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***Ph.D.* thesis**
Promocijas darbs

THE STUDY OF MILK POLAR LIPIDS COMPOSITION AND FUNCTIONALITY
PIENA POLĀRO LIPĪDU SASTĀVA UN FUNKCIONALITĀTES IZPĒTE

Thesis submitted for the degree of Doctor of Science (*Ph.D.*) in Food and Beverage Technologies

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ANNOTATION

Polar lipids of the milk fat globule membrane remain an important area of research, including the characterisation of composition, the development and improvement of analytical methods for determination, the investigation of technological properties, and the assessment of nutritional value, as scientific evidence on the composition and nutritional relevance of milk-derived polar lipids remains limited. Buttermilk is a dairy by-product containing milk fat globule membrane-associated polar lipids that are significant in human nutrition. These compounds are of interest because they may contribute to the nutritional and technological properties of dairy products and may be relevant to lipid metabolism in humans. Therefore, it is important to characterise the composition of polar lipids in milk and dairy products, evaluate how processing and season influence their distribution, and assess whether habitual dietary exposure to milk polar lipids is associated with the plasma lipid profile in perimenopausal women.

The Ph.D. thesis evaluates the functional properties of buttermilk by integrating food composition analysis, dietary assessment, and a clinical nutrition approach. The study includes composition analysis of raw milk and its processed products from the same milk batch in two seasons, evaluation of a commercially available cultured buttermilk, and assessment of the plasma lipid profile in perimenopausal women with moderately elevated low-density lipoprotein cholesterol concentrations. Special attention is given to determining the polar lipids present in milk and dairy products and to estimating the quantity of polar lipids from dairy products in the habitual diet, based on individual dairy product intake and experimentally determined product composition.

The hypothesis of the Ph.D. thesis: polar lipid concentration in milk and dairy products varies seasonally and with processing and may influence the plasma lipid profile in perimenopausal women with moderately elevated low-density lipoprotein cholesterol concentrations.

Theses:

1. the composition and concentration of polar lipids in milk and dairy products vary within seasons and with milk processing;
2. cultured buttermilk may contribute to habitual dietary exposure to milk polar lipids;
3. habitual dietary exposure to milk polar lipids requires integrating individual dietary intake data with product-specific polar lipid composition;
4. estimated habitual milk polar lipid exposure is associated with plasma lipid profile in perimenopausal women;
5. the effect of cultured buttermilk consumption on plasma lipid profile is associated with product composition, participants' baseline lipid profile, and habitual diet characteristics.

The aim of the Ph.D. thesis is to evaluate the composition and distribution of polar lipids in milk and dairy products and to assess the relationship between dietary exposure to polar lipids from cultured buttermilk and the plasma lipid profile in perimenopausal women.

The **research objectives** are set to achieve the aim of the study:

1. to analyse the chemical composition and polar lipid profile of milk and dairy products obtained during milk processing;
2. to evaluate seasonal differences in the concentration and relative distribution of the main milk polar lipid classes in milk and in the processed products studied;
3. to conduct a 28-day randomised controlled dietary intervention study evaluating the effect of daily consumption of cultured buttermilk on the plasma lipid profile in perimenopausal women with moderately elevated low-density lipoprotein cholesterol concentrations;

4. to assess dietary intake and estimate habitual milk polar lipid exposure by combining individual dietary intake data with season-specific dairy product composition;
5. to analyse associations between estimated habitual milk polar lipid exposure and plasma lipid profile of participants.

The Ph.D. thesis is structured into three chapters.

1. **Literature review.** The chapter summarises the scientific literature on milk lipids, milk fat globule membrane components and polar lipid classes in milk and dairy products. It describes the composition and functional properties of buttermilk, the technological relevance of milk polar lipids and their possible role in human nutrition. It focuses on phosphatidylcholine, phosphatidylethanolamine, and sphingomyelin, as well as evidence linking milk polar lipids to intestinal cholesterol absorption, plasma lipid profile, and cardiometabolic health in perimenopausal women.
2. **Materials and methods.** The chapter describes the overall research design and methodological approaches. It includes raw milk sampling and processing; chemical composition analysis of milk and dairy products; and quantification of polar lipids by LC-MS. The chapter also describes the selection and characterisation of cultured buttermilk for the intervention, the design of the clinical trial, ethical considerations, participant recruitment, inclusion and exclusion criteria, randomisation, intervention dose and duration, blood sampling, biochemical analyses, dietary assessment, estimation of habitual milk polar lipid exposure and statistical analysis.
3. **Results and discussion.** The chapter presents and interprets the thesis's main findings. First, the chemical composition of milk and dairy products is analysed in relation to processing and season. Second, the total polar lipid concentration and the main polar lipid classes in milk and dairy products are evaluated. Third, the selected cultured buttermilk is characterised as the intervention product. The clinical and dietary results are analysed, including participant recruitment, baseline characteristics, dietary habits, estimated habitual milk polar lipid exposure, changes in the plasma lipid profile after the 28-day intervention, and associations between estimated milk polar lipid exposure and the lipid profile.

The Ph.D. thesis was financially supported by the programme “Strengthening Research Capacity in the Latvia University of Life Sciences and Technologies”, project “The study of buttermilk polar lipids”, and ERDF project No. 1.1.1.8/1/24/I/002 “LBTU Doctoral Students Support and Development Initiative”.

The Ph.D. thesis is written in English and comprises 121 pages, excluding the list of references and annexes. The main text contains 12 figures and 29 tables. The thesis includes 7 annexes and cites 159 literature sources.

ANOTĀCIJA

Piena tauku lodīšu membrānas polārie lipīdi joprojām ir nozīmīgs pētniecības virziens, kas ietver to sastāva raksturošanu, analītisko noteikšanas metožu izstrādi un pilnveidošanu, tehnoloģisko īpašību izpēti un uzturvērtības novērtēšanu, jo zinātniskie pierādījumi par piena izcelsmes polāro lipīdu sastāvu un nozīmi uzturā joprojām ir ierobežoti. Paniņas ir piena pārstrādes blakusprodukts, kas satur piena tauku lodīšu membrānā esošos polāros lipīdus, kuriem ir nozīme cilvēka uzturā. Šie savienojumi ir nozīmīgi, var ietekmēt piena produktu uzturvērtību un tehnoloģiskās īpašības un tiem var būt nozīme lipīdu metabolismā cilvēka organismā. Tādēļ ir būtiski raksturot polāro lipīdu sastāvu pienā un piena produktos, izvērtēt pārstrādes un sezonas ietekmi uz to sadalījumu un novērtēt, vai piena polāro lipīdu daudzums uzturā ir saistīts ar plazmas lipīdu profilu sievietēm perimenopauzē.

Promocijas darbā paniņu funkcionālās īpašības vērtētas, integrējot pārtikas produktu sastāva analīzi, uztura novērtējumu un klīniskās uzturzinātnes pieeju. Pētījums ietver svaigpiena un no tās pašas piena partijas iegūto pārstrādes produktu sastāva analīzi divās sezonās, komerciāli pieejamu raudzētu paniņu novērtēšanu un plazmas lipīdu profila novērtēšanu sievietēm perimenopauzē ar mēreni paaugstinātu zema blīvuma lipoproteīnu holesterīna koncentrāciju. Īpaša uzmanība pievērsta pienā un piena produktos esošo polāro lipīdu noteikšanai un piena produktu polāro lipīdu daudzuma uzturā novērtēšanai, izmantojot individuālos piena produktu patēriņa datus un eksperimentāli noteikto produktu sastāvu.

***Promocijas darba hipotēze:** polāro lipīdu saturs pienā un piena produktos mainās atkarībā no sezonas un pārstrādes un var ietekmēt plazmas lipīdu profilu sievietēm perimenopauzē ar mēreni paaugstinātu zema blīvuma lipoproteīnu holesterīna koncentrāciju.*

*Aizstāvēšanai **izvirzītās tēzes**:*

1. *polāro lipīdu sastāvs un saturs pienā un piena produktos atšķiras atkarībā no sezonas un piena pārstrādes;*
2. *raudzētās paniņas var palielināt polāro lipīdu daudzumu uzturā;*
3. *lai novērtētu piena polāro lipīdu daudzumu uzturā, jāapvieno individuālie uztura patēriņa dati ar konkrētiem produktiem raksturīgo polāro lipīdu sastāvu;*
4. *piena polāro lipīdu daudzums uzturā ir saistīts ar plazmas lipīdu profilu sievietēm perimenopauzē;*
5. *raudzētu paniņu lietošanas ietekme uz plazmas lipīdu profilu ir saistīta ar produkta sastāvu, dalībnieču sākotnējo lipīdu profilu un uztura īpatnībām.*

***Promocijas darba mērķis** ir izvērtēt polāro lipīdu sastāvu un sadalījumu pienā un piena produktos un novērtēt saistību starp polāro lipīdu uzņemšanu ar raudzētām paniņām un plazmas lipīdu profilu sievietēm perimenopauzē.*

*Promocijas **darba uzdevumi**:*

1. *analizēt piena un piena pārstrādes procesā iegūto piena produktu ķīmisko sastāvu un polāro lipīdu profilu;*
2. *izvērtēt sezonālās atšķirības galveno piena polāro lipīdu klašu saturā un relatīvajā sadalījumā pienā un pētītajos pārstrādes produktos;*
3. *veikt 28 dienu randomizētu kontrolētu uztura intervences pētījumu, izvērtējot raudzētu paniņu ikdienas lietošanas ietekmi uz plazmas lipīdu profilu sievietēm perimenopauzē ar mēreni paaugstinātu zema blīvuma lipoproteīnu holesterīna koncentrāciju;*
4. *novērtēt uztura patēriņu un aprēķināt piena polāro lipīdu daudzumu uzturā, apvienojot individuālos uztura patēriņa datus ar sezonai raksturīgo piena produktu sastāvu;*
5. *analizēt saistības starp aprēķināto piena polāro lipīdu daudzumu uzturā un pētījuma dalībnieču plazmas lipīdu profilu.*

*Promocijas **darba novitāte un zinātniskais nozīmīgums**:*

1. *noteiktas fosfatidilholīna, fosfatidiletanolamīna un sfingomielīna satura un relatīvā sadalījuma likumsakarības pienā un piena produktos sezonas un piena pārstrādes procesa ietvaros;*
2. *izstrādāta integrēta pieeja piena polāro lipīdu daudzuma uzturā novērtēšanai, apvienojot dalībnieču individuālos uztura patēriņa datus ar eksperimentāli noteikto sezonai raksturīgo piena produktu sastāvu.*

Promocijas ***darba praktiskā nozīme****:*

1. *pētījuma atziņas palīdz skaidrot sezonālās izmaiņas polāro lipīdu sastāvā un saturā pienā un piena pārstrādes procesā;*
2. *darbā izstrādāto pieeju var izmantot pārtikas patēriņa un uztura epidemioloģijas pētījumos, lai novērtētu piena polāro lipīdu daudzumu uzturā, ņemot vērā produktu izvēli un sezonai raksturīgo sastāvu, nevis tikai kopējo piena produktu patēriņu;*
3. *iegūtie rezultāti nodrošina uz pētījumiem balstītu raudzētu paniņu uzturvērtības raksturojumu, nošķirot produkta ķīmiskā sastāva datus no pierādījumiem par ietekmi uz veselību.*

Promocijas darbs strukturēts trīs nodaļās.

1. ***Literatūras apskats.*** *Nodaļā apkopota zinātniskā literatūra par piena lipīdiem, piena tauku lodīšu membrānas sastāvdaļām un polāro lipīdu klasēm pienā un piena produktos. Raksturots paniņu sastāvs un funkcionālās īpašības, piena polāro lipīdu tehnoloģiskā nozīme un to iespējamā loma cilvēka uzturā. Īpaša uzmanība pievērsta fosfatidilholīnam, fosfatidiletanolamīnam un sfingomielīnam, kā arī pierādījumiem par piena polāro lipīdu saistību ar holesterīna absorbciju zarnās, plazmas lipīdu profilu un kardiometabolisko veselību sievietēm perimenopauzē.*
2. ***Materiāli un metodes.*** *Nodaļā raksturots kopējais pētījuma dizains un metodoloģiskās pieejas. Aprakstīta svaigpiena paraugu ņemšana un pārstrāde, piena un piena produktu ķīmiskā sastāva analīze un polāro lipīdu kvantitatīva noteikšana ar LC-MS. Nodaļā aprakstīta arī raudzēto paniņu atlase un raksturošana intervencei, klīniskā pētījuma dizains, ētiskie apsvērumi, dalībnieču piesaiste, iekļaušanas un izslēgšanas kritēriji, randomizācija, intervences deva un ilgums, asins paraugu ņemšana, bioķīmiskās analīzes, uztura novērtēšana, piena polāro lipīdu daudzuma uzturā aprēķināšana un statistiskā analīze.*
3. ***Rezultāti un diskusija.*** *Nodaļā izklāstīti un interpretēti promocijas darba galvenie rezultāti. Analizēts piena un piena produktu ķīmiskais sastāvs saistībā ar pārstrādi un sezonu. Izvērtēta kopējā polāro lipīdu koncentrācija un galvenās polāro lipīdu klases pienā un piena produktos. Izvēlētās raudzētās paniņas raksturotas kā intervences produkts. Analizēti klīniskie un uztura rezultāti, tostarp dalībnieču piesaiste, sākotnējie raksturlielumi, uztura paradumi, aprēķinātais piena polāro lipīdu daudzums uzturā, plazmas lipīdu profila izmaiņas pēc 28 dienu intervences un saistības starp aprēķināto piena polāro lipīdu uzņemšanu un lipīdu profilu.*

Promocijas darbs finansiāli atbalstīts ar programmas "Zinātniskās kapacitātes stiprināšana Latvijas Biozinātņu un tehnoloģiju universitātē" projekta "Paniņu polāro lipīdu izpēte" un ERAF projekta Nr. 1.1.1.8/1/24/I/002 "LBTU doktorantu atbalsta un attīstības iniciatīva" līdzekļiem.

Promocijas darbs ir sagatavots angļu valodā. Darba apjoms ir 121 lappuse, neskaitot izmantoto informācijas avotu sarakstu un pielikumus. Darba pamatdaļā ir 12 attēli un 29

tabulas. Darbam pievienoti 7 pielikumi. Promocijas darba izstrādē izmantoti 159 literatūras avoti.

Approbation of the Ph.D. thesis */ Zinātniskā darba aprobācija*

The results of the thesis have been published in three peer-reviewed scientific publications indexed in Scopus and/or Web of Science. / *Promocijas darba rezultāti publicēti trīs recenzētās zinātniskajās publikācijās, kas indeksētas datubāzēs Scopus un/vai Web of Science.*

1. Aleksejeva S., Radenkovs V., Zolovs M., Vilkoite I.; Meija L., Ciprovica I. (2026) Estimated habitual dairy polar lipid exposure and post-intervention plasma lipid outcomes in perimenopausal women in Latvia: A secondary exposure-based analysis of a 28-day fermented buttermilk randomised trial. *Nutrients*, 18, 2194; https://doi.org/10.3390/nu18132194
2. Aleksejeva S., Meija L., Zolovs M., Ciprovica I. (2025) Plasma lipid profile among perimenopausal Latvian women in relation to dietary habits. *Nutrients*, 17(2), 243; https://doi.org/10.3390/nu17020243
3. Aleksejeva S., Ciprovica I., Meija L. (2022) A review: Dairy phospholipids in human nutrition. *Research for Rural Development 2022: Proceedings of the 28th Annual International Scientific Conference*, 37, 89-92; https://doi.org/10.22616/rrd.28.2022.013

The results of the thesis have been published in two peer-reviewed conference abstracts indexed in Scopus and/or Web of Science. / *Promocijas darba rezultāti publicēti divās recenzētās konferenču tēzēs, kas indeksētas datubāzēs Scopus un/vai Web of Science.*

1. Aleksejeva S., Ciprovica I., Meija L. (2024). Seasonal variation in serum cholesterol level in perimenopausal women: A preliminary research. Clinical Nutrition ESPEN, 63, 1245^{-1}246. https://doi.org/10.1016/j.clnesp.2024.07.844
2. Aleksejeva S., Krasikova A., Ciprovica I., Meija L. (2023). The effect of fermented buttermilk on plasma lipid profile in perimenopausal women: A preliminary research. Clinical Nutrition ESPEN, 58, 723-724. https://doi.org/10.1016/j.clnesp.2023.09.851

The research findings were presented at seven international scientific conferences held in Latvia, Lithuania, France and Italy. / *Pētījuma rezultāti prezentēti septiņās starptautiskās zinātniskajās konferencēs Latvijā, Lietuvā, Francijā un Itālijā.*

1. Aleksejeva S., Radenkovs V., Zolovs M., Ciprovica I., Meija L. (2026) Seasonal variation in the lipid profile of dairy products. In: *Sustainable environmental development: innovative technologies*, 16 April 2026, Klaipeda, Lithuania (Oral presentation)
2. Aleksejeva S., Radenkovs V., Zolovs M., Meija L., Ciprovica I. (2026). Compositional dynamics of polar lipids in dairy products across processing. In: *FoodBalt 2026, 19th Baltic Conference on Food Science and Technology*, 13^{-1}5 May 2026, Jelgava, Latvia (Oral presentation)
3. Aleksejeva S., Ciprovica I., Meija L., Zolovs M. (2024) Lipid profile changes in perimenopausal women depending on buttermilk consumption in diet: a paired samples analysis. In: *4th International Conference Nutrition and Health*, 5 to 6 December 2024, Riga, Latvia (Oral presentation)
4. Aleksejeva S., Ciprovica I., Meija L. (2024) Seasonal variations in serum cholesterol level in perimenopausal women: a preliminary research. In: *46th ESPEN Congress on Clinical Nutrition & Metabolism 2024*, 7 to 10 September 2024, Milan, Italy (Poster presentation)
5. Aleksejeva S., Ciprovica I., Meija L., Krasikova A. (2023) The effect of fermented buttermilk on plasma lipid profile in perimenopausal women: a

preliminary research. In: *45th ESPEN Congress on Clinical Nutrition & Metabolism 2023*, 11 to 14 September 2023, Lyon, France (Poster presentation)

6. Aleksejeva S., Ciprovica I., Meija L., Krasikova A. (2023) Effect of buttermilk polar lipids on plasma lipid profile: a pilot study. In: *Foodbalt 2023, 16th Baltic Conference on Food Science and Technology*, 11 to 12 April 2023, Jelgava, Latvia (Oral presentation)
7. Aleksejeva S., Ciprovica I., Meija L. (2022) A review: dairy phospholipids in human nutrition. In: *28th Annual International Scientific Conference Research for Rural Development 2022*, 18 to 20 May 2022, Jelgava, Latvia (Oral presentation)

Results from other research activities were published during the preparation of the Ph.D. thesis. */ Promocijas darba izstrādes laikā publicēti arī citu pētniecisko darbu rezultāti.*

1. Aumeistere L., Majore K., Keke A., Driksna A., Aleksejeva S., Ciprovica I. (2026) Associations between human milk oligosaccharides and maternal nutrition: Latvian study. *Nutrients*. Vol.18(1); https://doi.org/10.3390/nu18010136
2. Ciprovica I., Aumeistere L., Majore K., Ķeke A., Zagorska J., Driksna A., Aleksejeva S. (2025) Datasets for human milk oligosaccharide comparative analysis. DataverseLV. - Version 1.0. https://doi.org/10.71782/DATA/RGML1E

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LIST OF THE ACRONYMS AND ABBREVIATIONS USED IN THE *PH.D.* THESIS */ PROMOCIJAS DARBĀ LIETOTIE SAĪSINĀJUMI UN SIMBOLI*

β - beta regression coefficient / *beta regresijas koeficients*
η² - eta squared / *eta kvadrāts*
ALAT - alanine aminotransferase / *alanīnaminotransferāze*
ANOVA - analysis of variance / *dispersijas analīze*
ApoB - apolipoprotein B / *apolipoproteīns B*
ApoA1 - apolipoprotein A1 / *apolipoproteīns A1*
ATP - adenosine triphosphate / *adenozīntrifosfāts*
BIOR - Institute of Food Safety, Animal Health and Environment "BIOR" / *Pārtikas drošības, dzīvnieku veselības un vides zinātniskais institūts "BIOR"*
BMI / *ĶMI* - body mass index / *ķermeņa masas indekss*
CDP - cytidine diphosphate / *citidīndifosfāts*
CFU / *KVV* - colony-forming units / *koloniju veidojošās vienības*
CI / *TI* - confidence interval / *ticamības intervāls*
CVD - cardiovascular diseases / *sirds un asinsvadu slimības*
df - degrees of freedom / *brīvības pakāpes*
DHA / *DHS* - docosahexaenoic acid / *dokozaheksaēnskābe*
DM - dry matter / *sausna*
E% - the percentage of total daily energy intake / *procentuālais dienā uzņemtais enerģijas daudzums*
EAS - European Atherosclerosis Society / *Eiropas Aterosklerozes biedrība*
EFSA - European Food Safety Authority / *Eiropas Pārtikas nekaitīguma iestāde*
EPA / *EPS* - eicosapentaenoic acid / *eikozapentaēnskābe*
ESC - European Society of Cardiology / *Eiropas Kardiologu biedrība*
ESI - electrospray ionisation / *elektroizsmidzināšanas jonizācija*
et al. - and others / *un citi*
EU - European Union / *Eiropas Savienība*
FA - fatty acid / *taukskābe*
FFA - free fatty acids / *brīvās taukskābes*
FFQ - food frequency questionnaire / *pārtikas patēriņa biežuma anketa*
Fig. / *att.* - figure / *attēls*
FMP - final menstrual period / *pēdējās menstruācijas*
g - Hedges' g, standardised between-group mean difference / *Hedžesa g, standartizēta atšķirība starp grupu vidējām vērtībām*
g_z - Hedges-corrected standardised within-group mean change / *standartizētas vidējās izmaiņas grupas ietvaros ar Hedžesa korekciju*
GIP - glucose-dependent insulinotropic polypeptide / *no glikozes atkarīgais insulinotropais peptīds*
GluCer - glucosylceramide / *glikozilkeramīds*
HDL-C - high-density lipoprotein cholesterol / *augsta blīvuma lipoproteīnu holesterīns*
HPAA - hypothalamic-pituitary-adrenal axis / *hipotalāma-hipofīzes-virsnieru ass*
HRT - hormone replacement therapy / *hormonu aizstājterapija*
IPA - isopropanol / *izopropanols*
ISO - International Organization for Standardization / *Starptautiskā Standartizācijas organizācija*
LacCer - lactosylceramide / *laktozilkeramīds*
LC-MS - liquid chromatography-mass spectrometry / *šķidruma hromatogrāfija-masspektrometrija*

LDL-C - low-density lipoprotein cholesterol / *zema blīvuma lipoproteīnu holesterīns*
LVS - Latvian Standard / *Latvijas standarts*
Max / *Maks* - maximal value / *maksimālā vērtība*
MFGM - milk fat globule membrane / *piena tauku lodīšu membrāna*
Min - minimal value / *minimālā vērtība*
MRM - multiple reaction monitoring / *vairāku reakciju monitorings*
MRS - de Man, Rogosa and Sharpe medium / *de Man, Rogosa un Sharpe barotne*
MUFA - monounsaturated fatty acids / *mononepiesātinātās taukskābes*
m/z - mass-to-charge ratio / *masas un lādiņa attiecība*
n - number of samples or participants / *paraugu vai dalībnieku skaits*
NA - not applicable / *nav attiecināms*
NCD - non-communicable disease / *neinfekcijas slimība*
NNR - Nordic Nutrition Recommendations / *Ziemeļvalstu uztura rekomendācijas*
non-HDL-C - non-high-density lipoprotein cholesterol / *ne-augsta blīvuma lipoproteīnu holesterīns (kopējais aterogēno lipoproteīnu holesterīns)*
OECD - Organisation for Economic Co-operation and Development / *Ekonomiskās sadarbības un attīstības organizācija*
OLS - ordinary least squares / *mazāko kvadrātu metode*
p-value - p-value / *p vērtība*
PC - phosphatidylcholine / *fosfatidilholīns*
PCA - principal component analysis / *galveno komponentu analīze*
PE - phosphatidylethanolamine / *fosfatidiletanolamīns*
PI - phosphatidylinositol / *fosfatidilinozitols*
PL - polar lipids / *polārie lipīdi*
PS - phosphatidylserine / *fosfatidilserīns*
PTFE - polytetrafluoroethylene / *politetrafluoretilēns*
PUFA - polyunsaturated fatty acids / *polinepiesātinātās taukskābes*
R^2 - coefficient of determination / *determinācijas koeficients*
RCT - randomised controlled trial / *randomizēts kontrolēts pētījums*
RSD - relative standard deviation / *relatīvā standartnovirze*
RSU - Rīga Stradiņš University / *Rīgas Stradiņa universitāte*
SD - standard deviation / *standartnovirze*
SFA - saturated fatty acids / *piesātinātās taukskābes*
SL - sphingolipids / *sfingolipīdi*
SM - sphingomyelin / *sfingomielīns*
STRAW+10 - Stages of Reproductive Aging Workshop + 10 (staging system of reproductive ageing) / *Reproduktīvās novecošanas stadiju darba grupas + 10 klasifikācija (reproduktīvās novecošanas posmu sistēma*
TC - total cholesterol / *kopējais holesterīns*
TFA - trans fatty acids / *trans-taukskābes*
TG - triglycerides / *triglicerīdi*
TLC - thin-layer chromatography / *plānslāņa hromatogrāfija*
TNF-α - tumour necrosis factor alpha / *audzēja nekrozes faktors alfa*
UHT - Ultra High Temperature / *ultra-augsta temperatūras apstrāde*
VLDL - very-low-density lipoprotein / *ļoti zema blīvuma lipoproteīns*
VLDL-TG - very-low-density lipoprotein triglycerides / *ļoti zema blīvuma lipoproteīnu triglicerīdi*
WHO - World Health Organization / *Pasaules Veselības organizācija*
WHR - waist-hip ratio / *vidukļa un gurnu apkārtmēra attiecība*

INTRODUCTION / *IEVADS*

Milk and dairy products contain, in addition to triacylglycerols, a quantitatively minor but structurally and nutritionally important fraction of polar lipids. They are located predominantly in the milk fat globule membrane, where they stabilise the fat globule and determine the functional properties of dairy emulsions; in bovine milk they consist mainly of phosphatidylcholine, phosphatidylethanolamine and sphingomyelin, with phosphatidylserine, phosphatidylinositol and glycosphingolipids at lower concentrations (Anto et al., 2020; Guan et al., 2020; Sprenger et al., 2024).

The concentration and class distribution of polar lipids in dairy products depend on the structure of the milk fat globule membrane and on processing. Separation, thermal treatment, cream maturation prior to churning and churning modify membrane integrity and redistribute membrane-associated lipids between cream, skimmed milk, butter and buttermilk, so that products with a similar total fat concentration do not contain equivalent amounts or proportions of individual polar lipid classes (Hokkanen et al., 2021; Huang et al., 2023; Jukkola et al., 2019).

Buttermilk is particularly relevant in this context: cream churning disrupts the fat globules and transfers membrane fragments into the aqueous phase, so that buttermilk contains membrane-associated lipids and proteins despite its low total fat concentration and represents a compositionally distinct dairy product rather than a residual stream of butter production (Bourlieu et al., 2018; Calvo et al., 2020; Iung et al., 2024; Venkat et al., 2024). The polar lipid composition of commercial cultured buttermilk, however, cannot be inferred from its declared fat content or product category, because it varies with the raw material, the butter-making process and the final formulation; product-specific analysis is therefore required (Calvo et al., 2020; Señoráns, Gallo, et al., 2025; Venkat et al., 2024).

In addition to processing, the concentration of milk polar lipids varies over the production season. In individual cows, most polar lipid classes were positively associated with milk fat concentration and negatively with fat globule size, and the profile was affected predominantly by lactation stage (Kostovska et al., 2025; Z. Liu et al., 2017). Raw milk of different seasons therefore introduces different concentrations and proportions of polar lipid classes into the same processing chain, and the composition of the resulting products reflects both effects (Venkat et al., 2024).

Seasonality also matters when milk polar lipid intake is estimated from dietary data, because the same reported quantity of a dairy product corresponds to different polar lipid intakes when its composition varies across seasons. In the present research, season is therefore treated primarily as a determinant of the composition values used to estimate habitual milk polar lipid exposure, not only as an independent factor affecting physiological outcomes.

Dietary exposure to milk polar lipids depends on both the polar lipid concentration of the products consumed and the amount and frequency of their consumption (Venkat et al., 2024; Yuzbashian et al., 2023) . Total dairy intake is not an adequate substitute, because milk, cream, butter, cheese, yoghurt and buttermilk differ in fat concentration, membrane proportion and polar lipid class distribution (Calvo et al., 2020; Haddadian et al., 2018). Estimating habitual exposure under real-life conditions therefore requires the integration of individual dietary intake data with season-specific product composition.

Most human intervention studies have used isolated milk fat globule membrane fractions, concentrated polar lipids or enriched products; supplementation with 3 or 5 g per day of milk polar lipids affected selected circulating lipid risk markers in overweight postmenopausal women (Vors et al., 2020). These findings support the biological plausibility of an effect on cholesterol metabolism, but effects observed at supplementation doses cannot be extrapolated to the substantially lower intakes of naturally occurring milk polar lipids from habitual dairy consumption, which remain little studied (Anto et al., 2020; Bruno et al., 2021; Venkat et al., 2024).

The choice of perimenopausal women as the study population follows from the physiology of the menopausal transition, during which total cholesterol (TC), low-density lipoprotein cholesterol (LDL-C), high-density lipoprotein cholesterol (HDL-C) and triglycerides (TG) change in a direction that raises cardiovascular disease (CVD) risk (El Khoudary et al., 2020; Sharma et al., 2022). In Latvia, this is a pressing public health issue: in 2023, the standardised mortality rate from diseases of the circulatory system was 726 per 100 000 inhabitants, exceeded in the European Union only by Bulgaria and Romania (Eurostat, 2026). The clinical part of the thesis therefore asks whether cultured buttermilk consumption and estimated habitual intake of milk polar lipids are related to the plasma lipid profile of perimenopausal women.

The research problem arises from several interconnected gaps. First, the concentration and relative distribution of the main polar lipid classes across milk and its processing products remain incompletely characterised. Second, processing-related redistribution and seasonal variation complicate comparisons of dairy products and assessments of their nutritional value. Third, the polar lipid composition of commercially available cultured buttermilk cannot be predicted from its declared fat content. Fourth, total dairy intake alone cannot estimate habitual exposure to milk polar lipids. Finally, evidence is limited on whether the estimated intake of naturally occurring milk polar lipids in the habitual diet is associated with the plasma lipid profile in perimenopausal women. To address these gaps, the thesis integrates compositional analysis of milk and its processing products, characterisation of a commercial cultured buttermilk, dietary assessment, and a 28-day randomised controlled intervention, linking the polar lipid composition of dairy products, the estimated habitual intake of milk polar lipids, and the plasma lipid profile of perimenopausal women.

The **hypothesis** of the study**:** Polar lipid concentration in milk and dairy products varies seasonally and with processing and may influence the plasma lipid profile in perimenopausal women with moderately elevated low-density lipoprotein cholesterol concentrations.

The hypothesis is supported by the following **theses**:

1. the composition and concentration of polar lipids in milk and dairy products vary within seasons and with milk processing;
2. cultured buttermilk may contribute to habitual dietary exposure to milk polar lipids;
3. habitual dietary exposure to milk polar lipids requires integrating individual dietary intake data with product-specific polar lipid composition;
4. estimated habitual milk polar lipid exposure is associated with plasma lipid profile in perimenopausal women;
5. the effect of cultured buttermilk consumption on plasma lipid profile is associated with product composition, participants' baseline lipid profile, and habitual diet characteristics.

The aim of the Ph.D. thesis is to evaluate the composition and distribution of polar lipids in milk and dairy products and to assess the relationship between dietary exposure to polar lipids from cultured buttermilk and the plasma lipid profile in perimenopausal women.

The **research objectives** are set to achieve the aim of the study:

1. to analyse the chemical composition and polar lipid profile of milk and dairy products obtained during milk processing;
2. to evaluate seasonal differences in the concentration and relative distribution of the main milk polar lipid classes in milk and in the processed products studied;
3. to conduct a 28-day randomised controlled dietary intervention study evaluating the effect of daily consumption of cultured buttermilk on the plasma lipid profile in perimenopausal women with moderately elevated low-density lipoprotein cholesterol concentrations;

4. to assess dietary intake and estimate habitual milk polar lipid exposure by combining individual dietary intake data with season-specific dairy product composition;
5. to analyse associations between estimated habitual milk polar lipid exposure and plasma lipid profile of participants.

The scientific significance of the study:

1. the study established processing-related and seasonal patterns in the concentration and relative distribution of phosphatidylcholine, phosphatidylethanolamine, and sphingomyelin across milk and dairy products within the same processing chain;
2. an integrated approach was developed to estimate habitual milk polar lipid exposure by combining participant-level dietary intake data with experimentally determined season-specific dairy product composition.

The **practical significance** of the study:

1. the findings help explain seasonal changes in polar lipid concentration and composition in milk and during milk processing;
2. the approach developed in the thesis can be applied in food consumption and nutritional epidemiology studies to estimate habitual daily milk polar lipid exposure, taking into account dairy product selection and season-specific product composition rather than total dairy intake alone;
3. the findings provide an evidence base for communicating the nutritional attributes of cultured buttermilk while distinguishing between data on its chemical composition and evidence of health effects.

The study was **financially supported** by the following projects:

1. the programme “Strengthening Research Capacity in the Latvia University of Life Sciences and Technologies” project “The study of buttermilk polar lipids”;
2. ERDF project No. 1.1.1.8/1/24/I/002 “LBTU Doctoral Students Support and Development Initiative”.



1. REVIEW OF THE LITERATURE / *LITERATŪRAS APSKATS*

1.1. Milk lipids and milk fat globule membrane-associated polar lipids / *Piena lipīdi un piena tauku lodīšu membrānas polārie lipīdi*

Owing to their compositional diversity and variability, milk lipids remain an important field of research for advancing knowledge of milk processing and technological and functional properties of dairy products. Although triacylglycerols are the dominant lipid fraction, increasing attention is now being paid to the quantitatively smaller polar lipid (PL) fraction, associated mainly with the milk fat globule membrane (MFGM). The structural organisation and distribution of these compounds in MFGM contribute to emulsion stability, processing behaviour, and the nutritional characteristics of dairy products. Raw milk composition, biological and seasonal factors, and the redistribution of MFGM compounds and membrane fragments during milk processing influence their concentration (Anto et al., 2020; Fox et al., 2015; Venkat et al., 2024).

The fat content of milk is usually 3-5 g 100 g^{-1} and depends on breed, lactation stage, feeding system, and the animal's physiological and environmental conditions. Triacylglycerols constitute 97-98 % of milk lipids and form the neutral core of the fat globule; the remainder consists of diacylglycerols, monoacylglycerols, free fatty acids, cholesterol and its esters, glycerophospholipids, sphingolipids, glycosphingolipids and minor lipid classes. The share of PL is typically 0.5^{-1}.0 % of total milk lipids and varies with milk origin, fat globule size, storage time and the processing applied (Bourlieu et al., 2018; Fox et al., 2015; Venkat et al., 2024).

Milk fat (in the study, the term used characterises all lipids present in the milk) is secreted as globules surrounded by a structurally complex membrane derived from mammary epithelial cell membranes. The MFGM is organised as an inner monolayer adjacent to the triacylglycerol core and an outer bilayer facing the aqueous milk phase. This multilayer arrangement contains PL, cholesterol, minerals, fat-soluble vitamins and membrane-associated proteins, providing a physicochemical interface between the hydrophobic lipid core and the surrounding aqueous phase. The MFGM maintains the integrity of the fat globule, governs its interfacial stability and response to processing, and determines the technological and nutritional properties of the membrane-associated lipid fraction (Anto et al., 2020; Hernell et al., 2016; Venkat et al., 2024) (Fig. 1.1).

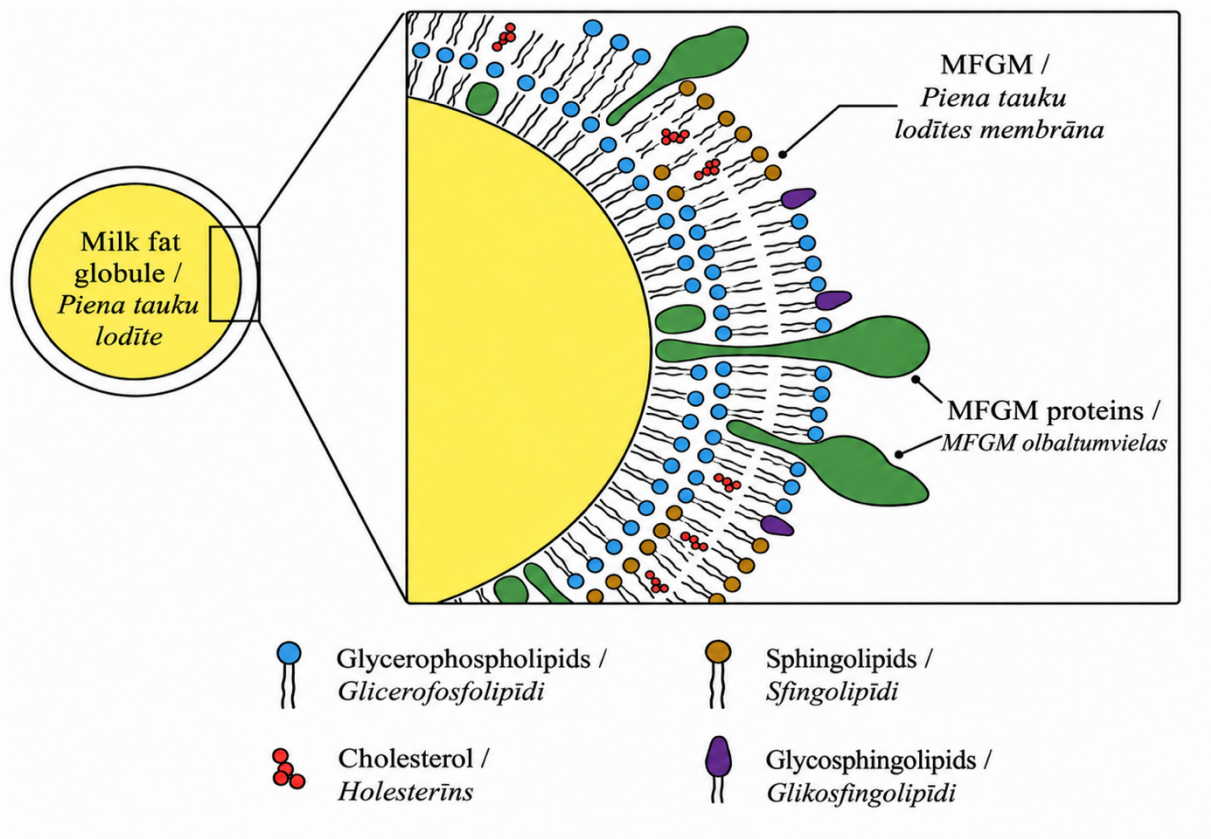


Fig. 1.1. **Composition of the milk fat globule membrane /** *1.1. att.* ***Piena tauku lodītes membrānas sastāvs***
(Hernell et al., 2016)

The MFGM is compositionally heterogeneous, and Table 1.1 presents selected literature-derived characteristics of isolated bovine MFGM compounds, retaining the original reference sources for the reported values.

Table 1.1. **The composition of isolated bovine milk fat globule membrane compounds /** *1.1. tabula* ***No piena tauku lodīšu membrānas izolētās sastāvdaļas***

(adapted from / *pielāgots pēc* Keenan et al. (1983), as reproduced in / *kas pārpublicēts no* Fox et al. (2015))

Component / *Sastāvdaļa*	Reported content / *Norādītais saturs*
Protein / *Olbaltumvielas*	25-60 % of dry matter / *no sausnas*
Total lipids / *Kopējie lipīdi*	0.50^{-1}.20 mg mg^{-1} protein / *olbaltumvielu*
Phospholipids / *Fosfolipīdi*	0.13-0.34 mg mg^{-1} protein / *olbaltumvielu*
Phosphatidylcholine / *Fosfatidilholīns*	34 % of total lipid phosphorus / *no kopējā lipīdu fosfora*
Phosphatidylethanolamine / *Fosfatidiletanolamīns*	28 % of total lipid phosphorus / *no kopējā lipīdu fosfora*
Sphingomyelin / *Sfingomielīns*	22 % of total lipid phosphorus / *no kopējā lipīdu fosfora*
Phosphatidylinositol / *Fosfatidilinozītols*	10 % of total lipid phosphorus / *no kopējā lipīdu fosfora*
Phosphatidylserine / *Fosfatidilserīns*	6 % of total lipid phosphorus / *no kopējā lipīdu fosfora*
Neutral lipids / *Neitrālie lipīdi*	56-80 % of total lipids / *no kopējiem lipīdiem*
Sterols / *Sterīni*	0.20-5.20 % of total lipids / *no kopējiem lipīdiem*
Glycerides / *Glicerīdi*	53-74 % of total lipids / *no kopējiem lipīdiem*
Free fatty acids / *Brīvās taukskābes*	0.60-6.30 % of total lipids / *no kopējiem lipīdiem*

Note. Reported ranges and proportions retain the reporting basis of the original source and describe isolated bovine MFGM material rather than whole milk or a commercial dairy product. / *Piezīme. Norādītie diapazoni un proporcijas ir izteikti atbilstoši oriģinālajā avotā izmantotajam rezultātu izteikšanas pamatam un raksturo no govs piena izolētu tauku lodīšu membrānas materiālu nevis no pilnpiena vai komerciāla piena produkta iegūtu.*

Within the MFGM lipid fraction, milk PL comprise glycerophospholipids and sphingolipids. Glycerophospholipids contain a glycerol backbone, two fatty acyl chains, a phosphate group, and a class-specific polar head group, and include phosphatidylcholine (PC), phosphatidylethanolamine (PE), phosphatidylserine (PS), phosphatidylinositol (PI), and minor phosphatidylglycerol (PG) classes. Sphingolipids are built on a sphingoid base and include sphingomyelin (SM), glucosylceramide (GluCer), lactosylceramide (LacCer), and gangliosides. SM contains a phosphocholine head group and is therefore frequently included among phospholipids, although it is structurally classified as a sphingophospholipid, whereas glycosphingolipids contain carbohydrate head groups rather than phosphate-containing head

groups (Anto et al., 2020; Venkat et al., 2024). The structural classification of the major PL classes in dairy products is summarised in Fig. 1.2.

