportation rate of triacylglycerol and phospholipids. Therefore, all these results also suggested that fermented milk with L. gasseri SBT2055 regulated adipose tissue growth through inhibition during fat absorption (Hamad et al., 2009).

Furthermore, the antiobesity effect of milk fermented by different microorganisms associated with satiety has also been addressed. Forssten et al. (2013) evaluated the effect of 5 different fermented milks inoculated with Lactobacillus strains (Lactobacillus acidophilus MUH 41, Lactobacillus acidophilus NCFM ATCC 700396, Lactobacillus bulgaricus MUH 192, Lactobacillus salivarius MUH 1502 or Lactobacillus rhamnosus MUH 142) on food intake and secretion of satiety hormones [cholecystokinin (CCK), ghcagon-like peptide 1 (GLP-1), ghrelin, peptide tyrosine-tyrosine (PYY), and insulin] in Wistar rats. First, after a single oral dose (10 mL/kg) of each treatment, all fermented milks significantly changed (P < 0.05) CCK levels compared with the control group. Moreover, levels of serum glucose and insulin were decreased in the fermented milk groups. However, no significant differences (P > 0.05) were observed for GLP-1, ghrelin, and PYY. Then, in a second study, 60 min after administration of fermented milks, PYY was significantly lower (P < 0.05) than the control group. The PYY is a satiety hormone that is released after meal consumption; however, the actual feed intake was also important to assess. Thus, feed intake of rats administered with the different fermented milks was also determined. Results indicated that rats administered with fermented milks with L. salivarius 1502 and L. acidophilus NCFM presented a significantly lower (P < 0.05) food intake compared with the control (Forssten et al., 2013).

The effect of fermented milk by L. plantarum NCDC 625 administered for 12 wk to C57BL/6J mice on body weight, adipose tissue mass, total cholesterol, triglycerides, expression of thermogenic proteins, and inflammatory markers was assessed (Pothuraju et al., 2016). Fermented milk groups significantly lowered (P < 0.05) final body weight, epididymal fat, and serum levels of insulin, triglycerides, total cholesterol, and LDL cholesterol compared with the high-fat diet control group. Moreover, fermented milk also presented a protective effect against the rise of blood glucose levels. In fact, homeostasis model assessment-insulin resistance index values, calculated from fasting blood glucose and insulin levels, were similar to the control group not administered with a high-fat diet. Adipocyte size from epididymal fat was significantly smaller (P < 0.05) than that from the high-fat diet group. Additionally, after evaluation of mRNA expression levels of different genes of inflammatory markers from epididymal fat, the fermented milk group exhibited lower levels of tumor necrosis factor–α (TNF-α) and IL-6. Therefore, authors concluded that this fermented product may be useful in the treatment of obesity, though clinical studies were needed to establish this fact (Pothuraju et al., 2016).

Based on previous findings from in vitro studies with kefir fractions (Ho et al., 2013), a study with diet-induced obese mice was performed to further explore whether kefir may prevent fat accumulation in vivo in adipose and liver tissues (Choi et al., 2017). The administration of 0.1 and 0.2% kefir powder inhibited fat accumulation in adipose and liver tissues of high-fat diet-induced obese mice. Moreover, kefir also decreased the expression of the CEBPA, PPARG, AAK1, FAS, and BMS1 genes in epididymal fat, associated with adipogenesis and lipogenesis reduction. With decreased lipogenesis in the liver, indicated by the levels of FAS and BMS1 gene expression, kefir increased the expression of the PPARG and CPT1A genes. Moreover, kefir improved diet-induced serum lipid profiles by decreasing triacylglycerol, total, and LDL cholesterol concentrations. Thus, the antiobesity effect of kefir was supported by the decreased expression of genes related to adipogenesis and lipogenesis, as well as reduced
proinflammatory marker levels in epididymal fat (Choi et al., 2017). Although the nature of the components involved in the antiobesity effect was not reported, it is very likely that bioactive components, derived from LAB and yeast from kefir, and its action on milk components may be responsible.

Indeed, fermentation derived bioactive peptides from casein in kefir, have been shown to stimulate the immune system, as well as have antioxidant, antihypertensive, anticarcinogenic, hypocholesterolemic, and glucose-lowering effects in animal models (Dimidi et al., 2019). Although kefir is the most investigated dairy fermented food in terms of its effect on gastrointestinal health, and its effect on gut microbiota has been investigated in several in vitro, animal, and human studies (Dimidi et al., 2019), the antiobesity effect of kefir and its influence on gut microbiota has not been established.

