

## Comparative meta-analysis of the effect of *Lactobacillus* species on weight gain in humans and animals

Matthieu Million, Emmanouil Angelakis, Mical Paul, Fabrice Armougom,  
Leonard Leibovici, Didier Raoult

► **To cite this version:**

Matthieu Million, Emmanouil Angelakis, Mical Paul, Fabrice Armougom, Leonard Leibovici, et al.. Comparative meta-analysis of the effect of *Lactobacillus* species on weight gain in humans and animals. *Microbial Pathogenesis*, Elsevier, 2012, 53 (2), pp.100-108. 10.1016/j.micpath.2012.05.007. hal-01773004

HAL Id: hal-01773004

<https://hal-amu.archives-ouvertes.fr/hal-01773004>

Submitted on 20 Apr 2018

**HAL** is a multi-disciplinary open access archive for the deposit and dissemination of scientific research documents, whether they are published or not. The documents may come from teaching and research institutions in France or abroad, or from public or private research centers.

L'archive ouverte pluridisciplinaire **HAL**, est destinée au dépôt et à la diffusion de documents scientifiques de niveau recherche, publiés ou non, émanant des établissements d'enseignement et de recherche français ou étrangers, des laboratoires publics ou privés.





## Comparative meta-analysis of the effect of *Lactobacillus* species on weight gain in humans and animals

Matthieu Million<sup>a,1</sup>, Emmanouil Angelakis<sup>a,1</sup>, Mical Paul<sup>b</sup>, Fabrice Armougom<sup>a</sup>, Leonard Leibovici<sup>c</sup>, Didier Raoult<sup>a,\*</sup>

<sup>a</sup>URMITE-CNRS UMR 7278 IRD 198, IFR 48, Faculté de Médecine, Université de la Méditerranée, 27 bd Jean Moulin, Marseille, France

<sup>b</sup>Sackler Faculty of Medicine, Tel-Aviv University, Tel-Aviv, Israel

<sup>c</sup>Rabin Medical Center, Beilinson Hospital, Petah-Tiqva, Israel

### ARTICLE INFO

#### Article history:

Received 27 April 2012

Received in revised form

11 May 2012

Accepted 16 May 2012

Available online 24 May 2012

#### Keywords:

Probiotics

*Lactobacillus*

Weight

Obesity

Meta-analysis

### ABSTRACT

**Background:** Obesity is associated with alteration of the gut microbiota. In order to clarify the effect of *Lactobacillus*-containing probiotics (LCP) on weight we performed a meta-analysis of clinical studies and experimental models. We intended to assess effects by *Lactobacillus* species.

**Methods:** A broad search with no date or language restriction was performed. We included randomized controlled trials (RCTs) and comparative clinical studies in humans and animals or experimental models assessing the effect of *Lactobacillus*-containing probiotics on weight. We primarily attempted to extract and use change from baseline values. Data were extracted independently by two authors. Results were pooled by host and by *Lactobacillus* species and are summarized in a meta-analysis of standardized difference in means (SMDs).

**Results:** We identified and included 17 RCTs in humans, 51 studies on farm animals and 14 experimental models. *Lactobacillus acidophilus* administration resulted in significant weight gain in humans and in animals (SMD 0.15; 95% confidence intervals 0.05–0.25). Results were consistent in humans and animals. *Lactobacillus fermentum* and *Lactobacillus ingluviei* were associated with weight gain in animals. *Lactobacillus plantarum* was associated with weight loss in animals and *Lactobacillus gasseri* was associated with weight loss both in obese humans and in animals.

**Conclusions:** Different *Lactobacillus* species are associated different effects on weight change that are host-specific. Further studies are needed to clarify the role of *Lactobacillus* species in the human energy harvest and weight regulation. Attention should be drawn to the potential effects of commonly marketed lactobacillus-containing probiotics on weight gain.

© 2012 Elsevier Ltd. Open access under [CC BY-NC-ND license](https://creativecommons.org/licenses/by-nc-nd/4.0/).

### 1. Introduction

The prevalence of obesity is increasing steadily among adults, adolescents and children and is now considered a worldwide epidemic [1]. The causes driving the obesity appear to be complex and include environmental, genetic, neural and endocrine factors [2] but infectious agents have also been proposed [3]. More recently obesity was associated with a specific profile of the bacterial gut microbiota [4] and was shown to be a transmissible phenotype by microbiota transplantation [5]. First studies on obesity reported a decrease in the *Bacteroidetes/Firmicutes* ratio [4] and a decrease in the archae *Methanobrevibacter smithii* [6]. Since these pioneering studies, significant associations were found between the increase of

some bacterial groups and human obesity (*Lactobacillus* [7], *Staphylococcus aureus* [8–10], *Escherichia coli* [10] and *Faecalibacterium prausnitzii* [11]). Conversely, other bacterial groups have been associated with lean status, mainly belonging to the *Bifidobacterium* genus [6,8–11]. We found recently that different *Lactobacillus* species may have a paradoxical effect with higher levels of *Lactobacillus reuteri* and lower levels of *Lactobacillus plantarum* and *paracasei* in obese gut microbiota [12]. In contrast, symbiotics (the combination of prebiotics and probiotics) have been proposed in the management of malnutrition with promising results on mortality [13].

As many probiotic strains of *Lactobacillus* and *Bifidobacterium* are marketed in products for human consumption, altering the intestinal flora [14], we hypothesized that widespread ingestion of probiotics may promote obesity by altering the intestinal flora [15]. However, this remains controversial [16]. On the other hand, manipulation of the gut microbiota by probiotics has been used for

\* Corresponding author. Tel.: +33 491 38 55 17; fax: +33 491 83 03 90.

E-mail address: [didier.raoult@gmail.com](mailto:didier.raoult@gmail.com) (D. Raoult).

<sup>1</sup> These authors contributed equally to this work.

growth promotion in farm animals for at least 30 years [17]. Indeed, *Lactobacillus acidophilus*, *L. plantarum*, *Lactobacillus casei*, *Lactobacillus fermentum*, *L. reuteri* are the most commonly used *Lactobacillus* spp. in agriculture [18]. All these data strongly suggest that *Lactobacillus* containing probiotics (LCP) may impact the weight regulation in humans and animals.

Many studies have reported the effects of *Lactobacillus* containing probiotics (LCP) on weight but according to recent data [12], this effect is at least species dependent. To our knowledge, no meta-analysis has been performed to confirm this difference among *Lactobacillus* containing probiotics. For this purpose, we pooled data from animal and human studies to obtain sufficient power to detect a significant effect at the species level.

