microorganisms

Review

Importance of lactobacilli for human health

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Citation: Lastname, F.; Lastname, F.; Lastname, F. Title. *Microorganisms* **2021**, *9*, x. https://doi.org/10.3390/xxxxx

Academic Editor: Firstname Lastname

Received: date
Accepted: date
Published: date

Abstract: As an extraordinarily diverse group of bacteria, lactobacilli are now classified into several genera, many of which still include "Lactobacillus" in their names. Despite the names, this group of lactic acid bacteria comprises microorganisms that are crucial for human health, especially during the early development of the human microbiota and immune system. The interactions between lactobacilli and components of the mucosal immunity lead to its shaping and development, which is possibly considered a prime mover in the advancement of the human immune system. Although much of the evidence backing the pivotal role of lactobacilli in maintaining human health comes from studies on probiotics aiming to elucidate mechanisms of their functional activities and studies on mucosal immunity on germ-free mice, it is justifiable to extend observations on properties of the individual probiotic Lactobacillus related to health benefits onto other strains sharing common characteristic of the species. In this review, we will discuss acquisition, presence and functions of lactobacilli in different human microbiota throughout the whole life including those arising in amnion and their interactions with mucosal and immune cells. Examples of immune system modulation by probiotic lactobacilli include their colonic competition for available nutrients, interference with colonization sites, competition for binding sites on gut epithelial cells, bacteriocin production, reduction of colonic pH, and nonspecific stimulation of the immune system.

Keywords: Lactobacillus; gut- vaginal- microbiota; neonates; innate immunity

1. Introduction

The *Lactobacillus* group (*sensu lato*) represented by over two hundred species and now classified into dozens of new genera [1] is among the most widely distributed taxa on Earth. It is present in practically all viable environments in different climate zones and comes into close interactions with multicellular host organisms, ranging from plants to primates. This unusual versatility of *Lactobacillus* bacteria is subsequent to its adaptability to various life conditions thanks to flexibility of their core metabolic pathways. These pathways include pyruvate metabolism, carbohydrate transport and metabolism, the proteolytic system, amino acid metabolism, and protein synthesis. This metabolic flexibility allows Lactobacilli to adjust protein levels in response to varying environmental conditions, enabling their survival and growth across diverse ecological niches [2]. Moreover, beyond general stress responses and regulatory mechanisms, specific metabolic pathways can be selectively activated or deactivated, leading to changes in the behavior of the strains [3]. One of the most well recognized *Lactobacillus* species found in different fermentation products but also in the human gut microbiome, thanks to its capabilities of adaptation and genome plasticity is *L. (Lactiplantibacillus) plantarum* [4].

Research on probiotics, aimed at uncovering their functional mechanisms, provides much of the evidence emphasizing the critical role of the *Lactobacillus* group in the housekeeping of human health. Although extending these findings to all *Lactobacillus* strains that inhabit human body surfaces or diverse ecological niches is challenging, it is reasonable to speculate that the beneficial properties observed in specific probiotic strains might be broadly characteristic of the species. Given that probiotic lactobacilli were historically isolated at random from different niches within the healthy human microbiome and subsequently characterized, it is likely that many other *Lactobacillus* strains with similar properties exist in the human microbiome but have yet to be identified or studied.

The human gastrointestinal (GI) tract is home to a diverse and ever-changing array of microorganisms, including the gut microbiota, which play a crucial role in maintaining host homeostasis and regulating metabolic functions. Analysis of various metagenomic studies has revealed a total of 2,172 distinct microbial species present in humans, categorized into 12 phyla. Of these, a significant majority—93.5%—are classified under the Proteobacteria, Firmicutes, Actinobacteria, and Bacteroidetes phyla. Within this population, 386 species are known to be strictly anaerobic and are predominantly located in the GI tract [5]. A comprehensive catalog of the gut microbiome's functional potential has recently been created, identifying a total of 9,879,896 genes from a combination of 249 newly sequenced and 1,018 previously published samples [6]. Despite not being the predominant group in the adult human gut microbiome, lactobacilli are essential for the early development of this microbiome.