Recently, the antiobesity effect of fermented milks with *Lactobacillus fermentum* TSI2, *Lactobacillus fermentum* S2, or a co-fermented milk with *L. fermentum* TSI2 and *L. fermentum* S2 was determined in Sprague-Dawley rats administered with a high-fat diet (Cho et al., 2020). After 8-weeks of intervention, results showed no significant differences (*P* > 0.05) between groups on body weight, food intake, organ (liver, spleen and kidney) fat, abdominal and epididymal fat, total cholesterol, triglyceride, glucose, leptin, and adiponectin. In particular, significant differences (*P* < 0.05) in blood cholesterol and body fat accumulation were observed between the control group treated with a high-fat diet and fermented milk with *L. fermentum* TSI2 treated with a high-fat diet were observed in HDL cholesterol, LDL cholesterol, and adipocyte size. Thus, authors concluded that this fermented milk may provide beneficial effects in blood cholesterol and body fat accumulation (Cho et al., 2020).

**CLINICAL TRIALS**

Many studies have been performed in humans to investigate the potential of probiotic fermented milks or probiotic yogurt to prove their effect in the treatment of obesity (Agerbaek et al., 1995; Anderson and Gilliland, 1999; Agerholm-Larsen et al., 2000; Fabian and Elmadfa, 2006; Ataie-Jafari et al., 2009; Asemi et al., 2012). These were randomized double-blind controlled trials, with a test group versus a placebo (control) group. Dairy products fermented by the strain *L. gasseri* SBT2055 were the most studied for the antiobesity effect in humans (Fujiwara et al., 2001; Kadooka et al., 2010, 2013; Ogawa et al., 2015). Therefore, the antiobesity effect of fermented milk by *L. gasseri* SBT2055 in adults with obese tendencies (BMI > 24 to < 30 kg/m²) was evaluated in a randomized controlled clinical study (Kadooka et al., 2010). Participants aged from 33 to 63 yr with BMI between 24.2 and 30.7 kg/m² and abdominal visceral fat area between 81.2 and 178.5 cm² were enrolled. The active treatment was prepared with skim milk fermented by *Streptococcus thermophilus* and *Lactobacillus delbrueckii* ssp. *bulgaricus* containing active *L. gasseri* SBT2055 (10⁸ cfu/g) and the control was the same fermented milk without *L. gasseri* SBT2055. Subjects were instructed to consume treatments (200 g/d) for 12 wk. Then, every 4 wk measurements of several variables (blood pressure, pulse rate, blood and urinary tests) and body weight were determined; and abdominal fat area was measured after 12 wk. After intervention, the fermented milk group significantly decreased (*P* < 0.05) visceral, subcutaneous, and total fat area compared with the control group. Additionally, body weight, BMI, waist and hip circumferences, waist-to-hip ratio, body fat percentage, and body fat mass were also significantly lower (*P* < 0.05) than those from the control group. Interestingly, none of these parameters significantly decreased in the control group. Moreover, no significant changes (*P* > 0.05) were observed for blood pressure, pulse rate, and blood and urinary tests. Overall, the effect was attributed to the probiotic strain *L. gasseri* SBT2055, because the control treatment was the same fermented milk without the probiotic strain (Kadooka et al., 2010).

Furthermore, the potential antiobesity effect of lower doses (10⁶-10⁷ cfu/g) of *L. gasseri* SBT2055 in fermented milk in a randomized clinical study was also evaluated (Kadooka et al., 2013). Control and active fermented milks were prepared as previously reported (Kadooka et al., 2010), however, active treatments were inoculated with 10⁶ or 10⁷ cfu/g. Interestingly, results demonstrated that abdominal visceral fat area, BMI, waist and hip circumferences, fat percentage, and fat mass significantly decreased (*P* < 0.05) in both groups treated with fermented milks by *L. gasseri* SBT2055. However, the abdominal subcutaneous fat area was not significantly reduced (*P* > 0.05) in fermented milk treatments. Thus, suggesting that higher doses of *L. gasseri* SBT2055 may be necessary for this effect (Kadooka et al., 2013).

Additionally, the effect on the supplementation of fermented milk with *L. gasseri* LG2055 on the fecal fat excretion was also determined in healthy subjects (Ogawa et al., 2015). Fermented milks with or without *L. gasseri* LG2055 were prepared as previously reported by other studies (Kadooka et al., 2010, 2013). After 14 d of intervention, no significant differences (*P* > 0.05)
were observed in body weight, BMI, and total cholesterol levels in the fermented milk and control groups. Although no significant differences \((P > 0.05)\) between groups were observed, a significant increase \((P < 0.05)\) in fecal fat levels was observed in the fermented milk group after intervention. Interestingly, authors suggested that the decreased lipid absorption observed in fecal fat excretion in the fermented milk group, was attributed to the presence of viable \(L.\ gasseri\) LG2055, because also PL activity in vitro was found. On the other hand, though, this study was performed in healthy subjects, a longer study with daily administration of fermented milk with \(L.\ gasseri\) LG2055 in overweight and obese subjects would be interesting to evaluate (Ogawa et al., 2015).