## 2. Methods

### 2.1. Data sources

According to PRISMA 2009 guidelines [19] (Table A.1), PubMed, Medline, ISI Web of knowledge, Google scholar, Google, Cochrane Central Register of Controlled Trials ([www.cochrane.org](http://www.cochrane.org)), meta-Register of Controlled Trials ([www.controlledtrials.com/mrct](http://www.controlledtrials.com/mrct)), [clinicaltrials.gov](http://clinicaltrials.gov) and a recent evidence report/technology assessment [20] were searched for articles, unrestricted by language, from 1950 to August 2011. Search terms included: probiotics, *Lactobacillus*, weight, weight gain, weight loss, weight change, growth, performance, randomized controlled trials, placebo-controlled and associated author names.

### 2.2. Study selection and data extraction

We retrieved the full text of studies including *Lactobacillus*-containing probiotics and looked for weight assessment as primary or secondary outcome. Inclusion was limited to experimental studies and randomized controlled trials in farm animals, experimental models and healthy humans. Authors were contacted when published data were incomplete. Exclusion criteria included hosts with underlying diseases (except for obesity) or pregnant women, probiotics given only to the mother, symbiotics (probiotics associated with prebiotics), other nutrients given exclusively to the intervention group, non-direct fed microbials (probiotics in silage), non viable probiotic administration, recombinant probiotics, hosts challenged prior to probiotic administration by viruses or bacteria, hosts with diarrhoea or colitis, before-after intervention studies, inappropriate control group (prebiotics, probiotics or antibiotics administration – traditional yogurt including *Lactobacillus delbrueckii* subsp. *bulgaricus* and *Streptococcus thermophilus* was accepted as control intervention), unavailable statistical data and double publications. Data were extracted independently by two authors (MM, EA).

### 2.3. Risk of bias assessment and outcome measures

The Jadad score [21] was used for the assessment of bias in evaluating human trials to determine studies to exclude and allowing sensitivity analysis based on this quality score. In animals, all studies were included except those with major methodological concerns, and a score was calculated with one point for each of these terms: dropouts mentioned, dropouts < 10%, outcome expressed as a weight difference (and not weight at the end) and absence of other risk of bias. Studies with a score > 2 were considered at low risk for bias.

The primary outcome was the effect on weight. Weight change from baseline, weight at the end of the study, daily weight change, weight/age ratio, delta-BMI (Body Mass Index) and weight percentile were considered as outcome measures. We primarily attempted to extract and use change from baseline values.

### 2.4. Statistical analysis and heterogeneity investigation

We used RevMan v5.1 [22] to carry out meta-analysis of the standardized difference in means (SMD) with 95% confidence interval for weight change after probiotics administration. When means were available and the *p*-value was described as > 0.05 (or “not significant”), a two-tailed *p*-value of 0.9 was attributed in order to increase the sensitivity of this pioneering work in this area. Each trial could contribute more than one comparison but comparisons were pooled for each study only if experimental conditions were similar. Heterogeneity was assessed by the *I*-squared value, 50% being considered as substantial. Summary measures were determined by a random effect model assuming significant clinical heterogeneity regardless of the *I*-squared value. We primarily investigated heterogeneity by stratifying results by *Lactobacillus* species including comparisons with only one *Lactobacillus* species in the probiotic product. In addition, subgroup analyses were planned a priori to discern weight changes by host category; overweight/obese animals or humans; and very low weight birth (VLWB) newborns. The effect of studies' risk of bias was assessed through sensitivity analysis. Funnel plot was used to identify outliers subsequently excluded and to assess small studies and publication bias. Classic fail-safe *N*, Egger's test for asymmetry were also used to assess small studies bias and Duval and Tweedie's Trim and Fill adjustment with random effects model was used to provide an estimate of the unbiased effect size. A standardized difference in means > 0.10 was considered clinically relevant as it correspond to a 1 kg weight difference for a 70 kg man based on data from a sample of 5000 healthy human individuals [23].

## 3. Results

The search yielded 200 studies of which 118 were excluded because of probiotic or host group definitions, study design or missing outcome data (Fig. 1). 82 studies, involving 153 comparisons, were included in the quantitative synthesis. Included human studies involved 15 double-blind randomized controlled trials and two open-labelled randomized trials (Table 1). Included animal studies involved 14 studies on experimental models and 51 on farm animals. After exclusion of two studies with high risk of bias (weight significantly different at baseline, open label design), LCPs were not associated with significant weight change in human adults (SMD = 0.18; 95%CI (-0.43–0.79)), infants (SMD = 0.004; 95%CI (-0.20–0.21)), or preterm newborn infants (SMD = -0.10; 95%CI (-0.32–0.12)). Meta-analysis of all comparisons in healthy humans and animals (134 comparisons, overweight and VLWB newborns excluded) resulted in weight gain but significant heterogeneity (SMD = 0.15; 95%CI (0.12–0.18); *p* < 0.001; *I*<sup>2</sup> = 85%) and thus we proceeded directly to the subgroup analyses, primarily assessing effects by species.

### 3.1. *Lactobacillus acidophilus*

The meta-analysis of 13 studies and 18 comparisons including 3307 subjects (879 humans) on *L. acidophilus* administration showed a significant weight gain effect (SMD = 0.15; 95%CI (0.05–0.25); *p* = 0.005; *I*<sup>2</sup> = 42%) (Fig. 2). Using classic fail-safe *N*, 34 unpublished studies would have been necessary to bring *p*-value > 0.05, Duval and Tweedie's trim and fill did not change this result, and Egger's asymmetry test was not significant (two-tailed *p*-value = 0.66) making this summary effect robust and publication bias unlikely. Direction of effect favouring weight gain was consistent in humans and animals. A sensitivity analysis including only studies with a quality score > 2 reduced heterogeneity and found a consistent and significant result (*I*<sup>2</sup> = 28%,

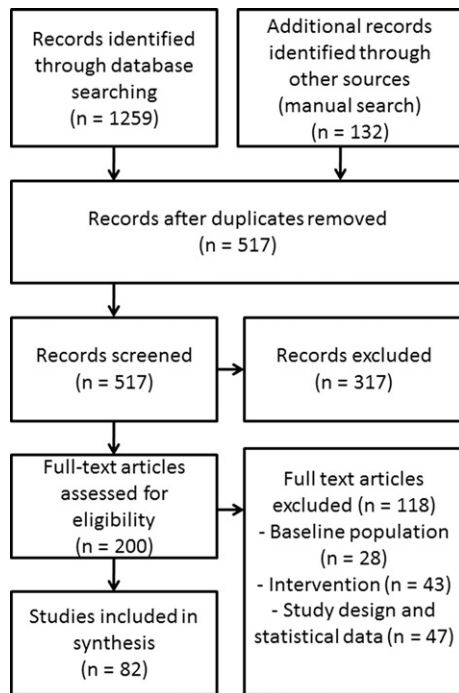


Fig. 1. Studies flow through meta-analysis according to PRISMA guidelines [19].