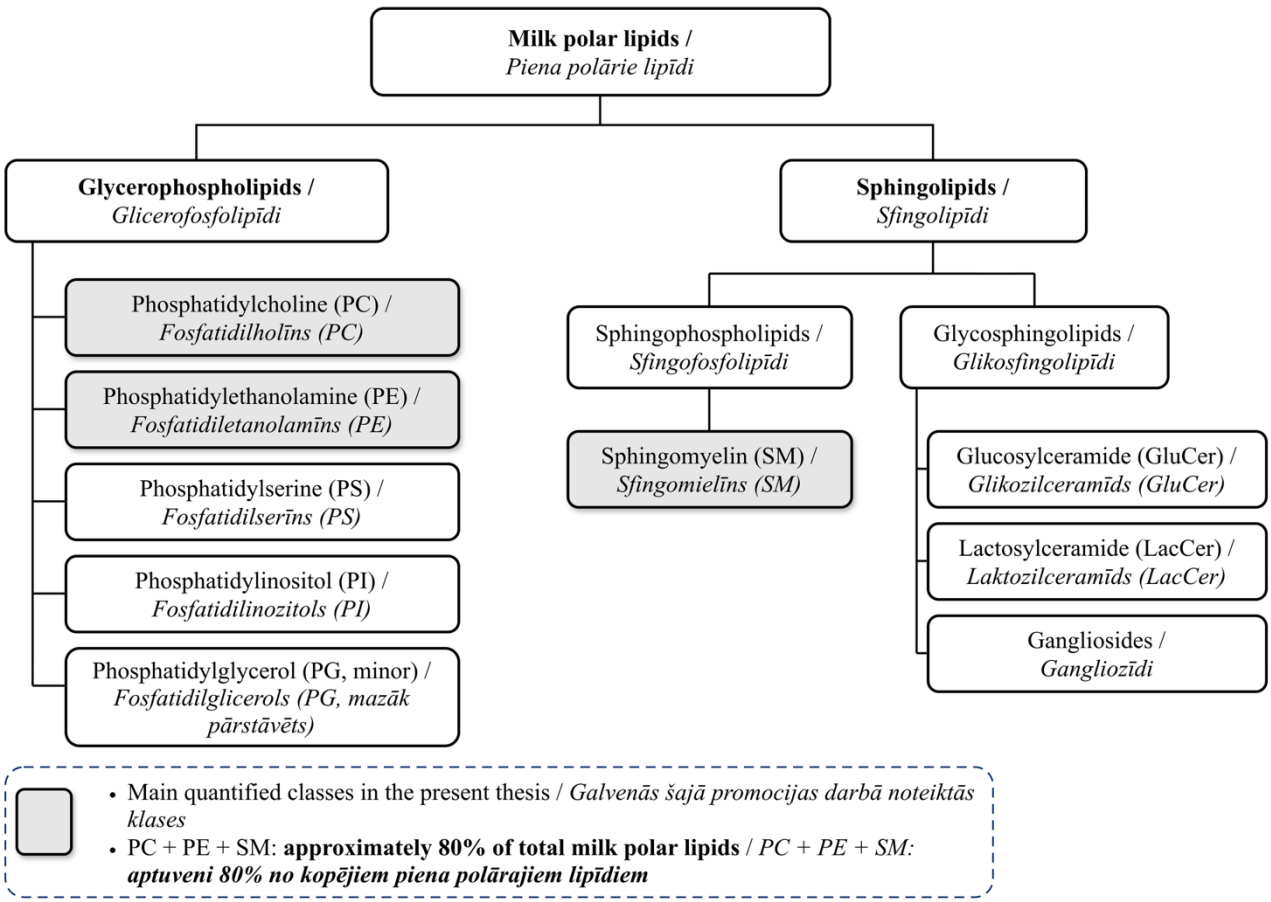


Fig. 1.2. **Classification of the major PL classes in milk /** *1.2. att.* ***Galveno PL klašu klasifikācija pienā***
(developed by the author based on / autora izstrādāts, balstoties uz Anto et al. (2020), Venkat et al. (2024))

PC, PE and SM, the classes quantified in the present thesis, are the predominant PL classes reported in milk and dairy products. PE and PC constitute major glycerophospholipid fractions, whereas SM is the principal sphingophospholipid. Their relative distribution varies with the dairy matrix, processing technology, season, and analytical method used (Calvo et al., 2020; Haddadian et al., 2018; Venkat et al., 2024).

Fig. 1.3 presents the molecular structures of the major milk PL classes: PC, PE and SM.

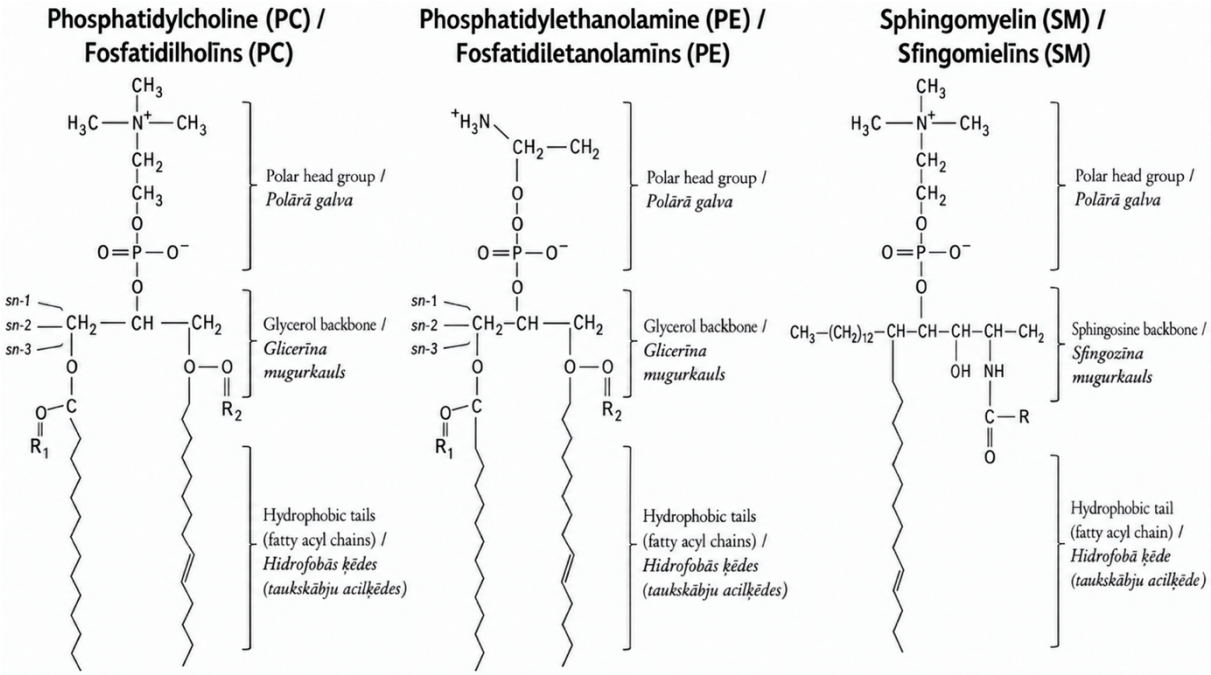


Fig. 1.3. **Molecular structures of the major PL classes** */ 1.3. att.* ***Galveno PL klašu molekulārās struktūras***

(developed by the author based on / autora izstrādāts, balstoties uz Morita et al. (2020))

The PC molecule comprises a glycerol backbone carrying two fatty acyl chains at the sn^{-1} and sn-2 positions and a phosphocholine group at the sn-3 position. A saturated fatty acid is usually located at sn^{-1} and an unsaturated fatty acid at sn-2, although the acyl composition differs between molecular species. The phosphocholine group constitutes the hydrophilic part of the molecule, and the two acyl chains constitute its hydrophobic part (Anto et al., 2020; Tavazzi et al., 2018).

PE shares PC's glycerol-based structural framework but has ethanolamine rather than choline as the polar head group. Its smaller head group favours membrane curvature and contributes to the structure and elasticity of the MFGM. SM differs more substantially, as its hydrophobic region comprises a sphingosine backbone linked with to a fatty acid via an amide bond; however, like PC, it contains a PC head group. SM is often associated with cholesterol-rich, more ordered membrane regions and contributes to membrane organisation and structural stability (Anto et al., 2022; Venkat et al., 2024).

The amphiphilic organisation of PC, PE, SM, and other PLs enables them to stabilise the interface between the triacylglycerol core and the aqueous phase. At the same time, the physicochemical behaviour of individual classes depends on the head group, fatty acid composition, degree of unsaturation, and interactions with cholesterol and proteins. PL containing highly unsaturated fatty acids are more susceptible to oxidative degradation, whereas the intact MFGM may also influence interfacial oxidation and protect the lipid core. These combined properties are relevant to the stability, emulsifying behaviour, and processing response of dairy products (Anto et al., 2022; L. Cui & Decker, 2016; Venkat et al., 2024).

Reliable characterisation of milk PLs requires methods that can distinguish structurally related lipid classes and molecular species. PC, PE, and SM differ in their fragmentation patterns, ionisation behaviour, and detector response; quantitative results may therefore be affected by extraction efficiency, chromatographic separation, matrix effects, calibration strategy, and the standards selected for each lipid class. Comparative analytical studies have shown that changes in extraction and chromatographic conditions can substantially influence the lipid classes detected and their measured abundance. Lipid-class-specific identification, appropriate calibration, method validation, and careful interpretation of response-factor

differences are required when quantifying milk PL by mass spectrometry (C. Li et al., 2023; Magnuson et al., 2022; Rund et al., 2024; Tavazzi et al., 2018).

Seasonal variation of milk fat and its fatty acid composition is well documented, whereas data on the seasonal variation of milk PL are scarce and are largely limited to raw milk. The most systematic evidence comes from a survey of 19 Holstein cows sampled over an entire milking season, in which LC-MS quantified six PL classes. The concentrations of all six classes increased gradually over the milking season and peaked in autumn. They were positively correlated with milk fat concentration and negatively correlated with fat globule size, while variation between cows was as large as variation between seasons. The proportions of the individual PL classes, however, remained constant throughout the season. Liu and colleagues interpreted this stability as evidence that PL synthesis in the mammary gland is tightly regulated. These findings suggest that, in the cows studied, seasonal variation primarily affected the amount of PL secreted with the fat globule membrane rather than the relative proportions of its PL classes (Z. Liu et al., 2017).

Season is not a single factor but a combination of feeding, lactation stage and environment, and those factors are difficult to separate. In Swedish raw milk collected in six different months, the lipid classes and fatty acid composition varied with the season, and the same seasonal pattern was found in the corresponding commercially processed UHT (Ultra High Temperature) milk, indicating that seasonal differences in raw milk composition are also present in the corresponding UHT milk (Lu et al., 2017). In an Irish spring-calving system, where bulk milk was sampled monthly from March to November, the PL profile was affected mainly by lactation stage, more than by pasture type or cow breed (Kostovska et al., 2025). In herds of this type, season and lactation stage coincide, so that the two effects cannot be distinguished; in herds with year-round calving, bulk milk pools cows at different stages of lactation, so that the lactation effect is averaged and seasonal differences are expected to reflect mainly the change between the indoor winter ration and pasture and the physiological state of the cows after the winter period, including body condition, metabolic state and udder health. Even with a broadly constant ration, spring milk may therefore differ from winter milk in its lipid and membrane-associated fractions (Venkat et al., 2024).

For this reason, the present thesis analysed raw milk and its processing products from the same batch in two seasons and used season-specific concentrations to estimate dietary PL exposure (Section 2.4).

Milk processing further alters the distribution of MFGM-associated PL. Cream separation distributes milk fat globules between cream and skimmed milk, while shear forces may modify membrane integrity. Thermal treatment can alter the interfacial composition of milk fat globules, and cream cooling, maturation, and churning distribute the membrane fragments among butter and buttermilk. Consequently, PL distribution across dairy products depends not only on the total fat concentration but also on fat globule disruption, membrane fragmentation, phase inversion, and the specific technological conditions applied (Hokkanen et al., 2021; Huang et al., 2023; Jukkola et al., 2019).

The contrast between the absolute PL concentration and the proportion of PL within the total lipid fraction is illustrated by the literature values in Table 1.2. Fat-rich products such as cream and butter contain the highest amounts of PL per 100 g, whereas low-fat processing streams such as skimmed milk and buttermilk contain a markedly higher proportion of PL relative to their total lipid content.

Table 1.2. **PL concentration and distribution of major PL classes in selected dairy products /** *1.2. tabula* ***PL saturs un galveno PL klašu sadalījums atsevišķos piena produktos***

(adapted from / *adaptēts no* Rombaut et al. (2006))

Product / *Produkts*	Total PL, mg 100 g^{-1} / *Kopējie PL, mg 100* g^{-1}	PL, % of total lipids / *no kopējiem lipīdiem*	PC, % of PL / *no PL*	PE, % of PL / *no PL*	SM, % of PL / *no PL*
Raw milk / *Svaigpiens*	29.4	0.7	19.1	42.0	17.9
Skimmed milk / *Vājpiens*	12.8	10.7	19.6	38.2	16.7
Cream (40 % fat) / *Saldais krējums (40 % tauku)*	138.9	0.4	20.7	40.0	13.9
Butter / *Sviests*	141.0	0.2	20.9	37.6	17.2
Fresh buttermilk / *Svaigas paniņas*	91.8	21.8	19.1	42.9	12.8
Skimmed milk curd / *Vājpiena biezpiens*	32.4	24.7	18.7	39.1	19.4
Whey from Mozzarella cheese / *Mocarellas siera sūkalas*	19.1	6.2	19.1	40.6	15.7

Note. PC, PE and SM are expressed as percentages of total PL; their sum is below 100 % because other PL classes are present. Buttermilk refers to the fresh product obtained during cream churning. / *Piezīme. PC, PE un SM īpatsvars izteikts procentos no kopējā PL satura; to summa nesasniedz 100 %, jo produktos ir arī citas PL klases. Paniņu vērtības attiecas uz svaigām paniņām, kas iegūtas krējuma kulšanas procesā.*

These data show that total fat concentration alone is an inadequate indicator of PL concentration in milk. Cream and butter contain the highest PL concentrations per 100 g of product, whereas fresh buttermilk and skimmed milk curd contain the highest proportions of PL relative to total lipids. The relative distribution of PC, PE, and SM also differs between products, indicating that processing affects both the concentration and composition of milk PL.

Buttermilk is particularly relevant as a processing-derived source of MFGM material. During cream churning, MFGM is disrupted, and some membrane material is released into the aqueous phase. PL composition depends on the raw material, cream treatment, churning conditions, subsequent fractionation, and final product formulation. Therefore, buttermilk should be considered a compositionally distinct dairy product with potential for value-added food applications rather than solely as a residual product of butter manufacture (Bourlieu et al., 2018; Calvo et al., 2020; Rombaut et al., 2005).

1.2. Buttermilk composition and functional properties / *Paniņu sastāvs un funkcionālās īpašības*

Among milk processing streams, buttermilk is of particular interest because churning disrupts milk fat globules and transfers part of the MFGM material into the aqueous phase.

Buttermilk contains caseins, whey proteins, lactose, minerals, and residual fat together with MFGM-associated proteins and PL (Ali, 2019; Haddadian et al., 2018). The gross composition of buttermilk is generally comparable with skimmed milk, and data are summarised in Table 1.3.

Table 1.3. **The composition of buttermilk /** *1.3. tabula* ***Paniņu sastāvs***

Source / *Avots*	Buttermilk type / *Paniņu veids*	Total solids, g 100 g^{-1} product / *Kopējā sausna,* g 100 g^{-1} *produkta*	Protein, g 100 g^{-1} product / *Olbaltumvielas,* g 100 g^{-1} *produkta*	Fat, g 100 g^{-1} product / *Tauki,* g 100 g^{-1} *produkta*	Lactose, g 100 g^{-1} product / *Laktoze,* g 100 g^{-1} *produkta*
Lambert et al. (2016)	Industrial sweet buttermilk / *Komerciālās* paniņas	8.70 ± 0.80	2.90 ± 0.30	0.90 ± 0.40	NR
Gebreselassie et al. (2016)	Naturally fermented traditional buttermilk / *Dabīgi raudzētas paniņas*	NR	3.04 ± 0.18	1.17 ± 0.68	3.61 ± 0.54
Sakkas et al. (2022)	Sweet buttermilk from non-frozen cream / *Paniņas, iegūtas no nesaldēta krējuma*	10.34 ± 0.26	3.28 ± 0.07	0.91 ± 0.35	4.99 ± 0.08
Dushkova et al. (2023)	Industrial sweet buttermilk / *Komerciālās paniņas*	8.93	2.70	0.75	NR

Note. Values follow the original studies, with mean ± SD where reported. NR - not reported. Differences in product type and analytical procedures limit direct comparison between studies. / *Piezīme. Vērtības norādītas atbilstoši oriģinālajiem pētījumiem; vidējā vērtība ± SD sniegta, ja tā pieejama avotā. NR - nav norādīts. Produktu veidu un analīzes metožu atšķirības ierobežo pētījumu rezultātu tiešu salīdzināšanu.*

The term buttermilk is used in the literature for technologically distinct dairy products. Sweet buttermilk is the aqueous phase released during the churning of non-acidified cream, whereas cultured buttermilk includes a fermentation step involving selected lactic acid bacteria and may be produced from cultured cream or from a standardised dairy base. Buttermilk powder is obtained by evaporating water, while MFGM-enriched ingredients undergo additional separation or concentration procedures. These differences in raw material, fermentation status, water content, and processing conditions may affect gross composition, acidity, residual fat, protein interactions, and the concentration of MFGM-associated components (Dushkova et al., 2023; Rose et al., 2023; Sakkas et al., 2022; Señoráns, Calvo, et al., 2025). The data show that buttermilk is not compositionally uniform. Differences in total solids, protein, fat, and lactose reflect both the product's origin and the efficiency of the butter manufacturing process.

Reported concentrations and relative proportions of PC, PE, and SM in buttermilk vary with product origin, processing conditions, and the analytical methods used. Published values for total PL and individual lipid classes in buttermilk are summarised in Table 1.4.

Table 1.4. **PL concentration of buttermilk and buttermilk-derived products /** *1.4. tabula* ***PL saturs paniņās un paniņu produktos***

Source / *Avots*	Buttermilk / *Paniņu veids*	PL concentration/ *PL saturs*	Reporting basis / *Rezultāti izteikti*	Notes / *Piezīmes*
Lambert et al. (2016)	Industrial sweet buttermilk / *Komerciālās paniņas*	1.2 ± 0.1 g 100 g^{-1}	Dry matter / *Sausnā*	NR
Sakkas et al. (2022)	Sweet buttermilk from non-frozen cream / *Paniņas, iegūtas no nesaldēta krējuma*	0.037 ± 0.024 g 100 g^{-1} product / *produkta*; 0.313 ± 0.171 g 100 g^{-1} dry matter / *sausnā*; 1.92 ± 0.54 g 100 g^{-1} fat / *tauku*	PL concentration expressed on three reference bases: on product weight, dry matter, and total fats / *PL saturs izteikts, izmantojot trīs atskaites sistēmas: produktā, sausnā un taukos*	NR
Calvo et al. (2020)	Buttermilk from butter and butter-oil manufacture / *Paniņas iegūtas sviesta un sviesta eļļas ražošanā*	12^{-1}6 % of total lipids / *no kopējiem lipīdiem*	Proportion of total lipids / *Procentuāli no kopējiem lipīdiem*	PC, PE, and SM are major analyzed PL classes / *PC, PE un SM ir galvenās analizētas PL klases*
Ferraris et al. (2020)	Commercial buttermilk powder / *Sausās paniņas*	1.71 ± 0.03 g 100 g^{-1}	Powder / *Sausā produktā*	Quantified phospholipids and sphingolipids / *Noteikti fosfolipīdi un sfingolipīdi*
Dushkova et al. (2023)	Industrial sweet buttermilk / *Komerciālās paniņas*	Total phospholipids / *Kopējie fosfolipīdi*: 123 ± 3 mg 100 g^{-1}	Product / *Produktā*	PC: 26.7 ± 0.5; PE: 28.5 ± 0.6; SM: 17.1 ± 0.2 mg 100 g^{-1}

Note. NR - not reported. Values retain the original reporting basis (product, dry matter, powder or total lipids); values expressed on different bases are not directly comparable. / *Piezīme. NR - nav norādīts. Vērtības izteiktas atbilstoši oriģinālajā avotā izmantotajam rezultātu izteikšanas pamatam (produktā, sausnā, sausajā produktā vai kopējos lipīdos); vērtības ar atšķirīgu izteikšanas pamatu nav tieši salīdzināmas.*

MFGM-associated components contribute to the technological properties of buttermilk and its products. Its emulsifying behaviour, emulsion stability, viscosity, and foaming properties reflect the combined effects of PL, MFGM proteins, casein, whey proteins, minerals, and their interactions at oil-water and air-water emulsions (Ramos de Paula et al., 2025; Sakkas et al., 2022).

Processing and fractionation can substantially modify the concentration basis of the reported PL values. In sweet buttermilk, ultrafiltration increased total phospholipid concentration by up to 2.8-fold relative to the initial liquid product. Similarly, extraction from buttermilk powder and the production of MFGM-enriched isolates generated fractions with markedly higher proportions of PL than untreated buttermilk. Therefore, PL concentrations reported for powders, ultrafiltration retentates, or isolated MFGM fractions cannot be directly applied to an untreated commercial cultured buttermilk (Dushkova et al., 2023; Señoráns, Calvo, et al., 2025; Ubeyitogullari & Rizvi, 2020).

These technological properties support the application of buttermilk in fermented dairy products, bakery products, beverages, emulsions, and other food matrices. Buttermilk also serves as a raw material for the recovery of MFGM compounds. Microfiltration, ultrafiltration, diafiltration, centrifugation, and related fractionation procedures can increase the concentration of membrane-associated proteins and PL, producing ingredients that differ compositionally from untreated buttermilk (Iung et al., 2024; Señoráns, Calvo, et al., 2025).

1.3. Milk polar lipids in nutrition */ Piena polārie lipīdi uzturā*

Milk PL are primarily associated with the MFGM and can be recovered from membrane-rich streams, particularly buttermilk and butter serum, to produce MFGM-enriched food ingredients. The major milk PL classes, PC, PE and SM, differ in structure, digestion and metabolic fate: PC and SM provide choline-containing metabolites, whereas PE provides ethanolamine and may contain polyunsaturated fatty acids (PUFA) in its acyl chains. These classes may influence intestinal lipid digestion, cholesterol absorption, membrane function and lipoprotein metabolism, but their individual effects are difficult to distinguish because most human studies have used whole milk PL fractions or MFGM-enriched products rather than isolated PC, PE or SM (Calvo et al., 2023; Le Barz et al., 2021; L.-Y. Lin et al., 2019; Pokala et al., 2023; Vors et al., 2020; Zajac et al., 2025).

Table 1.5 summarises human intervention studies with milk PL or MFGM in which plasma lipids were measured, identified through a structured literature search and narrative synthesis, and ordered by daily PL dose. Milk PL at 3-5 g day^{-1} in cream cheese reduced cholesterol and lipid risk markers in postmenopausal women after 4 weeks (Le Barz et al., 2021; Vors et al., 2020), and 1.7-4.0 g day^{-1} in fortified milk powder reduced TC and LDL-C in older adults after 16 weeks (Zajac et al., 2025), whereas 2-3 g day^{-1} in milk for 7-8 weeks had no effect on plasma lipids in overweight men (Weiland et al., 2016), 2.3 g day^{-1} for 2 weeks did not change TG in adults with the metabolic syndrome (Pokala et al., 2023), and about 0.4 g day^{-1} from a buttermilk-derived MFGM-enriched milk for 14 weeks did not change plasma lipids in adults over 65 years (Calvo et al., 2023).

Table 1.5. **Human intervention studies with milk PL or MFGM reporting plasma lipid outcomes: design, dose, duration and results /** *1.5. tabula* ***Intervences pētījumi cilvēkiem ar piena PL vai MFGM, kuros novērtēts plazmas lipīdu profils: dizains, deva, ilgums un rezultāti***

Reference / *Atsauce*	Design and population / *Dizains un populācija*	Product / *Produkts*	Daily PL dose / *PL deva dienā*	Duration / *Ilgums*	Lipid profile related and other outcomes / *Ar lipīdu profilu saistītie un citi rezultāti*
Vors et al. (2020)	Double-blind parallel RCT; 58 postmenopausal women with overweight / *Dubultakls paralēls RCT; 58 sievietes pēcmenopauzes periodā ar lieko ķermeņa masu*	Cream cheese enriched with milk PL / *Ar piena PL bagātināts krēmsiers*	0, 3 or 5 g milk PL / *0, 3 vai 5 g piena PL*	4 weeks / *4 nedēļas*	TC/HDL-C and ApoB/ApoA1 ratios reduced; fewer chylomicron particles at 5 g; intestinal cholesterol absorption reduced / *Samazinājās TC/HDL-C un ApoB/ApoA1 attiecība; pie 5 g mazāk hilomikronu daļiņu; samazinājās holesterīna absorbcija zarnās*
Le Barz et al. (2021)	Secondary analysis of Vors et al. (2020) trial; 58 postmenopausal women / *Vors et al. (2020) pētījuma sekundārā analīze; 58 sievietes pēcmenopauzes periodā*	Cream cheese enriched with milk PL / *Ar piena PL bagātināts krēmsiers*	0, 3 or 5 g milk PL / *0, 3 vai 5 g piena PL*	4 weeks / 4 nedēļas	Atherogenic serum ceramide and sphingomyelin reduction; changes correlated with the reduction of TC, LDL-C and ApoB; faecal ceramide excretion increased / *Samazinājās aterogēnie ceramīdi un sfingomielīns serumā; izmaiņas korelēja ar TC, LDL-C un ApoB samazinājumu; palielinājās ceramīdu izdalīšanās ar fēcēm*
Zajac et al. (2025)	Multicentre double-blind parallel RCT; 263 adults aged 55-75 y with subjective memory complaints / *Daudzcentru dubultakls paralēls RCT; 263 pieaugušie vecumā 55-75 g. ar subjektīvām sūdzībām par atmiņu*	Milk powder fortified with MFGM / *Ar MFGM bagātināts sausais piens*	1.7 or 4.0 g PL / *1.7 vai 4.0 g PL*	16 weeks / 16 nedēļas	TC and LDL-C reduced at both doses; TNF-α reduced and subjective alertness better maintained at 4.0 g; no effect on the primary cognitive outcome / *Abas devas samazināja TC un LDL-C; pie 4.0 g samazinājās TNF-α un labāk saglabājās subjektīvā modrība; primārais kognitīvais iznākums neuzlabojās*

Table 1.5 continuation / *1.5. tabulas turpinājums*

Reference / *Atsauce*	Design and population / *Dizains un populācija*	Product / *Produkts*	Daily PL dose / *PL deva dienā*	Duration / *Ilgums*	Main outcomes / *Galvenie rezultāti*
Weiland et al. (2016)	Two double-blind parallel RCTs; 62 and 57 men with overweight or obesity / *Divi dubultakli paralēli RCT; 62 un 57 vīrieši ar lieko ķermeņa masu vai aptaukošanos*	Milk enriched with milk PL versus milk fat (trial 1) or soya PL (trial 2) / *Ar piena PL bagātināts piens salīdzinājumā ar piena taukiem (1. pētījums) vai sojas PL (2. pētījums)*	2 g milk PL (trial 1); 3 g milk PL versus 2.8 g soya PL (trial 2) / *2 g piena PL (1. pētījums); 3 g piena PL salīdzinājumā ar 2.8 g sojas PL (2. pētījums)*	8 weeks; 7 weeks / *8 nedēļas; 7 nedēļas*	No effect on TC, LDL-C, HDL-C, TG, ApoB, ApoA1, glucose or insulin; waist circumference and γ-glutamyl transferase reduced versus control / *Nav ietekmes uz TC, LDL-C, HDL-C, TG, ApoB, ApoA1, glikozi vai insulīnu; samazinājās vidukļa apkārtmērs un γ-glutamiltransferāze salīdzinājumā ar kontroli*
Pokala et al. (2023)	Randomised crossover with controlled diet; 24 adults with the metabolic syndrome (11 men, 13 women) / *Randomizēts krusteniskais pētījums ar kontrolētu uzturu; 24 pieaugušie ar metabolo sindromu (11 vīrieši, 13 sievietes)*	MFGM-enriched milk versus a soya-lecithin beverage / *Ar MFGM bagātināts piens salīdzinājumā ar sojas lecitīnu dzērienu*	2.3 g milk PL / *2.3 g piena PL*	2 weeks per period / *2 nedēļas katrā periodā*	No effect on fasting or postprandial TG, glucose, incretins, endotoxaemia or intestinal permeability / *Nav ietekmes uz TG, glikozi, inkretīniem, endotoksēmiju vai zarnu caurlaidību tukšā dūšā un postprandiāli*

Table 1.5 continuation / *1.5. tabulas turpinājums*

Reference / *Atsauce*	Design and population / *Dizains un populācija*	Product / *Produkts*	Daily PL dose / *PL deva dienā*	Duration / *Ilgums*	Main outcomes / *Galvenie rezultāti*
Calvo et al. (2023)	Double-blind parallel pilot RCT; 44 adults aged over 65 y, healthy or with mild cognitive impairment / *Dubultakls paralēls pilota RCT; 44 pieaugušie vecumā virs 65 g., veseli vai ar vieglu kognitīvu traucējumu*	Buttermilk-derived MFGM-enriched milk, 200 mL day^{-1} (2.79 % fat, PL 6.77 % of fat), versus skimmed milk / *No paniņām iegūts ar MFGM bagātināts piens, 200 mL dienā (2.79 % tauku, PL 6.77 % no taukiem), salīdzinājumā ar vājpienu*	0.38 g milk PL / *0.38 g piena PL*	14 weeks / *14 nedēļas*	Short-delay cued recall improved in women; no between-group difference in TC, LDL-C, HDL-C, TG or anthropometry / *Sievietēm uzlabojās atmiņa ar norādi pēc īsa intervāla; nebija atšķirību starp grupām TC, LDL-C, HDL-C, TG vai antropometrijā*

Note. Findings summarise individual studies and are not pooled effect estimates. / *Piezīme. Apkopoti atsevišķu pētījumu rezultāti, nevis apvienoti efekta novērtējumi.*

The doses indicated in Table 1.5 range from 0.4 to 5 g PL day^{-1} and the durations from 2 to 16 weeks; lipid-lowering effects were observed only at 1.7 g day^{-1} and above, after 4 to 16 weeks of intake, whereas 0.4 g day^{-1} for 14 weeks did not change plasma lipids (Calvo et al., 2023). The 28-day period of the present study corresponds to the shortest duration at which a lipid-lowering effect of milk PL has been reported (Vors et al., 2020) and was chosen to detect early changes in the plasma lipid profile while limiting changes in dietary behaviour unrelated to the intervention. The daily portion of cultured buttermilk used in the present study provides 0.07-0.08 g of quantified PL (Section 3.2), which is one to two orders of magnitude below the doses of the enriched products; the intervention therefore tests a whole-food exposure at a habitual dietary level, not a supplementation effect.

Among the proposed mechanisms, the interaction of MFGM-associated SM with cholesterol in the intestinal lumen has the most direct human support: in the trial of Vors et al. (2020), ileostomy studies showed reduced intestinal cholesterol absorption and increased ileal cholesterol efflux, consistent with co-excretion of cholesterol and milk SM, and the secondary lipidomic analysis of the same trial associated the reductions in atherogenic circulating SM and ceramide species with the improvements in TC, LDL-C and ApoB (Le Barz et al., 2021). Effects on the intestinal barrier and the gut microbiota rest mainly on preclinical evidence: milk complex lipid fractions and milk SM improved selected barrier-related markers in Caco-2 cells and mice without consistent improvement of permeability assays (Anderson et al., 2018; Milard et al., 2019), milk PL improved the survival of *Bifidobacterium longum subsp. infantis* during simulated digestion (Kosmerl et al., 2021), and in an inflammatory mouse model milk PL altered tight junction proteins and hepatic lipid pathways without preventing the increase in gut permeability (Zhou & Ward, 2024). In humans, neither changes in major bacterial populations or faecal short-chain fatty acids nor improved gut barrier function have been demonstrated (Pokala et al., 2023; Vors et al., 2020).

The human trials have thus used MFGM- or milk PL-enriched products providing about 0.4-5 g PL day^{-1} in defined groups, mainly postmenopausal women, older adults and adults with overweight or the metabolic syndrome, whereas evidence on habitual exposure to naturally occurring milk PL from the whole products remains very limited, particularly in perimenopausal women. This gap supports the approach of the present thesis, which distinguishes between the experimentally determined PL composition of dairy products, the PL dose provided by the cultured buttermilk intervention product and the estimated habitual milk PL exposure derived from individual dairy intake; these measures are interpreted separately because product composition, processing-related redistribution, seasonal variation, habitual diet, baseline plasma lipid profile and individual variability may all influence the observed associations.

1.4. Women's health and dietary habits / Sievietes veselība un uztura paradumi

Women in perimenopause represent a clinically relevant population for CVD risk assessment because the menopausal transition coincides with changes in body composition and lipid metabolism. Perimenopause encompasses the menopausal transition and the first 12 months after the final menstrual period, although its duration varies between women (Harlow et al., 2012). Longitudinal evidence indicates that this period is associated with increasing central adiposity and adverse changes in TC, LDL-C, TG and vascular health, while the effects of ovarian ageing cannot be completely separated from chronological ageing (El Khoudary et al., 2020; Greendale et al., 2019). These changes provide a direct rationale for evaluating dietary exposures and plasma lipid outcomes in the perimenopausal women included in the present study.

The 2026 International Menopause Society recommendations continue to identify the Stages of Reproductive Aging Workshop + 10 criteria (STRAW+10; Harlow et al., 2012) as the gold-standard framework for menopause staging. The present literature review identified

no formally endorsed successor; STRAW+10 therefore remains the appropriate framework for this thesis. Nevertheless, STRAW+10 should be applied with its limitations clearly stated. Menstrual-cycle criteria may be unavailable or altered in women using hormonal contraception or menopausal hormone therapy, after hysterectomy, or in the presence of chronic menstrual irregularity. In such cases, clinical history and supportive endocrine criteria may assist staging, but no single hormone measurement should be treated as a definitive diagnostic test. Menopausal symptoms may also begin before the menstrual criteria for stage -2 are met (International Menopause Society, 2026). The simplified staging sequence is presented in Fig. 1.4.

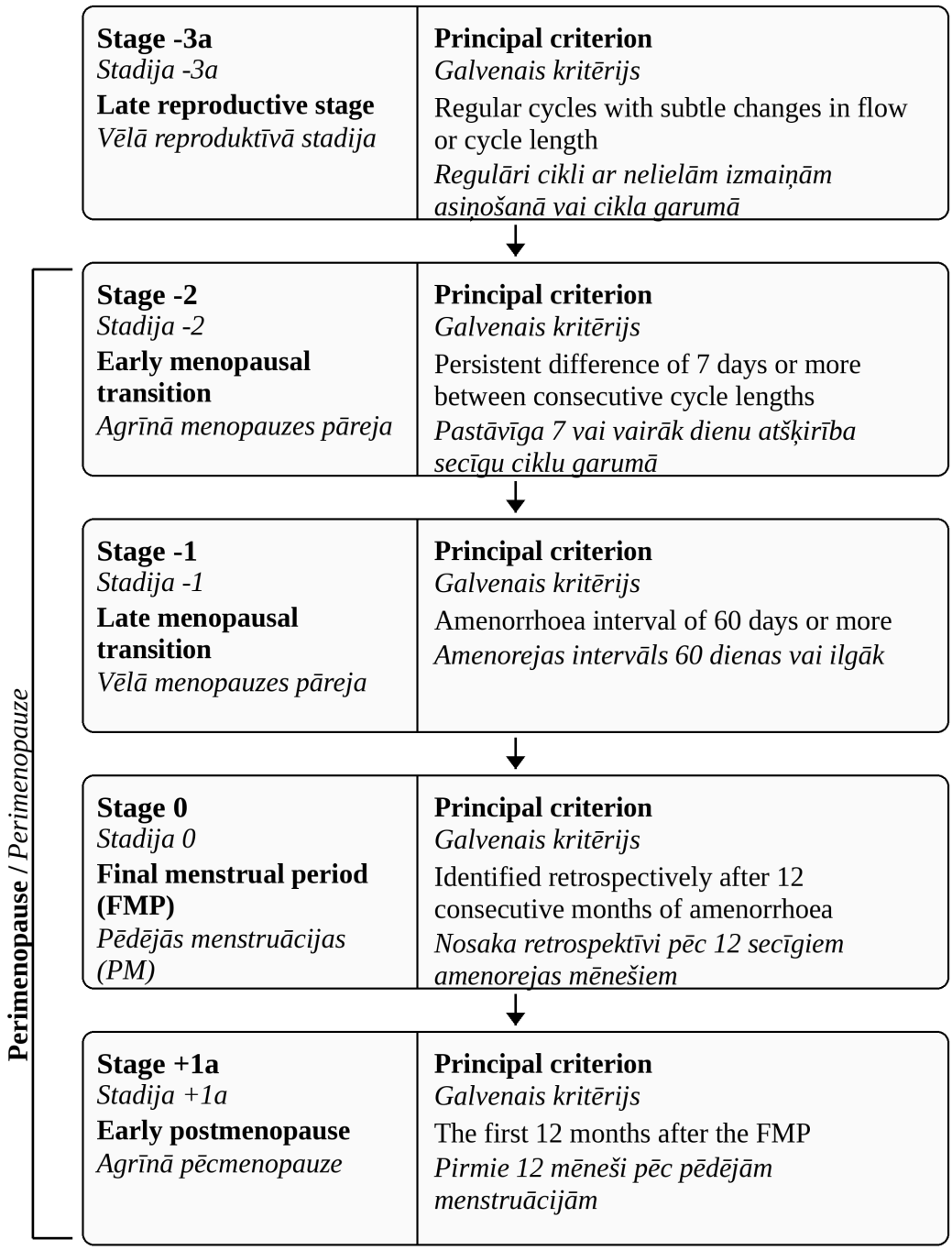


Fig. 1.4. **Simplified STRAW+10 staging of the menopausal transition and perimenopause** / *1.4. att.* ***Vienkāršota STRAW+10 menopauzes pārejas un perimenopauzes posmu shēma***

(adapted from / *pielāgots pēc* Harlow et al. (2012); International Menopause Society (2026))

CVD remains a major cause of mortality among women. In the European Union (EU-27), diseases of the circulatory system accounted for 1.68 million deaths, or 32.7 % of all registered deaths, in 2022. Ischaemic heart disease was the leading cause of death among women aged 65 years and older, with an age-standardised rate of 382.0 deaths per 100 000 women in this age group (Eurostat, 2025). Globally, approximately 275 million women were estimated to be living with CVD in 2019, and CVD accounted for approximately 8.9 million deaths and 35 % of all deaths among women (European Society of Cardiology, 2026; Eurostat, 2025; Vogel et al., 2021). These data describe mortality rather than disease prevalence and primarily reflect outcomes at older ages; nevertheless, they support the importance of identifying modifiable CVD risk factors earlier in women's lives.

The burden is particularly relevant in Latvia. According to the national cause-of-death register, 13 574 deaths from diseases of the circulatory system were recorded in 2024, corresponding to 51.6 % of all registered deaths in that year (Central Statistical Bureau of Latvia & Centre for Disease Prevention and Control, 2025). This national estimate includes both sexes and all age groups and should not be interpreted as the prevalence of coronary heart disease among perimenopausal women. A sufficiently recent nationally representative estimate of diagnosed coronary heart disease specifically among Latvian women aged approximately 45-54 years was not identified. European and global evidence confirms that CVD represents a substantial burden among women (European Society of Cardiology, 2026; Eurostat, 2025; Vogel et al., 2021).

The menopausal transition is considered a clinically relevant period for CVD prevention because longitudinal evidence demonstrates adverse changes in visceral adiposity, TC, LDL-C, TG and vascular health during midlife, although chronological ageing and ovarian ageing cannot be completely separated (El Khoudary et al., 2020). Latvian population data indicate that cardiometabolic risk factors are already common in the age group corresponding most closely to the present study. In the Health Behaviour among Latvian Adult Population survey conducted in 2024, measured anthropometry showed that 34.9 % of women aged 45-54 years had overweight and 29.4 % had obesity. Thus, 64.3 % were above the normal body mass index range (Grīnberga et al., 2025). In the national study on salt and iodine consumption in the Latvian adult population, conducted by BIOR in 2018-2020, 45 % of participating women aged 19-64 years reported that hypertension had been diagnosed at some point in their lives (Siksna et al., 2020). The age ranges and methods differ, but both sources show that excess body weight and elevated blood pressure are relevant background factors in Latvian women. Against this context, the elevated baseline TC, LDL-C and non-high-density lipoprotein cholesterol (non-HDL-C) concentrations observed in the present trial are clinically relevant markers of atherogenic lipid burden, even though the study was not designed to estimate population prevalence.