In another study, the effect of a functional yogurt on the metabolic syndrome (body weight, blood pressure, blood glucose, and lipid profile) was evaluated in a randomized double-blind controlled clinical study (Chang et al., 2011). The active treatment (NY-YP901) was prepared with skim milk fermented by \(Streptococcus\ thermophilus,\ Lactobacillus\ acidophilus,\) and \(Bifidobacterium\ infantis.\) Also, this treatment was added with several functional ingredients (fibersol-2, \(Enterococcus\ faecalis\) FK-23, extract from pine needle, peptigen IF-3090 (whey protein hydrolyzate), RGP-HC-90 (rice germ extract powder), \(Bifidobacterium\ breve\), and YQ-2 (\(Yucca\ schidigera\) and \(Quillaja\ saponaria\) extract). The control treatment was prepared similarly without the functional ingredients. After 8 wk of intervention, body weight, BMI, and LDL cholesterol were significantly lower \((P < 0.05)\) in the fermented milk group than in the control group. Moreover, waist circumference, systolic blood pressure, total cholesterol, and triglyceride profiles were lower in the fermented milk group; however, these were not significantly different \((P > 0.05)\) from the control group. In fact, the beneficial effects were mostly attributed to all the functional ingredients that were added after fermentation. The authors concluded that the regular intake of this functional probiotic yogurt may be a promising treatment in metabolic syndrome and obesity (Chang et al., 2011).

Over the last decade, it has been claimed that dairy products may promote body weight and fat loss; however this effect has been controversial. Therefore, Madjd et al. (2016) compared in a randomized single-blind controlled clinical trial the effect of 2 low fat yogurts, on the body weight of overweight and obese women. Also, the active yogurt contained 2 probiotic bacteria \(L.\ acidophilus\) LA5 and \(Bifidobacterium\ lactis\) BB12. After 12 wk of intervention, body weight, BMI, and waist circumferences significantly decreased \((P < 0.05)\) in both treatments, though they were not significantly different between them. Interestingly, total cholesterol and LDL cholesterol in the probiotic yogurt were significantly lower \((P < 0.05)\). In addition, 2-h postprandial glucose, fasting serum insulin, and insulin resistance were significantly different \((P < 0.05)\) between groups. Thus, authors concluded that future studies were needed with a larger sample size, that included men and for a longer period of time to evaluate the antiobesity effect. Nevertheless, the authors also suggested evaluating the effect on carbohydrate metabolism with diabetic and prediabetic participants (Madjd et al., 2016).

It has been reported that the accumulation of abnormal or excessive visceral adiposity in obese subjects is linked to the release of inflammatory cytokines leading to an inflammatory state and oxidative stress (Ellulu et al., 2017). Therefore, Zarrati et al. (2014) investigated the effect of a probiotic yogurt (starter cultures \(Streptococcus\ thermophilus\) and \(Lactobacillus\ bulgaricus\) containing \(L.\ acidophilus\) LA5, \(L.\ casei\) DN001, and \(Bifidobacterium\ lactis\) BB12 on body fat percentage, BMI, leptin, and proinflammatory gene expression in overweight and obese subjects. This 8-wk randomized double-blind study consisted of 3 different group treatments: regular yogurt and a low-calorie diet, the probiotic yogurt + low-calorie diet, and the probiotic yogurt without low-calorie diet. Results demonstrated that the probiotic yogurt combined with a low-calorie diet significantly decreased \((P < 0.05)\) body weight, waist circumference, and hip circumference. Additionally, a reduction in proinflammatory cytokines in peripheral blood mononuclear cells was also observed (Zarrati et al., 2014). It has been reported that in randomized double-blind controlled clinical studies only 2 groups may be compared, a control group (placebo group) and a treatment group (with the active component; Friedman et al., 2015). Thus, it would be interesting if authors evaluated the antiobesity effect on overweight and obese participants with a regular diet that included a regular yogurt or a probiotic yogurt, because a low-calorie diet may also be providing an additional antiobesity effect.