$p = 0.005$ ). The difference (SMD = 0.15) was clinically relevant as it corresponds to a weight gain of 1.5 kg for a 70 kg man.

### 3.2. *Lactobacillus fermentum*

The meta-analysis of 3 studies and 12 comparisons including 598 chicks, pigs and ducks but no humans on *L. fermentum* found a significant weight increase (SMD = 0.81; 95%CI (0.12–1.50);  $p = 0.02$ ;  $I^2 = 90\%$ ). After exclusion of one outlier, 34 unpublished studies would have been necessary to bring  $p$ -value > 0,05 using classic fail-safe  $N$ , Duval and Tweedie's trim and fill one study but found a consistent result (SMD = 0.53; 95% CI (0.18–0.87)) and Egger's asymmetry test was not significant (two-tailed  $p$ -value = 0.28) making this summary effect robust. All these studies have a quality score > 2.

### 3.3. *Lactobacillus ingluviei*

The meta-analysis of three studies and 11 comparisons including 198 chicks, ducks and mice on one *L. ingluviei* strain isolated from an ostrich found a significant weight increase effect (SMD = 0.97; 95%CI (0.49–1.45);  $p < 0.001$ ;  $I^2 = 59\%$ ). After exclusion of one outlier [24], 27 unpublished studies would have been necessary to bring a  $p$ -value > 0.05 using classic fail-safe  $N$ , Egger's asymmetry test was not significant ( $p = 0.59$ ) and Duval and Tweedie's trim and fill give a similar significant result (SMD = 0.76; 95% CI (0.48–1.03)). All these studies had a quality score > 2. No human trials included *L. ingluviei*.

### 3.4. *Lactobacillus plantarum*

Pooled analysis of three studies, three comparisons including 335 lean chicks, rats and mice on *L. plantarum* showed a weight loss effect direction but result was not significant (Fig. 3a). However, *L. plantarum* was associated with significant weight loss effect in overweight/obese animals in four studies and five comparisons including 64 mice and rats (SMD = -1.33; 95%CI (-2.50 to -0.16);

$p = 0.03$ ;  $I^2 = 74\%$ ) (Fig. 3b). No human studies included *L. plantarum*. Small studies bias was unlikely because 19 unpublished studies would have been necessary to bring the  $p$ -value to > 0,05, Duval and Tweedie's trim and fill did not change this result and Egger's asymmetry test was not significant (two-tailed  $p$ -value = 0.52). All these comparisons in experimental animals had a medium to low risk of bias.

### 3.5. *Lactobacillus gasseri*

*L. gasseri* was associated with a trend for weight loss in lean animals in three studies and four comparisons including 48 pigs and rats ( $p = 0.09$ ) (Fig. 3a). In obese animals and humans (Fig. 3b), three studies and three comparisons including 87 humans and 36 rats found an anti-obesity effect (SMD = -0.67; 95%CI (-1.17 to -0.16);  $p = 0.009$ ;  $I^2 = 29\%$ ). Using classic fail-safe  $N$ , six unpublished studies would have been necessary to bring the  $p$ -value to > 0,05, Duval and Tweedie's trim and fill did not change this result and Egger's asymmetry test was not significant (two-tailed  $p$ -value = 0.88) making this summary effect robust and small studies bias unlikely. This effect was consistent between humans and animals. Two *L. gasseri* strains (SBT2055 [25–27] and BNR17 [28]) have a significant anti-obesity effects in individual studies. All these studies had a medium to low risk of bias. In obese individuals, the difference (SMD = -0.57) was clinically relevant since it correspond to a weight loss of 6 kg in humans.

### 3.6. Other species

Other species (*L. reuteri*, *L. casei*, *Lactobacillus rhamnosus* and *Lactobacillus sporogenes*) were not associated with significant and consistent effects. Only *L. delbrueckii* was significantly associated with weight gain (five comparisons;  $I^2 = 0\%$ ; SMD = 0.39; 95%CI (0.06–0.71);  $p = 0.02$ ) but this effect was summarized from only two different studies in chicks and rats.

## 4. Discussion

### 4.1. Significant impact of *Lactobacillus*-containing probiotics on weight

In this meta-analysis, we showed that some *Lactobacillus* species were significantly associated with weight modifications in human and animals: *L. acidophilus*, *L. ingluviei*, *L. fermentum* were linked to weight gain whereas *L. gasseri* and *L. plantarum* were linked to weight loss or an anti-obesity effect. The latter effect seemed particularly evident in overweight or obese individuals. Wide variation in response was explained by probiotic species and host. Stratification only by probiotic species revealed significant and consistent results. In a second step, we showed that the host was a covariate explaining part of the heterogeneity found for a specific probiotic species (Table A.2). The differences found were clinically relevant as they correspond to a weight change that ranged from 1.5 kg gain in leans for *L. acidophilus* or 6 kg loss in overweight for *L. gasseri* based on the statistics of a population of >600 healthy men with an average weight of 70 kg (standard deviation of 9.8 kg) [23].

### 4.2. *Lactobacillus* species associated with weight gain

With the results of our meta-analysis, bacteria candidates for increasing energy efficiency in humans are *L. acidophilus* and *L. fermentum*. To our knowledge, *L. ingluviei* was not identified in the human digestive microbiota but only in the intestinal tract of pigeons, chickens and ostrich and is not contained in probiotics