Diet is a modifiable component of CVD prevention and should be evaluated primarily as an overall dietary pattern rather than solely based on individual foods or nutrient intakes. For the Latvian population, the national healthy eating guidelines provide the primary framework for assessing dietary adequacy. Dairy products are established components of the Latvian diet, but recent sex-specific individual-level intake data are limited. The most recent quantitative data come from the national dietary survey of 2018-2020, which included 1 011 adults aged 19-64 years, of whom 541 were women, selected using a stratified two-stage cluster sampling procedure (Siksna et al., 2020). Mean intake of milk and dairy products was 185.4 g day^{-1} among women and 204.1 g day^{-1} in the total sample; the report also indicated that younger women consumed the lowest amounts and the researchers concluded that dairy intake was generally insufficient relative to national recommendations (Siksna et al., 2020). These estimates combine different dairy products and do not provide a product-specific intake for cultured buttermilk. They should also not be directly compared with the intake derived from the food frequency questionnaire (FFQ) in the present thesis because the dietary assessment methods, food-group definitions and reference periods differed. The more recent Health

Behaviour among Latvian Adult Population survey of 2024 provides frequency data only: 68.9 % of women aged 45-54 years reported drinking no milk, 48.4 % consumed at least one 200 mL glass of fermented milk products (kefir, cultured buttermilk, yoghurt and similar products) daily, and 62.1 % ate cheese or curd on three or more days of the previous week (Grīnberga et al., 2025). Because that survey records neither portion weights nor individual products, it cannot quantify dairy intake.

Latvian healthy eating recommendations advise adults to consume two to three portions of milk or dairy products daily, preferably lower-fat products. One glass of an unsweetened fermented milk product, including kefir, cultured buttermilk or plain yoghurt, is defined as one portion (Veselības ministrija, 2020a). However, total dairy intake does not capture the nutritional heterogeneity of milk, fermented milk products, curd, cheese, cream and butter. These products differ in saturated fatty acid content, processing technology, milk fat globule membrane or its fragment content and PL composition. Consequently, total dairy intake cannot be assumed to represent exposure to milk PL. This distinction directly supports the analytical approach of the present thesis, which combines individual dairy intake with season-specific PL composition rather than treating all dairy products as nutritionally equivalent.

In the present thesis, the habitual dietary intake of the participants (Section 3.5) is interpreted against the Latvian healthy eating guidelines and the Nordic Nutrition Recommendations 2023, which serve as the reference framework for adults and are not menopause-specific treatment targets. The Latvian guidelines recommend, per day, five or more portions (approximately 500 g) of vegetables, fruit and berries and four to six portions of cereal products and potatoes with preference for whole grain, fish at least twice a week, regular consumption of pulses, lean meat, eggs, nuts and seeds, replacement of animal fats by vegetable oils, and restriction of foods high in salt, added sugar and saturated fat (Veselības ministrija, 2020a). The updated Latvian reference intakes set dietary fibre at not less than 25 g day^{-1} for women, SFA at no more than 10 % of energy and sugars below 10 % of energy, with a further reduction towards 5 % regarded as beneficial (Veselības ministrija, 2025). The Nordic Nutrition Recommendations 2023 follow the same direction and provide the quantitative reference ranges applied in Table 3.14: 500-800 g day^{-1} of vegetables, fruit and berries, 300-450 g $week^{-1}$ of fish and seafood, and 10-20 % and $5^{-1}0$ % of energy from MUFA and PUFA, respectively (Nordic Council of Ministers, 2023). These reference values are used in Section 3.5 to describe the adequacy of participants' habitual diet and, thus, the dietary background against which the intervention and the estimated milk PL exposure are interpreted.

For lipid metabolism, the type of fibre is as important as the total amount. Pooled data from 181 randomised trials with 14 505 participants indicate a modest, dose-dependent reduction in TC and LDL-C with soluble fibre (Ghavami et al., 2023); and a separate synthesis of 89 trials attributed to viscous fibre, at a median dose of 7.0 g day^{-1}, found decreases of 0.26 mmol L^{-1} in LDL-C, 0.33 mmol L^{-1} in non-HDL-C and 0.04 g L^{-1} in apolipoprotein B (ApoB) relative to non-viscous cereal fibre (Jovanovski et al., 2023). The Latvian and Nordic targets for total fibre should therefore be read alongside the food sources of viscous soluble fibre (oats, barley, pulses, fruit and certain seeds), bearing in mind that fibre is only one element of a lipid-lowering dietary pattern.

Fish and seafood, particularly fatty fish, are dietary sources of the omega-3 PUFA eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA). The Nordic Nutrition Recommendations 2023 recommend regular fish consumption, which is also characteristic of Mediterranean dietary patterns (Nordic Council of Ministers, 2023). In a meta-analysis of randomised controlled trials in postmenopausal women, omega-3 fatty acid supplementation reduced TG concentrations, did not significantly affect TC, and modestly increased HDL-C and LDL-C concentrations (J. Wang et al., 2023). A larger, continuous dose-response meta-analysis of 90 randomised trials found near-linear reductions in TG and non-HDL-C, with more pronounced effects at intakes above 2 g day^{-1} and among participants with hyperlipidaemia, overweight, or obesity (T. Wang et al., 2023). Because many trial doses exceed amounts

obtainable from habitual dietary intake, these findings should not be translated into a general recommendation for unsupervised high-dose supplementation.

During midlife, physical activity influences body composition, insulin sensitivity, blood pressure, and lipid metabolism. The World Health Organization (2020) recommends that adults accumulate 150-300 min of moderate-intensity or $75^{-1}50$ min of vigorous-intensity aerobic activity per week, or an equivalent combination of both, and train the major muscle groups on at least two days a week. In a 4-year longitudinal study of 298 women aged 48-55 years, higher physical activity was associated with a more favourable metabolic profile and body composition throughout the menopausal transition, although it did not eliminate all menopause-related changes (Hyvärinen et al., 2022). A meta-analysis of 38 randomised trials involving 1407 postmenopausal women found that exercise improved blood pressure and several lipid parameters, with effects varying by exercise modality (Xin et al., 2022). A further meta-analysis of resistance-training trials reported reductions in TC, LDL-C, and TG concentrations, whereas the effect on HDL-C was small and inconsistent (He et al., 2023).

Diet and physical activity occur within a broader lifestyle context. Smoking, alcohol intake, sleep duration and quality, psychological stress, and sedentary behaviour may influence weight regulation and cardiometabolic risk, and so may also cluster with dietary habits. A recent review of non-pharmacological interventions in menopausal health supports an integrated lifestyle approach that includes dietary quality, regular physical activity, sleep and stress management, tobacco avoidance, and moderation of alcohol intake (Anekwe et al., 2025). However, the magnitude and direction of associations with individual lipid parameters vary across studies, so these behaviours should be treated as potential covariates rather than assumed explanations for a specific lipid change.

Taken together, the substantial CVD burden in Latvia, the high prevalence of excess body weight among women aged 45-54 years, and the elevated baseline atherogenic lipid concentrations observed in the present study support the relevance of CVD risk assessment during perimenopause. Available Latvian data confirm that dairy products are regularly consumed but provide limited information on the intake of individual dairy products and do not quantify habitual milk PL exposure. This evidence gap supports the approach used in the present thesis, which combined individual dietary data with season-specific measurements of milk PL composition. However, the population statistics provide epidemiological context only and do not demonstrate that cultured buttermilk or naturally occurring milk PL reduce CVD risk or improve the plasma lipid profile.

Summary of literature review / *Literatūras apskata kopsavilkums*

Milk PL are quantitatively minor but structurally and functionally important components of the MFGM. Their concentration and relative distribution in milk and dairy products depend on the composition of raw milk, season, milk fat globule characteristics, and milk processing. Milk separation, cream thermal treatment, cooling, maturation, and churning redistribute membrane-associated lipids among skimmed milk, cream, butter, and buttermilk. Consequently, total fat concentration alone does not adequately characterise a dairy product as a PL source. Published values are also difficult to compare because PL studies use different analytical methods. Systematic data on the distribution of PC, PE, and SM across products from the same milk-processing chain and across different seasons remain limited, particularly in Latvia.

Buttermilk is a source of MFGM-associated PL because cream churning disrupts the MFGM, transferring material into the aqueous phase. Therefore, buttermilk contains appreciable amounts of PL despite its relatively low total fat concentration. However, published evidence is dominated by sweet buttermilk, buttermilk powders, and isolated or concentrated MFGM fractions. Because these products differ in fermentation status, water content, processing conditions, and degree of fractionation or concentration, their reported

compositional values should not be assumed to be directly transferable to commercially available cultured buttermilk. The PL concentration of cultured buttermilk cannot be reliably predicted from its declared fat concentration, product category, or literature values obtained for other buttermilk products. Evaluating cultured buttermilk as a dietary source of milk PL requires product- and season-specific determination of total PL, PC, PE, and SM.

Human studies provide biological plausibility that milk PL may influence intestinal cholesterol absorption and selected circulating lipid markers. Nevertheless, most intervention studies have used isolated PL fractions or MFGM-enriched products providing approximately 2.3-5.0 g of milk-derived PL or phospholipids per day and have mainly included postmenopausal women, individuals with overweight, or adults with metabolic syndrome. Results have varied across intervention products, study populations, doses, and measured outcomes. Therefore, these findings cannot be directly extrapolated to the lower and more variable milk PL exposure associated with habitual consumption of commonly consumed dairy products. Evidence on cultured buttermilk as a whole-food intervention for perimenopausal women remains particularly limited (Le Barz et al., 2021; Pokala et al., 2023; Venkat et al., 2024; Vors et al., 2020).

A further methodological gap concerns estimating habitual milk PL exposure. Dietary questionnaires provide information on the quantity and frequency of dairy product consumption but do not directly measure PL intake. Laboratory analyses provide product composition data but do not indicate how much of each product an individual consumes. Total dairy intake is therefore an inadequate proxy for milk PL exposure because dairy products differ in their PL concentrations, class distributions, fat concentration, and processing. Reliable exposure estimation requires integrating individual dietary intake data with product- and season-specific PL composition. In the literature reviewed for this thesis, no previous study in Latvia integrated these analytical and dietary data to estimate habitual intake of total milk PL, PC, PE, and SM.

During the menopausal transition, body composition, lipid metabolism and CVD risk change. The plasma lipid profile, however, is determined not by a single food or nutrient but by baseline lipid concentrations, the habitual diet, body composition, physical activity, season and other lifestyle factors acting together (El Khoudary et al., 2020; Sharma et al., 2022). The research uses an integrated design that links dairy product composition, processing and season effects, the PL composition of cultured buttermilk, habitual dietary intake, and the plasma lipid profile. This design allows evaluation of the compositional and nutritional relevance of cultured buttermilk without attributing plasma lipid-lowering or therapeutic properties to the product when the evidence does not support them.

Piena PL ir kvantitatīvi nelielas, taču strukturāli un funkcionāli nozīmīgas MFGM sastāvdaļas. To koncentrācija un relatīvais sadalījums pienā un piena produktos ir atkarīgs no svaigpiena sastāva, sezonas, piena tauku lodīšu īpašībām un piena pārstrādes. Piena separēšana, krējuma termiskā apstrāde, dzesēšana, nogatavināšana un kulšana izraisa ar membrānu saistīto lipīdu pārdali starp vājpienu, krējumu, sviestu un paniņām, tādēļ kopējā tauku koncentrācija vien nav pietiekama, lai raksturotu piena produktu kā PL avotu. Arī publicētās vērtības ir grūti salīdzināt, jo PL pētījumos izmantotas atšķirīgas analītiskās metodes. Sistemātisku datu par PC, PE un SM sadalījumu vienas piena pārstrādes ķēdes produktos dažādās sezonās joprojām ir maz, īpaši Latvijā.

Paniņas ir ar MFGM saistīto PL avots, jo krējuma kulšanas laikā MFGM tiek izjaukta un tās materiāls nonāk ūdens fāzē. Tādēļ paniņas satur ievērojamu PL daudzumu, neraugoties uz salīdzinoši zemo kopējo tauku koncentrāciju. Tomēr publicētajos pētījumos galvenokārt analizētas saldkrējuma paniņas, paniņu pulveri un izolētas vai koncentrētas MFGM frakcijas. Šie produkti atšķiras pēc tā, vai tie ir raudzēti, pēc ūdens satura, pārstrādes apstākļiem un frakcionēšanas vai koncentrēšanas pakāpes, tādēļ nevar pieņemt, ka publicētās to sastāva vērtības ir tieši attiecināmas uz tirdzniecībā pieejamām raudzētām paniņām. Raudzētu paniņu PL koncentrāciju nevar ticami prognozēt pēc deklarētās tauku koncentrācijas, produkta

kategorijas vai literatūrā norādītajām vērtībām par citiem paniņu produktiem. Vērtējot raudzētas paniņas kā piena PL avotu uzturā, kopējo PL, PC, PE un SM koncentrācija jānosaka konkrētajam produktam attiecīgajā sezonā.

Pētījumi ar cilvēkiem sniedz bioloģisku pamatojumu iespējamai piena PL ietekmei uz holesterīna uzsūkšanos zarnās un atsevišķiem cirkulējošo lipīdu rādītājiem. Tomēr lielākajā daļā intervences pētījumu izmantotas izolētas PL frakcijas vai ar MFGM bagātināti produkti, kas nodrošina aptuveni 2,3-5,0 g piena izcelsmes PL vai fosfolipīdu dienā. Šajos pētījumos galvenokārt piedalījušās sievietes pēcmenopauzes periodā, personas ar lieko ķermeņa masu vai pieaugušie ar metabolisko sindromu. Rezultāti ir atšķirušies atkarībā no intervencē izmantotā produkta, pētījuma populācijas, devas un vērtētajiem rādītājiem. Tādēļ šos rezultātus nevar tieši attiecināt uz mazāku un mainīgāku piena PL uzņemšanu, kas saistīta ar ikdienā lietotu piena produktu patēriņu ierastajā uzturā. Pierādījumi par raudzētu paniņu izmantošanu uztura intervencē sievietēm perimenopauzē joprojām ir īpaši ierobežoti (Le Barz et al., 2021; Pokala et al., 2023; Venkat et al., 2024; Vors et al., 2020).

Vēl viena metodoloģiska nepilnība ir saistīta ar piena PL uzņemšanas novērtēšanu ar ierasto uzturu. Uztura anketas sniedz informāciju par piena produktu patēriņa daudzumu un biežumu, taču tieši nenosaka uzņemto PL daudzumu. Laboratoriskās analīzes sniedz datus par produktu sastāvu, bet nenorāda, cik daudz katra produkta patērē konkrētais indivīds. Tādēļ kopējais piena produktu patēriņš nav pietiekams aizstājējrādītājs piena PL uzņemšanai, jo piena produkti atšķiras pēc PL koncentrācijas, PL klašu sadalījuma, tauku koncentrācijas un pārstrādes. Ticamai PL uzņemšanas novērtēšanai nepieciešams apvienot individuālos uztura patēriņa datus ar konkrētajam produktam un sezonai raksturīgo PL sastāvu. Šajā promocijas darbā analizētajā literatūrā netika identificēts neviens iepriekš Latvijā veikts pētījums, kurā šie analītiskie un uztura dati būtu apvienoti, lai novērtētu kopējo piena PL, PC, PE un SM daudzumu uzturā.

Pārejas periodā uz menopauzi mainās ķermeņa sastāvs, lipīdu vielmaiņa un sirds un asinsvadu slimību risks. Tomēr plazmas lipīdu profilu nosaka nevis viens pārtikas produkts vai uzturviela, bet gan sākotnējās lipīdu koncentrācijas, uztura, ķermeņa sastāva, fizisko aktivitāšu, sezonas un citu dzīvesveida faktoru kopējā ietekme (El Khoudary et al., 2020; Sharma et al., 2022). Pētījumā izmantots integrēts dizains, kas sasaista piena produktu sastāvu, pārstrādes un sezonas ietekmi, raudzētu paniņu PL sastāvu, ierasto uztura patēriņu un plazmas lipīdu profilu. Šāds dizains ļauj izvērtēt raudzētu paniņu sastāvu un nozīmi uzturā, nepiedēvējot produktam plazmas lipīdu līmeni pazeminošas vai terapeitiskas īpašības, ja pierādījumi tās neapstiprina.

2. MATERIALS AND METHODS / *MATERIĀLI UN METODES*

2.1. Overall research design / *Pētījuma struktūra*

The research was conducted from 2022 to 2026. Laboratory analyses were carried out in the laboratories of the Latvia University of Life Sciences and Technologies, including the Microbiology Laboratory, the Dairy Pilot Plant at the Food Institute, and the Research Laboratory of Biotechnology. Dietary data were processed in cooperation with the Institute of Food Safety, Animal Health and Environment "BIOR".

The research comprised five stages. **The first stage** was a compositional study of milk and dairy products in two seasons (winter and spring). Raw milk was processed into skimmed milk, cream, butter and buttermilk, and the chemical composition and the concentration of total PL and of the major PL classes were determined in all products. Attention was given to PC, PE and SM, as these classes represent the major MFGM-associated PL by quantity and are relevant to lipid metabolism.

The second stage involved selecting the intervention product: commercially available cultured buttermilks were evaluated against predefined criteria, and the nutritional value, lactic acid bacteria count, and PL concentration of the selected product were characterised.

The third stage was a randomised controlled, food-based clinical trial in perimenopausal women with moderately elevated LDL-C concentration, preceded by a pilot feasibility study; the intervention group consumed the selected cultured buttermilk daily for 28 days, whereas the control group maintained their usual diet and lifestyle, and plasma lipid profile parameters and dietary intake were assessed before and after the intervention.

The fourth stage combined the season-specific PL composition of dairy products from the first stage with the FFQ-derived dairy intake from the clinical trial to estimate each participant's habitual milk PL exposure.

The fifth stage comprised statistical analyses of the compositional, clinical, dietary, and exposure data, including product and season comparisons, regression models for plasma lipid profile outcomes, and exploratory analyses of PL composition patterns. The compositional study and the clinical trial were conducted as independent lines of work that converge in the estimation of habitual milk PL exposure and in the statistical analysis; the thesis was therefore structured not only to evaluate cultured buttermilk as an intervention product but also to examine how dairy product composition, habitual dairy intake and plasma lipid outcomes relate within a single research framework.

The general structure of the research is presented in Fig. 2.1.

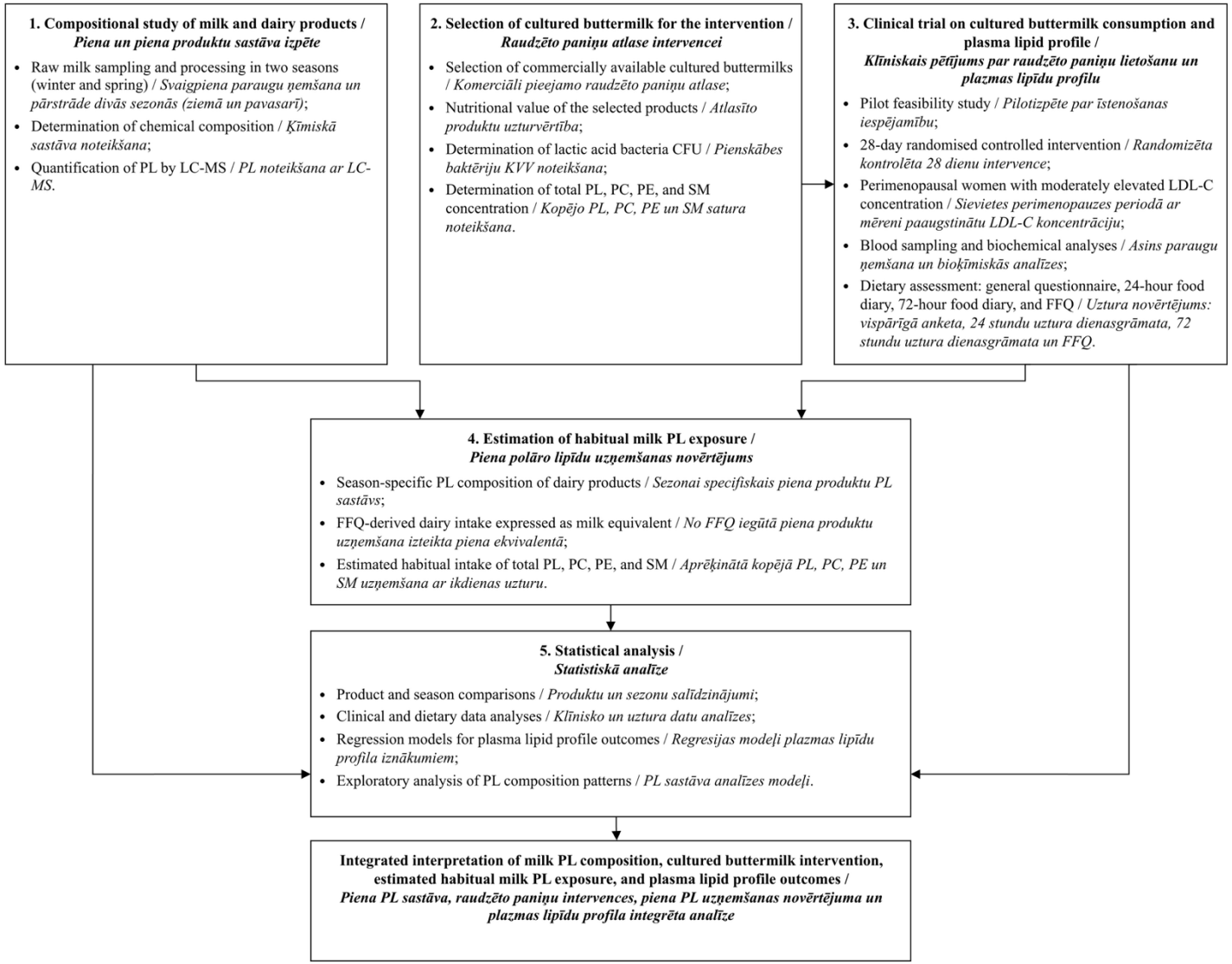


Fig. 2.1. **Overall research design of the study /** *2.1. att.* ***Pētījuma struktūra***

2.2. Compositional study of milk and dairy products / *Piena un piena produktu sastāva izpēte*

The compositional study characterised the distribution of MFGM PL among milk-processing products in two seasons, winter and spring. In each season, one batch of raw milk was processed into skimmed milk, cream, butter and buttermilk, and products were analysed. The buttermilk of the compositional study differs from the cultured buttermilk used as the intervention product (Section 2.3.1).

The compositional study provided the basis for the subsequent clinical and dietary-exposure sections. Since milk PL are primarily associated with the MFGM, their concentration in dairy products depends not only on the composition of raw milk but also on processing-related redistribution among processing products. Therefore, analysis of dairy products obtained during milk processing was necessary to understand the dietary PL exposure from consumed dairy products.

The results of this study were further used to support the selection and characterisation of cultured buttermilk for the intervention study and to estimate habitual exposure to milk PL among study participants. The methodological details of sampling, processing, chemical composition determination, and PL quantification are described in the following subsections.

2.2.1. Sampling and milk processing / *Paraugu ņemšana un piena pārstrāde*

Raw milk was obtained from Latvijas Piens, Ltd., a local dairy company, in two seasons: December 2023 (winter) and May 2024 (spring). In each season, the raw milk was processed at laboratory scale into the products used in the subsequent analyses: separation yielded skimmed milk and cream, and churning of the cream yielded butter and buttermilk. Because all products of a season originated from the same raw milk, the redistribution of milk components, including the lipid fraction and MFGM-associated PL, between the products could be followed directly.

Raw milk was warmed to 40 °C and passed through a laboratory cream separator (Armfield, Hampshire, UK), which yielded skimmed milk and cream. The cream was heat-treated at 90 ± 2 °C for 5 min, cooled and held at 6 ± 2 °C for 6 h for physical ripening. The ripened cream was brought to 12 °C and churned in a laboratory butter churn FJ 10 (Janschitz, Althofen, Austria) at $11^{-1}2$ °C until butter grains formed released buttermilk. The sweet buttermilk was drained off and used for analysis without any further treatment, and the grains were worked into a homogeneous butter mass (Fig. 2.2).

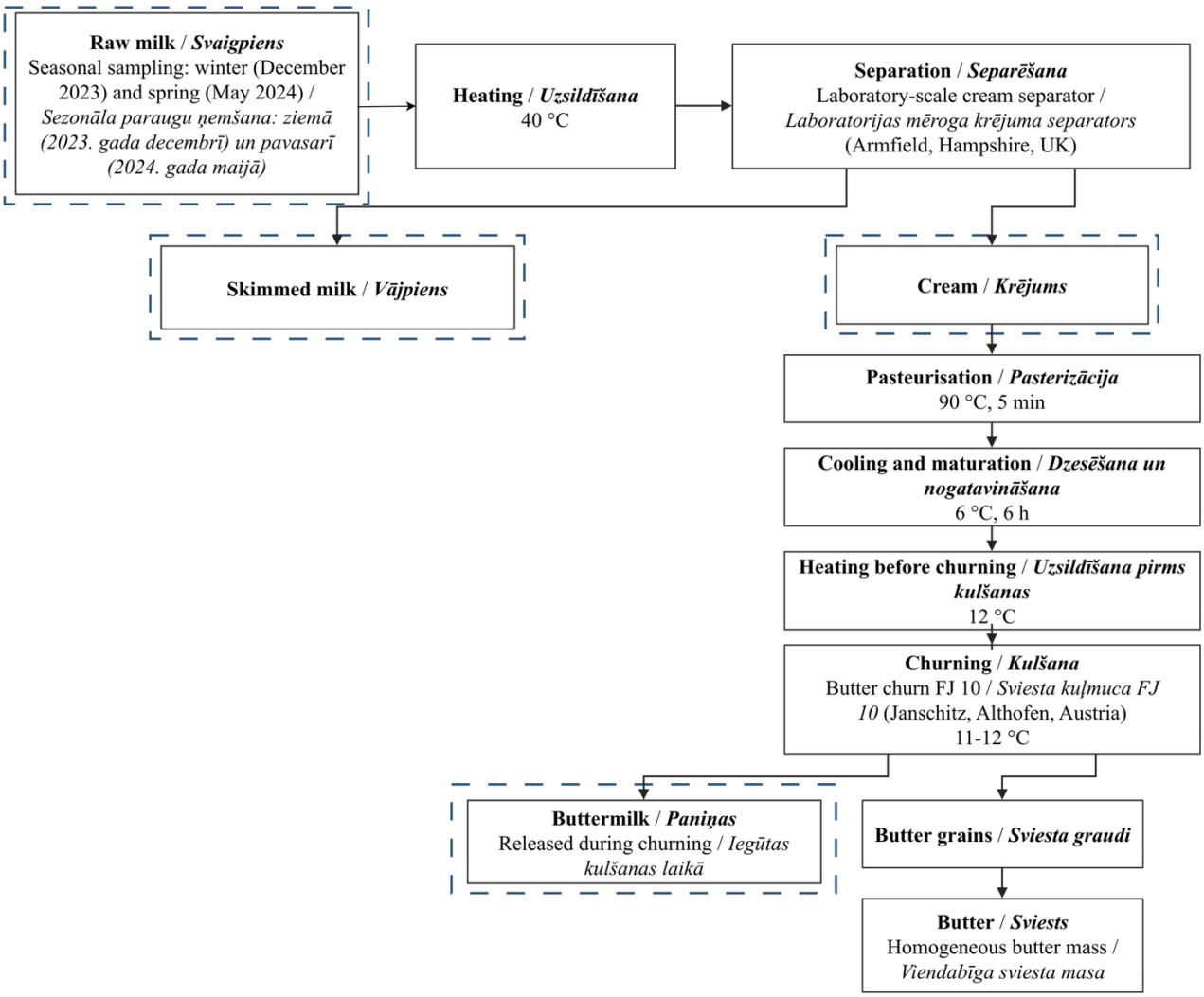


Fig. 2.2. **Milk processing scheme for the compositional study** / *2.2. att.* ***Piena pārstrādes shēma sastāva pētījumam***

Note. Dashed frames identify the products analysed experimentally. / *Piezīme. Ar pārtrauktas līnijas ietvaru apzīmēti eksperimentāli analizētie produkti.*

Five products: raw milk, skimmed milk, cream, buttermilk and butter. The first four formed the season-specific PL dataset, which was later used to estimate habitual milk PL intake; butter was characterised only within the wider compositional survey of products obtained during processing.

Processing technologies and corresponding compositional analyses were performed in triplicate within each season. Replicate measurements were used to describe product composition and to summarise results at the product-season level for further compositional and exposure-based analyses. All samples were handled under refrigerated conditions at 4 °C, where applicable, to minimise changes in product composition before analysis.

2.2.2. Determination of the chemical composition of milk and dairy products / *Piena un piena produktu ķīmiskā sastāva noteikšana*

The chemical composition of milk and dairy products obtained during pilot-scale processing was determined to describe the basic nutritional and technological characteristics of the analysed products before detailed lipid characterisation. The following parameters were determined: fat, protein, lactose, solids-not-fat, and total solid content. These parameters were selected because they capture the main compositional differences between dairy products and

provide a basis for interpreting the distribution of lipid components, including PL, across products with diverse fat and solid contents.

The chemical composition (fat, protein, lactose, solids-not-fat and total solids) of all liquid products was determined by mid-infrared spectrometry on a MilkoScan Mars milk analyser (Foss, Hillerød, Denmark). The same instrument was used for all products; only the built-in calibration profile was matched to the product type: the milk profile for raw milk and the buttermilk released during churning, the skimmed-milk profile for skimmed milk and the cream profile for cream. Each product was measured in triplicate in both seasons, in accordance with ISO 9622:2013.

2.2.3. Quantification of polar lipids / *Polāro lipīdu kvantitatīvā noteikšana*

PL were extracted from milk and dairy product samples using a modified Bligh and Dyer method adapted for LC-MS analysis (Breil et al., 2017). High-purity chemicals and LC-MS-grade solvents were used throughout sample preparation and analysis. Chloroform, methanol, water, acetonitrile, 2-propanol, formic acid, and ammonium formate were used for lipid extraction and chromatographic separation. Annex I, Table A1.1 presents the multiple reaction monitoring (MRM) transitions, collision energies, and retention times of the analytical standards.

Lipids were extracted from 1.0 g portions of each sample placed in 15 mL centrifuge tubes. After adding 3.0 mL chloroform and 6.0 mL methanol, the tube contents were vortexed for 1 min and then treated in an ultrasonic bath for 15 min at 20 ± 1 °C. To separate the phases, 3.0 mL of water was added, the mixture was vortexed again for 30 s and centrifuged at 9280 × g for 10 min at 4 °C. The lipid-containing lower chloroform layer was transferred to a clean vial and dried under a gentle nitrogen flow; residual moisture was then removed by freeze-drying for 24 h.

The dried extract was reconstituted in 3.0 mL of a 2-propanol:methanol mixture (50:50, v/v). Immediately before LC-MS analysis, the reconstituted extracts were vortex-mixed and ultrasonicated for 5 min to ensure complete solubilisation and homogeneity. When required, the extracts were diluted with the initial mobile-phase mixture (60 % A, 40 % B, 0 % C) to bring the analyte concentrations within the calibration range. Extracts were generally analysed within 12 h of preparation. When immediate analysis was not possible, they were stored at $^{-1}8$ ± 1 °C and analysed no later than 24 h after preparation.

PL were quantified on a Shimadzu Nexera UC liquid chromatography system connected to a Shimadzu LCMS-8050 triple quadrupole mass spectrometer with an electrospray ionisation interface. Separation took place on a Discovery HS F5-3 column (3.0 µm, 150 × 2.1 mm; Merck KGaA, Darmstadt, Germany) thermostated at 40 °C, using gradient elution at 0.25 mL min^{-1} and an injection volume of 3.0 µL. The mass spectrometer operated in positive electrospray mode and monitored analyte-specific MRM transitions.

Three mobile phases were used: phase A, LC-MS-grade water with 10 mM ammonium formate and 0.1 % formic acid; phase B, LC-MS-grade acetonitrile with 0.1 % formic acid; and phase C, LC-MS-grade 2-propanol with 10 mM ammonium formate and 0.1 % formic acid. Their composition and the gradient programme are given in Table 2.1 and Table 2.2, respectively.

Table 2.1. **Mobile phases used for PL analysis by LC-MS /** *2.1. tabula* ***Mobilās fāzes PL analīzei ar LC-MS***

Mobile phase / *Mobilā fāze*	Chemical / *Ķīmiskā viela*	Additives / *Piedevas*
A	Water, LC-MS grade / *LC-MS tīrības pakāpes ūdens*	10 mM ammonium formate and 0.1 % formic acid / *10 mM amonija formiāts un 0.1 % skudrskābe*
B	Acetonitrile, LC-MS grade / *LC-MS tīrības pakāpes acetonitrils*	0.1 % formic acid / *0.1 % skudrskābe*
C	2-Propanol, LC-MS grade / *LC-MS tīrības pakāpes 2-propanols*	10 mM ammonium formate and 0.1 % formic acid / *10 mM amonija formiāts un 0.1 % skudrskābe*

Note. Additive concentrations refer to the final concentration in each mobile phase. / *Piezīme. Piedevu koncentrācijas norādītas kā galīgās koncentrācijas attiecīgajā mobilajā fāzē.*

Mass spectrometric detection was performed using electrospray ionization (ESI) in positive ion mode and MRM. Compound identification was based on retention time and analyte-specific precursor-to-product ion transitions using analytical standards.

Table 2.2. **Gradient elution programme for PL analysis by LC-MS /** *2.2. tabula* ***Gradienta eluēšanas programma PL analīzei ar LC-MS***

Time, min / *Laiks, min*	A, %	B, %	C, %
0	60	40	0
3.0	50	30	20
8.0	30	10	60
15.0	10	0	90
20.0	10	0	90
23.0	60	40	0
25.0	60	40	0

Note. A, B and C correspond to the mobile phases in Table 2.1. / *Piezīme. A, B un C apzīmē 2.1. tabulā norādītās mobilās fāzes.*

PC, PE and SM were quantified against external calibration curves built from the respective standards dissolved in 2-propanol (50:50, v/v). Six calibration levels were analysed with two measurements per level (n = 12). The concentration ranges were 0.0156-0.5000 µg mL^{-1} for PC, 0.0172-0.5500 µg mL^{-1} for PE, and 0.0188-0.6000 µg mL^{-1} for SM. Linearity was accepted at $R^2 > 0.995$ and precision at RSD ≤ 15%. The calibration parameters, limits of detection, and limits of quantification are presented in Annex I, Table A1.2.

Concentrations of PC, PE and SM were calculated using the corresponding calibration equations and expressed as mg 100 g^{-1} of product. Their sum was taken as the total quantified PL, and the proportion of each class was expressed as a percentage of that sum. These values were used to characterise the milk and dairy products and subsequently to estimate habitual milk PL intake. The method also allowed the detection of phosphatidylinositol and phosphatidylserine, but these were not detected in the analysed samples.

Detailed MRM transitions, retention times, calibration parameters, limits of detection and quantification, and representative extracted ion chromatograms are presented in Annex I, Tables A1.1-A1.2 and Fig. A1.2.

The selection and characterisation of the fermented buttermilk used in the intervention were performed as a separate methodological step, linking the compositional part of the study to the clinical trial. Because the intervention was designed as a food-based study using a commercially available product, the selected fermented buttermilk had to be suitable for regular daily consumption, available in retail, compositionally similar, and nutritionally appropriate for a 28-day dietary intervention.

2.3. Clinical trial on cultured buttermilk consumption and plasma lipid profile / *Klīniskais pētījums par raudzēto paniņu lietošanu un plazmas lipīdu profilu*

This section describes the selection of the intervention product and the clinical trial's methodological framework, including ethical considerations, study design, pilot feasibility assessment, participant recruitment, eligibility criteria, randomisation procedures, intervention dose and duration, blood sampling, biochemical analyses, and dietary assessment. The clinical trial constituted the human intervention component of the thesis.

The clinical trial was retrospectively registered in the ISRCTN registry under identifier ISRCTN11974930 on 25 June 2026, in accordance with the CONSORT 2025 recommendations.

2.3.1. Selection of the intervention product / *Intervences produkta atlase*

A preliminary analysis of commercially available cultured buttermilk samples was conducted to select a suitable product for the intervention study. The analysis included cultured buttermilks available in the Latvian retail market during the study preparation period, including products from Latvian, Lithuanian, and Polish producers. In total, five products from five different producers were selected for evaluation.

The intervention product was selected according to the following predefined criteria: (1) ingredient composition, with preference for products consisting of buttermilk and starter culture only, without added skimmed milk, milk or milk proteins; (2) absence of flavouring ingredients; (3) declared fat and protein content; (4) viable mesophilic lactic acid bacteria count meeting the regulatory minimum of 10^7 CFU g^{-1}; (5) regular retail availability throughout the planned recruitment period; (6) analytically determined total quantified PL concentration, with preference for a higher concentration; and (7) feasibility of daily consumption during a 28-day intervention. Total quantified PL concentrations were measured in candidate products before the intervention product was selected. These analytical results were considered alongside ingredient composition, declared nutritional value, viable lactic acid bacteria counts, and retail availability. The evaluation of the products against these criteria is presented in Section 3.2.

Cultured buttermilk is produced by fermentation of pasteurised buttermilk with a mesophilic lactic acid bacteria starter culture. According to Cabinet Regulation No. 97/2011, Regulations on classification, quality and labelling requirements for milk products and composite milk products (Ministru kabinets, 2011), cultured buttermilk must contain not less than 10^7 viable lactic acid bacteria per 1 g of product. Therefore, microbiological analysis was performed to identify the product that complied with this regulatory requirement.

Viable mesophilic lactic acid bacteria were counted by the pour-plate method on MRS agar (Scharlau, Barcelona, Spain) in accordance with LVS ISO 15214:1998. Cultured buttermilk was diluted decimally in peptone water down to 10^{-7}, and the 10^{-6} and 10^{-7} dilutions were plated. After 72 h at 30 °C, plates bearing 30-300 colonies were counted with a SCAN 500 automatic colony counter (INTERSCIENCE, Saint-Nom-la-Bretèche, France), and the results were expressed as $\log_{10}$ CFU g^{-1} of product. The results are given in Section 3.2.

Fat and protein content of the commercial products, including the selected product, were taken from the nutrition declaration and were not analysed. Total PL and the individual PC, PE

and SM classes were determined in purchased samples, following the procedure described in Section 2.2.3.

Participants were instructed to consume 250 mL of cultured buttermilk daily. A volume-based portion provided clear, practical instructions for consumption under free-living conditions and did not require participants to weigh the product. To calculate and report PL intake, the 250 mL portion was assigned a nominal mass of 250 g. This was a simplifying approximation, as a cultured buttermilk density of $1020^{-1}022$ kg m^{-3} corresponds to a mass of approximately 255 g for a 250 mL portion (Walstra et al., 2005).

2.3.2. Study design and pilot feasibility study / *Pētījuma dizains un pilotpētījums*

The clinical part was designed as a randomised, controlled, food-based intervention study evaluating the effect of cultured buttermilk consumption on plasma lipid profile in perimenopausal women. The main clinical trial was conducted in Latvia from October 2023 to June 2024. The study compared an intervention group that consumed cultured buttermilk with a control group that maintained their habitual diet and lifestyle.

Ethical considerations / *Ētiskie apsvērumi*

The clinical trial followed internationally recognised ethical principles for biomedical research involving human participants. The Ethics Committee of Rīga Stradiņš University reviewed and approved the study protocol before participant recruitment and data collection (protocol code 2-PĒK-4/513/2022; approval date: 23 November 2022). The approval document is provided in Annex II.

The study was designed and conducted in line with the Declaration of Helsinki of the World Medical Association (2013 revision), the Council of Europe Convention on Human Rights and Biomedicine (ETS No. 164, 1997) and the EU General Data Protection Regulation, Regulation (EU) 2016/679 (European Parliament and Council of the European Union, 2016). Participation was voluntary, participants' autonomy was respected, risks were kept to a minimum, and confidentiality and personal data protection were maintained at every stage.

Before inclusion in the study, all participants received information on the study aim, procedures, duration, dietary intervention, blood sampling, dietary assessment, data handling, and their right to withdraw from the study at any time without giving a reason. We obtained written informed consent from all participants before performing any study-related procedures. The informed consent form is provided in Annex III.

Confidentiality of the participants was maintained at every stage of the study. Personal data were handled in compliance with Regulation (EU) 2016/679 (General Data Protection Regulation) and the Personal Data Processing Law of the Republic of Latvia. All study documents and datasets were anonymised by replacing personal identifiers with a unique four-digit participant code.

The intervention consisted of daily consumption of a commercially available cultured buttermilk and did not involve administering medication or isolated pharmacological compounds. Nevertheless, the study population was screened using predefined inclusion and exclusion criteria to reduce potential health risks and ensure that participants with conditions or treatments that could affect lipid metabolism or study safety were not included.

Before implementing the main clinical trial, we conducted a pilot feasibility study in Latvia between December 2022 and April 2023 to assess the feasibility of the study protocol, participant recruitment procedures, dietary assessment tools, intervention duration, and adherence to daily cultured buttermilk consumption. The pilot study included eight perimenopausal women aged 45-55 years, who were allocated to an intervention group (n = 4) and a control group (n = 4). The pilot protocol followed the same main methodological principles as the subsequent clinical trial, including the same target population, intervention duration, intervention product, and lipid profile assessment.

The pilot study provided preliminary information on the feasibility of the 28-day intervention and supported the continuation of the main clinical trial. After 28 days, LDL-C concentrations decreased by 0.18 ± 0.65 mmol L^{-1} in the pilot intervention group, whereas they increased by 0.09 ± 0.51 mmol L^{-1} in the pilot control group. These preliminary findings were not used as confirmatory evidence of efficacy, but they supported the methodological feasibility of evaluating cultured buttermilk consumption in perimenopausal women with moderately elevated LDL-C concentrations.

