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Effects of Probiotic-Enriched Yogurt on Lipid and Thyroid Profiles During Pregnancy; A 12-Week Randomized Controlled Trial

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ABSTRACT

Background: Probiotic-containing foods have attracted increasing attention as safe dietary approaches during pregnancy because of their potential role in supporting maternal metabolic balance. However, evidence regarding their influence on thyroid-related indices and lipid changes during gestation remains limited. This randomized controlled study included 200 women, comprising 100 pregnant participants and 100 age-matched non-pregnant controls. The pregnant women group was subdivided into two equal groups according to taking the probiotic dietary. Participants in the intervention group consumed probiotic yogurt daily for 12 weeks. Lipid profiles were assessed before and after the intervention period. Furthermore, the thyroid profile was assessed during the pregnancy period. **Results:** The results of the study revealed fluctuations in lipid and thyroid parameters between the control group and pregnant women during the first and third trimesters. Following the 12-week intervention, women who consumed probiotic yogurt showed significant reductions in triglycerides and LDL-C compared with their earlier values. Nevertheless, the observed biochemical shifts remained within expected physiological limits. Despite these changes, all measured parameters remained within the normal physiological range. **Conclusion:** The investigated probiotic yogurt contains various bioactive compounds beneficial for health. Thyroid and lipid profile screening during pregnancy is highly advised. Probiotic-enriched yogurt may help modulate pregnancy-related lipid changes, but its clinical significance requires further confirmation.

Keywords: Trimesters, dyslipidemia, Microbiota

1. Introduction

Pregnancy is accompanied by coordinated endocrine, metabolic, and microbial adaptations that support fetal growth but may also alter maternal thyroid activity, lipid handling, and intestinal

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microbial balance (Parrettini *et al*., 2020; Qi *et al.,* 2021). These changes are essential for fetal development but can also increase the risk of adverse gestational outcomes and sequelae of prolonged complications for both mother and child (Basak *et al.,* 2022). Rising gestational hormones, particularly estrogen and progesterone, can modify insulin sensitivity and lipid transport, leading to trimester-dependent changes in maternal lipid concentrations (Sharma *et al.,* 2022; Wild and Feingold 2023). This condition, often associated with metabolic syndrome, poses a significant cardiovascular risk factor (O'Kelly *et al.,* 2022). Concurrently, pregnancy impacts thyroid function, with potential consequences for maternal and fetal well-being(Ge *et al.,* 2020). While current guidelines recommend monitoring thyroid hormone levels, particularly thyroid-stimulating hormone (TSH) and adherence to these recommendations remains suboptimal.

Recent research suggests that microbes during pregnancy may influence thyroid hormone levels by modulating iodine uptake, degradation, and enterohepatic cycling. Current clinical guidance emphasizes TSH assessment when thyroid dysfunction is suspected during pregnancy, with FT4 and FT3 testing used as complementary measures when indicated. Furthermore, studies show that individuals with hypothyroidism and hyperthyroidism often have reduced levels of *Lactobacillaceae and Bifidobacteriaceae* (Stagnaro-Green *et al.,* 2011).

The maternal gut microbiome changes progressively across gestation, with alterations in major bacterial groups and a relative reduction in some taxa associated with metabolic and anti-inflammatory functions such as *Roseburia intestinalis* and *Faecalibacterium prausnitzii* (Miko *et al.,* 2022). These alterations have been linked to increased adiposity (Aron-Wisnewsky *et al*., 2021), insulin resistance (Dualib *et al*., 2021), and systemic inflammation (Di Vincenzo *et al*., 2024), potentially contributing to the development of gestational diabetes (Huang *et al*., 2021; Miko *et al*., 2022), cardiovascular disease (Xu *et al*., 2020), and metabolic syndrome (Stagnaro-Green *et al*., 2011).

Probiotics, live microorganisms offering health benefits, have emerged as a potential strategy to modulate the gut microbiota and mitigate these pregnancy-related risks (De Siena *et al*. 2021). Studies suggested that specific probiotic strains could influence lipid metabolism and thyroid function (Roshdy 2024; Shu *et al*., 2024), and might offer a promising avenue for improving maternal health outcomes (Rani *et al*., 2023). The gut microbiome is rich in Verrucomicrobia and maintains stability with Firmicutes, Actinobacteria, and Bacteroidetes as the primary taxa. Changes in T4 levels can lead to adverse effects such as impaired neurocognitive development in infants, increased risks of premature birth, low birth weight, miscarriage, and lower IQ levels in children.

Probiotic supplementation may help individuals at risk of dyslipidemia by modulating gut microbial composition and promoting a balanced microbiota, thereby positively influencing host metabolism. Probiotic recommendations vary across nations, and distinguishing between probiotics in food and those in pill form is essential. The scientific standards for evaluating the health advantages of probiotics are becoming increasingly rigorous (Zoumpopoulou *et al*., 2017). Generally, probiotic usage during pregnancy is safe and well-received by both the mother and the fetus, although adverse effects such as mild gastrointestinal discomfort, systemic infections, and skin issues have been minimally reported (Didari *et al*., 2014). A research study had suggested that certain nutrients serve as a preferred vehicle for probiotics compared to pills (Homayoni Rad *et al*., 2012).

Probiotic intake can potentially prevent infections, reduce cholesterol levels, stimulate vitamin and cytokine production, and inhibit cancer spread (He and Shi, 2017). Probiotics may help maintain or restore microbial homeostasis in both the gastrointestinal and genitourinary tracts (He and Shi 2017; Hill *et al*., 2014). Probiotic supplementation has been widely used during and after antibiotic therapy to support gut microbial balance (Homayoni Rad *et al*., 2012).