It has been acknowledged for over a century that the gut microbiome plays a crucial role in health, a concept first explored through Metchnikoff's groundbreaking research on lactic acid bacteria, particularly *Lactobacillus delbrueckii ssp. Bulgaricus* [7]. Recent years have brought an increasing number of data which causes appreciation of the infant gut microbiome and especially on the critical window of its highly dynamic maturation. Recent findings shed light on the significant influence of the gut microbiome on early life development, with a particular focus on how it affects the maturation of the immune system [8]. While the precise molecular processes involved in neonatal priming in humans remain unclear, recent animal research indicates that the way infants acquire their gut microbiota is shaped by the interplay between bacterial and host factors. Therefore, the timing of bacterial colonization seems to be crucial for the development of the gut microbiome. For instance, *Lactobacillus* species are abundant in the vaginal microbiota during the final stages of pregnancy and are also present in large numbers in human milk. Consequently, at birth, lactobacilli outnumber other genera, such as *Bifidobacterium*, when colonizing the mucosal and skin surfaces of newborns. This initial colonization happens through exposure to the *Lactobacillus*-rich environment of the vagina during delivery and is further supported by breastfeeding, which provides additional lactobacilli from the mother's milk.

Colonization of the neonate surfaces with Firmicutes with predominance of lactobacilli, but also bifidobacteria and coagulase-negative staphylococci has also an immediate effect on the neonate innate immunity by activation of its different mechanisms, exemplified by stimulation of production of the tight-junction proteins by gut epithelial cells, resulting in gut impermeability for proteins and other macroparticles but also bacteria and thus preventing bacterial translocation from the gut lumen to lymphatics and bloodstream. In preterm neonates, especially those with low and very low birth weights, the lack of gut tightness often leads to neonatal sepsis [9-11]. Current understanding of gut colonization suggests that the beneficial microbiota, transferred from the adult host to the neonate, is established during early infancy. This early microbial community has lasting effects, extending into adulthood and providing protection against systemic infections. Therefore, both the timing and selection of bacterial strains are critical for the effective artificial colonization of at-risk neonates. The success of this strategy is likely to improve as we identify these and other influential factors in humans and validate the outcomes of artificial colonization through clinical trials [12].

2. Early gut colonization: sources of lactobacilli

There is more and more evidence showing that DNA from *Lactobacillus*, *Bifidobacterium* and other mostly anaerobic bacteria is found in most placenta samples from apparently healthy labors. There are also studies that have found living bacteria in the placenta and amniotic fluid, although their presence in these tissues seems to be related to chorioamnionitis and in utero inflammation [13]. Thus, at the moment it is safer to accept that bacterial DNA is transferred horizontally from mother to fetus across the placenta under normal physiological conditions. Unmethylated CpG oligodeoxynucleotide motifs within bacterial DNA induce immune responses. Toll-like receptor 9 is activated by specific CpG motifs, which then trigger Th-1-type immune reactions. Even though newborns are generally viewed as immunologically underdeveloped, exposure to bacterial DNA might influence and shape their immune system during fetal development earlier than was once believed [14]. Although there are studies indicating a presence of viable bacteria, but not lactobacilli, in amniotic fluid, evidence is not strong enough to accept that this is a physiological mechanism of maternal – fetal transfer of the bacteria [15, 16].

From the moment of birth and in the hours that follow, through interactions with the external environment, such as breathing, breastfeeding, and contact with parents and healthcare providers, the baby's mouth is exposed to numerous microbes. This exposure initiates the process of permanent colonization of the oral cavity during the postpartum period. Within just twenty-four hours, the first microorganisms, known as pioneer species, begin to establish themselves in the infant's mouth. At this early stage, the oral cavity is most commonly colonized by Gram-positive cocci, particularly *Streptococcus*, *Staphylococcus*, and *Lactobacillus sensu lato* [17, 18].

In fact, literature on oral microbiota just after birth is based on studies done in the previous century, and what is even worse, using only qualitative cultures [18]. Despite rather simple and non-accurate methods it was possible to demonstrate that bacterial strains obtained from the mother's vagina and newborn's mouth were identical [19]. It seems that most of the organisms present in the newborn oral cavity represent only planktonic but not resident part of the microbiota and that some species which are extremely rarely found in a healthy vagina at birth may represent hospital pathogens. These bacteria do not form early gut microbiota found in meconium which is very similar to amniotic and vaginal microbiota. Using a modern sequencing approach, He et al. found various *Lactobacillus* operational taxonomic units (OTUs) together with diverse strict anaerobic taxons OTUs but not those of *Staphylococcus* [20].