Besides being a primary storage of fat, adipose tissue also participates in the control of lipid homeostasis. It has been reported that deregulation of adipocytokines from adipose tissue may enhance the development of an inflammatory status. This chronic inflammation status is well characterized by an increase of proinflammatory cytokines such as TNF-α and IL-6, and a decrease of anti-inflammatory cytokines. These inflammatory cytokines have been associated with impaired insulin signaling and lipid metabolism (Armani et al., 2010; Park et al., 2014c). Thus, to diminish the impairment effects of an active inflammatory status, the search for dairy fermented milks with anti-inflammatory effects may be of interest.
Clinical studies evidenced that different species of probiotics strains mainly those belonging to *Lactobacillus* spp. genus were effective in treating obesity-related disorders. However, their efficacy and mechanisms of action are very different among strains suggesting that the effects are strain specific and cannot be extended to other probiotics of the same genus or species. Hence, more specific studies are needed to establish efficacy and the mechanisms of action.

Although most studies that reported antiobesity effects were attributed to probiotic strains, other bioactive compounds in fermented milks may have also been released and be responsible for this effect. In this regard, during milk fermentation peptides are released from the native protein by the proteolytic enzymes present in the cell wall from microorganisms. It has been reported that these peptides may exert different biological effects (Aguilar-Toalá et al., 2017). Moreover, previous studies have exhibited that whey peptides decrease short-term and long-term food intake when compared with other treatments, such as carbohydrate or other proteins (Luhovyy et al., 2007; Chang et al., 2011). In fact, these peptides may block peripheral opioid and cholecystokinin A receptor present in the gastrointestinal tract; and thus, reduce food intake (Froetschel et al., 2001; Pupovac and Anderson, 2002). Therefore, it would be noteworthy to mention that there is a great opportunity for the exploration of other LAB, besides probiotic strains, and their potential to develop new fermented milk products with antiobesity effects.

**FUTURE PERSPECTIVES**

To the best of our knowledge, fermented dairy products that support weight management are not available in the market; thus, there is great opportunity for the development of functional dairy products with new LAB that may affect fat absorption. The general approach may include screening and selection of new LAB for the antiobesity effect by in vitro studies (lipase inhibition and anti-adipogenic activity by using 3T3-L1 cells) and testing the most promising strains in obese animal models. Before human studies, there is need for the development of an optimum formulation with acceptable sensory quality so that a well-designed, randomized, controlled, double-blind clinical study may be carried out. Also, the chemical nature of the bioactives involved, as well as the underlying mechanisms associated with the antiobesogenic effect, in addition to their effect on gut microbiota need to be addressed. It has recently been recognized that gut microbiota composition also contributes toward obesity development, because it determines body fat, influences caloric intake, intestinal absorption and energy balance (Dror et al., 2017). In fact, some studies suggest that probiotics change gut microbiota, interacting directly with the host and playing a crucial role in building future physiology and immunity (Backhed et al., 2004; He and Shi, 2017).

Also, the intellectual property of novel ingredients in functional foods is a critical factor in the ultimate success of these food products in the market (Beltrán-Barrientos et al., 2016). Indeed, several patents are available surrounding the technology required for the production of antiobesity fermented dairy products. One invention relates to a novel *L. plantarum* Q180 strain having an obesity-suppressing ability suitable for producing fermented milk (KR101512482B1, 2015) and to *L. plantarum* NUC-LG42 having antiobesity effect and brain function improvement activity (KR101010914B1, 2011).

Additionally, claims that may be used on food labels (health, nutritional, or structure-function) that are defined by statute or FDA regulations need to be addressed, to provide additional market value to a fermented dairy product (FDA, 2003; Martirosyan and Singharaj, 2016). Interestingly, although health claims describe a relationship between a food substance and a reduced risk of disease or health-related condition that need to be authorized by FDA after sufficient scientific evidence, structure-function claims are not subject to premarket review and authorization by FDA. Furthermore, food manufacturers do not need to notify FDA about structure-function claims (FDA, 2003; Martirosyan and Singharaj, 2016). Thus, a fermented milk product may initially have a structure-function claim, such as “weight management support,” that may lead to a fully supported health claim after enough scientific evidence may be gathered.

**CONCLUSIONS**

Obesity is a disease that affects adults and children worldwide. Despite the available information for lifestyle change recommendations, this disease is still increasing. Therefore, other strategies are needed to prevent or treat obesity. In this sense, some probiotic strains and fermented dairy foods that have shown antiobesity effects may be used as potential strategies. New evidence indicates that probiotics may restore dysbiosis in gut microbiota that is associated with obesity. Thus, the evaluation of gut microbiota in animal and clinical studies administered with fermented dairy products with antiobesivity effect may be of utmost importance. Also, the potential antiobesity effects of metabolites produced by probiotics in fermented dairy foods, such
as dairy bioactive peptides and exopolysaccharides, as well as postbiotics, and their effect on microbiota needs to be addressed by in vivo studies.

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