Due to conflicting findings, the cholesterol-lowering effects of probiotics have been investigated in controlled laboratory experiments as well as in real-life settings. Given the necessity and significance of innovative therapeutic approaches, it is crucial to manage and enhance patients' lipid profiles in the treatment of dyslipidemia. Accordingly, this trial examined whether daily intake of probiotic-enriched yogurt could influence lipid fractions and thyroid-related biochemical indices in pregnant women.

2. Patients and Methods

2.1. Study Populations

A single-center, parallel-group randomized controlled trial, Outpatient Clinic of Complementary Medicine, Medical and Scientific Center of Excellence, National Research Centre (NRC), Cairo, Egypt.

The study followed a two-arm design with 1:1 allocation. The trial enrolled 200 female participants, who were categorized into (control) and pregnant women (cases) in the first trimester. Pregnant participants were divided into two equal groups: one group received daily probiotic yogurt for 12 weeks, while the comparison group continued routine dietary intake without probiotic yogurt supplementation. The lipid and thyroid profiles were assessed pre- and post- the administration period of probiotic yogurt. The study timeline included a baseline visit during the first trimester (8–12 weeks gestation), a 12-week intervention period, and follow-up visits in the second and third trimesters.

Pregnant women between 18 and 40 years old and confirmed by blood test and ultrasound for gestational age identification at the enrollment, 8–12 weeks was considered as inclusion criteria. Also, Willingness to consume assigned yogurt daily, for 12 weeks and to attend scheduled follow-up visits.

However the non-pregnant control group was enrolled in study if they are age-matched women (18–40 yrs.), not pregnant, and without major chronic illnesses, Who consented to baseline blood tests.

While the exclusion criteria that were applied to the pregnant participants to be excluded from the study if they had known causes of recurrent miscarriages, structural uterine abnormalities affecting the cavity, or Structural uterine abnormalities expected to affect pregnancy outcome. Also if Autoimmune diseases (including rheumatoid arthritis), known thyroid or parathyroid disorders, adrenal disease, hepatic or renal insufficiency. Pre-existing diabetes mellitus or diagnosis of gestational diabetes mellitus at baseline and Known major obstetric complications (e.g., previous preeclampsia) or current use of lipid-lowering drugs statins) or thyroid medications. In addition to the use of antibiotic therapy or probiotic supplements, within four weeks prior to enrollment.

2.2. Data collection

Screening and baseline data collection: At screening, demographic data, obstetric history, medication and supplement use, smoking status, measured weight and height. In addition to baseline blood pressure were documented. Preexisting conditions that triggered exclusion were recorded. A baseline 24-hour dietary recall was collected to document habitual intake.

2.3. Probiotic Yogurt Source

Buffalo milk supplied by Cairo University Faculty of Agriculture was used for yogurt preparation. Its baseline nutritional composition was recorded before processing to ensure product standardization; (g per 100 g) was: protein 4.00, fat 7.00, ash 0.80, total solids 17.00, lactose 5.00, and water 82.00.
The starter culture consisted of an ABT culture supplied by Chr. Hansen (Denmark) containing *Lactobacillus acidophilus*, *Bifidobacterium bifidum*, and *Streptococcus thermophilus*. Exact strain designations and culture lots were recorded on a production log and retained for quality control.
Manufacturing Procedure for Probiotic Yogurt **(standardized protocol to reproduce the product)**

1. **Milk standardization and pre-treatment:** Buffalo milk was standardized to ~17% total solids (if necessary by partial skimming or reconstitution), then homogenized at 200/50 bar to improve texture.
2. **Thermal treatment:** Milk was heated to 85° for 30 minutes (or alternatively 95°C for 5 minutes, depending on plant SOP) to denature whey proteins and reduce microbial load; then rapidly cooled to the inoculation temperature (42°C ±1°C).
3. **Inoculation:** The cooled milk was inoculated with the ABT culture at a rate of 2% (v/v) according to the manufacturer's instructions to achieve an initial inoculum of approximately 10^6–10^7 CFU/g of each probiotic strain. The inoculum volume was documented and culture lot numbers was maintained.
4. **Fermentation:** Fermentation was performed at 42°C until the target pH 4.6–4.5 was achieved (within 4–6 hours). pH and temperature were monitored hourly.

5. **Packaging and cooling:** When the target pH was reached, the yogurt was immediately cooled to 4°C and aseptically packaged in to 150 g single-serve containers.
6. **Storage and distribution:** Finished product was stored at 4°C and delivered to participants twice per weekly insulated coolers with ice packs. Participants were instructed to refrigerate the yogurt at 4°C and consume one 150 g container per day for 12 consecutive weeks.

2.4. Target probiotic dose and microbiological QC

To ensure adequate probiotic delivery, the finished yogurt was prepared to contain not less than 10^8 viable cells per gram at distribution, corresponding to approximately 1.5 × 10^10 CFU per 150 g serving (i.e., ≥1.5 × 10^10 CFU per 150 g serving) for combined probiotic strains; actual viable counts were determined during production and periodically during shelf-life (days 0, 7, 14, and 21) by plate counts. For bacterial enumeration :serial dilutions were plated on de Man, Rogosa and Sharpe (MRS) agar for lactobacilli and on selective TOS-propionate agar or MRS supplemented as required for bifidobacteria; plates were incubated anaerobically at 37°C for 48–72 hours and colonies were counted. Microbial identification for quality control was performed by colony morphology and Gram staining; representative colonies were periodically confirmed by PCR or MALDI-TOF per lab capability.