After birth, breast milk is crucial for establishing bacterial colonization in the infant's gut. During the lactation period, the gut undergoes a progressive and sequential colonization by various microbial communities, transitioning from an aerobic, milk-focused microbiome in infancy to a more complex, adult-like microbiome as the child grows [21]. Human milk is a primary source of bacteria for breastfed infants; between 10^5 and 10^7 bacteria are ingested daily by a baby consuming about 800 mL/day of milk. Notably, viable *Lactobacillus* and *Bifidobacterium* species are common in the milk of women who have not been treated with antibiotics, with their DNA detectable in most milk samples [22]. Species such as *L. gasseri*, *L. salivarius*, *L. reuteri*, *L. fermentum*, and *B. breve* are found to be more frequent among the bacteria isolated from human milk compared to others. The origins of these bacteria in breast milk are still debated, with research exploring various potential pathways, including retrograde backflow and the possibility of a neonatal gut-to-mother mammary gland pathway [23, 24].

3. Further steps in the development of the gut microbiota

The gut microbiota of vaginally delivered neonates resembles the vaginal microbiome, with high populations of *Lactobacillus sensu lato* and *Prevotella* species [25]; other bacteria, such as the members of the Enterobacteriaceae family, that is *Escherichia* or *Klebsiella*, and Gram-positive cocci are also present. Occurrence of both last groups, which are facultative pathogens, poses a danger of the neonatal sepsis, especially in low-birth-weight newborns [11]. As individuals age, the diversity of the gut microbiota expands until it stabilizes into a mature adult composition. This adult microbiota is primarily characterized by dominance of three bacterial phyla and is shaped by factors such as genetics, environment, diet, lifestyle, and gut physiology. By around three years of age, the composition and diversity of a child's gut microbiota closely resemble those of adults [26]. As noted before, *Lactobacillus sensu lato* species found in the gut microbiota, although in relatively small numbers, appear to be crucial for supporting the overall health of the organism.

4. Lactobacilli in the adult gut microbiota

The human gut microbiome primarily consists of three bacterial phyla: Bacteroidetes (*Porphyromonas, Prevotella*), Firmicutes (*Ruminococcus, Clostridium*, and *Eubacterium*), and Actinobacteria (*Bifidobacterium*). While lactobacilli and other facultative anaerobic taxa are present in relatively low quantities within the gut, their regulatory functions in maintaining the gut microbiota are disproportionately significant. This importance has been highlighted by numerous studies focusing on probiotics that contain *Lactobacillus* [27]. Historically one of the mechanisms of the *Lactobacillus* – microbiota interactions involve exerting growth-promoting factors for the other microorganisms which was the first idea on probiotics as proposed by Lilly and Stilwell in 1965 [28]. *Lactobacillus sensu lato* bacteria, after successful early colonization of the gut, become long-life members of the gut microbiota although their numbers as well numbers of bifidobacteria decrease in elderly persons [29]. Again, there are multiple clinical trials with *Lactobacillus*- (and *Bifidobacterium*-) containing probiotics showing that supplementation of the elderly gut microbiota with such bacteria leads to restoration of the gut microbiota composition but may also prevent development of the gut carcinoma [30]. Besides genetic aberrations and non-coding RNA, disruptions in gut microbiota, known as dysbiosis, play a role in the development of colon cancer. Among various interventions, lactic acid bacteria, including *Lactobacillus sensu lato*, have been extensively researched as probiotics for their potential in cancer prevention [6].

After effectively colonizing the gut, lactobacilli engage in direct communication with human epithelial cells. For instance, they trigger transcriptional modifications in the gut epithelium, leading to changes in the expression of numerous genes within just a few hours following oral administration. Most of the human genes involved in down- or up-regulation by lactobacilli are coding for fatty acid metabolism and cellular growth and development [31]. In relation to regulation of the gut immune response, lactobacilli induce anti-inflammatory activities related to NF-kappa B complex [32]. Moreover, animal studies also indicate that presence of *Lactobacillus sensu lato* is associated with the growth of beneficial gut microbiota. The phyla Firmicutes and Bacteroidetes form the core microbiota, accounting for over 90% of the known phylogenetic groups present in the healthy gut of breastfed mice, a composition that closely mirrors the core microbiota in human infants [33]. Lactate produced by microbes strongly induces colonic hyperproliferation in mice, with this effect being most evident after a short-term period of starvation. Additionally, lactate serves as a crucial energy source for small intestinal stem cells, enabling them to maintain their capacity for proliferation and differentiation, as shown in organoid models [34].