**Table 1**  
Characteristics of included human studies.

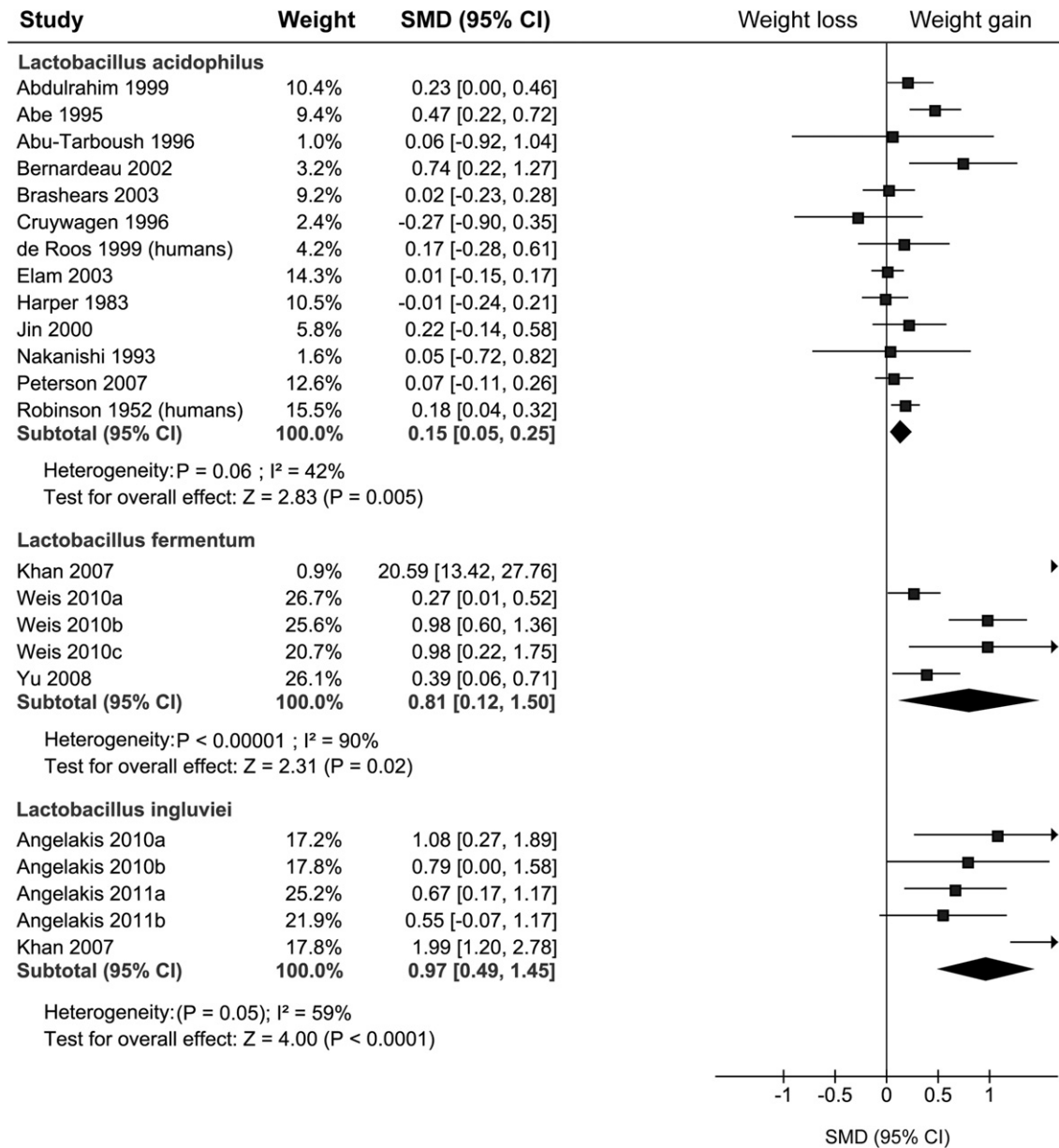
Study source	Location; period of inclusion; mono or multicentric; study design; risk of bias (Jadad score)	Subjects included; age and sex; sample size (subjects enrolled, dropped out, used); no of treated /control subjects	Exclusion criteria	Probiotic (dose); duration of treatment	Outcomes (primary in first); weight change assessment (unit)
<b>Lactobacillus probiotics in infants (&lt;2 years)</b>					
Chouraqui et al., 2008	France; 2004–2005; multicentric ( $n = 5$ ); prospective, double-blind, reference controlled, parallel-group, randomized trial; low (5)	Full term, singletons, exclusively formula fed healthy infants, with weight between 2500 and 4500 g; <15 d of age (284, 57, 227) – 2/4 groups using prebiotics were excluded from this meta-analysis; two comparisons: boys: 29/25, girls: 30/28	Major deformities or cardiovascular, GI, renal, neurologic, or metabolic illnesses, intensive care for $\geq 3$ days, mother with diabetes, parents having difficulties complying the feeding regimen	<i>B. longum</i> BL999 ( $1.3 \times 10^8$ cfu per 100 mL of reconstituted formula) and <i>L. rhamnosus</i> LPR ( $6.45 \times 10^8$ cfu per 100 mL of reconstituted formula) in powdered starter formula; 4 months	Weight gain; daily weight gain on 4 months (g/d)
Maldonado et al., 2010	Spain; period of inclusion not mentioned; monocentric; prospective, double-blind, placebo controlled, randomized trial; medium (3)	Healthy breast-fed infants fed exclusively with formula at the moment of recruitment, sixth month of life; boys 39, girls 41; (80, 0, 80); treated/control: 40/ 40	Frequent gastrointestinal disorders (frequent diarrheal, constipation episodes, gastroesophageal reflux), gastrointestinal surgery, cow's milk protein allergy, metabolic disease (diabetes or lactose intolerance), antibiotic treatment during the trial or within the preceding 3 wk	<i>L. salivarius</i> CECT5713 ( $2 \times 10^6$ cfu/g) on formula; 6 months	Multiple outcomes : antibiotic susceptibility of the strain, AEs', growth parameters, intestinal microbiota; weight gain on 6 months (g)
Robinson et al.1952	USA; period of inclusion not mentioned; two centers; prospective, randomized trial (no mentioned blinding); high (1)	~800 enrolled newborns, number of dropped out not mentioned, four groups (treated/control): Completely bottle fed (124/123), partially breast fed infants (79/69), completely bottle fed with folic acid (134/129), partially breast fed with folic acid (60/83) – sex ratio not mentioned	Infants who were obviously ill in the hospital, who had congenital irregularities or those found to have been ill and that did not gain at least 16 ounces during the first month	<i>L. acidophilus</i> ATCC4962 and ATCC4963 ( $>5 \times 10^8$ cfu), 1 ml to each quart of formula; from birth until hospital discharge (1–6 days)	Weight gain; weight gain at one month <sup>a</sup>
Scalabrin et al., 2009	USA; 2006–2007; multicentric; prospective, double-blind, randomized trial; low (4)	Healthy term infants (birth weight $\geq 2500$ g) enrolled at 14 d of age, solely formula fed; M/F = 94/94; (188, 55, 133) – one group using different casein formula was excluded; treated/control: 63/70	Underlying disease or congenital malformation, formula intolerance, weight at 14 d of age $\leq 98\%$ of birth weight, large for gestational age born from a mother diabetic at childbirth, immunodeficiency, fever, antibiotic within 7 d, systemic steroid since birth, LGG-suppl diet since birth, diarrhoea within 24 h	Extensively hydrolysed casein formula supplemented or not with <i>L. rhamnosus</i> strain GG ( $10^8$ cfu/g of formula powder); 120 days	Growth and tolerance; weight gain (g/d) on 120 d
Vendt et al., 2006	Finland; 2002; multicentric; prospective, double-blind, randomized trial; medium (3)	Healthy infants from 0 to 2 months on formula at least half of their daily feedings; M/F = 60/60; (120, 15, 105); treated/control: 51/54	Not mentioned (reasons for discontinuation: colic pain, cow's milk protein intolerance, constipation, diarrhoea, excessive breastfeeding)	<i>L. rhamnosus</i> strain GG ATCC53103 ( $1 \times 10^7$ cfu); till the age of 6 months	Growth <sup>b</sup> and fecal flora on 6 months
Weizman et al., 2006	Israel; 2006; monocentric; prospective, double-blind, randomized trial; low (4)	Full term healthy infants aged 3–65 days solely formula fed; M/F = 26/13; (39; 7;32); treated/control: 16/17.	<36 wks gestation, birth weight < 2500 g, congenital anomalies, chronic disease, failure to thrive (weight loss of > 2 percentiles), allergy or atopic disease and recent (less	<i>L. reuteri</i> ATCC55730 (BioGAIA AB, Sweden) ( $1 \times 10^8$ cfu); 4 weeks	Growth parameters, daily characteristics of feeding, stooling and behaviour and side effects.