2.5. Intervention adherence and participant monitoring

Participants in the probiotic were instructed to consume one 150 g container daily and to return the empty containers at weekly clinic visits. Adherence was assessed by (1) participants daily consumption logs, (2) returned container counts, and (3) twice-weekly telephone reminders. Adherence was calculated as the proportion of prescribed servings consumed; per-protocol analyses included participants who achieved ≥80% adherence. Missed doses and reasons were recorded. Safety monitoring, all Participants were advised to report any adverse events (AEs) such as gastrointestinal symptoms, allergic reactions, or infections. A routine obstetric care was continued and any pregnancy-related complications were recorded.

2.6. Biochemical assessment

Following an overnight fast of 8–12 hours, a 3-ml venous blood sample was collected in the morning and allowed to clot in a plain tube. After centrifugation at 5000 rpm for 10 minutes, the serum was separated and stored at -20 °C until analysis. The lowest limit of detection for the assay was 2.0 IU/ml. Total cholesterol and triglycerides (TG) were enzymatically quantified using a colorimetric method using a Biosystems BTS-302 photometer (Egypt)(Fossati and Medicci 1987) and a commercially available bio-diagnostic kit. High-density lipoprotein-cholesterol (HDL-C) was measured using the precipitation end-point method. Following precipitation, the supernatant was collected and analyzed using the same procedure as for total cholesterol. Low-density lipoprotein-cholesterol (LDL-C) levels were calculated using the Friedewald equation: LDL-C = total cholesterol – HDL-C – (TG/5)(Wt 1972). Furthermore, serum samples were investigated in all three trimesters for assessment of TSH, free T3, and free T4 by ELISA kit (using the quantitative sandwich immunoassay technique).

2.7. Statistical analysis

Numerical variables were expressed as mean ± SD, whereas categorical variables were summarized using frequencies and percentages. Statistical analyses were performed using Minitab 17.1.0.0 for Windows (Minitab Inc., 2013, Pennsylvania, USA). Data distribution was checked for normality before selecting the appropriate comparative tests. The independent t-test was employed to compare two numerical datasets, and the one-way ANOVA test was used for comparing more than two means. The paired *t*-test was used to compare means before and after treatment. All tests were two-tailed, with *p-v*alues < 0.05 was considering statistical significance.

3. Results

3.1. Primary Outcome

The pregnant and non-pregnant groups showed no relevant baseline difference in age or body mass index., as illustrated in Table 1. However, pregnant women showed alterations in thyroid hormone levels, showing elevated free T3 and T4 levels and reduced TSH levels, with statistical

significance (P<0.05 for all). All lipid parameters (total cholesterol, triglycerides, and LDL cholesterol) were significantly increased with the progression of the pregnancy, whereas HDL cholesterol levels were significantly lower. (P <0.05, for all).

Table 1: A descriptive comparison between the pregnant women group and control group according to the characteristics of participants

Factors	Control group (n=100) Mean ± SD	Pregnant women (n=100) Mean ± SD	*P* value
Age (Year)	28.40±5.30	27.10±5.10	0.07
BMI (Kgm/m^2)	25.50±4.10	25.60±4.20	0.43
T3 (nmol/ml)	1.49±0.48	1.79±0.31	**<0.001**
T4 (nmol/ml)	90.03±12.66	94.98±17.87	**0.02**
TSH (nmol/ml)	1.78±0.44	1.33±0.57	**<0.001**
Cholesterol (mg/dl)	126.01±15.79	119.34±13.87	**0.001**
Triglyceride (mg/dl)	157.98±35.99	125.14±14.37	**<0.001**
HDL(mg/dl)	45.02±8.89	49.93±18.89	**0.01**
LDL(mg/dl)	50.01±18.11	44.22±20.99	**0.03**
VLDL(mg/dl)	32.06±7.32	25.17±3.13	**<0.001**

The data is presented as mean ± standard deviation, n: number, SD: standard deviation, the test of significance: one-way ANOVA test with Tukey methods for multiple comparisons, P < 0.05 considered significant, and the bold line represents the significant data.

At the second-trimester assessment, the two pregnant subgroups showed comparable thyroid and lipid profiles, with no statistically significant differences between them for the measured parameters. Both pregnant groups exhibited expected pregnancy-related changes in thyroid hormones, characterized by higher FT3 and FT4 levels and lower TSH levels compared with the non-pregnant control group (Table 2).

Table 2: A comparison between the pregnant women groups and control group according to the thyroid and lipid profiles in the second trimester

Factors	Control group (n=100) Mean ± SD	Pregnant women with no probiotic use (n=50) Mean ± SD	Pregnant women Before probiotic use (n=50) Mean ± SD	p^1	p^2	*P3*
T3 (nmol/ml)	1.64±0.39	2.11±0.75	2.19±0.65	**<0.001**	**<0.001**	0.76
T4 (nmol/ml)	91.13±11.42	103.01±12.98	102.16±11.88	**<0.001**	**<0.001**	0.93
TSH (nmol/ml)	1.88±0.56	1.09±0.23	1.01±0.21	**<0.001**	**<0.001**	0.61
Cholesterol (mg/dl)	127.09±14.99	146.31±26.14	145.91±25.94	**<0.001**	**<0.001**	0.95
Triglyceride (mg/dl)	156.79±36.86	148.01±31.08	147.91±32.01	0.19		
HDL (mg/dl)	44.12±7.99	44.99±8.73	43.87±7.81	0.76		
LDL (mg/dl)	51.21±19.31	69.97±21.48	68.89±20.98	**<0.001**	**<0.001**	0.96
VLDL (mg/dl)	31.96±8.22	30.08±5.98	31.18±5.87	0.31		

The data is presented as mean ± standard deviation, n: number, SD: standard deviation, the test of significance: one-way ANOVA test with Tukey methods for multiple comparisons, p<0.05 considered significant, and Bold values indicate statistically significant differences. $p^1$is the p value for comparing between the control group and the pregnant women (with no probiotic use) group, P^2 is the p value for comparing between the control group and the pregnant women (with probiotic use) group, and P^3 is the p value for comparing between the pregnant group (with no probiotic use) and the pregnant women (with probiotic use).