2.3.3. Participant recruitment, screening and eligibility / *Dalībnieku atlase, skrīnings un atbilstības izvērtēšana*

The study design and participant flow are illustrated in Fig. 2.3. The figure provides an overview of the pilot feasibility study and the subsequent main clinical trial, including participant recruitment, screening, eligibility assessment, informed consent, baseline evaluation, and block randomisation prior to commencement of the 28-day intervention.

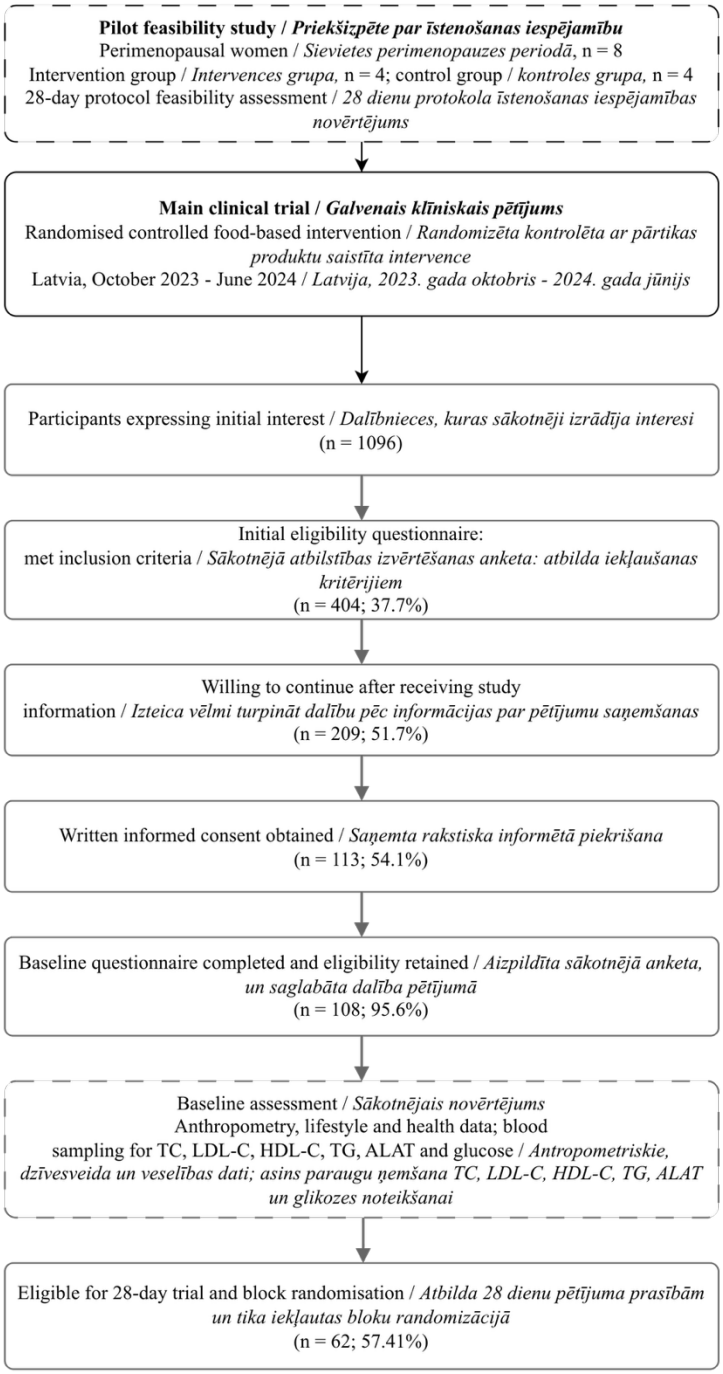


Fig. 2.3. **Participant recruitment, screening and eligibility /** *2.3. att.* ***Dalībnieču atlase, skrīnings un atbilstības izvērtēšana***

Fig. 2.4 shows the course of the 28-day trial: randomisation and allocation to the intervention and control groups, the intervention protocol, baseline dietary assessment, post-intervention biochemical assessment, participant retention, and the final analysed population, together with the seasonal cohorts used in the intake-based analyses. The figure illustrates the allocation of eligible participants to the intervention and control groups, the intervention protocol, baseline dietary assessment, post-intervention biochemical assessment, participant retention, and the final study population included in the statistical analyses, including the seasonal cohorts used for the exposure-based analyses.

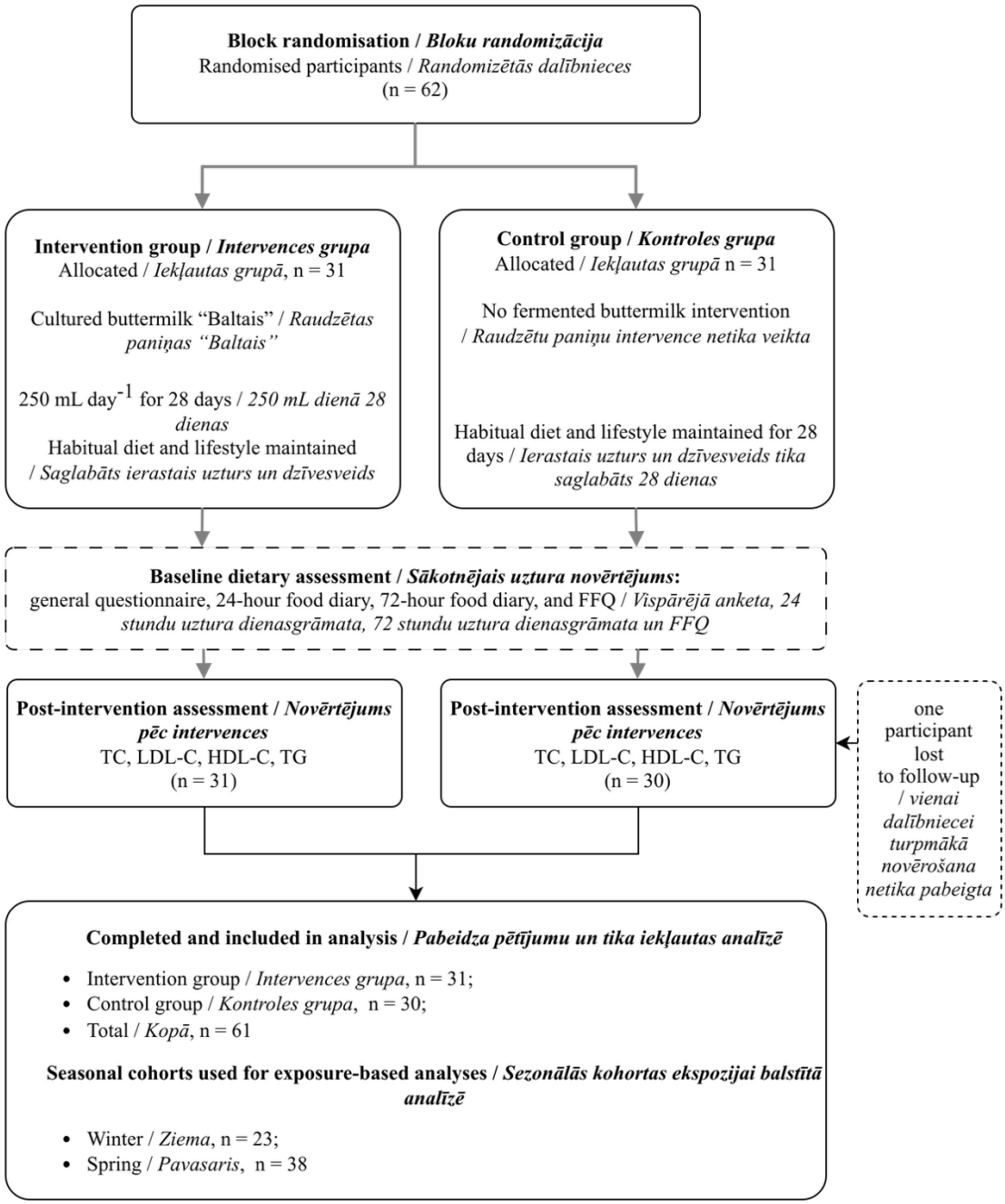


Fig. 2.4. **Randomisation, intervention and follow-up /** *2.4. att.* ***Randomizācija, intervence un novērošana***

The clinical trial target population consisted of perimenopausal women living in Latvia with moderately elevated LDL-C concentrations (3.01-4.12 mmol L^{-1}). Participants were recruited using convenience sampling through gynaecological practices, general practitioners, hospitals, and social media. Recruitment was carried out from October 2023 to June 2024.

The minimum sample size was derived using the feasibility-oriented approach of Viechtbauer et al. (2015), which estimates the number of participants needed to detect a problem with a chosen probability and confidence level. Under this approach, 29 participants suffice to detect a problem with an occurrence probability of 0.10 at 95 % confidence. On this basis, the target was set at roughly 30 women per group, that is, at least 60 in total (Viechtbauer et al., 2015).

The recruitment process consisted of several consecutive stages. Initially, 1096 women expressed interest in participating and completed a preliminary eligibility questionnaire. Of

these, 404 women met the initial eligibility criteria and were invited to proceed to the next recruitment stage. Detailed information about the study aim, procedures, duration, dietary intervention, and participant responsibilities was then provided to eligible women. Among those contacted, 209 women confirmed their willingness to continue participation.

Participants who expressed interest received written information on the study procedures together with the informed consent form; 113 signed forms were returned. Consenting participants then completed a baseline questionnaire covering demographic, anthropometric, lifestyle, health, physical activity, and dietary characteristics; based on this, 108 women remained eligible.

Eligibility was then verified by baseline blood testing, which also provided the initial clinical data. Three women did not attend the blood test, and a further 39 had LDL-C values outside the inclusion range, leaving 66 eligible participants. Of these, 62 finished all pre-randomisation procedures and were randomised. One control-group participant did not return for the follow-up assessment, so the final dataset comprised 61 women who completed the trial: 31 in the intervention group and 30 in the control group. To analyse seasonal effects on diet and plasma lipids, participants were also grouped into two cohorts according to the seasonal raw-milk composition data applied to their dietary estimates (Section 2.4): the winter cohort (n = 23; 11 intervention, 12 control), for which the December 2023 composition data were used, and the spring cohort (n = 38; 20 intervention, 18 control), for which the May 2024 data were used.

At baseline, participants completed a structured questionnaire including demographic, anthropometric, lifestyle, health-related, physical activity, and dietary information. The questionnaire included questions on age, body weight, height, waist and hip circumference, region of residence, education, occupation, marital status, medication and food supplement use, physical activity, smoking, alcohol intake, meal pattern, recent dietary changes, food exclusions, and disease history. Self-reported body weight and height were used to calculate body mass index (BMI), and waist and hip circumference values were used to calculate the waist-to-hip ratio.

Inclusion and exclusion criteria / *Iekļaušanas un izslēgšanas kritēriji*

Inclusion and exclusion criteria were established before participant recruitment to define a clinically homogeneous study population and to reduce the influence of medical conditions, medication use, or recent body weight changes on plasma lipid profile outcomes. The criteria were applied during the initial screening stage and verified using questionnaire data and baseline biochemical testing.

Inclusion criteria:

- residence in Latvia;
- female sex;
- age between 45 and 55 years;
- presence of at least one of the perimenopausal symptoms: vasomotor symptoms such as hot flushes or night sweats, infrequent menstrual periods;
- moderately elevated LDL-C concentration, defined as 3.01-4.12 mmol L^{-1} within the previous six months;
- stable body weight, defined as no change greater than ± 2 kg during the previous six months.

Exclusion criteria:

- history of primary hyperlipidemia;
- established CVD;
- endocrine disease (thyroid disease, type 1 or type 2 diabetes mellitus, or adrenal disease;

- liver disease, including non-alcoholic fatty liver disease, alcoholic liver disease, hepatitis, cirrhosis, or chronic liver failure;
- chronic kidney disease;
- history of gastrointestinal surgery;
- use of hormonal therapy;
- use of immunosuppressive therapy;
- use of lipid-lowering or antihypertensive medication;
- use of food supplements intended to lower blood lipids or blood pressure;
- known allergy to cow's milk proteins or clinically significant lactose intolerance.

These criteria were selected to minimise confounding from medical conditions and treatments known to affect lipid metabolism, glucose metabolism, inflammatory status, body weight, or CVD risk markers. The LDL-C inclusion range was used to identify women with moderately elevated LDL-C concentrations for whom a food-based dietary intervention could be evaluated without including participants who required pharmacological lipid-lowering treatment.

Randomisation and group allocation / *Randomizācija un dalībnieku sadalījums grupās*

After completion of participant screening, written informed consent, baseline questionnaire assessment, and baseline biochemical testing, 62 participants met the eligibility criteria and were enrolled in the randomisation procedure.

Allocation followed a randomisation list prepared before enrolment. Each participant entering the study received the next unused position in the sequence, and each block was filled completely before opening the next block. This blocked, sequential procedure was chosen to keep intervention and control group numbers balanced throughout recruitment.

The intervention group was allocated to consume cultured buttermilk daily for 28 days, whereas the control group was instructed to maintain their habitual diet and lifestyle without the cultured buttermilk intervention. At randomisation, 31 participants were allocated to the intervention group and 31 to the control group. One participant from the control group was lost to follow-up. Therefore, the final analysis included 61 participants: 31 in the intervention group and 30 in the control group.

Intervention dose and duration / *Intervences deva un ilgums*

The intervention was pragmatic and food-based: the test product was a cultured buttermilk available in retail, not an isolated or purified milk PL preparation. Women allocated to the intervention group consumed 250 mL of the product every day for 28 consecutive days and were asked not to change their usual diet or lifestyle in any other respect.

A daily portion of 250 mL was selected because it corresponds to an amount of cultured dairy product that can be consumed regularly under free-living conditions. With the product-specific PL composition this portion supplied 81.30 ± 1.95 mg of total quantified PL in winter and 69.73 ± 2.23 mg in spring, a standardised intake of the selected product that did not appreciably change total energy or fat intake. The trial thus tested a feasible addition of a whole food to the habitual diet and was not intended to reproduce the far higher PL doses used in studies with enriched foods or supplements.

Compared with interventions using milk PL-enriched products, the present study applied a lower-dose whole-food approach. The cultured buttermilk intervention provided 69.73-81.30 mg PL day^{-1}, equivalent to 1.4-2.7 % of the 3-5 g day^{-1} milk PL doses used in the enriched-product intervention by Vors et al. (2020). Related to the habitual milk PL intake estimated for the study participants (Table 3.15), the daily portion corresponded to about 31 % of the mean winter intake (81.30 of 264.25 mg day^{-1}) and 39 % of the mean spring intake (69.73 of 179.98 mg day^{-1}). The aim was not to reproduce supplement-level or pharmacological exposure, but to determine whether daily consumption of a commercially available cultured buttermilk product, at a dose compatible with habitual dietary practice, could influence the plasma lipid profile in

free-living participants. This distinction is important because cultured buttermilk represents a complex dairy product containing proteins, lactose, minerals, viable lactic acid bacteria, residual milk fat, MFGM-associated constituents, and naturally occurring PL.

Cultured buttermilk was supplied to participants throughout the intervention period. Product delivery was organised by the researcher or by courier, depending on participant availability and logistical requirements. Participants were instructed to consume the 250 mL portion each day and to report missed portions, difficulties with product consumption, adverse symptoms, or relevant changes in health status. The same daily portion was prescribed to all participants in order to standardise product exposure within the intervention group.

To interpret the intervention exposure, the analytically determined concentrations of total PL, PC, PE, and SM in the selected cultured buttermilk were recalculated for the daily 250 mL portion. The estimated daily amounts of total PL and individual PL classes supplied by the intervention product were calculated using season-specific, analytically determined concentrations.

Blood sampling and biochemical analyses / *Asins paraugu iegūšana un bioķīmiskās analīzes*

Blood samples were taken twice: immediately before the 28-day intervention and after its completion; the baseline sample confirmed eligibility, and both samples were used to follow the plasma lipid profile.

The main biochemical outcomes were lipid profile parameters. Additional biochemical parameters included alanine aminotransferase (ALAT) and glucose. These additional parameters were assessed to characterise general metabolic and liver-related status and to support participant screening, as disturbances in glucose metabolism or liver function could influence lipid metabolism and the interpretation of intervention outcomes.

All blood samples were analysed by Centrālā Laboratorija Ltd. (Rīga, Latvia), an accredited clinical laboratory (accreditation certificate LATAK-M-434-04-2011), following its standard operating procedures for sample collection, handling and biochemical measurement.

Plasma lipid profile parameters were measured using an enzymatic colourimetric method. ALAT activity was determined using a kinetic reaction method. Glucose concentration was measured in plasma using the hexokinase method.

Non-HDL-C and remnant cholesterol were calculated by Centrālā Laboratorija Ltd. as part of the laboratory's standard procedure. Non-HDL-C was calculated as the difference between TC and HDL-C, representing the cholesterol content of all atherogenic ApoB-containing lipoproteins. Remnant cholesterol represents cholesterol carried by triglyceride-rich lipoprotein remnants, including very-low-density lipoproteins and intermediate-density lipoproteins.

Biochemical analyses were performed at two time points. The first blood sample was collected at the baseline visit, before randomisation and before the start of the intervention. Women whose baseline LDL-C concentration was outside the predefined range of 3.01-4.12 mmol L^{-1} were not randomised. The second blood sample was collected after completion of the 28-day intervention period. At both time points, the same laboratory procedures were used to assess the lipid profile.

2.3.4. Dietary assessment / *Uztura novērtējums*

Dietary assessment was performed to characterise habitual dietary intake, describe food-group consumption patterns, and support the interpretation of plasma lipid profile outcomes in relation to diet. Dietary data were collected using an interviewer-administered 24-h dietary recall, a 72-h food diary and a semi-quantitative FFQ.

All three instruments were completed by all 61 participants who finished the trial (intervention group, n = 31; control group, n = 30), of whom 23 were assessed in the winter

cohort (intervention, n = 11; control, n = 12) and 38 in the spring cohort (intervention, n = 20; control, n = 18); no dietary data were missing.

Short-term dietary intake was assessed using a 24-h dietary recall and a separate 72-h food diary. The 24-h dietary recall was conducted by the researcher after randomisation: participants recalled all foods and beverages consumed during the preceding 24 hours, and the researcher clarified food items, mixed dishes, portion sizes, beverages, snacks, condiments, and dietary supplements to improve the completeness and accuracy of the recorded information.

The 72-h food diary was completed during the intervention period, after the 24-h dietary recall. It covered an uninterrupted 72-hour period corresponding to three consecutive 24-hour recording days. The 24-h recall was a separate dietary assessment instrument, and the recall day was not included among the three days of the 72-h diary. For the 72-h food diary, total food, beverage, energy, and nutrient intake were recorded over the three consecutive days.

The researcher briefed participants before they kept the dietary records; the briefing included a video tutorial and an opportunity to ask questions. Food identification and portion-size estimation were supported by the food product and portion atlas of the Institute of Food Safety, Animal Health and Environment "BIOR" (2018). The 24-h dietary recall form is reproduced in Annex V.Annex V

The cultured buttermilk supplied as the intervention product was excluded from the dietary intake calculations because the FFQ characterised habitual diet over the preceding 12 months and the 24-h recall and the 72-h diary served only to verify FFQ responses; the PL dose supplied by the intervention product was quantified separately from its measured composition (Sections 2.3.1 and 3.2) and compared with habitual intake in Section 3.6. Consequently, the recall and diary results reflected the participants' baseline dietary intake and did not include the additional food and nutrient contributions from the intervention product.

Habitual food intake was recorded with a semi-quantitative FFQ derived from the questionnaire of the Latvian National Dietary Survey (Pārtikas drošības, dzīvnieku veselības un vides zinātniskais institūts "BIOR", 2017). It covered the previous 12 months and listed about 260 foods, beverages and dietary supplements arranged in 26 categories. For every item, the respondent selected a consumption frequency from a fixed scale (from "never" up to "6 times per day or more") and indicated the portion size. The list of FFQ food categories is given in Annex VI.

The FFQ was completed during an online interview with researcher assistance. Clarification was provided when necessary to ensure consistent interpretation of food items, portion sizes, mixed dishes, and consumption-frequency categories. Although the FFQ was completed during the intervention period, it assessed habitual intake over the preceding 12 months and was therefore treated as a measure of the participants' habitual dietary background rather than short-term intervention-period intake.

The 24-h dietary recall, the 72-h food diary and the FFQ were all processed at the Institute of Food Safety, Animal Health and Environment "BIOR" in Microsoft Dynamics AX 2009, with nutrient values taken from the Latvian Food Composition Database (Pārtikas un veterinārā dienesta Pārtikas centrs, 2009).

The 24-h recall and the 72-h food diary were used as short-term reference records to assess the plausibility and internal consistency of the food items, consumption frequencies, and portion sizes reported in the FFQ. The recall and diary data were used to identify potentially omitted foods, implausible portion sizes, and inconsistencies between habitual intake reported in the FFQ and foods and quantities recorded during the short-term assessment periods (Q. Cui et al., 2023; Freedman et al., 2018; Yuan et al., 2017). Where clarification was required, the reported information was reviewed with the participant.

The recall- and diary-derived intake values were not combined with the FFQ data and were not used as independent dietary intake variables in the between-group comparisons, the nutritional adequacy assessment, the estimation of habitual milk PL exposure, or the exposure-based regression analyses. All food-group, beverage, energy, nutrient, and dairy-product intake

variables used in these analyses were derived from the semi-quantitative FFQ, which assessed habitual intake over the preceding 12 months. The FFQ-derived dairy-product intake data were also used to estimate habitual exposure to total milk PL, PC, PE, and SM.

2.4. Estimation of habitual milk polar lipid exposure / *Piena polāro lipīdu uzņemšanas novērtējums*

Habitual milk PL exposure was estimated by integrating each participant's FFQ-derived average daily intake across all reported milk and dairy product categories with the experimentally determined season-specific concentrations of total PL, PC, PE and SM in raw milk (Table 3.3). Because participants consumed a broader range of dairy products than those analysed directly, each FFQ-derived dairy product intake was first converted into a milk-equivalent amount (Table 2.3), that is, into the quantity of raw milk from which the product is made, and the summed milk equivalents were then combined with the raw-milk PL concentration of the corresponding season. Thus, all reported dairy product categories were included in the calculation, but the resulting values represent modelled exposure estimates rather than direct chemical measurements of the products consumed.

The winter and spring cohorts consisted of different participants: each woman took part in the study once, and her dietary assessment and blood sampling occurred either in winter or in spring. PL concentrations determined in December 2023 were applied to the winter cohort (n = 23) and those determined in May 2024 to the spring cohort (n = 38). Cohort allocation therefore followed the actual study schedule and the availability of corresponding seasonal compositional data rather than predefined calendar-season boundaries, and every seasonal comparison in the clinical part of the thesis compares two independent groups.

Conversion of dairy product intake to estimated milk equivalents / *Piena produktu patēriņa pārrēķins piena ekvivalentā*

To standardise the heterogeneous dairy product categories included in the FFQ, the participant-specific average daily intake of each category was converted to estimated milk equivalents using the predefined product-specific conversion coefficients presented in Table 2.3.

Table 2.3. **Product-specific coefficients used to convert FFQ-derived dairy product intake into estimated milk equivalents /** *2.3. tabula* ***Specifiskie koeficienti no FFQ iegūtā piena produktu patēriņa pārrēķināšanai piena ekvivalentā***

FFQ dairy product category / *FFQ piena produktu kategorija*	Milk-equivalent conversion coefficient, k_i / *Piena ekvivalenta pārrēķina koeficients, k_i*
Curd cream / *Biezpiena krēms*	6.00
Glazed curd snack with chocolate coating / *Biezpiena sieriņš šokolādes glazūrā*	6.00
Curd with 0.5 % fat / *Biezpiens ar 0.5 % tauku saturu*	8.00
Curd with 5 % fat / *Biezpiens ar 5 % tauku saturu*	7.00
Curd with 9 % fat / *Biezpiens ar 9 % tauku saturu*	6.00

FFQ dairy product category / *FFQ piena produktu kategorija*	Milk-equivalent conversion coefficient, k_i / *Piena ekvivalenta pārrēķina koeficients, k_i*
Fermented milk products without additives, <2 % fat / *Raudzēti piena produkti bez piedevām, ar tauku saturu <2 %*	1.00

Table 2.3 continuation / *2.3. tabulas turpinājums*

FFQ dairy product category / *FFQ piena produktu kategorija*	Milk-equivalent conversion coefficient, k_i / *Piena ekvivalenta pārrēķina koeficients, k_i*
Fermented milk products without additives, 2.1-5 % fat / *Raudzēti piena produkti bez piedevām, ar tauku saturu 2.1-5 %*	1.00
Fermented milk products with additives, 2.1-5 % fat / *Raudzēti piena produkti ar piedevām, ar tauku saturu 2.1-5 %*	1.00
Fermented milk products with additives, <2 % fat / *Raudzēti piena produkti ar piedevām, ar tauku saturu <2 %*	1.00
Ghee / *Gī sviests*	24.95
Cream with <20 % fat / *Skābais krējums ar tauku saturu <20 %*	5.00
Cream with >25 % fat / *Skābais krējums ar tauku saturu >25 %*	6.25
Soft cheese / *Mīkstais siers*	8.00
Buttermilk with additives, <1 % fat / *Paniņas ar piedevām, ar tauku saturu <1 %*	1.00
Buttermilk with additives, >1.5 % fat / *Paniņas ar piedevām, ar tauku saturu >1.5 %*	1.00
Buttermilk without additives, <1 % fat / *Paniņas bez piedevām, ar tauku saturu <1 %*	1.00
Buttermilk without additives, >1.5 % fat / *Paniņas bez piedevām, ar tauku saturu >1.5 %*	1.00
High-protein dairy products with additives / *Piena produkti ar palielinātu olbaltumvielu saturu un piedevām*	1.00
High-protein dairy products without additives / *Piena produkti ar palielinātu olbaltumvielu saturu bez piedevām*	1.00
Milk with 0.5 % fat / *Piens ar 0.5 % tauku saturu*	1.00
Milk with 1.5 % fat / *Piens ar 1.5 % tauku saturu*	1.00
Milk with 2.5 % fat / *Piens ar 2.5 % tauku saturu*	1.00
Milk with 3.2 % fat / *Piens ar 3.2 % tauku saturu*	1.00
Flavoured milk / *Piens ar garšas piedevām*	1.00
Sweet cream with <15 % fat / *Saldais krējums ar tauku saturu <15 %*	3.75

Sweet cream with >30 % fat / *Saldais krējums ar tauku saturu >30 %*	7.50
Hard cheese / *Cietais siers*	10.00
Semi-hard cheese / *Puscietais siers*	9.00
Fresh cheese or cream cheese / *Svaigais siers vai krēmsiers*	8.00

Table 2.3 continuation / *2.3. tabulas turpinājums*

FFQ dairy product category / *FFQ piena produktu kategorija*	Milk-equivalent conversion coefficient, k_i / *Piena ekvivalenta pārrēķina koeficients, k_i*
Butter / *Sviests*	20.50

Note. Each coefficient specifies the estimated milk equivalent per 1 g of the corresponding product. The coefficients are study-specific calculation inputs, not universal conversion constants. / *Piezīme. Katrs koeficients norāda aprēķināto piena ekvivalentu uz 1 g attiecīgā produkta. Koeficienti ir šī pētījuma aprēķinu ievaddati, nevis universālas pārrēķina konstantes.*

Milk-equivalent conversion may be based on the quantity of milk required to manufacture a dairy product, its milk-fat concentration, non-fat milk solids, or total solids content. Consequently, milk-equivalent coefficients are method-dependent and should not be regarded as universal constants. The selected calculation basis and the numerical conversion factors used can affect the resulting milk-equivalent estimates; for example, the USDA Economic Research Service calculates milk equivalents separately on milk-fat and non-fat milk solid bases, using product-specific composition estimates and typical conversion factors obtained from multiple sources (U.S. Department of Agriculture, Economic Research Service, 2026). For cream, sour cream, butter and ghee, the quantity of milk per kilogram of product follows from the mass balance of milk fat relative to raw milk with 4.0 % fat (for example, 3.75 kg of milk per kilogram of sweet cream with 15 % fat and 20.50 kg per kilogram of butter). For cheese and curd, it was estimated from the general cheese-yield relationships described by Fox et al. (2015)and Walstra et al. (2005): about 10, 9 and 8 kg of milk per kilogram of hard, semi-hard and soft or fresh cheese, respectively, and 8, 7 and 6 kg per kilogram of curd with 0.5 %, 5 % and 9 % fat, the last value being applied also to curd creams and glazed curd snacks, in which curd is the principal dairy ingredient.

For dairy product category *i*, estimated milk-equivalent intake was calculated as:

$$ME_i = D_i \times k_i, \quad (1)$$

where:

- ME_i - the estimated milk-equivalent intake of dairy product category *i*, expressed as g day^{-1};
- D_i - the FFQ-derived average daily intake of the corresponding dairy product, expressed as g day^{-1};
- k_i - the predefined product-specific milk-equivalent conversion coefficient (Table 2.3).

The coefficients were treated as fixed standardisation parameters specific to the present exposure model and were applied consistently to all participants in both seasonal cohorts. Seasonal variation was introduced through the analytically determined winter- and spring-specific PL concentrations rather than through the milk-equivalent conversion coefficients. Because individual FFQ categories may include products differing in composition, moisture content, fat concentration, and manufacturing yield, the resulting values were interpreted as

standardised model-based estimates of milk-equivalent intake rather than exact reconstructions of the quantity of raw milk used to manufacture each consumed product.

Habitual total milk PL, PC, PE, and SM exposure / *Kopējais piena PL, PC, PE un SM uzņemšanas novērtējums*

The individual daily intake of total milk PL was calculated as:

$$PL_{intake} = (\Sigma ME_i / 100) \times PL, \quad (2)$$

where:

PL_{intake} - the estimated habitual daily intake of total milk PL, expressed as mg day^{-1};

ΣME_i - the sum of the milk-equivalent intakes of all dairy product categories, expressed as g day^{-1} (Equation 1, Table 2.3);

PL - the season-specific concentration of total quantified PL in raw milk, expressed as mg 100 g^{-1} (Table 3.3).

The intakes of PC, PE and SM were calculated in the same way, with the respective season-specific raw-milk concentrations of PC, PE and SM substituted for PL.

For each participant, the FFQ intake of every dairy category was converted to milk equivalents (Table 2.3), and the milk equivalents were summed; the sum was multiplied by the PL concentration of raw milk of the corresponding season (Table 3.3: 27.89 mg 100 g^{-1} in winter and 20.12 mg 100 g^{-1} in spring) to obtain the estimated total milk PL intake, and by the raw-milk concentrations of PC, PE and SM to obtain the class-specific intakes. The raw-milk concentration was applied to all categories, including the composite category "Other dairy products", because the milk-equivalent coefficient already expresses each product as the quantity of raw milk from which it is made; the estimate therefore represents the PL concentration of the raw milk consumed as dairy products and does not account for the redistribution of PL between fractions during processing, which is discussed in Section 3.10.

Table 2.4 summarises the principal variables used to estimate habitual milk PL exposure. Habitual milk PL exposure was calculated separately from the PL dose provided by the intervention product. In the statistical analyses, habitual milk PL exposure, intervention allocation, season, and baseline plasma lipid concentrations were treated as separate analytical variables.

Table 2.4. **Variables used for the estimation of habitual milk PL exposure /** *2.4. tabula* ***Mainīgie, kuri izmantoti piena PL uzņemšanas novērtēšanai***

Variable / *Mainīgais*	Description / *Apraksts*	Unit / *Mērvienība*	Data source / *Datu avots*
ME_i	Intake of dairy product category i expressed as milk equivalent / *Piena produktu kategorijas i uzņemšana, izteikta piena ekvivalentā*	g day^{-1} / *g dienā*	FFQ-derived dietary intake data / *No FFQ iegūtie uztura uzņemšanas dati*
PL	Season-specific total PL concentration in raw milk (winter, spring) / *Sezonai specifiskais kopējo PL saturs svaigpienā (ziema, pavasaris)*	mg 100 g^{-1}	LC-MS analysis /*analīze*
PC	Season-specific PC concentration in raw milk (winter, spring) / *Sezonai specifiskais PC saturs svaigpienā (ziema, pavasaris)*	mg 100 g^{-1}	LC-MS analysis /*analīze*
PE	Season-specific PE concentration in raw milk (winter, spring) / *Sezonai specifiskais PE saturs svaigpienā (ziema, pavasaris)*	mg 100 g^{-1}	LC-MS analysis /*analīze*
SM	Season-specific SM concentration in raw milk (winter, spring) / *Sezonai specifiskais SM saturs svaigpienā (ziema, pavasaris)*	mg 100 g^{-1}	LC-MS analysis /*analīze*
Total PL intake / *Kopējā PL uzņemšana*	Estimated daily intake of total milk PLs / *Aprēķinātā kopējā piena PL uzņemšana dienā*	mg day^{-1} / *mg dienā*	Calculated variable / *Aprēķinātais mainīgais*
PC intake /*uzņemšana*	Estimated daily intake of PC / *Aprēķinātā PC uzņemšana dienā*	mg day^{-1} / *mg dienā*	Calculated variable / *Aprēķinātais mainīgais*
PE intake / *uzņemšana*	Estimated daily intake of PE / *Aprēķinātā PE uzņemšana dienā*	mg day^{-1} / *mg dienā*	Calculated variable / *Aprēķinātais mainīgais*
SM intake / *uzņemšana*	Estimated daily intake of SM / *Aprēķinātā SM uzņemšana dienā*	mg day^{-1} / *mg dienā*	Calculated variable / *Aprēķinātais mainīgais*

Note. The subscript i identifies an individual dairy product category. / *Piezīme. Apakšraksta indekss i apzīmē atsevišķu piena produktu kategoriju.*

2.5. Statistical analysis / *Statistiskā analīze*

Statistical analyses comprised four components: (1) descriptive and comparative analyses of the chemical and PL composition of milk and dairy products; (2) analysis of dietary intake and biochemical parameters; (3) estimation and statistical analysis of habitual milk PL exposure; and (4) exploratory multivariate analysis of PL composition patterns.

Continuous variables were summarised as mean ± standard deviation (SD) when their distributions were approximately normal and as median with the first and third quartiles (Q1; Q3) when marked departures from normality were observed (habitual food-group intakes, Table 3.13; the corresponding means ± SD are given in Annex VII). Categorical variables were presented as values and percentages. Distributional assumptions were assessed using the Shapiro-Wilk test together with visual inspection of histograms and quantile-quantile plots.

For the compositional study, one processing batch was analysed per season (December 2023 and May 2024). Proximate composition was measured in three analytical replicates, and total quantified PL and individual PC, PE and SM classes in two analytical replicates per product and season; values are reported as mean ± SD of these replicates. Two-way ANOVA with product type (four levels) and season (two levels) as fixed factors, including their interaction, was applied to the proximate composition (24 observations per parameter; degrees of freedom 3, 1 and 3 for product, season and interaction, and 16 for the residual term) and to total quantified PL, PC, PE and SM (16 observations per variable; residual degrees of freedom 8). A two-level factor has one degree of freedom by definition. Because the replicates are repeated measurements of the same batch and not independent samples, the residual variance reflects analytical repeatability only; the resulting F-values are correspondingly large, and the tests are exploratory: they characterise the analysed material and do not support inference about seasonal variation in raw milk or dairy products in general. This applies equally to the proximate composition and to the PL classes. Partial η^2 is reported as a descriptive measure of the share of variance attributable to each factor within the dataset. Cultured buttermilk (Section 3.2) was characterised descriptively.

Only FFQ-derived dietary intake variables were included in the descriptive and comparative dietary analyses reported in Section 3.5. The FFQ data were processed to estimate average daily intake of the predefined food groups and beverages, as well as energy, macronutrients, fatty acids, fibre, vitamins, and minerals. The 24-h dietary recall and the 72-h food diary were used only to assess the plausibility and internal consistency of the FFQ data and were not analysed as independent dietary outcome variables. The term “food group” was used consistently to refer to the predefined groups of foods included in the FFQ (Annex VI). Differences in dietary intake and exposure variables between the intervention and control groups, and between the winter and spring cohorts, which consisted of different participants (Section 2.4), were assessed with an independent-samples t-test (Welch's correction when variances were unequal) when the assumption of approximate normality was met, and otherwise with the Mann-Whitney U test. The distribution of the study groups between the two seasonal cohorts was compared with Fisher's exact test

Changes in biochemical parameters from baseline to the post-intervention assessment were analysed separately within each study group using a paired-samples t-test. The normality of the within-participant change values was assessed prior to testing. Results were reported for glucose, TC, LDL-C, HDL-C, TG, non-HDL-C, remnant cholesterol, and ALAT, where available.

Standardised effect sizes were reported for the continuous outcomes in Table 3.10, Table 3.12, Table 3.14, Table 3.15 and Table 3.17, irrespective of statistical significance. Between-group differences were expressed as Hedges’ g, calculated as the difference between group means divided by the sample-size-weighted pooled standard deviation and multiplied by the small-sample correction factor J. Within-group changes were expressed as Hedges-corrected

g_z, calculated as the mean individual change from baseline divided by the standard deviation of the individual changes and multiplied by J. The correction was calculated as J = 1 - 3/(4df - 1), with df = n_1 + n_2 - 2 for independent groups and df = n - 1 for paired observations, where n is the number of complete pairs (Hedges, 1981). The table notes specify the direction of each contrast. Within-group effect sizes describe standardised changes over time and do not estimate the between-group intervention effect. The 95 % confidence intervals accompanying mean changes were calculated using the t distribution and refer to changes in the original measurement units. Absolute between-group g values of approximately 0.20, 0.50, and 0.80 were used as descriptive benchmarks for small, medium, and large differences, respectively (Cohen, 1988); these benchmarks do not establish clinical importance.

For the exposure-based clinical trial analyses, separate ordinary least squares linear regression models were fitted for post-intervention TC, LDL-C, HDL-C, and TG. Each model included the corresponding baseline lipid concentration as a covariate, intervention allocation and season as categorical predictors, and estimated habitual milk PL exposure as a continuous predictor. Total PL exposure and the individual PC, PE, and SM exposure variables were examined in separate models to avoid collinearity between closely related exposure measures. Because the estimated exposure variables were right-skewed and potentially influenced by extreme values, natural logarithmic transformation was applied before modelling. Regression coefficients were presented with 95 % confidence intervals. Interaction terms between intervention allocation and the logarithmically transformed PL exposure variable were tested in separate models to evaluate whether the association between habitual milk PL exposure and the post-intervention lipid outcome differed between study groups.

The balance among the major PL classes was characterised by the relative proportions of PC, PE and SM (each as a percentage of their sum) and by the PC/PE ratio. Two graphical methods were used to display these proportions for the eight product-season observations. Principal component analysis (PCA) was computed from the correlation matrix of the standardised proportions, and scores and loadings were shown in a biplot together with the winter-to-spring displacement of each product in the score space. Because the three proportions sum to 100 %, at most two independent dimensions exist, and the first two components necessarily account for all of the variance; the percentages of variance attributed to PC1 and PC2 describe the orientation of the two-dimensional score space and are not a measure of model adequacy. PCA was used only to visualise the multivariate pattern and the direction of seasonal shifts; it was not used for inference. The same proportions were plotted without transformation in a ternary diagram, where winter-to-spring trajectories show the direction of the seasonal shift for each product.

All statistical tests were two-sided, and statistical significance was set at $p < 0.05$. No adjustment for multiple testing was applied. LDL-C, the parameter on which eligibility was based, was the primary lipid outcome; TC, HDL-C, non-HDL-C, remnant cholesterol, and TG were secondary outcomes, and the regression models for the individual PL classes and the group × exposure interaction were exploratory. Because the lipid outcomes are correlated and partly derived from one another (non-HDL-C = TC - HDL-C), the p-values for the secondary and exploratory analyses are nominal and should be interpreted descriptively rather than as confirmatory evidence.

Statistical analyses were carried out in jamovi 2.6; data management, calculations, tables and figures were prepared in Microsoft Excel 16.93; dietary data were processed at "BIOR" in Microsoft Dynamics AX 2009; LC-MS data were acquired and pre-processed in LabSolutions Insight LC-MS 3.7 SP3.

3. RESULTS AND DISCUSSION / *REZULTĀTI UN DISKUSIJA*

The chapter presents and discusses the main findings of the Ph.D. thesis in an integrated sequence. First, it describes the chemical composition of raw milk and the processing products obtained during pilot-scale processing. Second, total PL concentration and the distribution of major PL classes are evaluated by product, processing stage, and season. Third, the composition of the commercial cultured buttermilk used in the intervention is evaluated, with particular emphasis on its PL concentration and the amount of PL provided by the daily intervention portion. Finally, the clinical and dietary findings are presented, including participant characteristics, dietary habits, estimated habitual milk PL exposure, changes in the plasma lipid profile following the 28-day intervention, and associations between estimated habitual milk PL exposure and plasma lipid profile outcomes.

This structure enables interpretation within an integrated analytical framework linking milk and dairy product composition, estimated habitual milk PL exposure, and plasma lipid profile outcomes. The compositional findings provide the food-based evidence required to understand the distribution of PL across dairy products, whereas the clinical and dietary findings indicate how consumption of these products contributes to estimated habitual milk PL exposure and how this exposure is associated with the plasma lipid profile in perimenopausal women.

3.1. Chemical composition of milk and dairy products / *Piena un piena produktu ķīmiskais sastāvs*

The chemical composition of raw milk and the products obtained from it describes the basic nutritional and technological properties used in the compositional study. The proximate composition is presented in Table 3.1.