(continued on next page)

Table 1 (continued)

Study source	Location; period of inclusion; mono or multicentric; study design; risk of bias (Jadad score)	Subjects included; age and sex; sample size (subjects enrolled, dropped out, used); no of treated /control subjects	Exclusion criteria	Probiotic (dose); duration of treatment	Outcomes (primary in first); weight change assessment (unit)
			than four weeks) exposure to probiotics or antibiotics.		
<b>Lactobacillus probiotics in lean adults</b>					
De Roos et al., 1999	The Netherlands; period of inclusion not mentioned; monocentric; prospective, double-blind, randomized trial; medium (3)	Healthy adults between 18 and 65 years; BMI $24 \pm 3$ , at least 50% of the enrolled volunteers had serum cholesterol levels over 5 mmol/L, M/F = 22/56; (85,7,78); treated/control: 39/39	Heart disease, diabetes, liver or kidney disease, medications known to affect blood lipid metabolism, serum total cholesterol concentration higher than 8 mmol/L or a triacylglycerol concentration higher than 4 mmol/L	<i>L. acidophilus</i> L-1 ( $5 \times 10^9$ to $3 \times 10^{10}$ cfu d)	Lipid profile and body weight change; weight change difference (kg)
Fabian et al., 2007	Austria; period of inclusion not mentioned; monocentric; prospective, randomized trial (blinding not mentioned); high (2)	Healthy adults women (BMI: $21 \pm 3$ kg/m <sup>2</sup> ) – 22–29 years (33, 1, 32); treated/control : 16/16	Smoking, hypercholesterolemia, pregnancy, overweight or metabolic disease, allergies or intolerance, regular medications except oral contraceptive	Actimel® ( <i>L. paracasei</i> subsp. <i>Paracasei</i> ( <i>L. casei</i> DN-114 001) ( $3.6 \times 10^8$ cfu/g)); 4 weeks	Antioxidants and oxidant parameters in plasma; weight change (kg) <sup>b</sup>
Sadrzadeh-Yeganeh et al., 2010	Iran; period of inclusion not mentioned; prospective, double-blind, randomized trial; medium (3)	Healthy adults women (cholesterol < 6.2 mmol/l, TAG < 2.3 mmol/l, BMI < 30 kg/m <sup>2</sup> ) (90,1,89) – one group excluded (no yoghurt) Treated/control: 30/29	Smoking, kidney, liver or inflammatory intestinal disease, thyroid disorders, diabetes, immunodeficiency, lactose intolerance; taking supplements or medication, probiotic consumption in the last 2 months, elite athletes, pregnant or breastfeeding women	<i>L. acidophilus</i> La1 and <i>Bifidobacterium. lactis</i> Bb12 ( $4 \times 10^7$ cfu); 6 weeks	Lipid profile; weight change (kg)
<b>Lactobacillus probiotics in overweight/obese adults</b>					
Agerholm-Larsen et al., 2000	Denmark; period of inclusion not mentioned; monocentric; prospective, double-blind, randomized trial; low (4)	Healthy weight-stable overweight and obese ( $25 < \text{BMI} < 37.5$ kg/m <sup>2</sup> ); mean 38 years, M/F = 4/12, 5/9 and 4/10; (73,3,70); treated <i>L. acidophilus</i> 16/ <i>L. rhamnosus</i> 14/control 14	Diabetes, kidney or liver disease, high blood pressure, pregnancy, breastfeeding, elite athlete, chronic ethylism	2 strains of <i>L. acidophilus</i> ( $2 \times 10^7$ /ml) and 1 strain of <i>S. thermophilus</i> ( $10 \times 10^7$ /ml); 2 strains of <i>S. thermophilus</i> ( $8 \times 10^8$ /ml) and 1 strain of <i>L. rhamnosus</i> ( $2 \times 10^8$ /ml)	Lipid profile and body weight; weight change (kg)
Kadooka et al., 2010	Japan; 2008; multicentric ( $n = 10$ ); prospective, double-blind, randomized trial; medium (3)	Healthy adults with body mass index (BMI) between 24.2 and 30.7 kg/m <sup>2</sup> , abdominal visceral fat area between 81.2 and 178.5 cm <sup>2</sup> aged 33–63 years, M/F = 59/28; (87,0,87); treated/control: 43/44	Serious disorders, including internal organ diseases, diabetes and hypersensitivity to dairy products.	<i>L. gasseri</i> SBT2055 ( $5 \times 10^{10}$ cfu/100 g)–200 g/day; 12 weeks	Abdominal adiposity and body weight; weight change (kg)
Woodard et al., 2009	USA; 2006–2007; monocentric; prospective, double-blind, randomized trial; low (5)	Morbidly obese patients undergoing Roux-en-Y gastric bypass (RNYGB) (BMI ~ 45 kg/m <sup>2</sup> ); Age 40–50 yrs, M/F = 5/36; (44, 8, 35); treated/control:15/20	No exclusion criteria mentioned	Puritan's Pride® ( $2.4 \times 10^9$ cfu/pill) one pill a day – no characterization of the <i>Lactobacillus</i> strains; 6 months	Bacterial overgrowth, weight loss, quality of life; percent excess weight loss (%)

<sup>a</sup> g calculated from ounce,  $\times 28.35$ .<sup>b</sup> Data given by the authors under request – in this study, weight of treated group was significantly lower at baseline.