Moreover, after 12 weeks of the consumption of the prepared probiotic, Table 3 shows nearly normalized thyroid hormone profile parameters in the probiotic group compared with the recorded parameters of the non-probiotic group, including a reduction in both free T3 as well as T4, and even a decline of TSH levels compared with the assessed values of the control group, $P<0.001$.

The data is presented as mean ± standard deviation (Mean ± SD), n: number, SD: standard deviation, Statistical significance was assessed using one-way ANOVA followed by Tukey's multiple comparison test. $P< 0.05$ considered significant, and the bold line represents the significant data.

Table 3: A comparison between the pregnant women groups and control group according to the thyroid and lipid profile in the third trimester

Factors	Control group (n=100) Mean ± SD	Pregnant women (With no probiotic use) (n=50) Mean ± SD	Pregnant women (After probiotic use) (n=50) Mean ± SD	P^1	P^2	P^3
T3 (nmol/ml)	1.64±0.39	2.05±0.71	1.77±0.32	**<0.001**	0.16	**0.01**
T4 (nmol/ml)	91.13±11.42	112.01±11.99	94.98±17.87	**<0.001**	0.22	**<0.001**
TSH (nmol/ml)	1.88±0.56	2.01±0.56	1.33±0.57	0.37	**<0.001**	**<0.001**
Cholesterol (mg/dl)	127.09±14.99	152.41±31.49	119.34±13.87	**<0.001**	0.06	**<0.001**
Triglyceride (mg/dl)	156.79±36.86	199.16±48.80	125.14±14.37	**<0.001**	**<0.001**	**<0.001**
HDL (mg/dl)	44.12±7.99	45.00±7.98	49.93±18.89	0.91	**0.01**	0.08
LDL (mg/dl)	51.21±19.31	64.97±36.37	44.22±20.99	**0.004**	0.23	**<0.001**
VLDL (mg/dl)	31.96±8.22	40.61±9.94	25.17±3.13	**<0.001**	**<0.001**	**<0.001**

The data is presented as mean ± standard deviation, n: number, SD: standard deviation, the test of significance: one-way ANOVA test with Tukey methods for multiple comparisons, $P<0.05$ considered significant, and Bold values indicate statistically significant differences.(P^1 is the p value for comparing between the control group and the pregnant women (with no probiotic use) group, P^2 is the p value for comparing between the control group and the pregnant women (with probiotic use) group, and P^3 is the p value for comparing between the pregnant group (with no probiotic use) and the pregnant women (with probiotic use).

For lipid-related outcomes, the probiotic group displayed a more favorable pattern, with lower total cholesterol, LDL-C, triglycerides, and VLDL-C values than the non-probiotic pregnant group, $\boldsymbol{P<}$ 0.001. Additionally, the probiotic group exhibited notably elevated HDL levels compared with the control and non-probiotic groups, $\boldsymbol{P<}$0.001, as shown in Figure 1.

Comparison of the probiotic and non-probiotic pregnant groups showed expected pregnancy-related changes in thyroid indices, reflecting physiological thyroid adaptation during pregnancy. However, the probiotic group demonstrates a trend toward normalization in the third trimester, with T3 and T4 levels declining and TSH levels further decreasing compared to the non-probiotic group. These findings suggest that probiotic supplementation may modulate thyroid hormone homeostasis during pregnancy. Meanwhile, the results revealed fluctuations in lipid profiles, with both groups generally experiencing an increase in total cholesterol, triglycerides, LDL, and VLDL from the first to the second trimester, followed by a decrease in the third trimester after 12 weeks of probiotic supplementation in the probiotic group as shown in Figure 2.

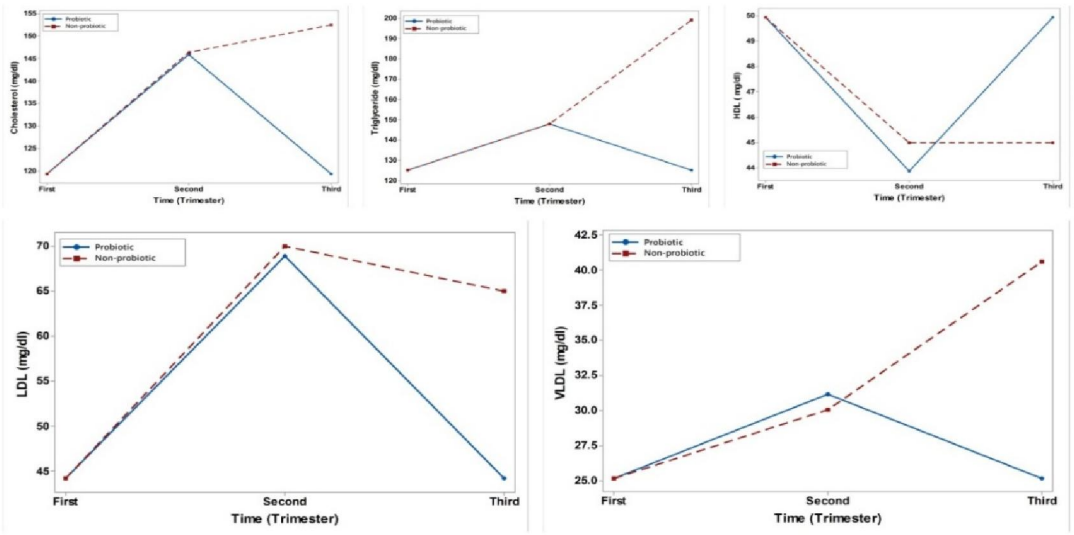

Fig. 1: Comparison of 1ipid parameters (total cholesterol, triglycerides, and LDL cholesterol) between the probiotic and non-probiotic group during the trimester.