Another significant, although not restricted to this group, function of *Lactobacillus* in human gut microbiota which positively influences health is production of vitamin K2 (menaquinone). This property was mentioned already 30 years ago in the fundamental work on its synthesis by Bentley and Meganathan [35]. Later on, it was revealed that yoghurt-fermenting lactobacilli produce menaquinone-7 [36]. However, contemporary data, although confirming the important role of the gut bacteria in vitamin K2 synthesis, do not link it to particular genera. This remains to be elucidated in the light of the actual bacterial taxonomy.

Alterations in gut microbiota composition are observed in various disease conditions, including cardiovascular disease, cancer, type 2 diabetes mellitus, obesity, colitis, asthma, psychiatric and inflammatory disorders, gut-brain axis disturbances, and numerous immune-related issues. Consequently, modulating the gut microbiota through probiotic bacteria, particularly those from the *Lactobacillus* genus, may help address a variety of health challenges. In fact, a range of degenerative diseases, such as obesity, diabetes, cancer, cardiovascular conditions, liver diseases, and inflammatory bowel disease (IBD), have been shown to be preventable by various *Lactobacillus sensu lato* strains from different species [27].

5. The role of Lactobacilli in gut innate and local immunity

Initial interactions with pioneer bacteria may play a crucial role in determining subsequent gut maturation, as well as in shaping metabolic and immune responses, ultimately influencing both short- and long-term health outcomes [37].

Newborns enter the world with an underdeveloped and naive acquired immune system, and several aspects of their innate mucosal immunity are not fully matured. As a result, the innate immune components found in human milk play a critical role in supporting the mucosal barrier of the infant's developing gut. Through breastfeeding, mothers supply their infants with numerous protective agents, some of which have been identified as *Lactobacillus sensu lato* strains in laboratory infection models [38].

As with other aspects of the interactions between lactobacilli and the human organism, most of the information on *Lactobacillus sensu lato* on immunity comes from studies on probiotic lactobacilli on animal models. There are multiple studies showing that probiotic lactobacilli contribute to the host's well-being by enhancing the balance of intestinal microflora and potentially strengthening the host's defense mechanisms. The mechanism by which innate immunity is stimulated in mammals and birds relies on NF-kappaB and p38 MAPK signaling pathways, as demonstrated on mice, cows, pigs and chickens [39-43]. It is obvious that this property is not restricted to probiotic strains of lactobacilli, although it is highly individualized among strains.

It is commonly agreed, especially in food science, that intestinal homeostasis in which local gut immunity plays an important role, can be reestablished by consuming supplements that contain fermented dairy products. These products often contain beneficial microbes, such as probiotics, predominantly from the *Lactobacillus sensu lato* genera. Multiple studies done on different *Lactobacillus sensu lato* strains commonly stress how crucial they are in modulating the gut immune response within both the innate and mucosal immune systems, such as T, B, and NK lymphocytes [44]. It has been shown that a very broad group of the *Lactobacillus* strains representing different species show common characteristics. These include the ability to activate an inflammatory response in macrophages in vitro through mechanisms involving the production of proinflammatory mediators, such as cytokines and reactive oxygen species (ROS), as well as involvement in signaling pathways, including NF-kappaB and TLR2 [45-46].

On the other hand, the regulatory functions of lactobacilli in the gut promote the production of anti-inflammatory cytokines, which has also been found [45] to positively impact chronic gastrointestinal issues. The work of probiotic lactobacilli in modulating the inflammatory response from the gut mucosa has also been demonstrated in patients with inflammatory bowel disease (IBD), highlighting their beneficial role in managing such conditions [47]. It should be noted that it is commonly accepted that IBD is linked to alterations in the composition and metabolism of the intestinal microbiota, known as dysbiosis, which interact with host genetic mutations and changed immune response to gut microbes. However, a definitive causal link between dysbiosis and IBD has not yet been established in humans [48].