**Fig. 2.** Forest plot of three *Lactobacillus* species associated with weight gain in humans and animals (a) *L. acidophilus*, (b) *L. fermentum*, (c) *L. ingluviei* (strain isolated from an ostrich [24]). A weight gain effect is represented by a deviation of the square (standardized difference in the means) to the right. The length of the horizontal line represents the 95% confidence interval and the diamond represents the summarized effect. Substantial heterogeneity was cancelled after sensitivity analysis for *L. acidophilus*. *L. acidophilus* and *L. fermentum*, when administered in overweight/obese humans or animals didn't have a significant anti-obesity effect (data not shown).

marketed for humans. The *L. ingluviei* comparisons included in this analysis involved only one strain, isolated from an ostrich gut, showing an astonishing weight gain effect both in farm animals and experimental models [24,29]. One candidate for the transmission of the obese phenotype, *L. acidophilus*, is widely present in many products for human consumption as the “acidophilus milk”, traditionally consumed in the United States or in other formulations such as freeze-dried products sold without any regulation on the internet. The consumption of this species is particularly prevalent in the United States [30] where the prevalence of obesity is particularly important.

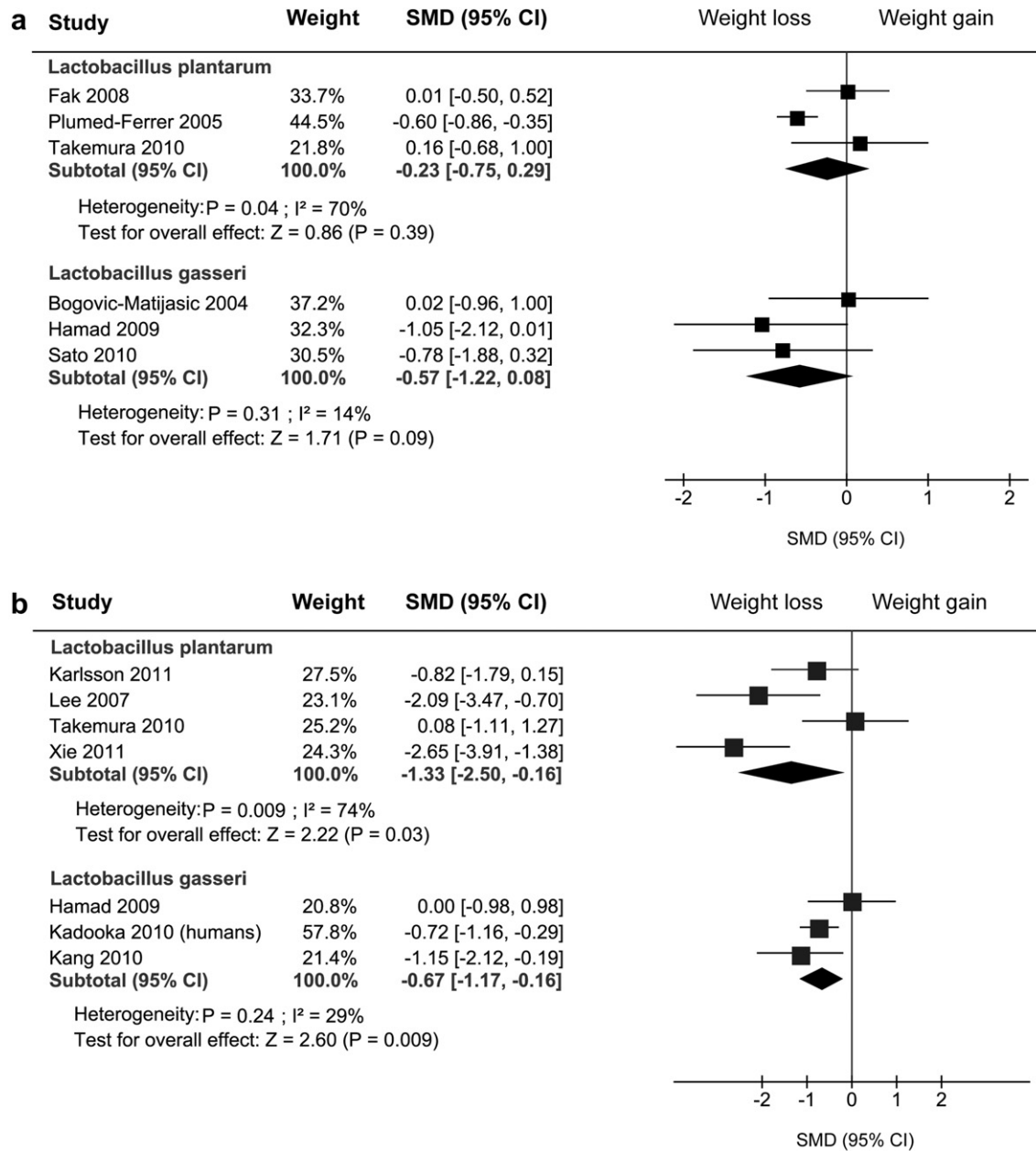
#### 4.3. *Lactobacillus* species associated with anti-obesity effect

On the other hand, many bacteria appear to be protective against obesity. In our study, a strong species dependent anti-obesity

effect was observed with two species having a significant anti-obesity effect; *L. gasseri* and *L. plantarum* (Fig. 3b). This anti-obesity effect was consistent with absence of significant weight-gain effect in lean individuals (Fig. 3a). A recent study confirmed our results showing a 6-months weight-loss effect of *L. plantarum* DSM 15313 in high-energy-dense diet rats when administered to mother and offspring [31]. This anti-obesity effect could be an important adjunct in the treatment of obesity, since, apart from surgery, no medical treatment can support efficiently the fight against obesity to date.

#### 4.4. Limitations

However, the paucity of data in individual hosts impelling us to the pool animal and human data limits the generalization of these



**Fig. 3.** Forest plot of three *Lactobacillus* species associated with an anti-obesity effect in humans and animals. *Lactobacillus plantarum* and *L. gasseri* and weight changes in (a) lean (b) and overweight/obese humans and animals. Weight loss effect is represented by a deviation of the square (standardized difference in the means) to the left. The length of the horizontal line represents the 95% confidence interval and the diamond represents the summarized effect. Anti-obesity effect was consistent for these two species.

data to humans. Moreover, effect size and standard deviation are probably very different in experimental models and in the general human population. Only few clinical studies have been conducted to test a weight gain effect assessing only one *Lactobacillus* species because, unlike animal studies, this effect was generally not sought in humans. *L. acidophilus* increased weight gain both in bottle-fed and breast-fed newborns but this effect was stronger in bottle-fed newborns [32], *L. gasseri* SBT2055 resulted in significant weight loss in human adults with obese tendencies [27].

#### 4.5. Conflict of interest in nutrition and obesity research

Finally, it is possible that the design and/or interpretation of the results of each individual study had been affected by a conflict of interest of each team. It has recently been shown that published

papers in nutrition and obesity research in which the authors were funded by industry were more likely than other papers to contain results or an interpretation that favored the industry or company that was producing the product or service that was being studied [33]. Furthermore, while a comprehensive search was performed using several strategies, we cannot be sure that all studies examining the effects of LCPs on weight have been identified.