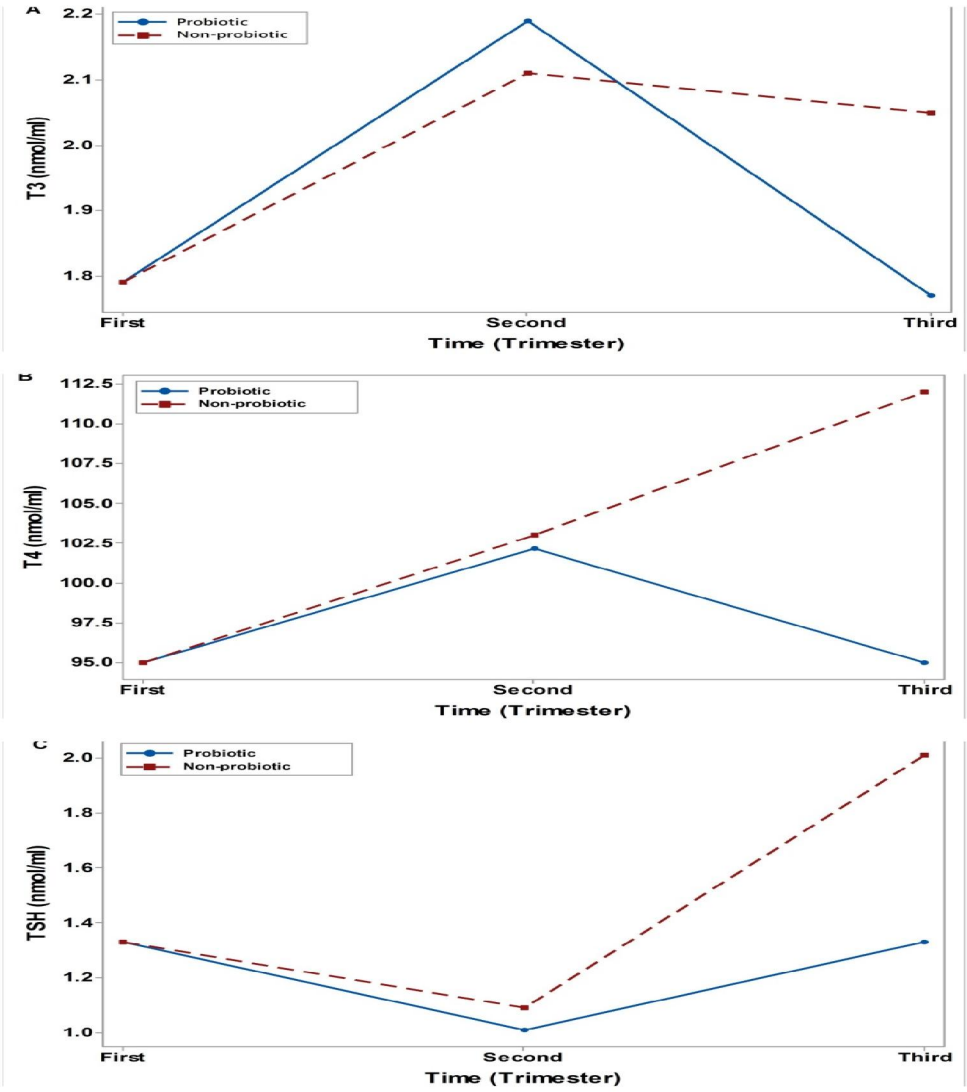

Fig. 2: Comparison of Thyroid parameters (TSH, Free T3, and Free T4) between the probiotic and non-probiotic group during the trimester.

4. Discussion

Yogurt is commonly considered a suitable food during pregnancy because it provides essential nutrients, particularly calcium and protein, in addition to its potential role as a carrier for probiotic cultures (Chakma *et al.,* 2025). Although larger randomized controlled trials are needed to strengthen the evidence, probiotic yogurt may represent a beneficial dietary component during pregnancy. It has been demonstrated that probiotic yogurt confers several health benefits during pregnancy, primarily through improved metabolic outcomes, immune function enhancement, and gut microbiota modulation. By enhancing insulin sensitivity and glucose metabolism, probiotic yogurt containing *Lactobacillus* and *Bifidobacterium* strains may reduce the risk of gestational diabetes mellitus (GDM), according to clinical research (Asemi *et al*., 2013; Fanelli *et al*., 2022). Additionally, there is evidence of a protective role against pre-eclampsia, most likely due to blood pressure-modulating and anti-inflammatory effects (Brantsæter *et al*., 2011). Furthermore, probiotics may reduce the risk of group B Streptococcus colonization and bacterial vaginosis in mothers, which could prevent complications such as preterm birth (Othman *et al*., 2007). Probiotic yogurt consumption by mothers has been associated with a lower risk of atopic dermatitis and other allergic disorders in their newborn children (Kalliomäki *et al*., 2001).