Table 3.1. **Proximate composition of milk and dairy products in winter and spring /** *3.1. tabula* ***Piena un piena produktu ķīmiskais sastāvs ziemā un pavasarī***

Product / *Produkts*	Fat / *Tauki, %*	Protein / *Olbaltumvielas, %*	Lactose / *Laktoze, %*	Solids-not-fat / *Beztauku sausna, %*	Total solids / *Kopējā sausna, %*
Winter / *Ziema*					
Raw milk / *Svaigpiens*	3.83 ± 0.01	3.35 ± 0.02	4.83 ± 0.02	8.87 ± 0.03	12.69 ± 0.03
Skimmed milk / *Vājpiens*	0.07 ± 0.00	3.42 ± 0.02	4.77 ± 0.01	9.09 ± 0.03	9.26 ± 0.02
Buttermilk / *Paniņas*	0.78 ± 0.01	2.90 ± 0.02	4.53 ± 0.01	8.27 ± 0.01	9.16 ± 0.01
Cream / *Krējums*	40.51 ± 0.06	1.99 ± 0.01	2.75 ± 0.01	5.62 ± 0.06	46.14 ± 0.07
Spring / *Pavasaris*					
Raw milk / *Svaigpiens*	3.81 ± 0.00	3.26 ± 0.01	4.64 ± 0.01	8.60 ± 0.01	12.41 ± 0.01
Skimmed milk / *Vājpiens*	0.05 ± 0.01	3.32 ± 0.00	4.59 ± 0.01	8.80 ± 0.01	9.01 ± 0.00

Table 3.1 continuation / *3.1. tabulas turpinājums*

Product / *Produkts*	Fat / *Tauki, %*	Protein / *Olbaltumvielas, %*	Lactose / *Laktoze, %*	Solids-not-fat / *Beztauku sausna, %*	Total solids / *Kopējā sausna, %*
Buttermilk / *Paniņas*	0.93 ± 0.00	2.83 ± 0.05	4.50 ± 0.01	8.24 ± 0.01	9.12 ± 0.00
Cream / *Krējums*	38.91 ± 0.09	2.01 ± 0.01	2.73 ± 0.01	5.59 ± 0.02	44.56 ± 0.10

Note. Mean ± SD; SD reflects analytical repeatability within the batch analysed in each season. An SD of 0.00 denotes zero at the displayed precision. / *Piezīme. Vidējā vērtība ± SD; SD raksturo analītisko atkārtojamību katrā sezonā analizētās partijas ietvaros. SD vērtība 0,00 norāda uz nulli izmantotajā noapaļošanas precizitātē.*

Similar methodological approaches have been used in recent studies (Hayes et al., 2023; Parmar et al., 2020) in which fat, protein, lactose, solids-non-fat, and total solids were measured to characterise raw milk and processing-related milk fractions before evaluating more detailed composition or technological properties.

A two-way ANOVA was performed to evaluate the effects of product, season, and the product × season interaction on the proximate composition in the analysed dataset. The results are presented in Table 3.2.

Table 3.2. **Two-way ANOVA results for chemical composition parameters /** *3.2. tabula* ***Divfaktoru dispersijas analīzes rezultāti ķīmiskā sastāva rādītājiem***

Parameter / *Rādītājs*	Variable / *Mainīgais*	df / *Brīvības pakāpes*	F	p-value / *p-vērtība*	*Partial η² / Parciālais η²*
Fat / *Tauki*	Product / *Produkts*	3, 16	1 663 182	**<0.001**	0.999
	Season / *Sezona*	1, 16	616.29	**<0.001**	0.975
	Product × season / *Produkts × sezona*	3, 16	764.71	**<0.001**	0.993
Protein / *Olbaltumvielas*	Product type / *Produkta veids*	3, 16	6 298	**<0.001**	0.999
	Season / *Sezona*	1, 16	65.15	**<0.001**	0.803
	Product × season / *Produkts × sezona*	3, 16	11.03	**<0.001**	0.674

Table 3.2. continuation / *3.2. tabulas turpinājums*

Parameter / *Rādītājs*	Variable / *Mainīgais*	df / *Brīvības pakāpes*	*F* / *F*	p-value / *p-vērtība*	*Partial η² / Parciālais η²*
Lactose / *Laktoze*	Product type / *Produkta veids*	3, 16	48 660	**<0.001**	0.999
	Season / *Sezona*	1, 16	569.48	**<0.001**	0.973
	Product × season / *Produkts × sezona*	3, 16	119.41	**<0.001**	0.957
Solids-not-fat / *Beztauku sausna*	Product type / *Produkta veids*	3, 16	21 143	**<0.001**	0.999
	Season / *Sezona*	1, 16	216.83	**<0.001**	0.931
	Product × season / *Produkts × sezona*	3, 16	44.94	**<0.001**	0.894
Total solids / *Kopējā sausna*	Product type / *Produkta veids*	3, 16	926 637	**<0.001**	0.999
	Season / *Sezona*	1, 16	863.12	**<0.001**	0.982
	Product × season / *Produkts × sezona*	3, 16	369.28	**<0.001**	0.986

Note. The df pairs give effect and residual degrees of freedom, respectively; F is the test statistic and partial η^2 is the effect-size measure. Bold p-values indicate $p < 0.05$. Results are exploratory and apply only to the analysed batches; residual variance reflects analytical repeatability. / *Piezīme. df pāros vispirms norādītas efekta, pēc tam atlikuma brīvības pakāpes; F ir testa statistika, bet parciālais η² - efekta lieluma rādītājs. Treknrakstā izceltas p vērtības < 0,05. Rezultātiem ir izpētes raksturs, un tie attiecas tikai uz analizētajām partijām; atlikuma dispersija raksturo analītisko atkārtojamību.*

In the analysed batches, product type, season and their interaction were associated with all five parameters ($p < 0.001$ in every case). Product type accounted for almost all the variance (partial $\eta^2 \geq 0.999$), reflecting the large compositional differences between skimmed milk, buttermilk, cream and raw milk relative to the very small analytical variation between replicates. The season and product × season show that winter-spring differences within the analysed batches were not the same across products.

Fat concentration showed the widest range among the analysed constituents. Raw milk had similar fat concentrations in both seasons, cream had the highest, skimmed milk the lowest, and buttermilk retained only a small fraction of the fat. The low total fat of buttermilk does not indicate a low PL concentration, because milk PL are associated with the MFGM material that is redistributed into buttermilk during cream churning rather than with the fat itself (Sakkas et al., 2022; Venkat et al., 2024).

Total solids followed the same pattern as fat concentration: they were highest in cream, intermediate in raw milk and comparable in skimmed milk and buttermilk, with a slightly lower level in spring than in winter for raw milk and cream. This pattern is consistent with reports that total solids in milk and dairy products are determined primarily by fat and protein content, with fat being the decisive component in cream and other fat-rich fractions (Hayes et al., 2023; Parmar et al., 2020).

Protein content varied less than fat concentration but still differed between products. Skimmed milk had the highest protein content, followed closely by raw milk; buttermilk contained less protein than either, and cream had the lowest content in both seasons. This pattern reflects the physical principles of milk separation: because fat globules are less dense than the aqueous phase, centrifugal force concentrates them in cream, whereas most proteins

remain in the serum phase, so that protein is slightly enriched in skimmed milk and diluted in cream. Separation efficiency is also influenced by milk viscosity, which affects fat-globule movement, and churning subsequently redistributes proteins and other non-fat solids between butter and buttermilk (Fox et al., 2015; Walstra et al., 2005)

Lactose and solids-not-fat varied little among the products. Both were highest in raw and skimmed milk and lowest in cream, reflecting the partition of the aqueous and lipid phases during separation, whereas buttermilk retained most non-fat constituents and was compositionally close to skimmed milk, in agreement with descriptions of sweet buttermilk in the literature (Jakopović et al., 2019; Sakkas et al., 2022).

Within the analysed material, the differences between winter and spring were consistent across the analytical replicates, but their size depended on the product and on the component considered. In raw and skimmed milk, protein, lactose, solids-not-fat and total solids were somewhat higher in winter; cream likewise contained more fat and total solids in winter. Buttermilk deviated from this pattern: its fat concentration was slightly higher in spring, while the other components stayed at the winter level or marginally below it. Seasonal variation of this modest magnitude agrees with recent reports on milk fat, protein, lactose and total solids, in which the size and direction of the changes in raw milk are governed by the production system, feeding regime, lactation stage and environment (Hayes et al., 2023; Parmar et al., 2020).

Overall, the data supported the observation that product was the dominant source of the variations in chemical composition within the analysed dataset. These results provide a compositional basis for further study of lipid fraction distribution, including total PL and individual PC, PE and SM (Y. Lin et al., 2017; Sakkas et al., 2022).

PC, PE, and SM together represent the predominant fraction of bovine milk PL. Published compositional data generally indicate that these classes account for approximately 80 % of total milk PL, although the exact proportion depends on the dairy matrix and analytical method (Z. Liu et al., 2015, 2020; Rombaut et al., 2005). Therefore, the targeted LC-MS determination captured the major MFGM-associated PL fraction relevant to the objectives of this thesis. However, selected molecular species within these classes were quantified; the term total quantified PL refers specifically to the sum of the measured PC, PE, and SM rather than to a complete untargeted lipidomic profile.

Table 3.3 presents the concentrations of total quantified PL and PC, PE, and SM in raw milk, skimmed milk, buttermilk, and cream in winter and spring, showing how PL are distributed among the products of processing.

Table 3.3. **The concentration of total quantified PL and major PL classes in milk and dairy products in winter and spring seasons /** *3.3. tabula* ***Kopējo PL un galveno PL pārstāvju saturs pienā un piena produktos ziemā un pavasarī***

Product / *Produkts*	Season / *Sezona*	Total PL /*PL saturs, mg 100 g^{-1}*	PC*, mg 100 g^{-1}*	PE*, mg 100 g^{-1}*	SM*, mg 100 g^{-1}*
Raw milk / *Svaigpiens*	Winter / *Ziema*	27.89 ± 1.93	11.23 ± 0.68	12.43 ± 0.94	4.24 ± 0.31
	Spring / *Pavasaris*	20.12 ± 0.13	9.03 ± 0.06	8.52 ± 0.06	2.57 ± 0.01

Table 3.3 continuation / *3.3. tabulas turpinājums*

Product / *Produkts*	Season / *Sezona*	Total PL /*PL saturs, mg 100 g^{-1}*	PC*, mg 100 g^{-1}*	PE*, mg 100 g^{-1}*	SM*, mg 100 g^{-1}*
Skimmed milk / *Vājpiens*	Winter / *Ziema*	11.97 ± 0.61	4.76 ± 0.27	5.05 ± 0.27	2.16 ± 0.07
	Spring / *Pavasaris*	10.84 ± 1.52	4.46 ± 0.78	4.46 ± 0.55	1.91 ± 0.18
Buttermilk / *Paniņas*	Winter / *Ziema*	51.65 ± 0.37	22.07 ± 0.16	23.87 ± 0.17	5.71 ± 0.04
	Spring / *Pavasaris*	44.51 ± 1.99	18.51 ± 0.13	20.88 ± 1.36	5.11 ± 0.49
Cream / *Krējums*	Winter / *Ziema*	110.98 ± 2.69	43.95 ± 0.37	59.35 ± 1.68	7.68 ± 0.64
	Spring / *Pavasaris*	110.05 ± 1.12	51.44 ± 0.71	50.53 ± 0.35	8.08 ± 0.06

Note. Mean ± SD of analytical measurements. Total quantified PL = PC + PE + SM; other PL classes are not included. / *Piezīme. Analītisko mērījumu vidējā vērtība ± SD. Kopējais kvantitatīvi noteiktais PL saturs = PC + PE + SM; citas PL klases nav iekļautas.*

The partitioning of fat and PL among the products obtained during processing is summarised in Fig. 3.1.

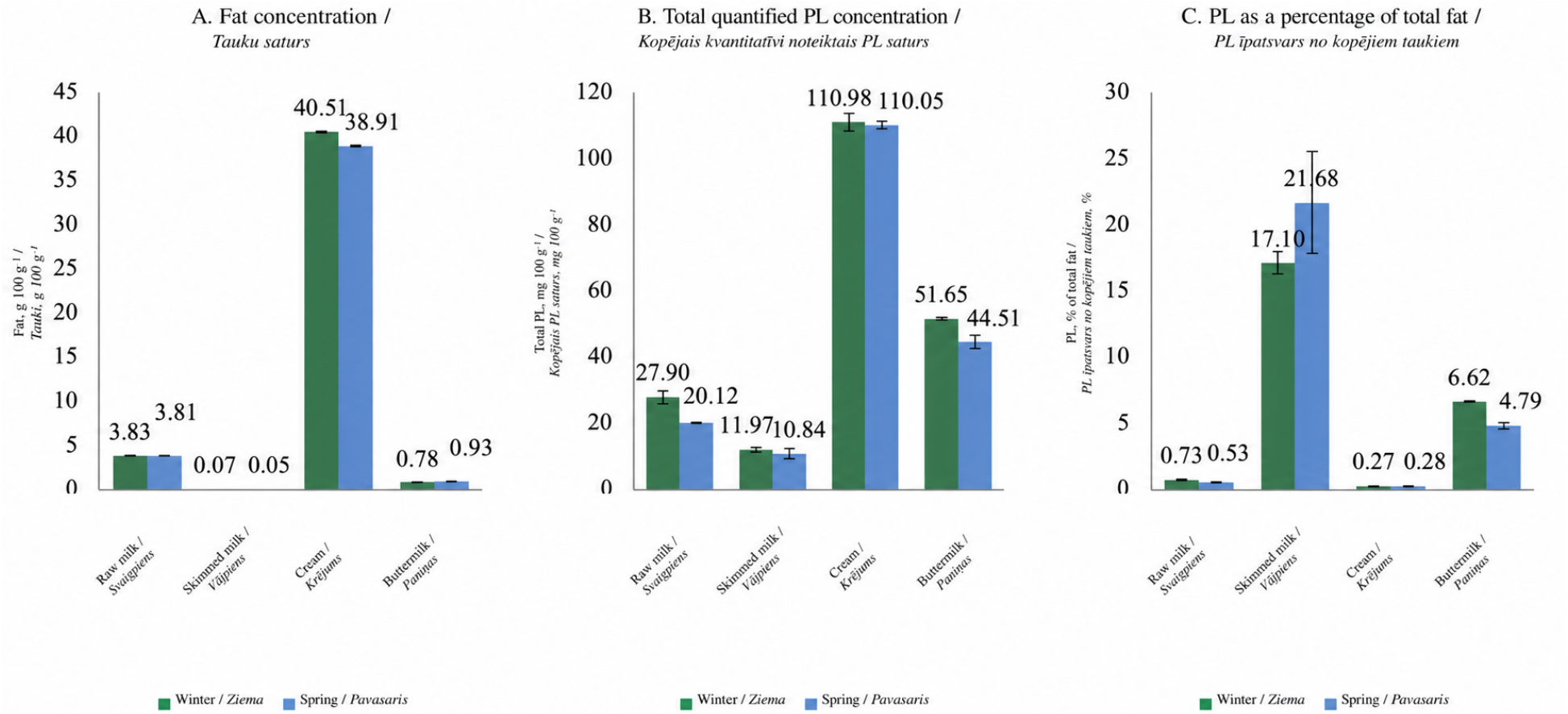


Fig. 3.1. **Fat concentration (A), total quantified PL concentration (B) and PL as a percentage of total fat (C) in raw milk and its processing products in winter and spring** / *3.1. att.* ***Tauku saturs (A), kopējais kvantitatīvi noteiktais PL saturs (B) un PL īpatsvars no kopējiem taukiem (C) svaigpienā un tā pārstrādes produktos ziemā un pavasarī***

Note. Panels A and B show mean concentrations with ± SD error bars. In panel C, PL as a percentage of total fat = [mean total quantified PL (mg 100 g^{-1}) / mean fat (g 100 g^{-1})] × 0.1. / *Piezīme. A un B panelī norādītas vidējās koncentrācijas; kļūdu joslas apzīmē ± SD. C panelī PL īpatsvars no kopējiem taukiem = [vidējais kopējais kvantitatīvi noteiktais PL saturs (mg 100 g^{-1}) / vidējais tauku saturs (g 100 g^{-1})] × 0,1.*

The concentration of quantified PL was determined by the technological distribution of MFGM material between the products and was not proportional to total fat concentration (Song et al., 2026). The highest concentration of total quantified PL was determined in cream ($110.05^{-1}10.98$ mg 100 g^{-1}), whereas the proportion of PL relative to fat in cream was the lowest (0.27-0.28 %). This distribution is attributed to centrifugal separation, which transfers predominantly large, intact fat globules into the fat phase; in these globules the triacylglycerol core constitutes the major lipid fraction, and membrane lipids constitute a minor fraction (Song et al., 2026; Venkat et al., 2024). The lowest concentration of total quantified PL was determined in skimmed milk ($10.84^{-1}1.97$ mg 100 g^{-1}), whereas the proportion of PL relative to fat was the highest (17.1-21.7 %), since the fat remaining after separation consists predominantly of small fat globules and MFGM fragments (Z. Liu et al., 2017; Pan et al., 2023). Because the fat concentration of skimmed milk (0.05-0.07 g 100 g^{-1}) is close to the quantification limit of the applied method, the calculated proportion is subject to considerable analytical uncertainty and is reported as an approximate value. Buttermilk contained 44.51-51.65 mg 100 g^{-1} of total quantified PL, corresponding to 4.79-6.62 % of fat. Disrupting the MFGM during churning releases membrane-associated lipids into the aqueous phase (Iung et al., 2022); consequently, PL concentration in buttermilk corresponded to 40-47 % of that in cream, whereas its fat concentration was 42-52 times lower. In raw milk, PL constituted 0.53-0.73 % of fat. An approximate mass balance illustrates this partitioning. A mass balance is the standard approach for describing the partitioning of milk components among processing fractions (Maniaci et al., 2026; Singh & Heldman, 2013); because the fractions were not weighed in the present study, an approximate balance was calculated from the measured concentrations. With the mass fraction of cream estimated from the fat balance (0.093 in winter and 0.097 in spring), 98-99 % of the raw-milk fat was recovered in cream, whereas the quantified PL was divided almost equally between cream (49-52 %) and skimmed milk (48-51 %).

The enrichment of PL in the dairy processing streams is in agreement with Rombaut et al. (2006), who reported an analogous distribution of phospho- and sphingolipids in buttermilk and butter serum, and with Song et al. (2026), who quantified the transfer of phospholipids into the skimmed milk and buttermilk fractions during byproduct separation and churning of milk.

Within the quantified PL fraction, PC and PE were the principal classes in all analysed products, whereas SM constituted a smaller proportion. This distribution corresponds to the composition of MFGM-rich fractions reported by Bourlieu et al. (2018) and Si et al. (2026); the relative proportions of the individual classes varied by product and processing stage (Si et al., 2026; Song et al., 2026).

Seasonal differences in PL composition were product-specific. In cream, the PC concentration increased and the PE concentration decreased from winter to spring, whereas total quantified PL remained unchanged (110.98 and 110.05 mg 100 g^{-1}); this indicates a seasonal redistribution between PL classes rather than a change in the total PL. In buttermilk, the proportion of PL relative to fat decreased from 6.62 % to 4.79 %, although fat concentration increased from 0.78 to 0.93 g 100 g^{-1}; this indicates that milk fat globule size and MFGM thickness vary with season. Both observations are consistent with the seasonal variation of raw milk lipid composition reported by Liu et al. (2017) and Lu et al. (2017). In the present study, seasonal changes originating in raw milk were further modified by separation, cream maturation, and churning, which affect fat globule size and membrane integrity in a product-specific manner (Y.-H. Liu et al., 2024; Lu et al., 2017). The physiological causes of seasonal variation in raw milk composition are discussed in Section 3.2.

A two-way ANOVA was conducted to assess the effects of product type, season, and the product × season interaction on total concentrations of PL and individual PC, PE, and SM classes in the analysed dataset. The results are presented in Table 3.4.

Table 3.4. **Two-way ANOVA results for total PL concentration and major PL classes /** *3.4. tabula* ***Divfaktoru dispersijas analīzes rezultāti kopējam PL saturam un galvenām PL klasēm***

Parameter / *Rādītājs*	Effect / *Efekts*	df / *Brīvības pakāpes*	F	p-value / *p vērtība*	*Partial η² / Parciālais η²*
Total PL / *Kopējie PL*	Product type / *Produkta veids*	3, 8	3273.48	**<0.001**	0.999
	Season / *Sezona*	1, 8	30.41	**<0.001**	0.792
	Product × season / *Produkts × sezona*	3, 8	5.84	**0.021**	0.686
PC	Product type / *Produkta veids*	3, 8	6412.98	**<0.001**	1.000
	Season / *Sezona*	1, 8	2.25	0.172	0.220
	Product × season / *Produkts × sezona*	3, 8	106.72	**<0.001**	0.976
PE	Product type / *Produkta veids*	3, 8	2637.97	**<0.001**	0.999
	Season / *Sezona*	1, 8	87.04	**<0.001**	0.916
	Product × season / *Produkts × sezona*	3, 8	15.66	**0.001**	0.855
SM	Product type / *Produkta veids*	3, 8	262.30	**<0.001**	0.990
	Season / *Sezona*	1, 8	11.37	**0.010**	0.587
	Product × season / *Produkts × sezona*	3, 8	7.60	**0.010**	0.740

Note. The df pairs give effect and residual degrees of freedom, respectively; F is the test statistic and partial η² is the effect-size measure. Bold p-values indicate $p < 0.05$. Results characterise the analysed batches only; residual variance reflects analytical repeatability, not variability between independent batches. / *Piezīme. df pāros vispirms norādītas efekta, pēc tam atlikuma brīvības pakāpes; F ir testa statistika, bet parciālais η² - efekta lieluma rādītājs. Treknrakstā izceltas p vērtības < 0,05. Rezultāti raksturo tikai analizētās partijas; atlikuma dispersija atspoguļo analītisko atkārtojamību, nevis mainību starp neatkarīgām partijām.*

In the exploratory two-way ANOVA, product type was associated with total PL and individual PC, PE and SM classes ($p < 0.001$), and the product × season interaction terms were significant for total PL ($F(3, 8) = 5.84$, $p = 0.021$), PC ($F(3, 8) = 106.72$, $p < 0.001$), PE ($F(3, 8) = 15.66$, $p = 0.001$) and SM ($F(3, 8) = 7.60$, $p = 0.010$). Within the analysed batches, winter-spring differences were therefore not uniform across products. Given the duplicate measurements and a single batch per season, this interaction terms indicate product-dependent seasonal patterns in the analysed material.

The differences among milk and dairy products are consistent with the technological redistribution of milk fat globules and MFGM-associated material during separation, cream maturation, and churning. Milk separation transfers large-sized fat globules into cream, whereas skimmed milk retains fewer globules (Hokkanen et al., 2021; Jukkola et al., 2019). Thus, the observed product effect reflects not only differences in total fat but also differences in membrane integrity and the partitioning of membrane fragments between dairy products.

The measured concentrations of total PL and individual PC, PE, and SM in raw and skimmed milk were broadly comparable to published compositional data, whereas the values for cream and buttermilk were lower than some estimates in the literature (Rombaut et al., 2005; Sakkas et al., 2022; Venkat et al., 2024). These differences may reflect variations in raw milk composition, processing conditions, sampling design, and analytical methodology. The reported PL values included only selected PC, PE, and SM classes rather than the glycerophospholipid and sphingolipid classes reported in broader lipidomic studies. Direct comparisons between studies should therefore be made cautiously, particularly when extraction procedures, standards, and result expressions differ.

Buttermilk contained a substantial quantified PL fraction despite its comparatively low total fat concentration, supporting the interpretation that total fat alone is not an adequate predictor of milk PL concentration. During churning, the MFGM is disrupted, and part of its material is released into the aqueous buttermilk phase, producing a low-fat stream that remains enriched in membrane-associated lipids and proteins. This finding is consistent with studies that describe buttermilk and butter serum as compositionally distinct sources of MFGM material, although their absolute PL concentrations vary within cream treatment prior butter production, churning conditions, subsequent butter grain fractionation, and analytical method for PL analysis (Bourlieu et al., 2018; Calvo et al., 2020; Sakkas et al., 2022).

Season was associated with total quantified PL, PE, and SM concentration, but the seasonal effect cannot be interpreted independently by product type. Seasonal changes in raw milk PL may result from differences in feeding system, pasture grass availability, stage of lactation, milk fat concentration, and fat-globule size, as previously reported in longitudinal bovine milk studies (Z. Liu et al., 2017; Venkat et al., 2024). The different responses among cream, buttermilk, and skimmed milk suggest that product-specific processing modified seasonal variations originating in raw milk composition.

For PC, the non-significant overall season effect ($p = 0.172$) combined with a product × season interaction ($p < 0.001$) indicates that PC did not change in the same direction across all products. In particular, PC increased in cream, while other products showed reductions or limited changes, indicating selective retention of PC-containing membrane fragments in the cream phase rather than a general spring-related increase. Such product-specific PC behaviour is consistent with reports showing that different PL classes respond differently to separation, shear forces, heating, and the source of the dairy fraction (Haddadian et al., 2018; Jukkola et al., 2019; Venkat et al., 2024).

PE generally declined from winter to spring, whereas SM varied by product, explaining the significant season and season-product interaction effects for both lipid classes. The predominance of PC and PE over SM was consistent with the established composition of MFGM (Bourlieu et al., 2018; Kanon et al., 2024; Venkat et al., 2024).

Similar compositional approaches have been used in recent lipidomic studies of MFGM-derived dairy fractions, in which the relative distribution of PL classes has been interpreted alongside matrix type, processing technology, and dairy product source (Calvo et al., 2020; Señoráns et al., 2023; Venkat et al., 2024).

The relative proportions of PC, PE and SM in the studied products in winter and spring are shown in Table 3.5.

Table 3.5. **Relative distribution of PC, PE, and SM and the PC/PE ratio in milk and dairy products in winter and spring /** *3.5. tabula* ***PC, PE un SM relatīvais sadalījums un PC/PE attiecība pienā un piena produktos ziemā un pavasarī***

Product / *Produkts*	Season / *Sezona*	PC, *%*	PE, *%*	SM, *%*	PC/PE ratio / *attiecība*
Raw milk / *Svaigpiens*	Winter / *Ziema*	40.30	44.50	15.20	0.91
	Spring / *Pavasaris*	44.90	42.30	12.80	1.06
Skimmed milk / *Vājpiens*	Winter / *Ziema*	39.80	42.20	18.10	0.94
	Spring / *Pavasaris*	41.20	41.10	17.60	1.00
Buttermilk / *Paniņas*	Winter / *Ziema*	42.70	46.20	11.10	0.92
	Spring / *Pavasaris*	41.59	46.92	11.49	0.89
Cream / *Krējums*	Winter / *Ziema*	39.60	53.50	6.90	0.74
	Spring / *Pavasaris*	46.70	45.90	7.30	1.02

Note. Each class is expressed as a percentage of PC + PE + SM. / *Piezīme. Katras klases īpatsvars izteikts procentos no PC, PE un SM summas.*

Across the analysed products, PC and PE were the major quantified PL classes by relative contribution, whereas SM accounted for a smaller proportion. In winter, PE was the dominant relative class in all analysed products, ranging from 42.20 % in skimmed milk to 53.50 % in cream. In spring, the relative balance shifted in some products: PC exceeded PE in raw milk and cream, while PC and PE were nearly equal in skimmed milk. Buttermilk retained a PE dominant profile in both seasons. These results show that the relative PL profile was not identical across product types or seasons, even when the same major PL classes were quantified.

Skimmed milk had the highest relative SM proportion, at 18.10 % in winter and 17.60 % in spring. Raw milk also showed a relatively higher SM proportion than buttermilk and cream, 15.20 % in winter and 12.80 % in spring. In contrast, cream indicated the lowest relative SM proportion - 6.90 % in winter and 7.30 % in spring, despite having the highest absolute SM concentration among studied products. This distinction illustrates why absolute concentrations and relative distributions provide complementary information. Absolute values describe the amount of quantified PL in the analysed products, whereas relative proportions describe the balance between PL classes within the quantified fraction. Similar differences between absolute PL concentration and relative PL class distribution have been reported in studies of buttermilk, butter serum and MFGM-enriched dairy fractions (Bourlieu et al., 2018; Calvo et al., 2020; Señoráns et al., 2023).

The PC/PE ratio was calculated to characterise the balance between the two main glycerophospholipid classes. In raw milk, the PC/PE ratio rose from 0.91 in winter to 1.06 in spring. A smaller increase was observed in skimmed milk, from 0.94 to 1.00. Cream showed the most pronounced shift, with the PC/PE ratio rising from 0.74 in winter to 1.02 in spring. By contrast, buttermilk showed a slight decrease, from 0.92 in winter to 0.89 in spring. These product-specific trajectories indicate that winter-spring shifts in the PC/PE balance were not uniform across the products studied. The increase in the relative contribution of PC in cream,

accompanied by a decrease in PE and an increase in the PC/PE ratio, indicates a shift towards a more PC-enriched PL profile in spring. From a consumer perspective, consumption of the same amount of cream in spring would therefore provide a greater amount of PC and a lower amount of PE, while total PL exposure would remain essentially unchanged. This compositional shift may be relevant because PL class balance can influence lipid digestion and membrane-related functionality. Studies of cream churning, butter and buttermilk have shown that processing conditions and MFGM partitioning during butter-making can influence the distribution of MFGM-associated PL classes, supporting the relevance of evaluating the PC/PE balance alongside the total PL concentration (Haddadian et al., 2018; Hokkanen et al., 2021; Señoráns et al., 2023).

SM is the principal sphingophospholipid of MFGM and is often discussed separately from glycerophospholipids because of its structural significance. Studies of buttermilk and butter serum have shown that these co-products can contain concentrated sphingolipid fractions, indicating that SM should be analysed separately from total PL (Bourlieu et al., 2018; Venkat et al., 2024).

The relative distribution of PC, PE and SM was additionally examined by PCA of the eight product-season observations. PCA served only to display the multivariate pattern and the direction of the winter-to-spring shift of each product; it was chosen because it places all products and both seasons in one plane and shows which PL class drives each displacement. With three closed proportions, two components describe the dataset completely (PC1 61.2 %, PC2 38.8 % of the variance), and these percentages characterise the orientation of the score space rather than the adequacy of a model. The loadings are given in Table 3.6 and the biplot Fig. 3.2.

Table 3.6. **Coordinates of PL variables in the PCA biplot derived from standardised compositional data /** *3.6. tabula* ***PL mainīgo koordinātes PCA divkomponentu grafikā, kas iegūts no standartizēta sastāva datiem***

Variable / *Mainīgais*	PC1 (61,2 %)	PC2 (38,8 %)
PC	0.304	1.025
PE	0.943	-0.504
SM	$^{-1}$.058	-0.155

Note. Values are variable coordinates derived from standardised relative proportions. PC1 and PC2 denote the first and second principal components. Their combined explained variance is 100 % because the three proportions sum to 100 %; this does not indicate model adequacy. / *Piezīme. Norādītas mainīgo koordinātes, kas iegūtas no standartizētiem relatīvajiem īpatsvariem. PC1 un PC2 apzīmē pirmo un otro galveno komponenti. To kopējā izskaidrotā dispersija ir 100 %, jo trīs īpatsvaru summa ir 100 %; tas neliecina par modeļa atbilstību.*

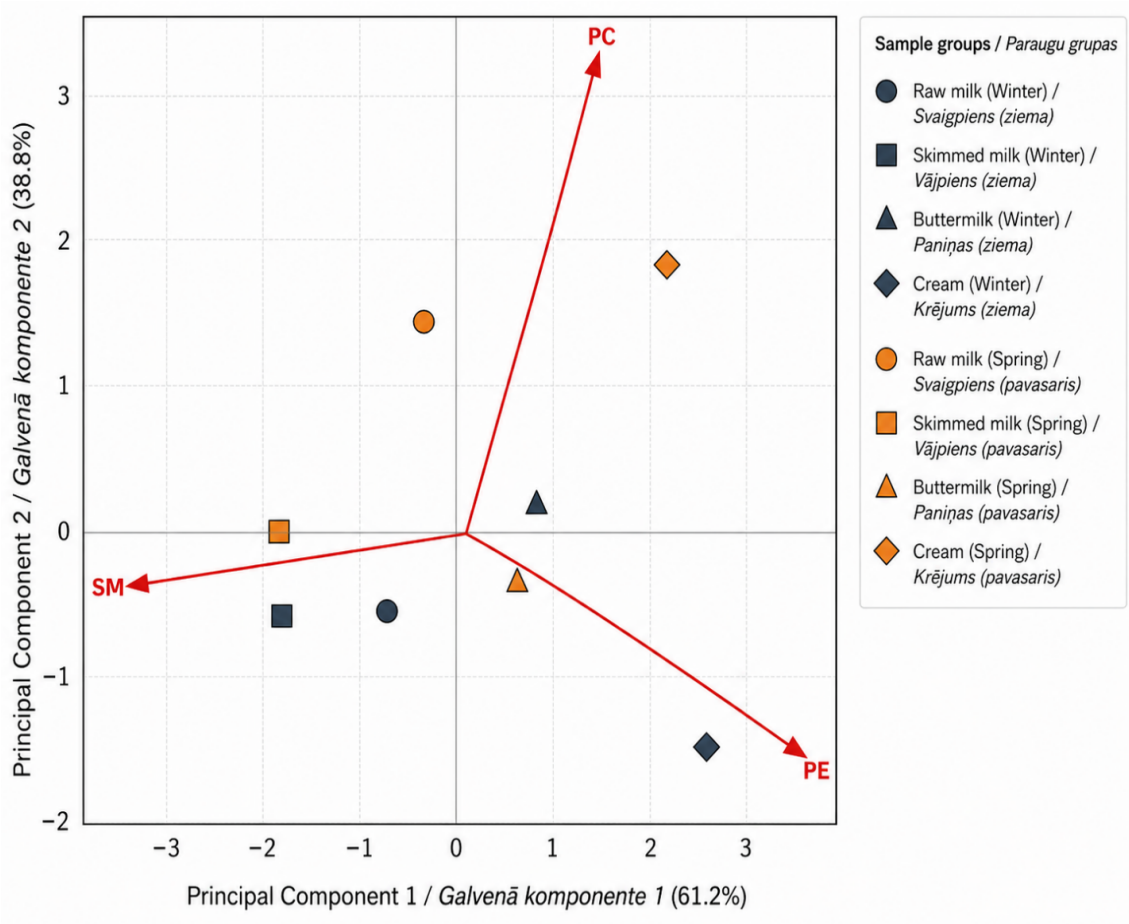


Fig. 3.2. **PCA biplot of the relative PL composition of dairy products /** *3.2. att.* ***Piena produktu relatīvā PL sastāva PCA divkomponentu grafiks***

Note. Points represent product-season combinations; vectors show the contribution of PC, PE and SM to the principal components. / *Piezīme. Punkti attēlo produktu un sezonu kombinācijas; vektori parāda PC, PE un SM ieguldījumu galvenajās komponentēs.*

PC1 primarily captured the contrast between PE and SM, with positive coordinates indicating a higher PE contribution and negative coordinates indicating a higher SM contribution. PC2 was largely aligned with PC, suggesting that variation along this component reflected changes in PC's relative contribution. The PCA supported the interpretation that analysed products differed not only in total PL concentration but also in the relative balance of major quantified PL classes.

To further highlight the direction of seasonal shifts, the same PCA score space was visualised as winter-to-spring trajectories for each product (Fig. 3.3).

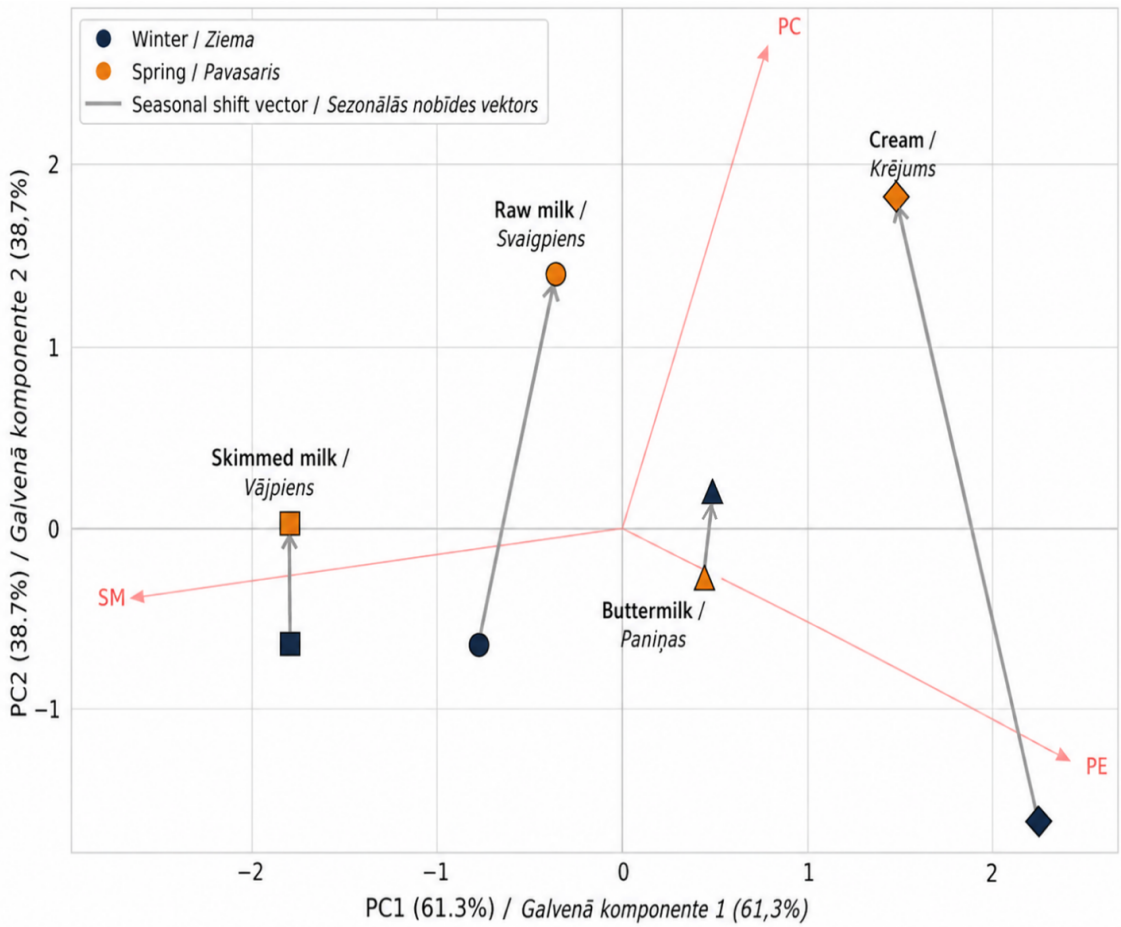


Fig. 3.3. **Seasonal trajectory analysis of relative PL composition in PCA score space /**
3.3. att. ***Piena produktu relatīvā PL sastāva sezonālo trajektoriju analīze PCA***

Note. Grey arrows connect the winter and spring observations for each product, pointing towards spring. / *Piezīme. Pelēkās bultiņas savieno katra produkta ziemas un pavasara novērojumus un ir vērstas uz pavasara novērojumu.*

In the PCA score space, the spring samples of raw milk and cream, in particular, shifted towards a higher relative share of PC, consistent with the increase in their PC/PE ratio. Buttermilk behaved differently: its PC/PE ratio decreased slightly, and PE remained the dominant class in both seasons. Within this dataset the seasonal shift in relative PL composition varied by product. Because the analysis rests on duplicate measurements and only eight product-season combinations, these shifts are exploratory observations on the analysed material rather than estimates for the dairy-product population.

In addition, ternary compositional trajectories were used to visualise seasonal shifts directly within the constrained PC-PE-SM compositional space Fig. 3.4.

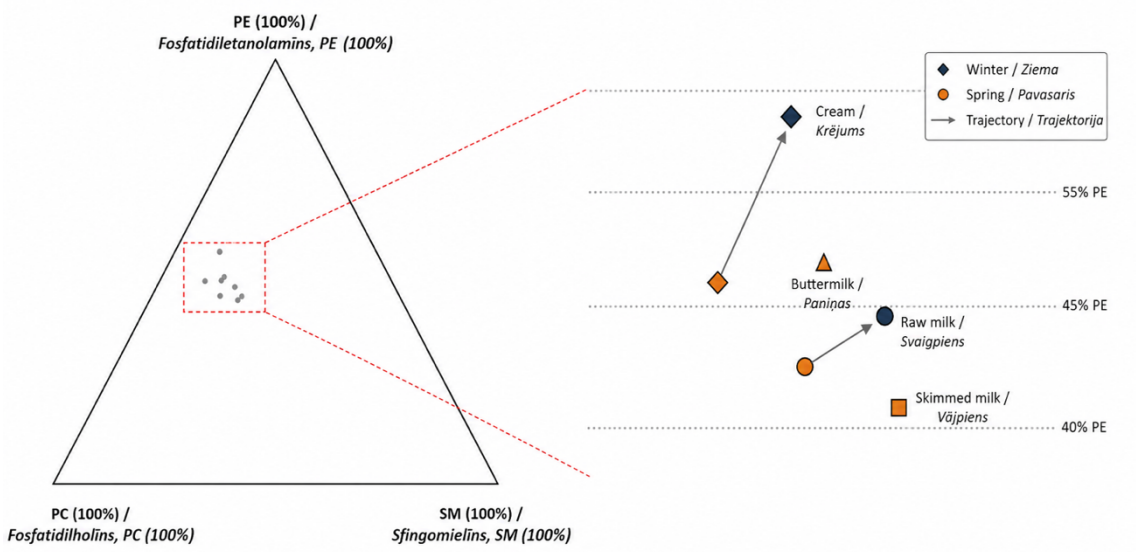


Fig. 3.4. **Ternary compositional trajectories of relative PC, PE and SM distribution in dairy products** / *3.4. att.* ***Relatīvā PC, PE un SM sadalījuma trīskomponentu sastāva trajektorijas piena produktos***

Note. Class proportions are expressed relative to PC + PE + SM. Dark symbols represent winter and orange symbols spring; the dashed red outline marks the area enlarged on the right. / *Piezīme. Klašu īpatsvari izteikti attiecībā pret PC, PE un SM summu. Tumšie simboli apzīmē ziemu, oranžie - pavasari; ar sarkanu pārtrauktu līniju apzīmētā daļa palielināta attēla labajā pusēa.*

The ternary trajectory approach offers a complementary visualisation of PL class balance. It indicates whether winter-spring changes shifted a product towards a more PC-, PE- or SM-enriched relative profile. In the analysed dataset, raw milk and cream shifted towards a higher PC proportion in spring, whereas buttermilk remained closer to the PE-dominant region. Skimmed milk showed comparatively little change, with PC and PE nearly balanced in spring. These results align with recent studies indicating that milk processing and MFGM redistribution can modify not only total PL concentration but also the relative distribution of PL classes within dairy products (Calvo et al., 2020; Haddadian et al., 2018; Hokkanen et al., 2021; Señoráns et al., 2023).