#### 4.6. Perspectives

In the next future, new double-blind randomized human trials should assess long-term growth in newborn infants receiving *Lactobacillus*-containing probiotics. A critical point is to stratify according to the initial weight [34]. For species associated here with a significant weight change and used for human consumption as



*L. acidophilus*, *L. fermentum*, *L. plantarum* and *L. gasseri*, trials evaluating weight gain as a primary outcome measure will be needed. The long-term evaluation to at least 3–5 years of age will be critical to identify a difference that could have been undetected by shorter studies [35]. According to the register [ClinicalTrials.gov](http://ClinicalTrials.gov), at least one current study is testing the average weight gain as a primary endpoint among newborns receiving a probiotic containing *L. fermentum* (trial number NCT01346644). This bacterium has been associated with obesity in our study. In addition, case–control studies comparing obese and lean children according to their consumption of *Lactobacillus*-containing probiotics in the first weeks of life should be carried out.

## 5. Conclusion

Food is a source of bacteria and viruses and changes in patterns of food consumption result in differences in human gut flora among different groups of people [36,37]. As a result, it is necessary to further investigate the effects of routinely adding high amounts of bacteria in food [38]. Our systematic analysis found that the manipulation of the gut microbiota by *L. acidophilus*, *L. ingluviei* or *L. fermentum* results in weight gain whereas specific strains of *L. gasseri* and *L. plantarum* used as food supplements presented an anti-obesity effect. Only two studies including these species were available in humans, one showing a significant weight gain effect of *L. acidophilus* in newborns whereas *L. gasseri* was found to have a significant anti-obesity effect in the first and only well-designed study to date assessing the impact of *Lactobacillus*-containing probiotics on overweight humans. *L. acidophilus* and *L. gasseri* were associated with the same effect direction both in animals and humans. *L. fermentum* and *L. ingluviei* were associated with an astonishing weight gain effect in ducks, chicks and mice but have never been studied in humans. Next-generation human probiotic species should contain *Lactobacillus* spp. that are not associated with weight gain in humans. Of note, on 24 August 2007, the FDA issued regulations that require current good manufacturing practice for dietary supplements to be phased in over the next few years [39]. These regulations should focus on weight assessment outcome according to probiotic species and strains. Finally, selection of specific *Lactobacillus* containing probiotics could take part in the future management of the two major health problems in the 21st century, malnutrition and obesity.

## Competing interest statement

All authors have completed the Unified Competing Interest form at [www.icmje.org/coi\\_disclosure.pdf](http://www.icmje.org/coi_disclosure.pdf) (available on request from the corresponding author) and declare that (1) no authors have support from probiotics companies for the submitted work; (2) no authors have relationships with probiotics companies that might have an interest in the submitted work in the previous 3 years; (3) their spouses, partners, or children have not financial relationships that may be relevant to the submitted work; and (4) no authors have non-financial interests that may be relevant to the submitted work.

## Contributors

DR conceived and designed the study, MM & EA extracted the data, MM, FA, MP, LL analysed the data. MM, EA and DR wrote the manuscript. MP & LL revised the paper. MM had full access to all of the data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis and is guarantor. MM and EA contributed equally to this work.

## Funding

URMITE –CNRS UMR 6236 IRD 198.

## Ethical approval

Not required.

## Acknowledgements

We thanks all authors that kindly answer and send us requested data and especially Asal Ataie, Wageha Awad, Jonna Aaltonen, Elisabeth Fabian, Kirsi Laitinen, Kostas Mountzouris, Denis Roy, Nancy Szabo, Neve Vendt and Christina West.

## Appendix A. Supplementary material

Supplementary material associated with this article can be found, in the online version, at [doi:10.1016/j.micpath.2012.05.007](https://doi.org/10.1016/j.micpath.2012.05.007).

## References

- [1] Yanovski SZ, Yanovski JA. Obesity. *N Engl J Med* 2002;346:591–602.
- [2] Tilg H, Moschen AR, Kaser A. Obesity and the microbiota. *Gastroenterology* 2009;136:1476–83.
- [3] Dhurandhar NV. A framework for identification of infections that contribute to human obesity. *Lancet Infect Dis* 2011;11:963–9.
- [4] Ley RE, Turnbaugh PJ, Klein S, Gordon JL. Microbial ecology: human gut microbes associated with obesity. *Nature* 2006;444:1022–3.
- [5] Turnbaugh PJ, Backhed F, Fulton L, Gordon JL. Diet-induced obesity is linked to marked but reversible alterations in the mouse distal gut microbiome. *Cell Host Microbe* 2008;3:213–23.
- [6] Schwartz A, Taras D, Schafer K, Beijer S, Bos NA, Donus C, et al. Microbiota and SCFA in lean and overweight healthy subjects. *Obesity (Silver Spring)* 2010;18:190–5.
- [7] Armougom F, Henry M, Viallettes B, Raccach D, Raoult D. Monitoring bacterial community of human gut microbiota reveals an increase in *Lactobacillus* in obese patients and Methanogens in anorexic patients. *PLoS One* 2009;4:e7125.
- [8] Kalliomaki M, Collado MC, Salminen S, Isolauri E. Early differences in fecal microbiota composition in children may predict overweight. *Am J Clin Nutr* 2008;87:534–8.
- [9] Collado MC, Isolauri E, Laitinen K, Salminen S. Distinct composition of gut microbiota during pregnancy in overweight and normal-weight women. *Am J Clin Nutr* 2008;88:894–9.
- [10] Santacruz A, Collado MC, Garcia-Valdes L, Segura MT, Martín-Lagos JA, Anjos T, et al. Gut microbiota composition is associated with body weight, weight gain and biochemical parameters in pregnant women. *Br J Nutr* 2010;104:83–92.
- [11] Balamurugan R, George G, Kabeerdoss J, Hepsiba J, Chandragunasekaran AM, Ramakrishna BS. Quantitative differences in intestinal *Faecalibacterium prausnitzii* in obese Indian children. *Br J Nutr* 2010;103:335–8.
- [12] Million M, Maraninchi M, Henry M, Armougom F, Richet H, Carrieri P, et al. Obesity-associated gut microbiota is enriched in *Lactobacillus reuteri* and depleted in *Bifidobacterium animalis* and *Methanobrevibacter smithii*. *Int J Obes (Lond)*; 2011. doi:10.1038/ijo.2011.153 [Epub ahead of print].
- [13] Kerac M, Bunn J, Seal A, Thindwa M, Tomkins A, Sadler K, et al. Probiotics and prebiotics for severe acute malnutrition (PRONUT study): a double-blind efficacy randomised controlled trial in Malawi. *Lancet* 2009;374:136–44.
- [14] Fujimoto J, Matsuki T, Sasamoto M, Tomii Y, Watanabe K. Identification and quantification of *Lactobacillus casei* strain Shirota in human feces with strain-specific primers derived from randomly amplified polymorphic DNA. *Int J Food Microbiol* 2008;126:210–5.
- [15] Raoult D. Human microbiome: take-home lesson on growth promoters? *Nature* 2008;454:690–1.
- [16] Delzenne N, Reid G. No causal link between obesity and probiotics. *Nat Rev Microbiol* 2009;7:901.
- [17] Fuller R. Probiotics in man and animals. *J Appl Bacteriol* 1989;66:365–78.
- [18] Anadon A, Martinez-Larranaga MR, Aranzazu MM. Probiotics for animal nutrition in the European Union. Regulation and safety assessment. *Regul Toxicol Pharmacol* 2006;45:91–5.
- [19] Liberati A, Altman DG, Tetzlaff J, Mulrow C, Gøtzsche PC, Ioannidis JP, et al. The PRISMA statement for reporting systematic reviews and meta-analyses of studies that evaluate health care interventions: explanation and elaboration. *PLoS Med* 2009;6:e1000100.
- [20] Hempel S, Newberry S, Ruelaz A, Wang Z, Miles JNV, Suttorp MJ, et al. Safety of probiotics to reduce risk and prevent or treat disease. Evidence report/technology assessment no. 200. Agency for Healthcare Research and Quality
- [21] Jadad AR, Moore RA, Carroll D, Jenkinson C, Reynolds DJ, Gavaghan DJ, et al. Assessing the quality of reports of randomized clinical trials: is blinding necessary? *Control Clin Trials* 1996;17:1–12.