There are at least two mechanisms by which *Lactobacillus sensu lato* may ameliorate chronic gut inflammation. Some *Lactobacillus* species possess anti-inflammatory enzymes decomposing ROS, like superoxide dismutase or catalase [49-50]. Observations on the gut microbiota composition in IBD patients and especially in pediatric patients with new-onset disease showed its altered composition with reduction of the bacteria belonging to phylum Firmicutes represented also by *Lactobacillus sensu lato*, support this idea [51-52].

On the other hand, increased populations of Proteobacteria are able to induce gut inflammation [53-54]. These data may also indicate an anti-inflammatory role of the lactobacilli *in vivo* related to their anti-oxidative properties [50]. There is also another explanation offered for the beneficial role of *Lactobacillus sensu lato* probiotics in IBD. Animal studies have shown that the NF-kappaB pathway is inhibited by probiotics, which promotes the activation of immune-modulating regulatory T cells and reduces the presence of proinflammatory effector-memory T cells in the intestinal mucosa [55].

Immune-related disorders have been connected to a decline in overall bacterial diversity, a reduced presence of *Lactobacillus sensu lato* in a healthy gut microbiota, and a rise in potentially harmful bacteria. Additionally, specific bacteria—not including lactobacilli—have been identified as contributors to allergic reactions in both animal studies and human research [56].

Thus, probiotics, especially those containing *Lactobacillus sensu lato* strains, are proposed to offer potential benefits in preventing and treating certain immune-related diseases through the modulation of gut microbiota and the regulation of host mucosal immune function. Nonetheless, their effectiveness has been variable, and additional research is needed to confirm these outcomes [56, 57].

6. Lactobacilli in the vaginal microbiota

Undoubtedly, the most completely documented leading role of the *Lactobacillus sensu lato* bacteria in keeping homeostasis and protecting healthy status occurs in human vagina where lactobacilli act solely as both pioneering and sentinel organisms forming and keeping normal vaginal microbiota composition in the majority of women of childbearing age [58]. Vaginal lactobacilli are a crucial defense against invading pathogens by producing bactericidal compounds and metabolites, as well as by modulating the innate immune responses in the vagina [59]. There are suggestions that not all *Lactobacillus* species residing in the vagina are equal in their protective role and that *L. crispatus* is the most active [60] in this respect. Conversely, a very recent study by Hassan *et al*. indicates that reduced species diversity, combined with an increased abundance of *L. iners, L. gasseri* and *G. vaginalis*, could be linked to female infertility [61].

The substantial need to discriminate among the species *Lactobacillus* in the vagina and roles of the new taxa and their taxonomic positions should be re-evaluated and in the light of the extensive studies on vaginal microbiota presented by Ravel *et al*., that there are at least several types of the microbiota with different predominant species of the *Lactobacillus sensu lato* [62]. The study by Ravel *et al.* documented that not all species of the *Lactobacillus* genera possess ability to colonize the vaginal epithelium, and *L. crispatus, L. iners, L. gasseri* and *L. jensenii* are listed as the most typical members of this community, however with different influences in vaginal health, as shown above. Still, there are very interesting differences in composition of the *Lactobacillus* populations related to a mix of cultural, behavioral, genetic and other as-yet-unknown factors [62]. Most probably these differences are caused by unknown variants in specific receptors in vaginal epithelium as well as by various foods since gut microbiota serve as a reservoir of the vaginal lactobacilli. In fact, the human vaginal microbiota is typically dominated by one or two of the five primary lactobacilli species and is distinguished by a pH level below 4.5 [63].

High populations of lactobacilli are constantly supplemented and supported by these strains which pass from GI tract over perianal, perineal and vulvar skin to the vaginal orifice thanks to unique mechanisms driven by specific genes [64, 65]. It is noteworthy that these mechanisms have been utilized in constructing and implementing vaginal probiotic products for the oral delivery [66-68]. The abundance of the vaginal lactobacilli is also regulated by hormonal interplay since it increases in puberty and further during pregnancy [69] and decreases during menopause. Most probably other factors are also involved in acquisition of the lactobacilli since it appeared recently that they appear before menarche. It was shown that the microbiota of most girls before menarche was predominantly composed of various *Lactobacillus* species, observed during the early to mid-puberty stages [70].