Summary of Chapter 3.1. / *3.1. nodaļas kopsavilkums*

This chapter evaluated the chemical composition, total quantified PL concentration, and relative distribution of PC, PE, and SM in milk and dairy products obtained during processing in winter and spring. Product type was the dominant source of variation in both chemical composition and PL concentration. Cream had the highest fat and total solids contents and the highest total quantified PL concentration, whereas skimmed milk had the lowest fat and PL concentrations. Buttermilk contained comparatively low total fat but a substantial quantified PL concentration, demonstrating that total fat concentration alone did not predict the distribution of MFGM-associated PL.

In the exploratory two-way ANOVA on duplicate measurements of one batch per season, product type was associated with total quantified PL and individual PC, PE and SM, and the product × season interaction terms were significant for all studied variables ($p \leq 0.021$), indicating that the winter-spring differences within the analysed batches were product-specific. PC and PE were the predominant quantified PL classes, whereas SM contributed a smaller proportion. The PC/PE ratio increased from winter to spring in raw milk, skimmed milk and cream but decreased slightly in buttermilk.

Šajā nodaļā izvērtēts piena un piena produktu, kas iegūti pārstrādes procesā ziemā un pavasarī, ķīmiskais sastāvs, tostarp PL saturs un PC, PE un SM relatīvais sadalījums. Produkta veids bija galvenais ķīmiskā sastāva un PL satura variāciju avots. Krējumā tika konstatēts augstākais tauku, kopējās sausnas un kvantificēto PL saturs, savukārt vājpienā bija mazākais tauku un PL saturs. Paniņās bija salīdzinoši zems kopējais tauku saturs, bet ievērojams kvantificēto PL saturs, apliecinot, ka kopējais tauku saturs neraksturo piena tauku lodīšu membrānā ietverto PL saturu un sastāvu.

Divfaktoru dispersijas analīzē, apstiprināja, ka produkta veids bija saistīts ar kopējo kvantificēto PL un individuālo PC, PE un SM saturu, un produkta × sezonas mijiedarbība bija statistiski nozīmīga visiem pētītajiem mainīgajiem ($p \leq 0,021$), kas norāda, ka atšķirības starp ziemu un pavasari bija atkarīgas no produkta veida. PC un PE bija dominējošās kvantificētās PL klases, savukārt SM bija mazāks īpatsvars, analizējot ziemas un pavasara sezonas datus. PC/PE attiecība palielinājās svaigpienā, vājpienā un krējumā, bet paniņās nedaudz samazinājās.

3.2. Characterisation of commercial cultured buttermilk selected for the intervention / *Intervencei izvēlēto komerciālo raudzēto paniņu raksturojums*

The compositional study provided two elements required for the clinical part of the thesis: the analytical procedure for PL quantification, which was applied without modification to the intervention product, and the season-specific PL concentrations of raw milk, which were subsequently used to estimate habitual milk PL intake of the participants (Section 2.4). The intervention was based on a commercially available food product rather than laboratory-produced buttermilk, an MFGM isolate, or a purified PL preparation; this section describes product selection and characterisation.

Cultured buttermilks available in the Latvian retail market during the study preparation period were screened; five products from five producers in Latvia, Lithuania and Poland were included. The products were compared against the selection criteria defined in Table 3.7.

Table 3.7. **Evaluation of commercially available cultured buttermilks against the selection criteria for the intervention product /** *3.7. tabula*
Komerciāli pieejamo raudzēto paniņu novērtējums atbilstoši intervences produkta izvēles kritērijiem

Parameter / *Parametrs*	Baltais / *Baltais*	Annele / *Annele*	Straupes paniņas / *Straupes paniņas*	Lazdonas paniņas / *Lazdonas paniņas*	Pilos / *Pilos*
Producer / *Ražotājs*	AS Tukuma piens, Latvia / *Latvija*	Pieno žvaigždės, Lithuania / *Lietuva*	PKS Straupe, Latvia / *Latvija*	AS Lazdonas piensaimnieks, Latvia / *Latvija*	Lidl Polska Sklepy Spożywcze sp. z o.o. private-label brand, Poland / *Polija*
Ingredients / *Sastāvs*	Buttermilk, lactic acid bacteria starter culture / *Paniņas, pienskābes baktēriju ieraugs*	Buttermilk, **skimmed milk**, lactic acid bacteria starter culture / *Paniņas, **vājpiens**, pienskābes baktēriju ieraugs*	Buttermilk, **skimmed milk**, lactic acid bacteria starter culture / *Paniņas, **vājpiens**, pienskābes baktēriju ieraugs*	Not declared / Nav deklarēts	50 % buttermilk, **pasteurised milk, milk proteins**, lactic acid bacteria / *50 % paniņas**, pasterizēts piens, piena olbaltumvielas**, pienskābes baktērijas*
Flavouring ingredients / *Aromatizējošas sastāvdaļas*	None / *Nav*	None / *Nav*	None / *Nav*	None / *Nav*	None / *Nav*
Declared fat, g 100 g^{-1} / *Deklarētais tauku saturs, g 100 g^{-1}*	0.5	0.3	0.5	0.5	**1.5**
Declared protein, g 100 g^{-1} / *Deklarētais olbaltumvielu saturs, g 100 g^{-1}*	3.2	3.0	3.3	3.2	3.5
Total PL, mg 100 g^{-1} / *Kopējie PL, mg 100 g^{-1}*	30.18 ± 2.40	16.33 ± 0.19	26.25 ± 0.18	24.65 ± 0.93	15.33 ± 0.21

Table 3.7 continuation / *3.7. tabulas turpinājums*

Parameter */ Parametrs*	Baltais */ Baltais*	Annele */ Annele*	Straupes paniņas */ Straupes paniņas*	Lazdonas paniņas */ Lazdonas paniņas*	Pilos */ Pilos*
Mesophilic lactic acid bacteria, CFU g^{-1} */ Mezofilās pienskābes baktērijas, KVV g^{-1}*	$(1.07 \pm 0.03) \times 10^9$	$(1.95 \pm 0.07) \times 10^9$	**$(9.20 \pm 0.12) \times 10^8$**	**$(6.37 \pm 0.37) \times 10^8$**	**$(3.57 \pm 0.74) \times 10^8$**
Selected for the intervention */ Izvēlēta intervencei*	Yes / *Jā*	No / *Nē*	No / *Nē*	No / *Nē*	No / *Nē*

Note. Ingredients and declared composition are taken from product labels; PL concentrations and bacterial counts are measured values, reported as mean ± SD. The ingredient list for "Lazdonas paniņas" was unavailable at evaluation. Bold text marks compositional features less consistent with the intervention aims, not statistical significance or regulatory non-compliance. / *Piezīme. Informācija par sastāvdaļām un deklarēto sastāvu iegūta no produktu marķējuma; PL saturs un baktēriju skaits ir mērījumu rezultāti, izteikti kā vidējā vērtība ± SD. Izvērtēšanas laikā "Lazdonas paniņas" sastāvdaļu saraksts nebija pieejams. Treknrakstā izceltas sastāva pazīmes, kas mazāk atbilst intervences mērķiem; izcēlums nenorāda uz statistisko nozīmīgumu vai neatbilstību normatīvajām prasībām.*

The declared fat ($0.3^{-1}.5$ g 100 g^{-1}) and protein content (3.0-3.5 g 100 g^{-1}) of the five products corresponded to the composition range reported for buttermilk. According to Sakkas et al. (2022), sweet-cream buttermilk contains 8.88-9.16 % total solids, 0.45-0.54 % fat and 2.78-2.94 % protein, and commercially available buttermilk contains 8.7 ± 0.8 % total solids, 0.9 ± 0.4 % total lipids and 2.9 ± 0.3 % protein. Direct comparison is limited, since the evaluated products were fermented and commercially standardised, whereas most published data refer to non-fermented sweet buttermilk obtained during butter manufacture.

According to Cabinet Regulation No. 97/2011, cultured buttermilk must contain at least 10^7 viable lactic acid bacteria per gram (Ministru kabinets, 2011). All five products complied with this requirement: the viable mesophilic lactic acid bacteria count ranged from (3.57 ± 0.74) × 10^8 to (1.95 ± 0.07) × 10^9 CFU g^{-1}. The microbiological examination verified product functionality.

Cultured buttermilk "Baltais" was selected for the intervention based on the combined assessment of ingredient composition, analytically determined total quantified PL concentration, declared fat content, microbiological characteristics and regular retail availability. PL concentrations were measured in all candidate products before selection. "Baltais" had the highest observed mean total quantified PL concentration (30.18 ± 2.40 mg 100 g^{-1}), while its declared fat content was 0.5 g 100 g^{-1}. Its ingredient list included buttermilk and starter culture, with no added skimmed milk, milk, or milk proteins, consistent with the aim of studying a buttermilk-based intervention.

The pre-selection comparison of the five commercial products is presented in Table 3.7. Following selection, samples of "Baltais" purchased during the winter and spring intervention periods were analysed using the LC-MS procedure described in Section 2.2.3. These season-specific measurements were used to estimate the PL dose supplied by the daily intervention portion (Table 3.8).

Table 3.8. **PL concentration of the selected cultured buttermilk and estimated PL dose provided by the daily intervention portion /** *3.8. tabula*
Izvēlēto raudzēto paniņu PL saturs un aprēķinātā PL deva intervences porcijā dienā

Season / *Sezona*	Concentration, mg 100 g^{-1} / *Saturs, mg 100 g^{-1}*				Estimated dose, mg 250 g^{-1} / *Aprēķinātā deva, mg 250 g^{-1}*			
	Total PL / *Kopējie PL*	PC	PE	SM	Total PL / *Kopējie PL*	PC	PE	SM
Winter / *Ziema*	32.52 ± 0.78	13.91 ± 0.30	14.84 ± 0.44	3.77 ± 0.05	81.30 ± 1.95	34.78 ± 0.75	37.10 ± 1.10	9.43 ± 0.13
Spring / *Pavasaris*	27.89 ± 0.89	11.77 ± 0.26	14.55 ± 0.61	1.57 ± 0.05	69.73 ± 2.23	29.43 ± 0.65	36.38 ± 1.53	3.93 ± 0.13

Note. Mean ± SD. Total quantified PL = PC + PE + SM. / *Piezīme. Vidējā vērtība ± SD. Kopējais kvantitatīvi noteiktais PL saturs = PC + PE + SM.*

The daily portion supplied 81.30 ± 1.95 mg of total quantified PL in winter and 69.73 ± 2.23 mg in spring. Because PC, PE, and SM are the predominant milk PL classes, this estimate characterises the nutritionally relevant part of the intervention dose, although it does not cover the full diversity of milk PL classes.

The total quantified PL concentration of "Baltais" (27.89-32.52 mg 100 g^{-1}) was 37 % lower than that of the sweet-cream buttermilk produced in the pilot plant (44.51-51.65 mg 100 g^{-1}). Several technological differences account for this: the commercial product is manufactured from industrially separated and pasteurised cream; it is standardised and fermented; its fat concentration is lower (0.5 g 100 g^{-1} according to the nutrition declaration versus 0.78-0.93 g 100 g^{-1} determined in the pilot-plant buttermilk), and it was analysed after distribution rather than immediately after churning. The total PL concentration of the selected cultured buttermilk was within the broad range reported for buttermilk and buttermilk-derived fractions, although direct quantitative comparison is constrained by differences in raw material, fermentation applied, analytical methodology for PL quantification and quantified PL classes (Calvo et al., 2020; Castro-Gómez et al., 2017; Jukkola et al., 2019).

The concentration determined in "Baltais" (0.028-0.033 g 100 g^{-1}) lies within the range reported for buttermilk by Sakkas et al. (2022): 0.037 ± 0.024 g 100 g^{-1} in sweet buttermilk from bovine milk cream, 0.037 ± 0.010 g 100 g^{-1} in sweet buttermilk from sheep milk cream and 0.026 ± 0.012 g 100 g^{-1} in buttermilk from cheese-whey cream. Considerably higher PL concentration are obtained only after MFGM enrichment: in the industrial streams compared by Calvo et al. (2020), PL constituted 12-16 % of the quantified lipid fraction in buttermilk and butter serum and about 40 % after isolation of an MFGM-enriched fraction, with PE, PC and SM as the predominant classes, and Bourlieu et al. (2018) reported 1400 ± 200 mg 100 g^{-1} of PL in buttermilk versus 8500 ± 1100 mg 100 g^{-1} in butter serum. The commercial cultured buttermilk used in the present study is therefore a whole-food dairy matrix containing naturally occurring PL and viable lactic acid bacteria.

The PL dose supplied by the intervention product differed between seasons: the total quantified PL dose was 14 % lower in spring than in winter, owing to lower PC (-15 %) and, in particular, SM (-58 %) concentrations, whereas PE remained comparable (-2 %). This difference is considered when interpreting the intervention results. These differences should also be interpreted descriptively, as the intervention product samples represented season-specific commercial product measurements rather than a broad market-level sampling frame.

Summary of Chapter 3.2. / *3.2. nodaļas kopsavilkums*

This chapter characterised the commercial cultured buttermilk selected for the 28-day dietary intervention. Five products from Latvia, Lithuania and Poland were evaluated according to ingredient composition, declared nutritional value, absence of flavouring ingredients, analytically determined PL concentration, microbiological characteristics and retail availability. PL concentrations were measured before product selection. “Baltais” was selected because it combined the highest observed mean total quantified PL concentration among the evaluated products (30.18 ± 2.40 mg 100 g^{-1}) with a declared fat content of 0.5 g 100 g^{-1}, an ingredient list comprising buttermilk and starter culture without added milk ingredients, and regular retail availability. Its mesophilic lactic acid bacteria count was (1.07 ± 0.03) × 10^9 CFU g^{-1}.

Season-specific analyses of the selected product showed total quantified PL concentrations of 32.52 ± 0.78 mg 100 g^{-1} in winter and 27.89 ± 0.89 mg 100 g^{-1} in spring. The daily intervention portion was 250 mL. Using the nominal mass of 250 g adopted for dose calculations, the estimated total quantified PL dose was 81.30 ± 1.95 mg in winter and 69.73 ± 2.23 mg in spring. PC and PE were the predominant quantified PL classes, whereas SM was present at a lower concentration. The lower estimated PL dose in spring mainly reflected lower PC and SM concentrations, while PE remained comparatively stable. Total quantified PL

concentration was approximately 37 % lower than in the pilot-plant buttermilk; differences in raw material and fermentation may have contributed to this contrast.

The selected product provided naturally occurring milk PL in a commercially available cultured buttermilk suitable for daily consumption. Its measured PL composition differed between the winter and spring intervention periods, resulting in different estimated PL doses from the same daily portion volume. These findings characterise the analysed product samples and do not establish general differences between brands or seasons.

Šajā nodaļā raksturotas 28 dienu uztura intervencei izvēlētās komerciāli pieejamās raudzētās paniņas. Pieci produkti no Latvijas, Lietuvas un Polijas tika izvērtēti, ņemot vērā sastāvdaļas, deklarēto uzturvērtību, aromatizējošu sastāvdaļu neesamību, analītiski noteikto PL saturu, mikrobioloģiskos rādītājus un pieejamību mazumtirdzniecībā. PL koncentrācija tika noteikta pirms produkta izvēles. Paniņas "Baltais" tika izvēlētas, jo tām bija lielāks kopējo PL saturs starp izvērtētajiem produktiem (30.18 ± 2.40 mg 100 g^{-1}), deklarētais tauku saturs bija 0.5 g 100 g^{-1}, sastāvā bija paniņas un ieraugs bez pievienotām piena sastāvdaļām, un produkts bija regulāri pieejams mazumtirdzniecībā. Mezofilo pienskābes baktēriju skaits šajā produktā bija (1.07 ± 0.03) × 10^9 KVV g^{-1}.

Izvēlētā produkta kopējo kvantitatīvi noteikto PL saturs ziemā bija 32.52 ± 0.78 mg 100 g^{-1}, bet pavasarī - 27.89 ± 0.89 mg 100 g^{-1}. Intervences produkta dienas porcija bija 250 mL. Izmantojot devas aprēķiniem pieņemto nominālo porcijas masu 250 g, aprēķinātā kopējo kvantitatīvi noteikta PL deva ziemā bija 81.30 ± 1.95 mg, bet pavasarī - 69.73 ± 2.23 mg. PC un PE bija dominējošās kvantitatīvi noteiktās PL klases, savukārt SM saturs bija mazāks. Mazākā aprēķinātā PL deva pavasarī galvenokārt bija saistīta ar mazāku PC un SM saturu, bet PE saturs saglabājās salīdzinoši stabili. Kopējo kvantitatīvi noteikto PL saturs bija aptuveni par 37 % mazāks nekā pilotražotnē iegūtajās paniņās; šo atšķirību varēja ietekmēt atšķirības izejvielā un raudzēšanā.

Izvēlētais produkts nodrošināja dabīgi sastopamos piena PL komerciāli pieejamu, ikdienas patēriņam piemērotu raudzētu paniņu veidā. Analītiski noteiktais PL sastāvs ziemas un pavasara intervences periodos atšķīrās, tādēļ vienāds dienas porcijas tilpums nodrošināja atšķirīgas aprēķinātās PL devas. Šie rezultāti raksturo analizētos produktu paraugus un neļauj vispārināt secinājumus par atšķirībām starp zīmoliem vai sezonām.

3.3. Participant recruitment and baseline characteristics / *Dalībnieču atlase un to raksturojums*

The final study population comprised 61 perimenopausal women aged 45-55 years with moderately elevated LDL-C concentrations. The intervention group included 31 participants, and the control group included 30 participants. Participants were also classified into two seasonal cohorts of different women according to the assessment period (Section 2.3.3). The seasonal distribution by study group is presented in in Table 3.9.

Table 3.9. **Distribution of participants by study group and season /** *3.9. tabula* ***Dalībnieču sadalījums pētījuma grupās un sezonās***

Study group / *Pētījuma grupa*	Winter / *Ziema, n (%)*	Spring / *Pavasaris, n (%)*	Total / *Kopā, n*
Intervention group / *Intervences grupa*	11 (35.5 %)	20 (64.5 %)	31
Control group / *Kontroles grupa*	12 (40.0 %)	18 (60.0 %)	30
Total / *Kopā*	23 (37.7 %)	38 (62.3 %)	61

Note. Percentages are calculated within each row. / *Piezīme. Procentuālais sadalījums aprēķināts katras rindas ietvaros.*

Baseline characteristics of participants who completed the study are presented in Table 3.10. The table also includes anthropometric measurements, alcohol use, smoking status, daily physical activity, and meal-frequency indicators. These variables were included because they may influence the plasma lipid profile and therefore provide relevant context for interpreting the dietary and biochemical outcomes.

Table 3.10. **Baseline characteristics of participants /** *3.10. tabula* ***Dalībnieču sākotnējais raksturojums***

Characteristic / *Raksturlielums*	Intervention group / *Intervences grupa (n = 31)*	Control group / *Kontroles grupa (n = 30)*	p-value / *p-vērtība*	Hedges' g / *Hedžesa g*
Age, years / *Vecums, gadi*	49.16 ± 2.60	49.63 ± 3.17	0.528	0.16
Weight, kg / *Ķermeņa masa, kg*	69.50 ± 11.5	71.8 ± 14.3	0.499	0.17
Body mass index, kg m^{-2} / *Ķermeņa masas indekss, kg* m^{-2}	25.25 ± 4.15	25.31 ± 4.85	0.954	0.01
Waist circumference, cm / *Vidukļa apkārtmērs, cm*	83.52 ± 9.10	85.20 ± 13.23	0.566	0.15
Waist-to-hip ratio / *Vidukļa un gurnu apkārtmēra attiecība*	0.81 ± 0.05	0.82 ± 0.09	0.918	0.03
Alcohol use, yes / *Alkohola lietošana, jā*	19 (61.29 %)	17 (56.67 %)	0.797	NA
Smoking, yes / *Smēķēšana, jā*	1 (3.23 %)	2 (6.67 %)	0.612	NA
Daily physical activity / *Ikdienas fiziskā aktivitāte*	26 (83.87 %)	25 (83.33 %)	1.000	NA
Main meals per day: <2 / *Galvenās ēdienreizes dienā: <2*	8 (25.81 %)	4 (13.33 %)	0.322	NA
Main meals per day: 2-3 / *Galvenās ēdienreizes dienā: 2-3*	22 (70.97 %)	23 (76.67 %)		
Main meals per day: 4-5 / *Galvenās ēdienreizes dienā: 4-5*	1 (3.23 %)	3 (10.00 %)		

Table 3.10 continuation / *3.10. tabulas turpinājums*

Characteristic / *Raksturlielums*	Intervention group / *Intervences grupa (n = 31)*	Control group / *Kontroles grupa (n = 30)*	p-value / *p-vērtība*	Hedges' g / *Hedžesa g*
Snacks per day: no snacks / *Uzkodas dienā: nav*	2 (6.45 %)	0 (0.00 %)	0.245	NA
Snacks per day: 1 / *Uzkodas dienā: 1*	13 (41.94 %)	7 (23.33 %)		
Snacks per day: 2 / *Uzkodas dienā: 2*	10 (32.26 %)	13 (43.33 %)		
Snacks per day: 3 / *Uzkodas dienā: 3*	5 (16.13 %)	7 (23.33 %)		
Snacks per day: ≥4 / *Uzkodas dienā: ≥4*	1 (3.23 %)	3 (10.00 %)		

Note. Values are mean ± SD or n (%). p-values compare the study groups; positive Hedges' g values indicate higher means in the control group. Daily physical activity includes walking, active transport, stair climbing and housework. / *Piezīme. Vērtības norādītas kā vidējā vērtība ± SD vai n (%). p vērtības attiecas uz pētījuma grupu salīdzinājumu; pozitīvas Hedžesa g vērtības norāda uz lielākām vidējām vērtībām kontroles grupā. Ikdienas fiziskās aktivitātes ietver iešanu kājām, aktīvo pārvietošanos, kāpšanu pa kāpnēm un mājsaimniecības darbus.*

The intervention and control groups showed no statistically significant differences in baseline demographic, anthropometric, or lifestyle characteristics. Age, body weight, body mass index, waist circumference, and waist-to-hip ratio were comparable between the groups, indicating a generally similar baseline anthropometric profile. Likewise, no significant between-group differences were identified in alcohol use, smoking status, self-reported daily light physical activity, or the reported frequency of main meals and snacks. These findings provide no evidence of a systematic baseline imbalance in the characteristics assessed and support the comparability of the groups before the intervention.

The distribution of BMI categories is shown in Table 3.11.

Table 3.11. **BMI categories of participants by study group** / *3.11. tabula* ***Dalībnieču ĶMI sadalījums pa pētījuma grupām***

BMI category / *ĶMI kategorija*	Intervention group / *Intervences grupa* (n = 31)	Control group / *Kontroles grupa* (n = 30)	Total / *Kopā* (n = 61)	p-value / *p-vērtība*
Underweight, <18.5 kg m^{-2} / *Nepietiekama ķermeņa masa, <18.5 kg m^{-2}*	2 (6.45 %)	0 (0.00 %)	2 (3.28 %)	p = 0.092
Normal weight, 18.5-24.9 kg m^{-2} / *Normāla ķermeņa masa, 18.5-24.9 kg m^{-2}*	12 (38.71 %)	17 (56.67 %)	29 (47.54 %)	
Overweight, 25.0-29.9 kg m^{-2} / *Lieka ķermeņa masa, 25.0-29.9 kg m^{-2}*	13 (41.94 %)	6 (20.00 %)	19 (31.15 %)	
Obesity class I, 30.0-34.9 kg m^{-2} / *I pakāpes aptaukošanās, 30.0-34.9 kg m^{-2}*	4 (12.90 %)	7 (23.33 %)	11 (18.03 %)	

Note. Values are n (%), with percentages calculated within each column. The p-value refers to the overall comparison of BMI category distributions between the study groups. Categories follow the WHO adult classification. / *Piezīme. Vērtības norādītas kā n (%); procenti aprēķināti katras kolonnas ietvaros. p vērtība attiecas uz kopējo ĶMI kategoriju sadalījuma salīdzinājumu starp pētījuma grupām. Kategorijas noteiktas atbilstoši WHO pieaugušo klasifikācijai.*

A comparison with national data indicates that the BMI distribution of the study participants differed from that observed among women in the general Latvian population. In the present study, 49.18 % of participants had a BMI of at least 25.0 kg m^{-2}, including 31.15 % classified as overweight and 18.03 % as having class I obesity. Eurostat data for 2022 showed that 56.7 % of females aged 16 years or older in Latvia were classified as overweight, including 23.9 % with obesity. Thus, the prevalence of both excess body weight and obesity was lower in the total study sample than in the broader Latvian female population (Eurostat, 2024).

More recent age-specific national data provide a more appropriate comparison. According to the representative *Health Behaviour among Latvian Adult Population, 2024* survey, 34.9 % of Latvian women aged 45-54 years were classified as overweight and 29.4 % as having obesity. Therefore, 64.3 % had a BMI of at least 25.0 kg m^{-2}, whereas 34.0 % had a BMI within the normal-weight range. In comparison, 49.18 % of the present study participants had overweight or obesity, while 47.54 % were classified as having normal body weight. These findings indicate a lower prevalence of excess body weight, particularly obesity, in the study sample than in the age-matched national female population (Grīnberga et al., 2025).

The lower prevalence of overweight and obesity in the study sample may also partly reflect the recruitment and eligibility procedures. Participants were recruited via convenience sampling through gynaecological practices, general practitioners, hospitals, and social media. Women who encountered information about the study through healthcare services or health-related social media and subsequently volunteered for a dietary intervention may have been

more health-conscious and more motivated to participate than women in the general population. This suggests a plausible healthy-volunteer or self-selection effect, although health motivation was not directly assessed and this explanation therefore cannot be confirmed in the present study. Previous research has shown that volunteers in health-related cohort studies may have more favourable health characteristics, including a lower prevalence of obesity, than the population from which they were recruited (Fry et al., 2017).

In the present study, 54.84 % of participants in the intervention group and 43.33 % in the control group were classified as overweight or obese. The prevalence of obesity was 12.90 % in the intervention group and 23.33 % in the control group. The prevalence observed in the control group was closer to the Eurostat estimate for Latvian females aged 16 years or older and to the age-specific national estimate than that observed in the intervention group (Eurostat, 2024; Grīnberga et al., 2025). Conversely, the control group included a higher proportion of participants with normal body weight, whereas underweight was observed only in the intervention group. The overall distribution of BMI categories did not differ significantly between the intervention and control groups ($p = 0.092$).

Waist circumference and waist-to-hip ratios of participants / *Dalībnieču vidukļa apkārtmērs un vidukļa-gurnu attiecība*

The mean BMI of participants in both groups was close to the overweight threshold, indicating a moderate degree of excess body weight in the study population. This interpretation was supported by mean waist circumferences of 83.52 ± 9.10 cm in the intervention group and 85.20 ± 13.23 cm in the control group. Although the mean waist-to-hip ratio remained below the WHO cut-off of 0.85 for abdominal obesity in women, waist circumference provided additional information on central adiposity and should be considered when interpreting baseline cardiometabolic characteristics (World Health Organization, 2011).

Waist circumference and waist-to-hip ratio are clinically relevant because they reflect body fat distribution, which BMI alone does not fully capture. In women, a higher waist-to-hip ratio has been associated with an increased likelihood of dyslipidaemia, while menopausal status and obesity have been associated with to a higher prevalence of lipid profile abnormalities (Kindie et al., 2025; Nikparvar et al., 2021). Recent evidence also indicates that abdominal obesity and menopausal status contribute to a less favourable cardiometabolic risk profile in women (Orhan et al., 2025). In addition, a recent systematic review and meta-analysis found that an elevated waist-to-hip ratio was associated with a higher risk of myocardial infarction, with a stronger association observed in women than in men, supporting the relevance of waist-to-hip ratio as a CVD risk marker (Zhang et al., 2024).

Waist circumference and waist-to-hip ratio were somewhat higher in the control group than in the intervention group, but neither difference was statistically significant ($p = 0.566$ and $p = 0.918$), so the groups were comparable at baseline for these indicators of central adiposity. The two measures are nonetheless reported because central fat distribution contributes to variability in lipid metabolism between individuals and is relevant background for interpreting the lipid data.

Physical activity of participants / *Dalībnieču fiziskā aktivitāte*

Daily light physical activity was high in both study groups. In the intervention group, 26 of 31 participants reported daily light physical activity (83.87 %). In the control group, 25 of 30 participants reported daily light physical activity (83.33 %). Overall, 51 of 61 participants reported daily light physical activity (83.61 % of the total study population).

The between-group comparison showed no statistically significant difference in the proportion of participants reporting this type of activity ($p = 1.000$). Therefore, the available data provide no evidence that daily light physical activity constituted a baseline lifestyle imbalance between the study groups. This descriptive similarity suggests that measured daily light physical activity was unlikely to be a major source of baseline lifestyle imbalance between the groups. However, this interpretation should be approached with caution because the study

recorded only daily light physical activity and did not assess structured exercise, intensity, duration, or sedentary time in detail.

Compared with national data, the study cohort appeared substantially more active. According to the *Health behaviour among the Latvian adult population report*, 43.5 % of women aged 45-54 reported daily light physical activity (Grīnberga et al., 2023). In the present study, the corresponding proportion was 83.61 %, compared with 43.5 % among Latvian women in the same age group, a difference of 40.1 percentage points. This difference suggests that the study population may represent a more health-conscious or more active subgroup of perimenopausal women rather than the general population of Latvian women aged 45-54.

Physical activity is relevant to interpreting lipid profile outcomes because regular activity has been associated with more favourable lipid metabolism. Recent reviews indicate that physical activity and aerobic exercise are generally associated with higher HDL-C and lower TG concentrations, with possible additional benefits for TC and LDL-C concentrations depending on exercise type, intensity and volume (Franczyk et al., 2023; Y. Li et al., 2024). The high prevalence of daily light physical activity in the present cohort may therefore have contributed to a generally more favourable cardiometabolic background and should be considered when interpreting baseline lipid profile characteristics and intervention-related changes.

Overall, self-reported daily light physical activity was common in both groups, and the proportions were closely comparable, with no statistically significant difference between the intervention and control groups ($p = 1.000$). This finding supports baseline comparability between the groups for the measured physical activity variable. At the same time, the substantially higher prevalence of daily light physical activity than that reported in national data may indicate that the study cohort differed from the broader Latvian female population in health-related lifestyle behaviours. However, this comparison should be interpreted cautiously if the national survey used different definitions, response categories, or methods for assessing physical activity.

Alcohol consumption and smoking / *Alkohola lietošana un smēķēšana*

Alcohol consumption was reported by 19 of the 31 women in the intervention group and by 17 of the 30 women in the control group. The slightly higher proportion in the intervention group was not statistically significant ($p = 0.797$), so the groups did not differ at baseline in this respect. It should be noted, that alcohol use was recorded only as a yes/no characteristic; frequency, amount, drinking pattern and beverage type, all of which can affect cardiometabolic risk and the lipid profile, were not captured.

This difference should be interpreted descriptively because alcohol consumption was recorded as a general lifestyle variable and did not include detailed information on frequency, dose, beverage type or binge-drinking pattern. Therefore, the available data do not allow classification of alcohol intake as low, moderate or excessive. This distinction is important because the association between alcohol consumption and lipid metabolism is complex. Recent evidence indicates that alcohol intake may be associated with higher HDL-C and LDL-C concentrations, whereas heavy or high-intensity drinking patterns have been associated with less favourable lipid and cardiometabolic markers, including higher TG and cholesterol concentrations (Minzer et al., 2020; Rosoff et al., 2019; Suzuki et al., 2025).

Smoking prevalence was low in both groups. One participant in the intervention group and two participants in the control group reported smoking. The between-group difference was not statistically significant ($p = 0.612$). Therefore, the available data provide no evidence of a baseline imbalance in smoking status between the groups. However, smoking status may still be relevant when interpreting individual plasma lipid profiles.

In the European region, tobacco use among women remains relatively common, and national and European reports continue to identify tobacco use as an important contributor to preventable cardiometabolic morbidity and mortality (OECD/European Observatory on Health Systems and Policies, 2025). The lower smoking prevalence in the present cohort may therefore

indicate a more health-conscious study population or a selection effect related to participation in a dietary intervention study.

Smoking status is relevant to lipid profile interpretation because current smoking has been associated with a more atherogenic lipid profile, including lower HDL-C and higher TG, TC, or LDL-C concentrations in recent observational studies (Momayyezi et al., 2024; Sousa et al., 2024). Although only a small number of participants in the present study reported smoking, this variable should still be considered when interpreting individual variability in plasma lipid concentrations.

Overall, alcohol use was common in both groups and slightly more frequent in the intervention group, whereas smoking prevalence was low and slightly higher in the control group. The small between-group differences suggest that alcohol use and smoking did not represent major descriptive baseline imbalances. However, both behaviours may influence lipid metabolism and cardiometabolic risk; they provide relevant background for interpreting baseline biochemical characteristics and subsequent changes in lipid profiles.

Meal frequency and snacking / *Ēdienreižu biežums un uzkodu lietošana*

Meal frequency and snacking patterns were assessed as background dietary behaviour variables relevant to interpreting dietary intake and plasma lipid profile outcomes. Most participants in both groups reported consuming two to three main meals per day. Although the proportions in the individual meal-frequency categories differed between groups, the overall distribution of main meal frequency did not differ significantly between the intervention and control groups ($p = 0.322$). Thus, the data provide no evidence of a baseline imbalance in the number of main meals consumed per day.

Participants in the intervention group more often reported consuming fewer than two main meals per day, whereas the control group reported four to five main meals per day somewhat more often. These category-level differences should be interpreted descriptively, as the overall comparison was not statistically significant and several categories had few participants. Moreover, meal frequency alone does not indicate diet quality, as meal timing, portion size, energy intake per meal, and the distribution of energy and nutrients across the day were not evaluated in this subsection.

Snacking patterns differed between groups. Participants in the intervention group more frequently reported no daily snacks or one snack per day, whereas participants in the control group more often reported two, three, or at least four snacks per day. However, the overall distribution of daily snacking frequency did not differ significantly between the intervention and control groups ($p = 0.245$). Therefore, the observed pattern does not provide statistical evidence of a baseline difference in snacking frequency, although the control group showed a descriptive tendency towards more frequent snacking.

This finding should be interpreted with caution because several response categories had few participants, which limits the precision and statistical power of the comparison. In addition, snack frequency alone does not provide information on snack type, portion size, energy content, nutrient composition, or snacks' contribution to total daily dietary intake.

The relevance of meal frequency and snacking to cardiometabolic outcomes remains debated. The American Heart Association scientific statement on meal timing and frequency concluded that irregular eating patterns may be less favourable for cardiometabolic health, but evidence on the optimal number of eating occasions remains inconsistent (St-Onge et al., 2017). A systematic review drawn for the 2020 Dietary Guidelines Advisory Committee found insufficient evidence to establish a clear association between eating frequency and CVD risk (Heymsfield et al., 2020). More recent observational data suggest that meal skipping and shorter meal intervals may be associated with higher all-cause and mortality from CVD, although these findings do not establish causality and may partly reflect broader lifestyle patterns (Sun et al., 2022). Recent chrononutrition literature also suggests that the timing, regularity and distribution of food intake may influence glucose and lipid metabolism, but findings across populations and study designs remain heterogeneous (Raji et al., 2024).

In the present study, meal frequency and snacking variables were used descriptively to characterise baseline dietary behaviour rather than to test their independent associations with lipid outcomes. The observed differences between groups may be relevant when interpreting later dietary intake data, particularly total energy intake, macronutrient distribution, and habitual dairy consumption.

Overall, the baseline characteristics indicate that the intervention and control groups were broadly similar in age, BMI, waist-to-hip ratio, smoking prevalence and daily light physical activity. Descriptive differences were observed in BMI category distribution, alcohol use, waist circumference and snacking patterns, but these should be interpreted as background variability rather than evidence of major baseline imbalance. The high prevalence of daily light physical activity and low smoking prevalence suggest that the study cohort may represent a relatively health-conscious subgroup of perimenopausal women. These characteristics should be considered when interpreting subsequent outcomes for dietary intake, habitual dairy consumption, and biochemical and lipid profiles.

Summary of Chapter 3.3. / *3.3. nodaļas kopsavilkums*

This chapter described participant recruitment, randomisation, retention, and baseline characteristics. 62 participants were randomised and 61 completed the study, including 31 participants in the intervention group and 30 in the control group. The final study population consisted of perimenopausal women aged 45-55 years with moderately elevated LDL-C concentrations.

Overall, the results support the baseline comparability between the intervention and control groups across the assessed demographic, anthropometric, lifestyle, and eating-behaviour variables. However, non-significant p-values indicate that no between-group differences were detected and should not be interpreted as proof of complete equivalence, particularly given the limited sample size and the small numbers of participants in some categorical subgroups. The high prevalence of self-reported daily light physical activity and low smoking prevalence may also indicate that the cohort differed from the broader population of perimenopausal women in selected health-related behaviours, which should be considered when evaluating the generalisability of the subsequent dietary and plasma lipid profile findings.

Šajā nodaļā tika aprakstīta dalībnieču atlase, randomizācija, dalības saglabāšana pētījumā un sākotnējais raksturojums. 62 dalībnieces tika randomizētas, un 61 dalībniece pētījumu pabeidza, tostarp 31 dalībniece intervences grupā un 30 dalībnieces kontroles grupā. Pētījuma populāciju veidoja sievietes perimenopauzē vecumā no 45 līdz 55 gadiem ar vidēji paaugstinātu LDL-C koncentrāciju.

Kopumā rezultāti apstiprina intervences un kontroles grupas līdzību attiecībā uz izvērtētajiem demogrāfiskajiem, antropometriskajiem, dzīvesveida un ēšanas paradumu mainīgajiem. Lai gan statistiski nozīmīgas p vērtības starp grupām netika konstatētas, tās nevajadzētu interpretēt kā identiskas grupas, ņemot vērā ierobežoto izlases lielumu un nelielo dalībnieču skaitu dažās vērtētajās apakšgrupās. Augstā pašnovērtētā ikdienas fiziskā aktivitāte un zemā smēķēšanas izplatība dalībnieču vidū var liecināt, ka kohorta pēc atsevišķiem ar veselību saistītiem dzīvesveida paradumiem atšķīrās no vispārējās sieviešu perimenopauzē populācijas, kas jāņem vērā, izvērtējot uztura un plazmas lipīdu profila rezultātus.

3.4. Baseline plasma biochemical profile of the study participants / *Pētījuma dalībnieču sākotnējais asins bioķīmiskais profils*

The biochemical assessment included TC, LDL-C, HDL-C, TG, non-HDL-C, remnant cholesterol, glucose and ALAT. These variables were selected because they reflect lipid

metabolism, atherogenic cholesterol burden, glucose metabolism and liver enzyme status, all of which are relevant to interpreting plasma lipid profile outcomes in a dietary intervention study.

Baseline biochemical characteristics of participants in the intervention and control groups are presented in Table 3.12.

Table 3.12. **Baseline plasma biochemical profile of participants /** *3.12. tabula* ***Dalībnieču sākotnējais asins bioķīmiskais profils***

Parameter / *Rādītājs*	Intervention group / *Intervences grupa* (n = 31)	Control group / *Kontroles grupa* (n = 30)	Laboratory reference interval / *Laboratorijas references intervāls*	p-value / *p-vērtība*	Hedges' g / *Hedžesa g*
TC, mmol L^{-1}	5.93 ± 0.61	5.93 ± 0.48	0.0-5.0	0.996	0.00
HDL-C, mmol L^{-1}	1.92 ± 0.55	1.78 ± 0.34	>1.2	0.244	-0.30
LDL-C, mmol L^{-1}	3.59 ± 0.31	3.62 ± 0.29	1.7-3.0	0.634	0.12
TG, mmol L^{-1}	1.13 ± 0.65	1.17 ± 0.64	0.1^{-1}.7	0.811	0.06
non-HDL-C, mmol L^{-1}	4.01 ± 0.44	4.15 ± 0.47	≤3.9	0.223	0.31
Remnant cholesterol, mmol L^{-1} */ Remnanta holesterīns, mmol L^{-1}*	0.43 ± 0.26	0.53 ± 0.31	≤0.9	0.190	0.34
ALAT, U L^{-1}	19.52 ± 10.52	22.53 ± 9.81	10-35	0.251	0.29
Glucose, mmol L^{-1} */ Glikoze, mmol L^{-1}*	4.62 ± 0.51	4.71 ± 0.82	4.1-5.9	0.607	0.13

Note. Mean ± SD. Laboratory reference intervals are provided for clinical orientation. p-values compare the study groups; positive Hedges' g values indicate higher means in the control group. / *Piezīme. Vidējā vērtība ± SD. Laboratorijas references intervāli sniegti klīniskai orientācijai. p vērtības attiecas uz pētījuma grupu salīdzinājumu; pozitīvas Hedžesa g vērtības norāda uz lielākām vidējām vērtībām kontroles grupā.*

Although no between-group difference was statistically significant, the control group had higher baseline non-HDL-C and remnant cholesterol and lower HDL-C (absolute g values 0.30-0.34, small to moderate), a pattern relevant to interpreting the within-group changes in Section 3.7.