In this randomized trial, the daily ingestion of probiotic-enriched yogurt during pregnancy resulted in modest but statistically significant meaning improvements in several lipid fractions and with attenuation of pregnancy-associated thyroid perturbations compared with pregnant controls who did not receive the product. Specifically, participants consuming the enriched yogurt exhibited greater reductions in triglycerides and LDL-cholesterol and a trend toward normalization of thyroid hormone indices by the third trimester relative to the non-probiotic group. These changes were observed within physiological pregnancy ranges but suggest that dietary probiotic supplementation can favorably influence maternal metabolism during gestation.

Metabolic functioning has been noticed to be altered during pregnancy, resulting in changes in the lipid profile to accommodate the developing fetus. Altered lipid and thyroid indices during pregnancy have been linked in previous studies to complications such as gestational diabetes, hypertensive disorders of pregnancy, and abnormal fetal growth (Wild and Feingold, 2000).

The *Weissella* genus encompasses a group of lactic acid bacteria (LAB) that have garnered heightened interest over the past decade, primarily for their biotechnological and probiotic capabilities(Herrera 2002). Furthermore, the scientific standards for evaluating the health advantages of probiotics are progressively becoming more rigorous (Butte *et al*., 2000). In general, pregnant women tolerate the consumption of probiotics well, and it is considered safe.

Recent studies have shown potential health benefits of probiotic administration, including in managing metabolic diseases (Asemi *et al*., 2013; Fanelli *et al*., 2022). Additionally, research indicates that certain beneficial gut microbiota species play a role in modulating adiposity (Di Gioia *et al*., 2014; Gøbel *et al*., 2012).

Studies by Pusukuru *et al.* (2016); Vastrad and Vastrad (2025) suggested that the observed increase in lipid parameters could be attributed to elevated insulin levels or enhanced insulin sensitivity during pregnancy, leading to increased activity of adipose tissue lipoprotein lipase (LPL), which contributes to both lipogenesis and hyperlipidemia. Moreover, the increased level of triglyceride-rich VLDL particles is more dependent on an increased rate of synthesis caused by estrogen than on a decrease in the rate of removal. Consequently, the dysregulation of LPL resulted in a dyslipidemic lipid profile.

The significant anatomical and physiological changes begin shortly after conception and continue throughout pregnancy, including the delivery phase. During this time, maternal physiology is heavily influenced by placental hormones, particularly during the final trimester. These hormonal fluctuations have a notable impact on glucose and lipid metabolism, ensuring that the fetus receives sufficient nutrients for development (Kazma *et al*., 2020). The considerable increase in triglyceride levels is thought to be attributed to two main factors: increased hepatic lipase activity, which promotes the synthesis of triglycerides in the liver, and decreased activity of lipoprotein lipase (LPL), leading to reduced breakdown of adipose tissue (Chatterjee and Sparks 2011).

Yadav *et al*. (2023) were consistent with our results documenting a significant rise in TSH levels among pregnant women during the third trimester compared to the second trimester. Furthermore, a notable positive correlation was established between TSH levels and lipid profile.

Our results were also in parallel with other study (Wang *et al*., 2024), concerning the pregnant women with subclinical hypothyroidism group, who were associated with dyslipidemia alteration. Moreover, Wang *et al*. (2018) had confirmed the aforementioned outcome results via a retrospective survey in Beijing: the increase of TC, TG, and LDL-C and decreased levels of HDL-C in early, mid, and late pregnancy are related to the maternal disturbance of fat metabolism. Nevertheless, there is currently no consensus regarding the definition of normal ranges for maternal fat levels during pregnancy.

Several studies have suggested the normal reference values for serum TC, TG, and LDL-C in early and middle pregnancy be less than the 95th percentile, while HDL-C should be greater than the 5th percentile, i.e., early in pregnancy, TC <5.64 mmol/L, TG <1.95 mmol/L, HDL-C >1.23 mmol/L, LDL-C <3.27 mmol/L, and at mid-gestation, TC <7.50 mmol/L, TG <2.56 mmol/L, HDL-C >1.41 mmol/L, LDL-C <4.83 mmol/L. Large amounts of fat outside the range during early and mid-pregnancy have also been associated with an increased risk of adverse pregnancy outcomes.

Keeping on, Kumari *et al*. (2018) had performed an investigation involving analyzing the association between lipid profile and thyroid hormones at both 16 and 32 weeks of gestation. They observed significant increases in T3, T4, and TSH among healthy pregnant women. A significant positive correlation was seen between TSH and triglycerides and also between T3 and cholesterol.

Mousa *et al*. (2019) concluded that the range of normal thyroid function during pregnancy differed ethnically according to geographic regions and trimester of pregnancy; therefore, a special reference must be established for the third trimester and the population of the geographical regions

During pregnancy, estrogen-induced increases in thyroxine-binding globulin, together with changes in renal iodine clearance, can influence circulating thyroid hormone patterns., leading to reduced levels of triiodothyronine (T3) and tetraiodothyronine (T4). This reduction in T3 and T4 prompts the pituitary gland to release more thyroid-stimulating hormone (TSH). Moreover, heightened glomerular filtration in pregnancy enhances the clearance of iodine from the kidneys, resulting in a decrease in circulating inorganic iodine levels. This loss prompts a compensatory increase in thyroidal iodine clearance and uptake. Thus, the combined effects of estrogen, TBG, and renal clearance contribute to the hypersecretion of TSH by the thyroid gland (Brown *et al*., 2023).