7. Innate immunity in the vagina

Many studies continue to indicate that a vaginal bacterial community dominated by lactobacilli plays a significant role in driving the innate immune response [71]. The health of both pregnant and non-pregnant women is supported by a higher concentration of vaginal lactobacilli. Their resistance to infections is also enhanced under such conditions [72].

When established in the vaginal epithelium, lactobacilli successfully inhibit other bacteria using several well-known factors such as lowering pH, thanks to production of a variety of the organic acids, bacteriocin production and dissolving biofilm structures [73-74]. Low pH of the vaginal secretion is linked to the lactic acid produced by all *Lactobacillus* species, but, in fact, except for lactic acid, there are multiple organic acids produced by lactobacilli in the vagina at lower amounts but possessing much stronger antimicrobial activities [75, 76]. It is generally accepted that lactobacilli produce lactic acid from vaginal glycogen, but this theory is not supported with high quality studies. Remarkably, despite more than a hundred years since the strong association between glycogen, lactobacilli, and lactic acid in the vagina was established, we still have limited knowledge about how lactobacilli metabolize glycogen [77]. Even with the intensive sequencing studies, molecular mechanisms of *Lactobacillus* predominance in vaginal microbiota remain unexplored.

Other elements that play a role in vaginal defense are mannose-binding lectin (MBL), vaginal antimicrobial peptides (AMPs), and immunoglobulins A and G (IgA and IgG).

The presence of protective *Lactobacillus* species in the vagina leads to reduced activation of vaginal epithelial cells. Numerous epidemiological studies have shown that women with a vaginal microbiota predominantly consisting of *L. crispatus* have lower levels of pro-inflammatory cytokines, such as interleukin (IL)-1α and IL-1β, as well as chemokines like IL-8, interferon-gamma inducible protein (IP-10), and macrophage inflammatory protein (MIP)-3α in their cervicovaginal secretions [78].

8. Adaptive immunity

The female genital tract contains most elements of both the adaptive and innate mucosal immune systems. Unlike other mucosal areas, the vagina has a limited amount of local lymphoid tissue. While other mucosal sites mainly produce IgA and IgM, the predominant immunoglobulin secreted in the vagina and cervix is IgG [79]. Vaginal epithelial cells generate various antimicrobial substances and are equipped with membrane-bound Toll-like receptors that detect pathogen-associated molecular patterns. This detection triggers the production of pro-inflammatory cytokines and antigen-specific immune responses. Additionally, the endocervix and vagina can locally produce IgG and IgA antibodies in response to infections [69, 80].

We need to agree with Vaneechoutte [63] that, despite over a century of research, significant debates persist on fundamental issues, with ongoing publication of controversial findings. Given the substantial effects of vaginal microbiology on women's health and pregnancy outcomes, it is crucial to address these controversies. Doing so will enhance our understanding of vaginal microbiology and its relationship with vaginal immunity, leading to improvements in vaginal probiotics and approaches aimed at helping to maintain or restore *Lactobacillus* dominance.

9. Conclusion

The name *Lactobacillus* is broadly recognized and utilized by the medical community, the food and health-related industries, and the general public, and is also featured in national and international regulations. Despite being a traditional term that encompasses multiple genera and numerous species, it remains widely accepted. The bacteria hindered efforts to study the ecology, physiology, evolution, and practical uses of this significant group of organisms. In reality, these bacteria are genetically distinct and exhibit considerable diversity in their metabolism, ecology, and functions. As a result, they can no longer be grouped within the same genus and thus a new taxonomy has been proposed for them, and its adoption will stimulate a new wave of research on relations between taxonomic position and characteristics of the bacterial strains formerly placed in *Lactobacillus* genus. This will undoubtedly yield new insights into the significance of these bacteria for our health, even though many of them will be assigned new names.

Author Contributions: P.B.H. conceptualized and designed the study and data collection forms, performed the initial searches, extracted the data, conducted the literature review on lactobacilli, drafted the initial manuscript, and approved the final manuscript as submitted; M.G and W.P. conducted the literature review, drafted, and edited the manuscript; M.S. conceptualized and designed the study, reviewed and revised the manuscript, and approved the final manuscript as submitted.

Funding: This work was supported by the National Centre for Research and Development, grant no. RPMP.01.02.01-12-0413/17.

Acknowledgments: The authors wish to thank Doctor Piotr Kochan for his help in correcting the style of this manuscript.

Conflicts of Interest: The authors declare no conflict of interest.

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