Baseline biochemical profiles showed no statistically significant differences between the intervention and control groups for TC, HDL-C, LDL-C, TG, non-HDL-C, remnant cholesterol, ALAT, or glucose ($p > 0.05$). TC concentrations were essentially identical between the groups, whereas the remaining parameters showed only numerical differences between groups. Thus,

no statistically detectable baseline imbalance was identified in the measured biochemical variables. However, the absence of statistically significant differences should not be interpreted as evidence of complete biochemical equivalence.

In both groups, the baseline lipid profile was characterised by mean TC, LDL-C, and non-HDL-C concentrations above the corresponding laboratory reference thresholds. Elevated LDL-C concentrations were consistent with the study eligibility criteria and provided clinically relevant baseline context for the subsequent evaluation of dietary factors, milk PL exposure, and post-intervention lipid outcomes. Rather than indicating an adverse pattern across all lipid parameters, these findings specifically demonstrate elevated atherogenic cholesterol measures at baseline.

Recent Baltic and international evidence provides context for the baseline lipid profile observed in the present cohort. In a population-based Latvian survey, elevated TC and LDL-C were common among women aged 45-54 years, whereas low HDL-C and elevated TG were less prevalent. Although this survey did not classify participants by menopausal stage and reported threshold-based prevalence rather than mean concentrations, it indicates that elevated atherogenic cholesterol is common among women of comparable age in Latvia (Ērglis et al., 2022).

More directly comparable evidence is available from the Core-ERMA prospective cohort of perimenopausal women. In that study, more than half of the participants had LDL-C concentrations above 3.0 mmol L^{-1}, while low HDL-C and elevated TG were relatively uncommon. This pattern is broadly consistent with the present findings, in which elevated TC, LDL-C, and non-HDL-C were accompanied by mean HDL-C and TG concentrations within the corresponding laboratory reference intervals (Karppinen et al., 2022).

Compared with the SWAN longitudinal cohort, the present participants had higher mean TC and LDL-C concentrations, whereas TG concentrations were similar and HDL-C was higher. These differences are most plausibly attributable to the study eligibility criteria, as the present trial specifically recruited women with moderately elevated LDL-C, whereas the SWAN cohort was not selected based on lipid concentration (W. Yu et al., 2022).

LDL-C is a central marker in the assessment and management of dyslipidaemia, whereas non-HDL-C provides complementary information by representing the cholesterol content of atherogenic ApoB-containing lipoproteins, including LDL and triglyceride-rich lipoproteins and their remnants (Mach et al., 2020; Visseren et al., 2021). According to the WHO cholesterol indicator metadata, non-HDL-C can be calculated by subtracting HDL-C from TC and predicts ischaemic heart disease risk similarly to LDL-C concentrations (World Health Organization, 2021).

Mean TC concentrations in the present cohort were higher than the reference threshold. Mean HDL-C concentrations were above the laboratory lower reference threshold, while group mean concentrations of TG and remnant cholesterol were within their respective laboratory reference intervals. Mean glucose and ALAT concentrations were also within the corresponding reference intervals in both groups. These results describe group-level averages and do not exclude the possibility that individual participants had values outside the stated reference intervals.

Overall, the biochemical results indicate that the intervention and control groups were broadly comparable at baseline with respect to the measured variables. The cohort was characterised primarily by elevated TC, LDL-C, and non-HDL-C concentrations, while mean TG, remnant cholesterol, glucose, and ALAT concentrations remained within the corresponding laboratory reference intervals. Given the limited sample size and inter-individual variability, the non-significant between-group comparisons indicate that baseline differences were not detected, rather than demonstrating complete equivalence between the groups.

Summary of Chapter 3.4. / ***3.4. nodaļas kopsavilkums***

The baseline plasma lipid profile of the participants was characterised in this chapter. TC, HDL-C, LDL-C, TG, non-HDL-C, remnant cholesterol, ALAT and glucose did not differ significantly between the intervention and control groups (all $p > 0.05$). The measured parameters therefore provide no indication of a systematic imbalance between the groups at baseline.

Overall, the baseline biochemical profiles of the intervention and control groups were comparable for the measured parameters. The study population was characterised primarily by elevated TC, LDL-C, and non-HDL-C concentrations, while mean TG, remnant cholesterol, glucose, and ALAT concentrations remained within the respective laboratory reference intervals. This biochemical pattern provides the necessary baseline context for interpreting subsequent changes in plasma lipid profile outcomes.

Šajā nodaļā raksturots pētījuma dalībnieču sākotnējais plazmas lipīdu profils. Starp intervences un kontroles grupām netika konstatētas statistiski nozīmīgas atšķirības TC, HDL-C, LDL-C, TG, non-HDL-C, remnanta holesterīna, ALAT vai glikozes saturā, un visos gadījumos p vērtība pārsniedza 0.05. Tādējādi izvērtētie bioķīmiskie rādītāji neliecina par sistemātisku sākotnējo nelīdzsvarotību starp pētījuma grupām.

Kopumā intervences un kontroles grupas sākotnējais bioķīmiskais profils bija salīdzināms. Pētījuma populāciju galvenokārt raksturoja paaugstināta TC, LDL-C un non-HDL-C koncentrācija, savukārt vidējā TG, remnanta holesterīna, glikozes un ALAT koncentrācija saglabājās laboratorijas references intervālos. Šāds bioķīmiskais profils nodrošina nepieciešamo sākotnējo kontekstu turpmāko plazmas lipīdu profila izmaiņu interpretācijai.

3.5. Dietary habits and nutritional adequacy of the study participants / *Pētījuma dalībnieču uztura paradumi un uzturvielu nodrošinājuma atbilstība*

The dietary habits and nutritional adequacy of the study participants were evaluated to characterise their habitual dietary background and to identify potential dietary factors that could independently influence the plasma lipid profile, irrespective of the cultured buttermilk intervention.

The estimated habitual daily intake of food products and beverages is presented in Table 3.13 as medians with quartiles, because intake of most food groups was right-skewed; the corresponding means and standard deviations are given in Annex VII.

Table 3.13. **Estimated average daily intake of food products and beverages, median (Q1; Q3), g day^{-1} /** *3.13. tabula* ***Aprēķinātais vidējais pārtikas produktu un dzērienu patēriņš dienā, mediāna (Q1; Q3), g dienā***

Food products or beverages / *Pārtikas produkti vai dzērieni*	Total (n = 61), g day^{-1} / *Kopā (n = 61), g diena^{-1}*	Intervention group (n = 31), g day^{-1} / *Intervences grupa (n = 31), g diena^{-1}*	Control group (n = 30), g day^{-1} / *Kontroles grupa (n = 30), g diena^{-1}*	p-value / *p-vērtība*
Grains, grain-based products / *Graudaugi un graudaugu produkti*	75.15 (41.19; 97.96)	68.47 (41.02; 93.21)	80.31 (45.57; 103.93)	0.276

Table 3.13 continuation / *3.13. tabulas turpinājums*

Food products or beverages / *Pārtikas produkti vai dzērieni*	Total (n = 61), g day^{-1} / *Kopā (n = 61), g diena^{-1}*	Intervention group (n = 31), g day^{-1} / *Intervences grupa (n = 31), g diena^{-1}*	Control group (n = 30), g day^{-1} / *Kontroles grupa (n = 30), g diena^{-1}*	p-value / *p-vērtība*
Bread / *Maize*	63.34 (33.10; 89.37)	54.85 (32.41; 73.52)	67.07 (41.60; 116.67)	0.147
Potatoes / *Kartupeļi*	32.88 (20.30; 52.52)	34.52 (21.25; 59.88)	32.51 (20.08; 47.18)	0.778
Meat, poultry / *Gaļa un mājputnu gaļa*	71.73 (45.21; 125.97)	71.73 (48.22; 127.33)	73.23 (42.64; 116.86)	0.569
Meat offal / *Gaļas subprodukti*	4.03 (1.03; 8.36)	4.03 (1.62; 9.37)	3.74 (0.41; 8.14)	0.233
Meat products / *Gaļas izstrādājumi*	11.42 (5.42; 23.12)	12.99 (6.55; 26.41)	10.44 (5.18; 18.40)	0.139
Eggs / *Olas*	47.12 (25.64; 60.00)	26.96 (21.45; 54.16)	47.23 (25.73; 60.11)	0.101
Fish / *Zivis*	31.89 (15.70; 48.60)	32.22 (19.00; 44.73)	28.38 (14.86; 53.12)	0.988
Fish products / *Zivju izstrādājumi*	3.95 (2.36; 8.08)	4.19 (2.63; 8.67)	3.25 (2.29; 7.34)	0.629
Milk, milk products / *Piens un piena produkti*	277.63 (186.29; 369.26)	253.10 (153.97; 366.15)	299.23 (223.97; 368.88)	0.282
Vegetable oils, plant- and animal-based fats, dressings / *Augu eļļas, augu un dzīvnieku valsts izcelsmes tauki un mērces*	24.90 (14.72; 34.32)	24.90 (14.85; 35.63)	22.46 (14.60; 32.74)	0.692
Substitutes for meat products, plant-based / *Augu valsts izcelsmes gaļas produktu aizstājēji*	0.00 (0.00; 0.00)	0.00 (0.00; 0.00)	0.00 (0.00; 0.41)	0.256
Dairy substitutes, plant-based / *Augu valsts izcelsmes piena produktu aizstājēji*	4.11 (0.41; 15.89)	4.11 (0.41; 4.93)	6.58 (0.82; 21.68)	0.187
Vegetables / *Dārzeņi*	166.33 (127.53; 255.60)	191.51 (133.27; 263.44)	160.01 (116.47; 225.59)	0.493

Table 3.13 continuation / *3.13. tabulas turpinājums*

Food products or beverages / *Pārtikas produkti vai dzērieni*	Total (n = 61), g day^{-1} / *Kopā (n = 61), g diena^{-1}*	Intervention group (n = 31), g day^{-1} / *Intervences grupa (n = 31), g diena^{-1}*	Control group (n = 30), g day^{-1} / *Kontroles grupa (n = 30), g diena^{-1}*	p-value / *p-vērtība*
Fruits, berries / *Augļi un ogas*	205.56 (130.63; 289.84)	185.18 (122.58; 259.66)	235.95 (134.57; 343.03)	0.182
Mushrooms / *Sēnes*	4.11 (1.64; 7.12)	4.11 (2.88; 4.11)	4.11 (1.64; 7.12)	1.000
Legumes / *Pākšaugi*	10.19 (8.96; 15.67)	12.05 (7.81; 29.75)	9.62 (8.96; 14.92)	0.582
Nuts, seeds / *Rieksti un sēklas*	13.84 (7.97; 26.88)	13.84 (8.26; 26.64)	14.26 (8.01; 32.06)	0.880
Sweets / *Saldumi*	32.25 (26.05; 54.27)	30.12 (19.68; 47.36)	37.62 (27.65; 55.07)	0.108
Sugars and related foods / *Cukurs un saldvielas*	3.97 (2.08; 7.85)	3.52 (1.47; 6.95)	4.21 (2.42; 10.31)	0.197
Fast food, salty snacks / *Uzkodas un sāļās uzkodas*	26.22 (16.47; 35.71)	27.04 (16.53; 35.75)	26.16 (16.58; 34.46)	0.880
Soft drinks, energy drinks / *Limonādes un enerģijas dzērieni*	8.22 (2.05; 22.60)	8.22 (2.05; 21.92)	9.25 (4.11; 22.09)	0.686
Juices / *Sulas*	32.88 (8.22; 45.21)	24.66 (8.22; 40.07)	35.62 (11.13; 53.42)	0.273
Alcoholic beverages / *Alkoholiskie dzērieni*	27.12 (9.97; 52.93)	22.52 (9.82; 61.07)	28.86 (14.01; 47.28)	0.708
Coffee, tea / *Kafija un tēja*	693.49 (642.47; 910.62)	688.29 (548.66; 928.42)	752.81 (660.14; 896.58)	0.658
Water / *Ūdens*	1160.62 (1125.00; 1502.05)	1145.55 (1125.00; 1502.05)	1160.62 (1125.00; 1502.05)	0.745

Note. Q1 and Q3 are the first and third quartiles. p-values compare the study groups using the Mann-Whitney U test, except for coffee and tea, for which Welch's t-test was used. Zero medians for plant-based meat substitutes reflect the small proportion of consumers. Food categories are defined in Annex VI; means ± SD are provided in Table A7.1. / *Piezīme. Q1 un Q3 ir pirmā un trešā kvartile. p vērtības iegūtas, salīdzinot pētījuma grupas ar Manna-Vitnija U testu; kafijas un tējas kategorijai izmantots Velča t-tests. Nulles mediānas augu izcelsmes gaļas aizstājējiem atspoguļo nelielo šo produktu lietotāju īpatsvaru. Pārtikas produktu kategorijas definētas VI pielikumā; vidējās vērtības ± SD sniegtas A7.1. tabulā.*

Habitual food and beverage intake was comparable between the intervention and control groups at baseline. No statistically significant between-group differences were identified for any assessed food or beverage category, with all p-values exceeding 0.05. Therefore, the study groups showed no statistically significant differences in habitual food-group intake at baseline.

Although variations were observed, no statistically significant differences in habitual food-group intake were found between the study groups at baseline. The wide interquartile ranges of several episodically consumed categories, particularly plant-based dairy substitutes, eggs, fruits and berries, and alcoholic beverages, reflect the fact that these foods were consumed regularly by some participants and rarely or never by others; for such categories the median describes the typical intake, whereas the mean is pulled upwards by a few frequent consumers.

The combined mean intake of vegetables, fruits and berries was 436.47 g day^{-1} in the total sample, 401.27 g day^{-1} in the intervention group and 472.84 g day^{-1} in the control group; the medians were 166.33 g day^{-1} for vegetables and 205.56 g day^{-1} for fruits and berries (Table 3.13). Although the mean values were below the Latvian recommendation of approximately 500 g day^{-1} and the Nordic recommendation of 500-800 g day^{-1} or more, the observed intake was relatively high in the Latvian population context, where only 7.2 % of adults reported consuming at least 500 g of vegetables, fruits and berries per day (Central Statistical Bureau of Latvia, 2021; Nordic Council of Ministers, 2023; Veselības ministrija, 2025). The lower intake may be nutritionally relevant because vegetables, fruits, and berries provide dietary fibre, potassium, folate, vitamin C, polyphenols, and other bioactive compounds that may lower CVD risk by improving blood pressure, endothelial function, dietary fibre intake, and overall diet quality. The analysis that included 66719 women found that an intake of approximately five servings per day was associated with lower mortality from CVD, while dose-response meta-analyses have reported progressively lower CVD risk with increasing fruit and vegetable intake (Aune et al., 2017; Miller et al., 2017; D. Wang et al., 2021).

Grain, bread, and potato intake indicates low consumption of cereal products and potatoes compared with Latvian food-based recommendations. The FFQ food group classification did not allow a detailed distinction between whole-grain and refined cereal products. Therefore, conclusions regarding whole-grain adequacy should be made with caution. The cardiometabolic effect of carbohydrate-containing foods depends not only on total intake but also on quality, degree of processing, fibre content, and glycaemic properties. The CVD relevance of cereal intake depends primarily on cereal quality rather than on the quantity of grains, bread, and potatoes. Higher whole-grain intake has been associated with a lower risk of coronary heart disease and CVD mortality, and one randomised trial conducted predominantly in women reported reduced diastolic blood pressure when refined grains were replaced with whole grains. However, evidence from randomised trials regarding effects on lipid profile and blood pressure remains inconsistent and of limited certainty (Hu et al., 2023; Kelly et al., 2017; Kirwan et al., 2016).

The combined mean intake of fish and fish products was 44.90 g day^{-1} in total, 42.61 g day^{-1} in the intervention group and 47.26 g day^{-1} in the control group, corresponding to approximately 314, 298 and 331 g $week^{-1}$; so that half of the participants consumed less than about 225 g of fish per week. The mean estimated weekly intakes in the total sample and control group were within the lower part of the Nordic recommendation of 300-450 g of fish and seafood per week, whereas the intervention-group estimate was close to its lower boundary (Nordic Council of Ministers, 2023). Latvian dietary guidelines recommend consuming fish at least twice weekly (Veselības ministrija, 2020b); however, the estimated weekly amounts alone do not establish whether participants met this frequency-based recommendation.

Fish and processed fish products should be considered separately, as processed fish products may contain added salt and fat and may not provide the same nutritional profile as minimally processed fish. Recent evidence supports the CVD relevance of moderate fish consumption. In a large cohort of 26 034 healthy women, higher habitual fish intake was associated with lower TG concentrations, although changes in LDL-C concentrations were not uniformly favourable. Meta-analyses of prospective studies indicate that approximately 2-3 fish servings per week are associated with a modestly lower risk of CVD (Amigó et al., 2020; Giosuè et al., 2022; Ricci et al., 2023).

Milk and dairy product intake, expressed as median (Q1; Q3), was 277.63 (186.29; 369.26) g day^{-1} in the total sample, 253.10 (153.97; 366.15) g day^{-1} in the intervention group and 299.23 (223.97; 368.88) g day^{-1} in the control group, with no statistically significant between-group difference ($p = 0.282$). The mean intake in the total sample was 305.71 ± 185.57 g day^{-1} and exceeded the median, consistent with a right-skewed distribution. Nevertheless, dairy intake is particularly relevant to this thesis because dairy products are sources of MFGM, especially PL. Intervention studies support the potential relevance of dairy intake to lipid metabolism. In overweight postmenopausal women, milk-PL-enriched dairy products reduced several fasting and postprandial cholesterol-related risk markers. A recent meta-analysis of randomised trials also reported modest reductions in TC, LDL-C, ApoB, and the TC/HDL-C ratio following MFGM- or milk-PL-containing interventions (Borkar et al., 2026; Le Barz et al., 2021; Vors et al., 2020). However, these findings were obtained mainly with MFGM- or milk-PL-enriched interventions providing substantially higher PL doses than those typically obtained from habitual dairy intake. Therefore, habitual dairy intake should be considered not only as a conventional food group but also as a source of background exposure to milk PL.

To assess nutritional adequacy, the observed intake values were compared with Latvian dietary recommendations and the Nordic Nutrition Recommendations 2023 (Nordic Council of Ministers, 2023; Veselības ministrija, 2025). Average daily energy and nutrient intake is presented in Table 3.14.

Table 3.14. **Average daily energy and nutrient intake** / *3.14. tabula* ***Vidējā enerģijas un uzturvielu uzņemšana dienā***

Nutrient / *Uzturviela*	Total (n = 61) / *Kopā (n = 61)*	Intervention group (n = 31) / *Intervences grupa (n = 31)*	Control group (n = 30) / *Kontroles grupa (n = 30)*	p-value / *p-vērtība*	Hedges' g / *Hedžesa g*	Reference value / range / *Atsauces vērtība / intervāls*	Source / *Avots*
Energy, kcal day^{-1} / *Enerģija, kcal dienā*	1921.67 ± 519.79	1804.86 ± 488.08	2042.37 ± 531.88	0.075	0.46	1840-2360 kcal day^{-1} (Latvia) 2000-2300 kcal day^{-1} (NNR 2023)	The Ministry of Health of the Republic of Latvia (2025); Nordic Council of Ministers (2023)
Protein, g day^{-1} / *Olbaltumvielas, g dienā*	94.06 ± 30.41	90.01 ± 28.83	98.25 ± 31.90	0.294	0.27	NA	NA
Protein, E% / *Olbaltumvielas, E%*	19.70 ± 3.79	19.99 ± 3.36	19.39 ± 4.22	0.540	-0.16	10-20 E%	The Ministry of Health of the Republic of Latvia (2025); Nordic Council of Ministers (2023)
Carbohydrates, g day^{-1} / *Ogļhidrāti, g dienā*	169.81 ± 55.42	150.58 ± 45.80	189.67 ± 58.15	**0.005**	0.74	NA	NA
Carbohydrates, E% / *Ogļhidrāti, E%*	35.61 ± 7.75	33.81 ± 7.43	37.46 ± 7.76	0.066	0.47	45-60 E%	The Ministry of Health of the Republic of Latvia (2025); Nordic Council of Ministers (2023)

Table 3.14 continuation / 3.14. *tabulas turpinājums*

Nutrient / *Uzturviela*	Total (n = 61) / *Kopā (n = 61)*	Intervention group (n = 31) / *Intervences grupa (n = 31)*	Control group (n = 30) / *Kontroles grupa (n = 30)*	p-value / *p-vērtība*	Hedges' g / *Hedžesa g*	Reference value / range / *Atsauces vērtība / intervāls*	Source / *Avots*
Total fibre, g day^{-1} / *Kopējās šķiedrvielas, g dienā*	22.39 ± 8.69	20.11 ± 6.32	24.76 ± 10.18	**0.038**	0.54	25-35 g day^{-1} / *g dienā*	The Ministry of Health of the Republic of Latvia (2025); Nordic Council of Ministers (2023)
Soluble fibre, g day^{-1} / *Šķīstošās šķiedrvielas, g dienā*	6.82 ± 2.61	6.11 ± 1.99	7.55 ± 2.97	**0.031**	0.56	NA	NA
Total fat, g day^{-1} / *Kopējais tauku daudzums, g dienā*	90.55 ± 30.83	88.42 ± 31.12	92.75 ± 30.90	0.588	0.14	NA	NA
Total fat, E% / *Kopējais tauku daudzums, E%*	42.07 ± 6.71	43.63 ± 7.20	40.46 ± 5.85	0.064	-0.48	25-40 E%	The Ministry of Health of the Republic of Latvia (2025)
						25-40 E%	Nordic Council of Ministers (2023)
Saturated fatty acids, g day^{-1} / *Piesātinātās taukskābes, g dienā*	28.86 ± 11.85	28.71 ± 13.90	29.02 ± 9.52	0.920	0.03	NA	NA

Table 3.14 continuation / 3.14. *tabulas turpinājum*

Nutrient / *Uzturviela*	Total (n = 61) / *Kopā (n = 61)*	Intervention group (n = 31) / *Intervences grupa (n = 31)*	Control group (n = 30) / *Kontroles grupa (n = 30)*	p-value / *p-vērtība*	Hedges' g / *Hedžesa g*	Reference value / range / *Atsauces vērtība / intervāls*	Source / *Avots*
Saturated fatty acids, E% / *Piesātinātās taukskābes, E%*	13.30 ± 3.12	13.84 ± 3.75	12.74 ± 2.21	0.169	-0.35	<10 E%	The Ministry of Health of the Republic of Latvia (2025); Nordic Council of Ministers (2023)
Monounsaturated fatty acids, g day^{-1} / *Mononepiesātinātās taukskābes, g dienā*	34.42 ± 13.69	33.89 ± 13.95	34.98 ± 13.62	0.759	0.08	NA	NA
Monounsaturated fatty acids, E% / *Mononepiesātinātās taukskābes, E%*	16.00 ± 4.41	16.84 ± 5.26	15.13 ± 3.17	0.131	-0.39	10-20 E%	The Ministry of Health of the Republic of Latvia (2025); Nordic Council of Ministers (2023)
Polyunsaturated fatty acids, g day^{-1} / *Polinepiesātinātās taukskābes, g dienā*	15.73 ± 5.77	14.89 ± 5.22	16.60 ± 6.26	0.251	0.29	NA	NA
Polyunsaturated fatty acids, E% / *Polinepiesātinātās taukskābes, E%*	7.38 ± 1.75	7.47 ± 1.75	7.30 ± 1.78	0.709	-0.09	5^{-1}0 E%	Nordic Council of Ministers (2023)

Table 3.14 continuation / 3.14. *tabulas turpinājums*

Nutrient / *Uzturviela*	Total (n = 61) / *Kopā (n = 61)*	Intervention group (n = 31) / *Intervences grupa (n = 31)*	Control group (n = 30) / *Kontroles grupa (n = 30)*	p-value / *p-vērtība*	Hedges' g / *Hedžesa g*	Reference value / range / *Atsauces vērtība / intervāls*	Source / *Avots*
Total sugars, g day^{-1} / *Kopējie cukuri, g dienā*	87.02 ± 33.69	76.03 ± 28.30	98.38 ± 35.46	**0.009**	0.69	Added/ free sugars: ≤10 E%, preferably ≈5 E% / *Pievienotie/brīvie cukuri: ≤10 E%, vēlams ≈5 E%*	The Ministry of Health of the Republic of Latvia (2025)
						Added/ free sugars: <10 E%, preferably lower / *Pievienotie/ brīvie cukuri: <10 E%, vēlams mazāk*	Nordic Council of Ministers (2023)

Note. Mean ± SD. p-values compare the study groups; values < 0.05 are in bold. Positive Hedges' g values indicate higher means in the control group, negative values in the intervention group. Energy reference values provide population-level guidance. Sugar recommendations apply to added and/or free sugars and are not directly comparable with the reported total sugar intake. / *Piezīme. Vidējā vērtība ± SD. p vērtības attiecas uz pētījuma grupu salīdzinājumu; vērtības < 0,05 izceltas treknrakstā. Pozitīvas Hedžesa g vērtības norāda uz lielākām vidējām vērtībām kontroles grupā, negatīvas - intervences grupā. Enerģijas atsauces vērtības sniedz orientieri populācijas līmenī. Cukuru rekomendācijas attiecas uz pievienotajiem un/vai brīvajiem cukuriem un nav tieši salīdzināmas ar norādīto kopējo cukuru uzņemšanu.*

Compared with the national study on salt and iodine consumption in the Latvian adult population conducted by BIOR in 2018-2020 (Siksna et al., 2020), in which women aged 19-64 years (n = 541) recorded a two-day food diary and one 24-h recall, the participants had a higher mean combined intake of vegetables, fruits and berries (436.47 versus 404.6 g day^{-1}), of milk and dairy products (305.71 versus 185.4 g day^{-1}) and of fish (44.90 g day^{-1} of fish and fish products versus 26.3 g day^{-1} of fish and seafood), and a lower mean combined intake of grains, bread and potatoes (188.10 versus 273.9 g day^{-1} of cereal products and potatoes). This comparison, is indicativ, because the present study used a 12-month FFQ in a selected cohort of perimenopausal women and the food-group definitions were not identical.

No statistically significant between-group differences in baseline energy and nutrient intake were found for most assessed variables. However, the control group had significantly higher intakes of carbohydrates, total fibre, soluble fibre and total sugars. The standardised between-group differences were moderate for total fibre (g = 0.54) and soluble fibre (g = 0.56), and moderate-to-large for carbohydrates (g = 0.74) and total sugars (g = 0.69).

The higher absolute carbohydrate intake in the control group should be interpreted alongside total energy intake and the proportional contribution of carbohydrates to energy intake. Mean energy intake and carbohydrate intake expressed as E% were higher in the control group, but neither difference reached statistical significance. Therefore, the difference in absolute carbohydrate intake may partly reflect the higher total energy intake rather than a marked difference in the proportional composition of macronutrients, although the standardised between-group difference in absolute carbohydrate intake remained appreciable. Recent meta-analytic evidence from energy-matched randomised trials suggests that higher-carbohydrate diets may produce slightly greater reductions in LDL-C, whereas lower-carbohydrate diets tend to have more favourable effects on TG and HDL-C (Anagnostou et al., 2026). However, total fat and SFA intake did not differ significantly between the groups; it cannot be established whether the higher carbohydrate intake displaced dietary fat or independently influenced the subsequent lipid-profile outcomes.

No statistically significant between-group differences were identified for protein, total fat, SFA, MUFA, or PUFA. Although only the carbohydrate-related differences reached statistical significance, the differences in energy intake and in the proportions of energy from carbohydrates and fat were of moderate size (absolute g values 0.46-0.48) and in the same direction: the control group consumed more energy, a higher proportion of carbohydrates and a lower proportion of fat.

Mean energy intake in the total study population was within the Latvian reference range for women aged 31-60 years. The intervention-group mean was below the lower Latvian reference value, whereas the control-group mean was within the range

Protein contributed 19.70 ± 3.79 E% in the total study population and was within the recommended range of 10-20 E%. Mean proportional protein intake in both groups was close to the upper boundary of this range. Carbohydrates contributed 35.61 ± 7.75 E% of total energy and were below the recommended range of 45-60 E% in both groups. Thus, despite the higher carbohydrate intake in the control group, both groups had a lower-than-recommended proportion of carbohydrate intake.

Mean total fibre intake was below the recommended minimum of 25 g day^{-1} for adult women. The intervention-group mean was below the recommendation, whereas the control-group mean was close to, but remained below, the reference value. The control group consumed 4.65 g day^{-1} more total fibre and 1.44 g day^{-1} more soluble fibre than the intervention group. This baseline difference may be relevant to the interpretation of subsequent lipid-profile outcomes because viscous soluble fibre can reduce intestinal cholesterol absorption and increase bile-acid excretion, while meta-analytic evidence indicates a dose-dependent LDL-C-lowering effect of soluble fibre (Ghavami et al., 2023; Kirkpatrick et al., 2023; Reynolds et al., 2020). Although the observed differences were relatively modest, the higher total and soluble fibre intakes may have provided a more favourable dietary background for lipid metabolism in

the control group and should therefore be considered as potential background modifiers when interpreting the subsequent intervention outcomes.

Total fat contributed 42.07 ± 6.71 E% in the total study population, exceeding the upper limit of the Latvian and Nordic reference range of 25-40 E%. Mean total fat intake was above 40 E% in both groups, with a greater numerical contribution in the intervention group. SFA contributed 13.30 ± 3.12 E% in the total sample and exceeded the recommended maximum of less than 10 E% in both groups. This shared dietary characteristic is relevant to the interpretation of elevated baseline LDL-C and non-HDL-C concentrations, because replacing SFA with unsaturated fatty acids or fibre-rich carbohydrate sources can improve the plasma lipid profile (Kirkpatrick et al., 2023; Nordic Council of Ministers, 2023; Schoeneck & Iggman, 2021).

At the macronutrient level, the present findings were consistent with previous Latvian data. In the national study of Siksna et al. (2020), protein, carbohydrates and fat contributed 17.0, 39.9 and 40.8 E%, respectively, and in 52 postmenopausal Latvian women aged 49-69 years assessed with 72-h food diaries the corresponding baseline values were 18.2-19.0, 37.0-38.2 and 39.7-40.7 E% (Aumeistere et al., 2023). In the present cohort, protein contributed 19.70 E%, carbohydrates 35.61 E% and fat 42.07 E%. The proportional contribution of fat was thus similar across the three datasets, whereas the present cohort had the highest protein and the lowest carbohydrate contribution. This consistency is compatible with, but does not establish, a broader Latvian dietary pattern characterised by a relatively high contribution of fat and a comparatively low contribution of carbohydrates to total energy intake; direct equivalence is limited by differences in age, participant selection and dietary assessment methods (Aumeistere et al., 2023; Siksna et al., 2020).

Mean MUFA intake was within the recommended range of 10-20 E% in both groups, and mean PUFA intake was within the recommended range of 5-10 E%. The fat profile was therefore not uniformly unfavourable. The principal deviations were the excessive proportional contribution of total fat and SFA rather than an inadequate mean contribution of MUFA or PUFA.

Total sugar intake was statistically significantly higher in the control group than in the intervention group ($p = 0.009$; $g = 0.69$). Fruits and berries were the principal contributors to estimated total sugar intake in both study groups, followed by milk and dairy products and sweets. Together, these food categories accounted for approximately two-thirds of the estimated total sugar intake in the total sample and in each study group. Thus, differences in total sugar intake reflected sugars from a broad range of foods, including both naturally occurring sugars and sugars in sweetened products.

Direct comparison of the calculated total sugar intake with dietary recommendations is limited because the Latvian and Nordic recommendations refer to added and/or free sugars rather than total sugars. The calculated FFQ variable included all mono- and disaccharides, including naturally occurring lactose, fructose, and glucose from milk, dairy products, fruits, berries, and other foods, in addition to sugars contributed by sweets and beverages (Nordic Council of Ministers, 2023; Veselības ministrija, 2025). Furthermore, Regulation (EU) No 1169/2011 requires nutrition declarations to report total "sugars" but does not require a separate quantitative declaration of added sugars. Consequently, the available food-composition data and product information could not reliably determine the proportion of total sugars attributable specifically to added or free sugars (European Parliament, Council of the European Union, 2011).

To provide additional information on potentially less favourable dietary sources of sugars, the combined contribution of selected food categories commonly associated with added sugars was calculated. Total sugars contributed by sweets, sugars and related foods, and lemonades and energy drinks amounted to 20.43 ± 14.89 g day^{-1} in the total sample, 17.83 ± 12.61 g day^{-1} in the intervention group, and 23.11 ± 16.71 g day^{-1} in the control group. Although the mean value was higher in the control group, the between-group difference was not statistically significant ($p = 0.177$). These values represent total sugars contributed by the

selected food categories and should not be interpreted as a direct quantitative estimate of added- or free-sugar intake, because some products within these categories may also contain naturally occurring sugars.

In a cross-sectional analysis, more frequent consumption of sugar-sweetened beverages among women was associated with higher TG concentrations, a higher TC/HDL-C ratio, and lower HDL-C concentrations after adjustment for demographic, medical, dietary, and lifestyle factors. However, no significant overall association with LDL-C was reported (Z. Yu et al., 2018). However, in the present study, the selected categories commonly associated with added sugars did not differ significantly between the groups.

Accordingly, the higher total sugar intake in the control group should not be interpreted automatically as evidence of a less favourable sugar-related dietary pattern, its relevance to subsequent lipid-profile outcomes should be considered alongside the underlying food sources, total energy intake, carbohydrate intake, and dietary fibre intake. The difference in total sugars should therefore be treated as a background dietary characteristic.

Overall, the habitual dietary pattern was characterised by protein, MUFA, and PUFA contributions within the corresponding reference ranges, a lower-than-recommended proportion of energy from carbohydrates, insufficient fibre intake, and total fat and SFA intakes above the recommended upper limits. The groups did not differ significantly in any individual food category and were similar across most energy and macronutrient variables. However, higher intakes of carbohydrates, total fibre, soluble fibre, and total sugars in the control group should be considered when interpreting subsequent changes in plasma lipid outcomes. Between the study groups, energy and macronutrient intakes did not differ; the only statistically significant baseline differences were the higher total sugar intake in the control group, attributable mainly to fruit, berries and dairy products, and the higher total and soluble fibre intake in the same group, the latter representing a more favourable rather than a less favourable dietary background. Together with the comparable baseline plasma lipid concentrations, this indicates that the control group did not start from a less favourable dietary or lipid status.

This pattern is explained by the food-group data of participants. Intake of grains, bread, and potatoes was low (about 170 g day^{-1} compared with 274 g day^{-1} among Latvian women in the national study (Siksna et al., 2020), which accounts for the low proportion of energy from carbohydrates and the shortfall in dietary fibre. The intake of milk and dairy products, 278 g day^{-1} compared with 185 g day^{-1} nationally, and of eggs, 47 g day^{-1} compared with 31 g day^{-1}, was high, whereas meat intake was below the national level; the proportions of total fat and SFA above the recommended limits therefore derive mainly from dairy products, including cheese, high-fat curd, sour cream and butter, rather than from meat. A similar structure, with fat contributing about 41 E% and carbohydrates about 40 E%, characterises the habitual diet of Latvian women in the national data (Siksna et al., 2020), and of postmenopausal Latvian women (Aumeistere et al., 2023), so the observed pattern reflects the national dietary background rather than a feature of the study sample alone. In addition, the participants were selected for moderately elevated LDL-C, for which a diet high in SFA and low in fibre is a recognised dietary correlate.

Summary of Chapter 3.5. / *3.5. nodaļas kopsavilkums*

Overall, the dietary pattern of the study participants was characterised by mean energy intake within the updated Latvian reference range for women aged 31-60 years, protein intake close to the upper recommended limit, a lower-than-recommended proportion of carbohydrates, insufficient dietary fibre intake, and total fat and SFA intakes above the corresponding recommended limits. This pattern reflects the low intake of cereal products and potatoes and the high intake of dairy products and eggs in the study sample and corresponds to the habitual diet of Latvian women in national data.

No statistically significant between-group differences were detected for any assessed food group. Most energy and macronutrient variables were also similar between the groups. However, the control group had significantly higher absolute carbohydrate intake ($p = 0.005$), total fibre intake ($p = 0.038$), soluble fibre intake ($p = 0.031$), and total sugar intake ($p = 0.009$).

No statistically significant differences were observed for protein, total fat, SFA, MUFA, or PUFA. SFA intake exceeded the recommended maximum in both groups and was a shared dietary characteristic rather than a between-group imbalance. Total sugars could not be evaluated against added- or free-sugar recommendations because the FFQ-based estimate included naturally occurring sugars, and available European food-labelling information does not require separate quantitative declaration of added sugars.

Higher intakes of carbohydrates, total fibre, soluble fibre, and total sugars in the control group should be considered when interpreting subsequent changes in plasma lipid outcomes.

Kopumā pētījuma dalībnieču uzturu raksturoja vidēja enerģijas uzņemšana aktualizētā Latvijas references intervāla robežās sievietēm vecumā no 31 līdz 60 gadiem, olbaltumvielu uzņemšana tuvu ieteicamā intervāla augšējai robežai, zemāks par ieteikto ogļhidrātu īpatsvars kopējā enerģijas uzņemšanā, nepietiekama šķiedrvielu uzņemšana, kā arī kopējā tauku un piesātināto taukskābju uzņemšana virs ieteicamajām robežvērtībām. Īstenotais uztura modelis atspoguļo zemu graudaugu produktu un kartupeļu patēriņu un augstu piena produktu un olu patēriņu pētījuma izlasē un atbilst Latvijas sieviešu uzturam, atspoguļotam nacionālajos datos.

Netika konstatētas statistiski nozīmīgas atšķirības starp intervences un kontroles grupu nevienā no analizētajām pārtikas produktu grupām. Arī lielākā daļa enerģijas un makroelementu uzņemšanas rādītāju starp grupām bija līdzīgi. Tomēr kontroles grupā bija statistiski nozīmīgi lielāka ogļhidrātu uzņemšana ($p = 0.005$), kopējā šķiedrvielu uzņemšana ($p = 0.038$), šķīstošo šķiedrvielu uzņemšana ($p = 0.031$) un kopējo cukuru uzņemšana ($p = 0.009$).

Starp grupām netika konstatētas statistiski nozīmīgas atšķirības olbaltumvielu, kopējā tauku, piesātināto, mononepiesātināto vai polinepiesātināto taukskābju uzņemšanā. Piesātināto taukskābju uzņemšana abās grupās pārsniedza ieteicamo maksimālo robežvērtību. Kopējo cukuru uzņemšanu nevarēja tieši salīdzināt ar pievienoto vai brīvo cukuru uzņemšanu, jo FFQ aprēķins ietvēra arī dabīgus cukurus un Eiropas pārtikas marķējuma prasības neparedz obligātu pievienoto cukuru daudzuma norādi.

Interpretējot izmaiņas plazmas lipīdu rādītājos, jāņem vērā lielāks ogļhidrātu, kopējo šķiedrvielu, šķīstošo šķiedrvielu un kopējo cukuru patēriņš kontroles grupā.

3.6. Estimated habitual milk PL exposure / *Aprēķinātā piena PL uzņemšana*

Estimated habitual milk PL exposure was calculated as described in Section 2.4 (Equations 1 and 2). For each participant, the FFQ-derived average daily intakes of dairy product categories were converted to milk equivalents and summed, and the total milk equivalent was multiplied by the season-specific concentration of total quantified PL, PC, PE, or SM in raw milk (Table 3.3).

The resulting estimates represent milk-equivalent milk PL exposure rather than direct analytical measurements of PL intake from each dairy product category consumed. Estimated habitual intakes of total milk PL and individual PL classes, including PC, PE, and SM, for the total sample and stratified by season are presented in Table 3.15.

Table 3.15. **Estimated habitual milk PL intake in the total study sample and by season /** *3.15. tabula* ***Aprēķinātā piena PL uzņemšana pētījuma izlasē un sezonā***

PL / *PL*	Total (n = 61) / *Kopējā pētījuma izlase (n = 61)* mg day^{-1} / *mg dienā*	Winter (n = 23) / *Ziema (n = 23)* mg day^{-1} / *mg dienā*	Spring (n = 38) / *Pavasaris (n = 38)* mg day^{-1} / *mg dienā*	p-value / *p-vērtība*	Hedges' g / *Hedžesa g*
Total PL / *Kopējie PL*	211.76 ± 138.82	264.25 ± 171.07	179.98 ± 105.36	**0.041**	0.62
PC	90.47 ± 57.24	106.40 ± 68.88	80.83 ± 47.31	0.124	0.45
PE	91.82 ± 61.36	117.68 ± 76.18	76.18 ± 44.59	**0.024**	0.70
SM	29.46 ± 20.74	40.17 ± 26.01	22.98 ± 13.45	**0.006**	0.89

Note. Modelled intake estimates are presented as mean ± SD. p-values compare the independent winter and spring cohorts; values < 0.05 are in bold. Positive Hedges' g values indicate higher estimated intake in winter. / *Piezīme. Modelētās uzņemšanas aplēses norādītas kā vidējā vērtība ± SD. p vērtības attiecas uz neatkarīgo ziemas un pavasara kohortu salīdzinājumu; vērtības < 0,05 izceltas treknrakstā. Pozitīvas Hedžesa g vērtības norāda uz lielāku aprēķināto uzņemto daudzumu ziemā.*

Mean estimated habitual milk-derived PL intakes were higher in winter than in spring across all quantified PL classes. Total milk-derived PL intake was significantly higher in winter and showed a moderate standardized between-season difference (p = 0.041; g = 0.62). Estimated PE intake was also significantly higher in winter, with a moderate standardized difference (p = 0.024; g = 0.70). The largest seasonal difference was observed for SM intake, which was significantly higher in winter and showed a large standardized difference (p = 0.006; g = 0.89). Estimated PC intake was also higher in winter, with a small-to-moderate standardized difference (p = 0.124; g = 0.45), but the difference did not reach statistical significance. Overall, seasonal differences were most pronounced for SM, followed by PE and total milk-derived PL intake, whereas the evidence for a seasonal difference in PC intake was weaker.