The enriched yogurt administered to the pregnant probiotic group demonstrated a significant decrease in triglyceride and LDL-cholesterol levels compared to the pregnant control group not receiving the prepared probiotics, particularly among pregnant individuals at risk of dyslipidemia. This might be explained by the beneficial effects of probiotic administration, which increase adipose tissue lipolysis. This process may trigger the secretion of anorexigenic peptides like glucagon-like peptide-1 (GLP-1) and peptide YY (PYY), consequently improving glucose tolerance and promoting increased energy expenditure. Moreover, probiotic intervention was observed to downregulate the expression of key molecules associated with intestinal microflora inflammation and bacterial translocation, namely nucleotide-binding oligomerization domain-containing protein 1 (NOD-1) and CD-14 A (Asemi *et al*., 2013; Fanelli *et al*., 2022).

A meta-analysis revealed that administering Lactobacillus acidophilus alone, a combination of Lactobacillus acidophilus and Bifidobacterium lactis, as well as Lactobacillus plantarum for durations ranging from 3 to 12 weeks resulted in reductions in both total and low-density lipoprotein (LDL) cholesterol levels when compared to a placebo (Zikou *et al*., 2023). Conversely, *Lactobacillus helveticus* and *Enterococcus faecium* showed no significant impact on cholesterol levels according to Cho and Kim (2015). However, consumption of yogurt normalized lipid parameters.

Thus, probiotic supplementation has resulted in attenuating the aforementioned risks through complementary mechanisms. One possible explanation is that some probiotic strains possess bile salt hydrolase activity, which may increase bile acid loss and reduce intestinal cholesterol uptake, thereby lowering circulating LDL and triglycerides (Babadi *et al*., 2019; Sun *et al*., 2024). In parallel, microbial interactions promote short-chain fatty acid (SCFA) generation, which improves insulin sensitivity and modulates lipogenesis via gut hormone signaling (Fanelli *et al*., 2022; Lin *et al.,* 2026). Probiotics also strengthen the intestinal barrier and downregulate inflammatory signaling, reducing cytokine-driven hepatic lipid synthesis (Cao *et al*., 2024). Importantly, emerging evidence links probiotic intake with improved thyroid function, providing additional regulation of lipid metabolism through the thyroid–lipid axis (Brown *et al*., 2023; Nishiyama *et al.,* 2016). Collectively, these mechanisms provide a biologically plausible explanation for the observed improvements in lipid and

thyroid profiles in our cohort, underscoring the clinical potential of probiotic yogurt to support maternal metabolic health and reduce pregnancy-related complications.

Another mechanism that may contribute to probiotic activity is the ability of selected strains to adhere to the intestinal mucosa and persist within biofilm-like communities. When organized into biofilm communities, probiotic bacteria demonstrate improved survival under gastric acidity and bile exposure, as well as greater persistence in the gut compared with their free-living counterparts (Gao *et al*., 2025). Structural evaluations of probiotic biofilms have revealed heterogeneous architectures, which provide enhanced stability and resilience during colonization (Liu *et al*., 2024). This property has positioned biofilm-state probiotics as a promising next-generation alternative to conventional probiotic formulations due to their superior stress tolerance and functional durability (Yao *et al*., 2024). The interaction with intestinal surfaces is facilitated by adhesion molecules such as exopolysaccharides, pili, and specific surface proteins, which strengthen mucosal attachment and promote modulation of host immune responses (Gorreja and Walker, 2022). Supporting this, recent ex vivo studies have directly shown that selected probiotic strains maintain a strong capacity to adhere to human intestinal mucosa, confirming their role in sustaining long-term gut colonization and barrier support (Phùng *et al*., 2024). Taken together, these findings suggest that biofilm formation and mucosal adhesion may represent additional mechanisms by which probiotic yogurt contributes to the prevention of risk of hyperlipidemia and thyroid dysregulation during pregnancy.

Although our findings demonstrated favorable modulation of lipid and thyroid profiles with probiotic yogurt intake, studies have reported contrasting outcomes. For instance, Okesene-Gafa *et al*. (2020) observed only a minimal decline in triglycerides and total cholesterol in pregnant women receiving probiotics, while Gadelha and Bezerra (2019) showed no significant reduction in LDL-C following supplementation with *Lactobacillus salivarius*. Similarly, Cho and Kim (2015) noted that *Lactobacillus helveticus* and *Enterococcus faecium* did not exert meaningful effects on cholesterol levels, suggesting that probiotic efficacy may vary according to strain and study conditions. More recently, a meta-analysis of eight randomized controlled trials concluded that probiotics and prebiotics generally did not significantly alter TSH, FT3, or FT4 levels, although a slight reduction in thyroid receptor antibodies was reported in patients with Graves' disease (Shu *et al*.,2024). Furthermore, Ramezani *et al*. (2023) demonstrated in a randomized, double-blind, placebo-controlled clinical trial of hypothyroid patients that a 10-week symbiotic regimen did not significantly affect TSH levels compared with placebo, despite modest changes in FT4. Together, these findings highlight that probiotic effects on lipid and thyroid function are inconsistent and appear to depend on factors such as strain specificity, dosage, host physiology, and baseline metabolic or endocrine status.

Moreover, Babadi *et al.* (2019) demonstrated in their study that supplementation with probiotic capsules containing *Lactobacillus acidophilus, Lactobacillus casei, Bifidobacterium bifidum,* and *Lactobacillus fermentum* resulted in a decrease in triglyceride (TG) levels, very low-density lipoprotein (VLDL) cholesterol, and the total/high-density lipoprotein (HDL) cholesterol ratio while elevating HDL cholesterol levels.