- [22] The Cochrane Collaboration. Review Manager (RevMan) [computer program]. Version 5.1. Copenhagen: The Nordic Cochrane Centre; 2011.
- [23] Khosla T, Lowe C. Indices of obesity derived from body weight and height. *Br J Prev Soc Med* 1967;21:122–8.
- [24] Khan M, Raoult D, Riche H, Lepidi H, La Scola B. Growth-promoting effects of single-dose intragastrically administered probiotics in chickens. *Br Poult Sci* 2007;48:732–5.
- [25] Hamad EM, Sato M, Uzu K, Yoshida T, Higashi S, Kawakami H, et al. Milk fermented by *Lactobacillus gasseri* SBT2055 influences adipocyte size via inhibition of dietary fat absorption in Zucker rats. *Br J Nutr* 2009;101:1–9.
- [26] Sato M, Uzu K, Yoshida T, Hamad EM, Kawakami H, Matsuyama H, et al. Effects of milk fermented by *Lactobacillus gasseri* SBT2055 on adipocyte size in rats. *Br J Nutr* 2008;99:1013–7.
- [27] Kadooka Y, Sato M, Imaizumi K, Ogawa A, Ikuyama K, Akai Y, et al. Regulation of abdominal adiposity by probiotics (*Lactobacillus gasseri* SBT2055) in adults with obese tendencies in a randomized controlled trial. *Eur J Clin Nutr* 2010;64:636–43.
- [28] Kang JH, Yun SI, Park HO. Effects of *Lactobacillus gasseri* BNR17 on body weight and adipose tissue mass in diet-induced overweight rats. *J Microbiol* 2010;48:712–4.
- [29] Angelakis E, Raoult D. The increase of *Lactobacillus* species in the gut flora of newborn broiler chicks and ducks is associated with weight gain. *PLoS ONE* 2010;5:e10463.
- [30] Chandan RC. History and consumption trends. In: Chandan RC, White CH, Kilara A, Hui YH, editors. *Manufacturing Yogurt and Fermented Milks*. Ames: Blackwell Publishing; 2006. p. 3–15.
- [31] Karlsson C, Molin G, Fak F, Johansson Hagslåt ML, Jakesevic M, Håkansson Å, et al. Effects on weight gain and gut microbiota in rats given bacterial supplements and a high-energy-dense diet from fetal life through to 6 months of age. *Br J Nutr* 2011;106:887–95.
- [32] Robinson EL, Thompson WL. Effect on weight gain of the addition of *Lactobacillus acidophilus* to the formula of newborn infants. *J Pediatr* 1952;41:395–8.
- [33] Thomas O, Thabane L, Douketis J, Chu R, Westfall AO, Allison DB. Industry funding and the reporting quality of large long-term weight loss trials. *Int J Obes (Lond)* 2008;32:1531–6.
- [34] Vendt N, Grunberg H, Tuure T, Malminiemi O, Wuolijoki E, Tillmann V, et al. Growth during the first 6 months of life in infants using formula enriched with *Lactobacillus rhamnosus* GG: double-blind, randomized trial. *J Hum Nutr Diet* 2006;19:51–8.
- [35] Chouraqui JP, Grathwohl D, Labaune JM, Hascoet JM, de Montgolfier I, Leclaire M, et al. Assessment of the safety, tolerance, and protective effect against diarrhea of infant formulas containing mixtures of probiotics or prebiotics and prebiotics in a randomized controlled trial. *Am J Clin Nutr* 2008;87:1365–73.
- [36] Raoult D. The globalization of intestinal microbiota. *Eur J Clin Microbiol Infect Dis* 2010;29:1049–50.
- [37] Gordon JL, Klaenhammer TR. A rendezvous with our microbes. *Proc Natl Acad Sci U S A* 2011;108(Suppl. 1):4513–5.
- [38] Raoult D. Obesity pandemics and the modification of digestive bacterial flora. *Eur J Clin Microbiol Infect Dis* 2008;27:631–4.
- [39] Sanders ME. Probiotics: definition, sources, selection, and uses. *Clin Infect Dis* 2008;46(Suppl. 2):S58–61.

## Glossary

**Probiotics:** Probiotics are defined as “Live microorganisms which when administered in adequate amounts confer a health benefit on the host.” According to FAO/WHO.

**Obesity:** According to the WHO, Obesity is defined by a BMI > 30 kg/m<sup>2</sup> and a massive expansion of fat, and is associated with a significant increase in morbidity and mortality.

**Lactobacillus:** *Lactobacillus* is a genus of Gram-positive facultative anaerobic or microaerophilic rod-shaped bacteria. They are a major part of the lactic acid bacteria group, named as such because most of its members convert lactose and other sugars to lactic acid. In humans they are present in the vagina and the gastrointestinal tract. They are largely present in food products for human and animal consumption as probiotics.

**Meta-analysis with random effect model:** A meta-analysis combines the results of several studies by identification of a common measure of effect size, of which a weighted average might be calculated. A random effect model assumes that heterogeneity is due at least in part to the different experimental conditions between individual studies.