The potential mechanisms at play may synergistically enhance the lipid profile. Various genera producing bile salt hydrolases could facilitate the de-conjugation of bile salts, resulting in decreased absorption and enterohepatic recirculation, alongside heightened excretion. This process also increases the demand for cholesterol to synthesize new molecules, thereby offsetting losses. Moreover, de-conjugation modifies the solubilization capacity of cholesterol, consequently reducing its absorption. Additionally, cholesterol incorporation into cell membranes during microbial growth and the inhibition of hepatic synthesis of cholesterol and fatty acids via short-chain fatty acid production are described in the literature as contributing factors (Babadi *et al*., 2019).

These findings were corroborated by those of Babadi *et al.* (2019) and Ismail *et al*. (2020), who reported that probiotic strain and starter culture viable counts increased over storage times. Whereas Assem *et al*. (2019) who discovered that the acidity rose with storage time, corroborated these findings. Furthermore, El-Shafei *et al*. (2018) reported that all samples' pH values dropped over the course of storage. In conclusion, the effects of gut microbiota on pregnancy are comparable to those of metabolic syndrome. That being said, not every study produced the same outcomes. An imbalance in the gut flora during pregnancy might cause increased inflammation, an increase in energy content, and a decrease in insulin sensitivity, similar to metabolic dysfunction (DiGiulio *et al*., 2015). Elevated insulin resistance has the potential to elevate levels of glucose and free fatty acids (FAAs),

as well as impact fat oxidation during the latter stages of pregnancy. Dysbiosis of the gut microbiota in obese pregnant women can cause metabolic abnormalities and gestational diabetes mellitus (GDM) (Homayoni Rad *et al*., 2012). Therefore, to reduce the risk of illness and mortality in the mother and baby, dysbiosis could be prevented and perhaps treated(Gomez-Arango *et al*., 2016).

It is crucial to acknowledge that probiotic efficacy is not solely dependent on the ingestion of probiotics themselves; rather, factors influencing their bioavailability should also be taken into consideration, future research should explore additional variables that could impact probiotic absorption and consequently enhance the overall health of the gut microbiota. The variability in findings across different studies may stem from various factors, such as the specific strains and combinations used, the dosages administered, the duration of the studies, and other extraneous variables. This highlights the importance of thoroughly evaluating these parameters to elucidate the diverse outcomes observed in probiotic research.

Despite the probiotic-yogurt intake demonstrated favorable effects on maternal lipid parameters, its impact should not be equated with pharmacological agents such as statins, which are associated with robust and predictable reductions in LDL cholesterol but are generally contraindicated during pregnancy due to teratogenic concerns (Sharma *et al*., 2022; O'Kelly *et al*., 2022). Instead, probiotic yogurt represents a safe, dietary-based adjunct that may contribute to modest improvements in lipid metabolism and thyroid regulation (Fanelli *et al*., 2022; Shu *et al*., 2024).

The current study had certain limitations. Firstly, we did not evaluate the impact of probiotic administration on fecal short-chain fatty acids (SCFA). Secondly, the follow-up duration of our study was relatively short, which means that some changes in lipid concentrations that were initially non-significant may have become statistically significant with a longer follow-up period. However, these benefits may have both direct and indirect impacts on metabolic health and overall well-being over a prolonged duration. The variations in findings across studies could be attributed to factors such as the specific strains and combinations used, dosage levels, study durations, and other external variables.

5. Conclusion

Daily intake of locally prepared ABT-culture probiotic yogurt for 12 weeks was associated with favorable shifts in selected maternal lipid parameters and thyroid-related indices during pregnancy. However, because the measured values largely remained within physiological pregnancy ranges, these findings should be interpreted as a modest dietary modulatory effect rather than evidence of a direct therapeutic lipid-lowering action. Probiotic-enriched yogurt may be considered a safe and feasible dietary adjunct during pregnancy, but larger double-blind trials including women with clinically abnormal lipid or thyroid profiles and relevant maternal–neonatal outcomes are required to clarify its clinical value.

Limitations

The current study has some limitations. First, fecal short-chain fatty acids (SCFAs), gut microbiota composition, inflammatory markers, and other mechanistic biomarkers were not assessed; therefore, the biological pathways underlying the observed changes could not be directly confirmed. Second, the follow-up period was relatively short, and longer monitoring may be required to determine whether the observed lipid and thyroid-related changes are sustained throughout pregnancy and after delivery. Third, because of ethical considerations in pregnant women, it was not appropriate to intentionally enroll participants with clinically abnormal lipid or thyroid profiles and withhold standard medical care during the study period in order to evaluate probiotic yogurt as the only intervention. Accordingly, this study was not designed to assess the therapeutic effect of probiotic yogurt in pregnant women with established dyslipidemia or thyroid dysfunction. Instead, the findings should be interpreted within a physiological context, reflecting a modest dietary modulatory effect on pregnancy-related lipid and thyroid changes, particularly because the measured parameters largely remained within expected pregnancy ranges. Finally, larger double-blind, placebo-controlled trials including clinically relevant maternal and neonatal outcomes are required to clarify the clinical significance of probiotic-enriched yogurt during pregnancy.

Declarations

Conflicts of interest

The authors declare there is no conflicts of interest.

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Ethical considerations

The researches informed the participants about the aim of the study. Written illustrative consent forms were signed by all participants. This study was performed according to the ethical rules for medical research involving human participants of the Declaration of Helsinki (1964). The ethical approval of the study was obtained from the medical ethical committee of the National Research Center, subject No. 0943

Consent for publication

The authors approved for publication

Data availability

All data is provided within the manuscript file.

Competing interests

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