The estimates in Table 3.15 refer to PC, PE and SM supplied by milk and dairy products only and are therefore not comparable with estimates of total dietary phospholipid intake. Total phospholipid intake in Western diets is usually quoted as 2-8 g day^{-1}, about 1^{-1}0 % of fat intake, with eggs, meat, fish and soy contributing in addition to dairy products (Anto et al., 2020; Yang & Chen, 2022); this range is widely cited but derives from earlier food-composition estimates rather than from recent population surveys. The mean estimated milk PL intake of the present participants, 180-264 mg day^{-1}, corresponds to about 2^{-1}3 % of that range. For SM, for which dairy products are the main dietary source, the estimated intake of 23-40 mg day^{-1} corresponds to roughly 6^{-1}3 % of the 300-400 mg day^{-1} of dietary sphingolipids estimated for the United States (Calzada et al., 2023; Yang & Chen, 2022). The daily intervention portion (69.73-81.30 mg PL) added about one third to the habitual milk PL intake (31 % in winter, 39 % in spring) but only 1-4 % of the total dietary PL range, whereas the enriched-product trials that reported reductions of TC and LDL-C supplied 1.7-5 g day^{-1} of milk PL (Kanon et al., 2024; Vors et al., 2020).

The estimated contributions of dairy products (as assessed by FFQ) consumed by participants to total milk PL exposure by season are presented in Table 3.16.

Table 3.16. **Estimated contributions of FFQ dairy categories to total milk PL exposure by season /** *3.16. tabula* ***Aprēķinātais FFQ piena produktu kategoriju ieguldījums kopējā piena PL saturā sezonās***

Nr.	FFQ dairy product / *FFQ piena produkts*	Intake expressed as milk equivalent, g day^{-1} / *Patēriņš izteikts piena ekvivalentā, g dienā*	Estimated PL contribution, mg day^{-1} / *Aprēķinātais PL ieguldījums, mg dienā*	Contribution / *Ieguldījums, %*
Winter / *Ziema (n = 23)*				
1	Curd, 0.5 % fat / *Biezpiens ar 0,5 % tauku saturu*	193.40 ± 415.13	53.94 ± 115.78	20.41
2	Semi-hard cheese / *Puscietais siers*	104.70 ± 141.20	29.20 ± 39.38	11.05
3	Butter / *Sviests*	59.63 ± 85.24	16.63 ± 23.77	6.29
4	Milk, 2.5 % fat / *Piens ar 2,5 % tauku saturu*	56.10 ± 82.51	15.65 ± 23.01	5.92
5	Curd, 5 % fat / *Biezpiens ar 5 % tauku saturu*	53.53 ± 84.84	14.93 ± 23.66	5.65
6	Other dairy products / *Citi piena produkti*	480.10 ± 199.71	133.90 ± 55.70	50.67
Total / *Kopā*		947.47 ± 613.39	264.25 ± 171.07	100.00
Spring / *Pavasaris (n = 38)*				
1	Semi-hard cheese / *Puscietais siers*	113.17 ± 133.07	22.78 ± 26.79	12.66
2	Curd, 0.5 % fat / *Biezpiens ar 0,5 % tauku saturu*	104.63 ± 185.89	21.06 ± 37.42	11.70
3	Soft cheese / *Mīkstais siers*	96.38 ± 143.96	19.40 ± 28.98	10.78
4	Curd, 9 % fat / *Biezpiens ar 9 % tauku saturu*	65.10 ± 108.55	13.11 ± 21.85	7.28
5	Milk, 2.5 % fat / *Piens ar 2,5 % tauku saturu*	57.00 ± 118.46	11.47 ± 23.85	6.37
6	Other dairy products / *Citi piena produkti*	457.82 ± 301.66	92.16 ± 60.72	51.21
Total / *Kopā*		894.11 ± 523.38	179.98 ± 105.36	100.00

Note. Modelled estimates are presented as mean ± SD. Each percentage is the ratio of the category's seasonal mean PL contribution to the seasonal mean total PL intake. "Other dairy products" combines categories not listed separately. Category differences were not tested. / *Piezīme. Modelētās aplēses norādītas kā vidējā vērtība ± SD. Procentuālais ieguldījums aprēķināts, attiecīgās kategorijas sezonas vidējo PL ieguldījumu attiecinot pret sezonas vidējo kopējo PL uzņemšanu. "Citi piena produkti" apvieno atsevišķi neuzrādītās kategorijas. Atšķirības starp kategorijām nav statistiski pārbaudītas.*

The distribution of estimated contributions across FFQ dairy products differed between seasons, indicating a seasonal shift in the structure of dairy consumption. The large standard deviations reflect substantial inter-individual variability because participants did not regularly consume individual dairy products.

The seasonal difference in estimated habitual milk PL exposure cannot be attributed solely to total dairy product consumption. It reflects the combined influence of dairy product selection and season- and processing-related variation in MFGM-associated PL composition. Milk processing, including milk separation, cream churning, and the production of butter, redistributes MFGM-associated lipids among products and byproducts. Consequently, total dairy intake may yield different estimated milk PL exposures depending on the categories consumed and their corresponding PL compositions (Anto et al., 2020; Z. Liu et al., 2020; Venkat et al., 2024).

The seasonal variation in the estimated contributions of dairy products further supports using a composition-adjusted exposure estimate rather than total dairy intake alone. Frequently consumed dairy categories contributed substantially to estimated total milk PL intake even when their PL concentrations were lower than those of fat-rich dairy products. Thus, the main contributors to estimated milk PL intake were determined by the combined effect of product-specific PL concentration and habitual consumption, rather than by fat concentration or PL concentration alone.

This finding indicates that the principal contributors to estimated milk PL intake were not exclusively the dairy products with the highest fat or PL concentrations. Although fat-rich products such as butter and cream may contain concentrated lipid fractions, frequently consumed products such as curd and milk also contributed substantially to total estimated PL exposure. This is important because dietary exposure depends on the combination of concentration and consumption frequency. A product with moderate PL concentration may become a major contributor if consumed regularly, whereas a product with higher PL concentration may contribute less if consumed in small amounts.

In the present study, total milk-equivalent dairy intake did not differ significantly between winter and spring, whereas estimated total PL exposure was significantly higher in winter, reflecting the seasonal variation in raw-milk PL composition (Sections 1.1 and 3.1). Therefore, relying solely on total dairy intake would have undervalued the seasonal variation in exposure to lipids derived from MFGM. This reinforces the core methodological approach of the present thesis: integrating season-specific milk PL composition with individual dietary intake data provides a more informative estimate of biologically relevant dairy lipid exposure than assessing dairy intake alone.

The results are also relevant to perimenopausal women. During the menopausal transition, hormonal changes are associated with adverse shifts in lipid metabolism and increased cardiometabolic risk. Dietary factors that may influence intestinal cholesterol absorption, lipoprotein metabolism, and HDL-C concentration are therefore of interest in this population. Milk PL have been proposed to influence cholesterol homeostasis, lipid digestion, micellar cholesterol solubilisation, intestinal lipid absorption, and gut-lipid interactions, although evidence from habitual dietary exposure studies remains limited compared with studies using isolated or enriched PL fractions (Anto et al., 2020; Bruno et al., 2021; Vors et al., 2020).

Summary of Chapter 3.6. / *3.6. nodaļas kopsavilkums*

Estimated total PL, PE, and SM intakes were significantly higher in winter than in spring. The magnitude of the winter-spring difference was moderate for total PL and PE and large for SM. Although estimated PC intake was higher in winter, the difference was not statistically significant.

The main dairy product contributors to estimated PL exposure varied by season. In winter, curd with 0.5 % fat, semi-hard cheese, butter, and milk with 2.5 % fat made the largest individual contributions, whereas in spring, semi-hard cheese, curd with 0.5 % fat, soft cheese, curd with 9 % fat, and milk with 2.5 % fat were the principal contributors. The large standard deviations indicate substantial inter-individual variation in dairy product consumption.

Overall, the findings demonstrate that total dairy intake does not adequately represent milk PL exposure. Estimating habitual milk PL intake by combining individual dietary data with season-specific product composition provides a more specific and biologically relevant measure of exposure than evaluating dairy consumption alone. This approach is particularly relevant for investigating potential associations between habitual milk PL exposure and plasma lipid profile in perimenopausal women.

Aprēķinātā kopējo PL, PE un SM uzņemšana ziemā bija statistiski nozīmīgi lielāka nekā pavasarī. Standartizētās atšķirības starp sezonām bija vidējas kopējiem PL un PE un lielas SM.

Galvenie piena produkti, kas veidoja aprēķināto PL uzņemšanu, atšķīrās atkarībā no sezonas. Ziemā lielāko individuālo ieguldījumu nodrošināja biezpiens ar 0,5 % tauku saturu, puscietais siers, sviests un piens ar 2,5 % tauku saturu, savukārt pavasarī galvenie avoti bija puscietais siers, biezpiens ar 0,5 % tauku saturu, mīkstais siers, biezpiens ar 9 % tauku saturu un piens ar 2,5 % tauku saturu. Lielās standartnovirzes norāda uz izteiktām piena produktu patēriņa atšķirībām starp dalībniecēm.

Kopumā rezultāti liecina, ka kopējais piena produktu patēriņš nepietiekami raksturo piena PL uzņemšanu. Piena PL uzņemšanas aprēķināšana, apvienojot individuālos uztura datus ar sezonai raksturīgo produktu sastāvu, nodrošina specifiskāku un bioloģiski nozīmīgāku ekspozīcijas novērtējumu nekā tikai piena produktu patēriņa izvērtēšana. Šī pieeja ir īpaši nozīmīga, pētot iespējamās saistības starp piena PL uzņemšanu un plazmas lipīdu profilu sievietēm perimenopauzē.

3.7. Plasma lipid profile changes after the 28-day fermented buttermilk intervention / *Asins lipīdu profila izmaiņas pēc 28 dienu raudzētu paniņu lietojuma*

The changes in plasma lipid profile after the 28-day intervention period were evaluated separately in the intervention and control groups using paired sample analysis. This approach allowed assessment of within-group changes from baseline to post-intervention. The results are presented in Table 3.17.

Table 3.17. **Changes in plasma lipid profile after the 28-day intervention period, mmol L^{-1} /** *3.17. tabula* ***Plazmas lipīdu profila izmaiņas pēc 28 dienu intervences perioda, mmol L^{-1}***

Parameter / *Rādītājs*	Group / *Grupa*	Baseline / *Sākotnēji*	After 28 days / *Pēc 28 dienām*	Mean change [95 % CI] / *Vidējās izmaiņas [95 % TI]*	*p*-value / *p-vērtība*	Hedges-corrected g_z / *g_z ar Hedžesa korekciju*
TC	Intervention / *Intervence*	5.93 ± 0.61	5.88 ± 0.77	-0.05 [-0.33; 0.23]	0.717	-0.06
	Control / *Kontrole*	5.93 ± 0.48	5.44 ± 1.07	-0.50 [-0.90; -0.09]	**0.018**	-0.44
HDL-C	Intervention / *Intervence*	1.92 ± 0.55	1.87 ± 0.44	-0.05 [-0.15; 0.05]	0.285	-0.19
	Control / *Kontrole*	1.78 ± 0.34	1.67 ± 0.39	-0.11 [-0.23; -0.001]	**0.049**	-0.36
LDL-C	Intervention / *Intervence*	3.59 ± 0.31	3.63 ± 0.61	0.04 [-0.16; 0.24]	0.696	0.07
	Control / *Kontrole*	3.62 ± 0.29	3.34 ± 0.66	-0.28 [-0.53; -0.03]	**0.027**	-0.41
Non-HDL-C	Intervention / *Intervence*	4.01 ± 0.44	4.02 ± 0.63	0.01 [-0.22; 0.24]	0.933	0.01
	Control / *Kontrole*	4.15 ± 0.47	3.77 ± 0.91	-0.38 [-0.71; -0.06]	**0.023**	-0.43
Remnant cholesterol / *Remnanta holesterīns*	Intervention / *Intervence*	0.43 ± 0.26	0.41 ± 0.22	-0.01 [-0.11; 0.09]	0.778	-0.05
	Control / *Kontrole*	0.53 ± 0.32	0.43 ± 0.45	-0.09 [-0.22; 0.03]	0.140	-0.27
TG	Intervention / *Intervence*	1.13 ± 0.65	1.11 ± 0.47	-0.02 [-0.21; 0.16]	0.803	-0.04
	Control / *Kontrole*	1.17 ± 0.64	1.08 ± 0.61	-0.09 [-0.25; 0.07]	0.258	-0.21

Note. Baseline and day 28 values are mean ± SD. Change = day 28 minus baseline; negative changes and negative g_z values indicate decreases. p-values refer to changes within each group; values < 0.05 are in bold. These comparisons do not estimate the between-group intervention effect. / *Piezīme. Sākotnējās un 28. dienas vērtības norādītas kā vidējā vērtība ± SD. Izmaiņas = 28. dienas vērtība mīnus sākotnējā vērtība; negatīvas izmaiņas un negatīvas g_z vērtības norāda uz samazinājumu. p vērtības attiecas uz izmaiņām katrā grupā; vērtības < 0,05 izceltas treknrakstā. Šie salīdzinājumi neraksturo intervences efektu starp grupām.*

The intervention group showed no statistically significant within-group changes in TC, HDL-C, LDL-C, non-HDL-C, remnant cholesterol or TG after daily consumption of cultured buttermilk. The absolute standardised within-group changes were small (absolute g_z values ≤ 0.19), and all corresponding 95 % confidence intervals for the mean changes included zero. In the control group, TC, LDL-C, non-HDL-C and HDL-C decreased significantly, whereas remnant cholesterol and TG did not change significantly. The standardised within-group changes for the significant decreases ranged from g_z = -0.44 to -0.36. The corresponding 95 % confidence intervals for the mean changes excluded zero, although the upper limit for HDL-C was close to zero (-0.001 mmol L^{-1}).

The lack of statistically significant changes in the intervention group's lipid profile should be interpreted cautiously. The findings indicate that, under the conditions of this study, 28 days of consuming 250 mL day^{-1} of cultured buttermilk did not produce statistically detectable within-group changes in TC, LDL-C, HDL-C, non-HDL-C, remnant cholesterol or TG. Several factors may explain this result. Firstly, the intervention duration was short. Secondly, the intervention product was a commercially available fermented buttermilk containing naturally occurring PL, rather than a concentrated or purified MFGM or milk PL preparation. The daily portion supplied 70-81 mg of quantified PL, about one third of participants' habitual milk PL intake and 1-5 % of the 1.7-5 g day^{-1} used in the enriched-product trials in which TC and LDL-C decreased (Section 1.3, Table 1.5). Thirdly, habitual dietary background and milk PL exposure varied among individuals and may have influenced the plasma lipid profile independently of the assigned intervention. Fourthly, the paired analysis presented here does not adjust for baseline lipid concentration or estimated milk PL exposure.

The distinction between a food-based and an enriched-product intervention is important because most recent studies on milk PL used enriched or standardised MFGM fractions rather than fermented dairy products consumed in the diet. Controlled studies and reviews suggest that milk PL may influence intestinal cholesterol absorption, lipid digestion, SM-cholesterol interactions, bile acid metabolism and circulating sphingolipid species (Anto et al., 2020; Bruno et al., 2021; Kanon et al., 2024; Nilsson et al., 2021; Venkat et al., 2024; Vors et al., 2020). The present findings should therefore be understood as evidence from a short-term, food-based intervention at a habitual dietary dose rather than as a test of supplement-level milk PL exposure.

Several biological mechanisms have been proposed by which milk PL could influence lipid metabolism. Milk PL may interact with cholesterol during intestinal micelle formation, reduce cholesterol solubilisation and absorption, and promote intestinal cholesterol excretion. Milk PL may also affect the formation and metabolism of intestine-derived lipoproteins, bile acid pathways, lipid digestion, and gut-lipid interactions. However, evidence for these mechanisms comes from a combination of experimental studies, mechanistic human investigations, and interventions using purified or enriched PL fractions. Biological plausibility alone does not demonstrate that a short-term, low-dose, food-based intervention will produce measurable changes in fasting TC, LDL-C, HDL-C, or TG (Anto et al., 2020; Nilsson et al., 2021; Vors et al., 2020).

Interpret the statistically significant within-group changes in the control group with caution: they indicate neither an independent benefit of the control condition nor, on their own, that the control condition produced a better response than cultured buttermilk. In a parallel-group randomised trial, assess the intervention effect by directly comparing groups, preferably with adjustment for the baseline outcome, rather than by determining whether within-group p-values fall on different sides of the significance threshold. A significant change in one group and a non-significant change in the other does not imply that the changes differ significantly between the groups (Hopewell et al., 2025; Kypri, 2020).

The number of significant findings should not be interpreted as evidence of several independent physiological effects. TC, LDL-C, HDL-C, and non-HDL-C are biologically and mathematically related, with non-HDL-C calculated as the difference between TC and HDL-C.

Accordingly, reductions in TC, LDL-C, and non-HDL-C likely reflect different aspects of a common shift in circulating cholesterol rather than three independent metabolic responses. In line with the outcome hierarchy defined in Section 2.5, LDL-C is the primary outcome and the remaining parameters are secondary; no adjustment for multiple testing was applied, so the p-values of the secondary outcomes are nominal, and the HDL-C result ($p = 0.049$) in particular should be regarded as exploratory. CONSORT 2025 recommends explicitly addressing multiplicity when multiple outcomes and analyses are evaluated and cautions against drawing conclusions by comparing separate within-group significance tests (Hopewell et al., 2025).

Normal within-person biological variation in lipid concentrations may also have contributed. A systematic evaluation of biological-variation studies confirms that TC, LDL-C, HDL-C, and TG fluctuate within individuals, independent of a sustained treatment effect. A recent repeated-measures study estimated the within-person coefficient of variation for directly measured LDL-C at approximately 8.7 %. However, such variation estimates describe individual measurement variability and should not be used to dismiss a statistically significant group mean change (Alkan Baylan et al., 2026; Díaz-Garzón et al., 2019). In the present control group, the SD increased from 0.48 to 1.07 mmol L^{-1} for TC, from 0.29 to 0.66 mmol L^{-1} for LDL-C, and from 0.47 to 0.91 mmol L^{-1} for non-HDL-C. This increased dispersion suggests that the observed mean reductions were accompanied by heterogeneous individual responses and may have been influenced by a subset of participants rather than reflecting a uniform response across the control group.

Research-participation effects provide another possible explanation. Although the control group did not receive cultured buttermilk, participants underwent biochemical screening, detailed dietary assessment, researcher-assisted interviews, and repeat blood testing. Awareness of being observed and repeated assessment may increase attention to health-related behaviour and lead to temporary, unrecorded changes during a study. Contemporary methodological research describes assessment reactivity and other research-participation effects as potential sources of behavioural change in control groups. However, the evidence is heterogeneous and comes predominantly from behavioural research rather than lipid trials, so this mechanism should be presented as plausible but unconfirmed (Ulfsdotter Gunnarsson et al., 2025). Because equivalent end-of-study measurements of habitual diet, body weight, physical activity, sleep, and other lifestyle behaviours were unavailable, the present data cannot determine whether an unplanned behavioural change occurred preferentially in the control group.

Short-term hormonal variation may also have influenced the repeated lipid measurements. In an analysis of 8694 regularly menstruating women, menstrual-cycle phase showed non-linear associations with TC, LDL-C, HDL-C, and the TC-to-HDL-C ratio, but no statistically significant association with TG. This pattern is potentially relevant because the control group in the present study showed changes in several cholesterol-containing fractions, while TG remained comparatively stable (MacGregor et al., 2023). Nevertheless, that study included regularly menstruating women, and its results cannot be directly generalised to women in perimenopause. In the present trial, menstrual-cycle phase and sex-hormone concentrations were not standardised at the baseline and post-intervention blood-sampling visits. Hormonal timing should therefore be considered a potential source of within-person variability, but not a demonstrated mechanism explaining the control-group findings.

Reductions in LDL-C and non-HDL-C are generally consistent with a lower measured concentration of atherogenic cholesterol. However, the simultaneous reduction in HDL-C indicates that the control-group response was not uniformly favourable. The observed pattern may reflect a broader decrease in circulating cholesterol across several lipoprotein fractions rather than a selective LDL-C-lowering mechanism. Moreover, HDL-C concentration measures the cholesterol carried by HDL particles but does not directly assess HDL particle number, cholesterol-efflux capacity, reverse cholesterol transport, or other functional properties of HDL-C (von Eckardstein et al., 2023). Therefore, the clinical relevance of the small reduction in HDL-C cannot be determined from the HDL-C concentration alone.

A further potential contributor was the higher habitual intake of total and soluble fibre in the control group. At baseline, the control group consumed 4.65 g day^{-1} more total fibre and 1.44 g day^{-1} more soluble fibre than the intervention group. Soluble fibres, particularly viscous fibres, can reduce the incorporation of cholesterol into intestinal micelles, increase faecal bile-acid excretion, and promote hepatic conversion of cholesterol into bile acids, thereby increasing hepatic clearance of circulating LDL particles. In a dose-response meta-analysis of 181 randomised trials, each additional 5 g day^{-1} of soluble fibre reduced LDL-C and TC by approximately 0.14 and 0.16 mmol L^{-1}, respectively. A separate meta-analysis found that viscous fibre produced greater reductions in LDL-C, non-HDL-C, and ApoB than non-viscous cereal fibre (Ghavami et al., 2023; Jovanovski et al., 2023). This higher fibre intake may have modestly contributed to the more favourable dietary background in the control group but would be insufficient to explain the full extent of the observed reductions in TC and LDL-C. Moreover, the FFQ quantified total soluble fibre without distinguishing highly viscous fibres from those with weaker cholesterol-lowering properties. The control group's higher fibre intake should therefore be considered a plausible partial modifier of the observed changes.

The control group's higher absolute carbohydrate intake may also have influenced the direction of the lipid changes, although its independent contribution remains uncertain. Energy-matched randomised trials indicate that higher-carbohydrate diets may produce slightly greater reductions in LDL-C, whereas lower-carbohydrate diets tend to produce greater reductions in TG and increases in HDL-C. In a recent meta-analysis of 18 randomised trials, higher-carbohydrate diets produced a modestly greater reduction in LDL-C, while lower-carbohydrate diets were more favourable for TG and HDL-C (Anagnostou et al., 2026). The pattern in the present control group, characterised by reductions in LDL-C, a small reduction in HDL-C, and no significant change in TG, is directionally consistent with macronutrient-related effects, but this correspondence does not establish causality. Carbohydrate intake expressed as E% did not differ significantly between the study groups. Total energy intake was higher only in the control group, and total fat and SFA intake did not differ significantly. It is therefore not possible to demonstrate that carbohydrates displaced dietary fat or directly produced the observed lipid changes. The higher absolute carbohydrate intake may instead have reflected a combination of greater energy intake and greater consumption of fibre-containing carbohydrate sources. Accordingly, higher carbohydrate and fibre intakes may have modestly favoured cholesterol reduction in the control group, but they should be interpreted alongside regression towards the mean, biological variability, research-participation effects, and other unmeasured factors rather than as a sufficient causal explanation.

No single mechanism can be identified as the cause of the statistically significant within-group changes in the control group. The most defensible explanation is a combination of regression towards the mean, normal biological and analytical variation, heterogeneous individual responses, possible research-participation-related behavioural changes, hormonal timing, and chance arising from multiple correlated analyses. The findings should be presented as exploratory changes during follow-up rather than as a beneficial effect produced by the control condition. The primary interpretation of the intervention should rely on the direct, baseline-adjusted comparison between the randomised groups, while the control-group changes provide important context for understanding variability in the 28-day response.

Summary of Chapter 3.7. / *3.7. nodaļas kopsavilkums*

Overall, the paired-sample analysis showed no statistically significant within-group changes in TC, LDL-C, HDL-C, non-HDL-C, remnant cholesterol, or TG in the intervention group. In the control group, TC, LDL-C, HDL-C, and non-HDL-C decreased significantly, whereas TG and remnant cholesterol remained unchanged.

These within-group findings do not establish a causal effect of cultured buttermilk. The control-group changes may reflect biological variability, heterogeneous individual responses,

and hormonal or behavioural factors. Higher baseline intakes of carbohydrates, total fibre, and soluble fibre in the control group may also have influenced the lipid response but cannot fully explain the observed changes. The results should be interpreted alongside the direct baseline-adjusted between-group analyses presented in Section 3.8.

Kopumā saistīto izlašu analīze intervences grupā neuzrādīja statistiski nozīmīgas TC, LDL-C, HDL-C, non-HDL-C, remnanta holesterīna vai TG koncentrācijas izmaiņas grupas ietvaros. Kontroles grupā statistiski nozīmīgi samazinājās TC, LDL-C, HDL-C un non-HDL-C koncentrācija, savukārt triglicerīdu un remnantu holesterīna koncentrācija būtiski nemainījās.

Šie grupu ietvaros iegūtie rezultāti neapstiprināja raudzētu paniņu lietošanas cēloņsakarīgu efektu. Kontroles grupā novērotās izmaiņas varēja ietekmēt bioloģiskās mainības, heterogēnu individuālo reakciju, hormonālo un uzvedības faktoru kopums. Lielāka sākotnējā ogļhidrātu, kopējo šķiedrvielu un šķīstošo šķiedrvielu uzņemšana kontroles grupā varēja ietekmēt lipīdu profila dinamiku, taču tā nevar pilnībā izskaidrot novērotās izmaiņas. Tādēļ rezultāti jāinterpretē kopā ar 3.8. sadaļā sniegtajām tiešajām starpgrupu analīzēm, kas koriģētas pēc sākotnējiem rādītājiem.

3.8. Associations between estimated milk PL exposure and lipid profile / *Saistības starp aprēķināto piena PL uzņemšanu un lipīdu profilu*

The paired-sample analysis in this section describes within-group changes in the lipid profile following the 28-day intervention. However, paired comparisons alone do not account for baseline lipid concentrations, intervention allocation, seasonal cohort, or background milk PL exposure. Therefore, adjusted regression models were used to assess whether estimated habitual milk PL exposure was associated with the post-intervention lipid profile.

Post-intervention TC, LDL-C, HDL-C and TG were each modelled by ordinary least squares regression. Each model included the baseline value of the same lipid parameter as a covariate, along with three additional predictors: study group, season, and the ln-transformed estimate of total milk PL intake. This specification tested whether estimated milk PL intake explained variation in outcomes beyond baseline lipid status, group allocation, and season (Table 3.18).

Table 3.18. **Adjusted regression models for associations between estimated total milk PL exposure and post-intervention lipid profile /** *3.18. tabula* ***Koriģētie regresijas modeļi saistībām starp aprēķināto kopējo piena PL uzņemšanu un lipīdu profilu pēc intervences***

Predictor / *Rādītājs*	TC after intervention, β [95 % CI], p-value / *TC pēc intervences, β [95 % TI], p-vērtība*	LDL-C after intervention, β [95 % CI], p-value / *LDL-C pēc intervences, β [95 % TI], p-vērtība*	HDL-C after intervention, β [95 % CI], p-value / *HDL-C pēc intervences, β [95 % TI], p-vērtība*	TG after intervention, β [95 % CI], p-value / *TG pēc intervences, β [95 % TI], p-vērtība*
Baseline value of corresponding lipid parameter / *Attiecīgā lipīdu rādītāja sākotnējā vērtība*	0.752 [0.532; 0.973], **p < 0.001**	0.750 [0.448; 1.052], **p < 0.001**	0.769 [0.653; 0.885], **p < 0.001**	0.614 [0.453; 0.775], **p < 0.001**
Intervention group / *Intervences grupa*	0.485 [0.023; 0.948], **p = 0.040**	0.330 [0.024; 0.636], **p = 0.035**	0.099 [-0.034; 0.231], p = 0.143	0.074 [-0.129; 0.277], p = 0.468
Spring season / *Pavasara sezona*	-0.183 [-0.665; 0.298], p = 0.449	-0.091 [-0.417; 0.235], p = 0.578	-0.016 [-0.151; 0.118], p = 0.808	-0.001 [-0.206; 0.204], p = 0.993
ln(total milk PL intake) / *ln(kopējais piena PL patēriņš)*	0.206 [-0.030; 0.442], p = 0.085	0.130 [-0.067; 0.327], p = 0.192	0.059 [0.020; 0.099], **p = 0.004**	0.067 [0.014; 0.119], **p = 0.014**
Number of participants / *Dalībnieču skaits*	61	61	61	61

Note. β values are unstandardised coefficients; for milk PL intake, β refers to a one-unit increase in ln-transformed intake, with the other predictors held constant. Reference categories are the control group and winter. Bold p-values indicate p < 0.05. / *Piezīme. β vērtības ir nestandartizēti koeficienti; piena PL uzņemšanai β raksturo izmaiņas, uzņemtā daudzuma naturālajam logaritmam palielinoties par vienu vienību un pārējiem prognozētājiem paliekot nemainīgiem. Atsauces kategorijas ir kontroles grupa un ziema. Treknrakstā izceltas p vērtības < 0,05.*

In every adjusted model, the covariate with the largest and most stable contribution was the baseline value of the same lipid parameter: post-intervention TC depended on baseline TC, LDL-C on baseline LDL-C, HDL-C on baseline HDL-C, and TG on baseline TG. Such dominance of baseline values is not surprising when a dietary exposure lasting only a few weeks is evaluated against a lipid profile shaped over years by age, hormonal state, body fat, genetic background, long-term eating habits, and lifestyle. For women in the menopausal transition, this point carries particular weight, because this period is characterised by unfavourable shifts in lipid metabolism, rising TC, LDL-C and TG together with altered HDL-C concentration and function (El Khoudary et al., 2020).

After adjustment for baseline lipid concentrations, season and estimated total milk PL exposure, intervention group allocation was positively associated with post-intervention TC and LDL-C. The intervention group showed higher adjusted post-intervention TC ($\beta = 0.485$; 95 % CI: 0.023-0.948; $p = 0.040$) and LDL-C ($\beta = 0.330$; 95 % CI: 0.024-0.636; $p = 0.035$) than the control group. This result should not be interpreted as evidence that cultured buttermilk increased TC or LDL-C. Rather, it indicates that the intervention group had higher adjusted post-intervention TC and LDL-C than the control group. No statistically significant association was observed between intervention allocation and HDL-C or TG.

After adjustment for baseline lipid concentration, group allocation and ln-transformed milk PL intake, season was not an independent predictor of post-intervention TC, LDL-C, HDL-C or TG: the coefficient of the spring indicator was negative for TC, LDL-C and HDL-C and close to zero for TG, and none reached significance. This does not imply that season was biologically unimportant. Season shaped the estimated milk PL intake through the choice of dairy products and their season-specific composition, so its influence on the lipid profile, if any, was exerted indirectly through diet rather than as a separate factor in the regression models.

Estimated total milk PL intake (ln-transformed) showed a positive association with post-intervention HDL-C ($\beta = 0.059$; 95 % CI: 0.020-0.099; $p = 0.004$): women with a higher habitual intake of milk PL had higher HDL-C at the end of the study, independently of their baseline HDL-C, group and season. This is an associative finding and does not demonstrate that milk PL raised HDL-C. It does indicate, however, that in this cohort milk PL intake was more closely connected with HDL-related lipid transport than with reductions in atherogenic cholesterol markers.

No comparable association was observed for TC or LDL-C: the coefficients for ln-transformed total milk PL intake were positive, with confidence intervals crossing zero, so the adjusted models provide no support for an inverse association between habitual milk PL intake and either parameter. This contrasts with several controlled trials of PL-enriched milk or MFGM preparations, in which TC or LDL-C decreased (Kanon et al., 2024; Vors et al., 2020). The discrepancy has several plausible sources: the present study quantified habitual intake of PL naturally present in dairy foods rather than administering a concentrated PL product; intake was estimated from FFQ data combined with season-specific product composition; the observation period was short and the sample size moderate; and milk PL were consumed as part of a whole diet in which saturated fat, fibre, body composition and lifestyle also affect the lipid profile.

In the TG model, ln-transformed total milk PL intake was positively associated with post-intervention TG ($\beta = 0.067$; 95 % CI: 0.014-0.119; $p = 0.014$). Because baseline TG lay within the laboratory reference range and neither group showed a significant within-group change in TG in the paired analysis, this association is regarded as an exploratory observation and not as evidence that milk PL intake adversely affects TG metabolism.

Because total PL intake comprises PC, PE, and SM, additional exploratory models were used to evaluate whether individual estimated PL classes showed a similar pattern of association with lipid outcomes. Each model replaced ln(total PL intake) with ln(PC), ln(PE) or ln(SM), while retaining the same adjustment structure Table 3.19.

Table 3.19. **Exploratory adjusted associations between estimated milk PL classes and post-intervention lipid profile** / *3.19. tabula* ***Izpētē koriģētās saistības starp aprēķināto atsevišķo piena PL klašu uzņemšanu un lipīdu profilu pēc intervences***

Exposure variable / *Uzņemšanas mainīgais*	TC after intervention, β [95 % CI], p-value / *TC pēc intervences, β [95 % TI], p-vērtība*	LDL-C after intervention, β [95 % CI], p-value / *LDL-C pēc intervences, β [95 % TI], p-vērtība*	HDL-C after intervention, β [95 % CI], p-value / *HDL-C pēc intervences, β [95 % TI], p-vērtība*	TG after intervention, β [95 % CI], p-value / *TG pēc intervences, β [95 % TI], p-vērtība*
ln(PC intake) / *ln(PC uzņemšana)*	0.211 [-0.040; 0.463], p = 0.098	0.132 [-0.073; 0.338], p = 0.203	0.067 [0.023; 0.112], **p = 0.004**	0.074 [0.013; 0.136], **p = 0.019**
ln(PE intake) / *ln(PE uzņemšana)*	0.211 [-0.039; 0.460], p = 0.097	0.132 [-0.073; 0.337], p = 0.201	0.066 [0.022; 0.110], **p = 0.004**	0.073 [0.013; 0.134], **p = 0.018**
ln(SM intake) / *ln(SM uzņemšana)*	0.214 [-0.056; 0.484], p = 0.118	0.132 [-0.082; 0.345], p = 0.221	0.077 [0.025; 0.129], **p = 0.004**	0.083 [0.009; 0.158], **p = 0.028**

Note. Each PL class was modelled separately, with adjustment for baseline lipid concentration, study group and season. β is the unstandardised difference in post-intervention concentration (mmol L^{-1}) per one-unit increase in ln-transformed intake. Bold p-values indicate $p < 0.05$; these exploratory p-values are unadjusted for multiple testing. / *Piezīme. Katra PL klase modelēta atsevišķi, ņemot vērā sākotnējo lipīdu koncentrāciju, pētījuma grupu un sezonu. β ir nestandartizētā koncentrācijas atšķirība pēc intervences (mmol L^{-1}), uzņemtā daudzuma naturālajam logaritmam palielinoties par vienu vienību. Treknrakstā izceltas p vērtības < 0,05; šīs izpētes analīzes p vērtības norādītas bez daudzkārtējo salīdzinājumu korekcijas.*

The exploratory specific PL-class models showed a pattern broadly similar to that of the overall PL model. Estimated PC, PE and SM intakes were positively associated with post-intervention HDL-C, while no statistically significant inverse associations were observed for TC or LDL-C concentrations. Therefore, the individual PL-class models do not indicate that any single PL class clearly explains a cholesterol-lowering association in this cohort. Instead, the findings suggest that the observed association with HDL-C concentrations reflects the broader pattern of estimated milk PL exposure.

The positive association between estimated milk PL exposure and HDL-C concentrations is biologically plausible but should be interpreted carefully. Milk PL may influence lipid digestion, intestinal cholesterol absorption, micellar solubilisation, bile acid metabolism and lipoprotein metabolism (Anto et al., 2020; Bruno et al., 2021; Nilsson et al., 2021; Venkat et al., 2024). SM has attracted particular interest because of its interactions with cholesterol in the intestinal lumen and at biological membranes. However, findings from human studies on the effects of milk PL or MFGM-enriched products on TC, LDL-C, and HDL-C concentrations remain inconsistent. Some controlled interventions with milk PL- or MFGM-enriched fractions have reported reductions in TC and LDL-C, whereas effects on HDL-C have been less consistent (Kanon et al., 2024; Vors et al., 2020). The present study differs from those trials because it estimated habitual exposure to naturally occurring milk PL from real-life dairy consumption rather than assigning a standardised enriched PL dose.

To evaluate whether the association between estimated milk PL exposure and lipid outcomes differed by study group, interaction terms between intervention allocation and ln(total milk PL intake) were tested. The results are presented in Table 3.20.

Table 3.20. **Interaction between study group and estimated total milk PL exposure in adjusted models of post-intervention lipid profile /** *3.20. tabula* ***Pētījuma grupas un aprēķinātās kopējās piena PL uzņemšanas mijiedarbība koriģētajos lipīdu profila modeļos pēc intervences***

Post intervention outcome / *Iznākuma rādītājs pēc intervences*	Group × ln(total PL intake), β [95 % CI] / *Grupa × ln(kopējais PL patēriņš), β [95 % TI]*	p-value / *p-vērtība*
TC	-0.429 [-1.057; 0.198]	0.176
LDL-C	-0.154 [-0.510; 0.203]	0.391
HDL-C	-0.025 [-0.216; 0.166]	0.795
TG	-0.138 [-0.336; 0.060]	0.169

Note. The interaction β is the difference between the intervention and control groups in the slope for ln-transformed total PL intake. Its p-value tests whether this association differs between groups. Models account for baseline lipid concentration and season, with the control group and winter as reference categories. / *Piezīme. Mijiedarbības koeficients β raksturo regresijas taisnes slīpuma atšķirību starp intervences un kontroles grupām saistībai ar kopējās PL uzņemšanas naturālo logaritmu. Tā p vērtība pārbauda, vai šī saistība abās grupās atšķiras. Modeļos ņemta vērā sākotnējā lipīdu koncentrācija un sezona; atsauces kategorijas ir kontroles grupa un ziema.*

No statistically significant group × total PL interaction was observed for TC, LDL-C, HDL-C or TG concentrations. Therefore, the available data did not show evidence that the association between estimated total milk PL exposure and lipid outcomes differed significantly between the intervention and control groups. This supports the interpretation that habitual milk PL exposure should be considered as background dietary exposure rather than as an effect operating only within the intervention group.

Taken together, the adjusted models show that a participant's lipid profile after 28 days was determined mainly by where it started. Estimated milk PL intake was positively related to HDL-C only; it was not associated with lower TC or LDL-C, and season had no independent

effect once the other variables were controlled. The higher adjusted post-intervention TC and LDL-C in the intervention group must be viewed against the decrease observed within the control group and does not indicate a harmful effect of cultured buttermilk. The models for PC, PE and SM mirrored the total PL model, and no group × PL interaction was significant. These data suggest a plausible association between habitual milk PL intake and HDL-C in perimenopausal women, but it is correlational and needs confirmation in larger, longer, and more tightly controlled studies.

Summary of Chapter 3.8. / *3.8. nodaļas kopsavilkums*

The chapter examined how estimated habitual milk PL intake related to the post-intervention plasma lipid profile. Baseline values were the strongest predictors of post-intervention TC, LDL-C, HDL-C, and TG. After adjustment, allocation to the intervention group was associated with to higher TC and LDL-C, but not to HDL-C or TG; this suggests higher adjusted TC and LDL-C in the intervention group than in the control group, without implying that cultured buttermilk consumption caused the increase. Season had no independent effect on any post-intervention lipid parameter.

Overall, the findings suggest that estimated habitual milk PL exposure may be associated with plasma lipid profile parameters, particularly HDL-C, but the observed associations were exploratory and do not demonstrate a causal or general cholesterol-lowering effect.

Šajā nodaļā tika izvērtētas saistības starp aprēķināto piena PL uzņemšanu un plazmas lipīdu profilu pēc intervences. Sākotnējās lipīdu koncentrācijas bija izteiktākie TC, LDL-C, HDL-C un TG koncentrācijas prognozētāji pēc intervences. Pētījuma beigās intervences grupas dalībniecēm bija statistiski nozīmīgi augstāka TC un LDL-C koncentrācija nekā kontroles grupas dalībniecēm. HDL-C un TG koncentrācijai statistiski nozīmīgas atšķirības starp grupām netika konstatētas. Šie rezultāti norāda uz augstāku koriģēto TC un LDL-C koncentrāciju intervences grupā salīdzinājumā ar kontroles grupu, tomēr tie nav interpretējami kā pierādījumi, ka raudzētu paniņu lietošana izraisīja šo lipīdu rādītāju palielināšanos. Sezona nebija saistīta ne ar vienu no lipīdu profila rādītājiem pēc intervences.

Kopumā rezultāti liecina, ka aprēķinātā piena PL uzņemšana var būt saistīta ar atsevišķiem plazmas lipīdu profila rādītājiem, īpaši HDL-C, tomēr novērotās saistība bija asociatīvas un izpētes rakstura un neapliecina cēloņsakarību vai vispārēju holesterīna līmeni pazeminošu iedarbību.

3.9. Strengths, limitations and future research directions / *Pētījuma stiprās puses, ierobežojumi un turpmāko pētījumu virzieni*

The principal strength of the thesis is its integrated design, which connects food composition analysis, dietary assessment, and a clinical intervention within one framework, so processing, seasonality, product composition, habitual intake, and plasma lipid outcomes were examined as linked components rather than as separate studies. The compositional part analysed raw milk and its processing products from the same batch in two seasons, allowing direct evaluation of processing-related redistribution and product-specific seasonal differences in PC, PE, and SM, and showing that total fat is not an adequate indicator of PL composition. The intervention product was characterised analytically, so the daily PL dose is based on season-specific measured concentrations rather than label or literature values, and the intervention is correctly interpreted as a whole-food exposure to naturally occurring PL. The clinical part addressed an understudied group, perimenopausal women with moderately elevated LDL-C, with a randomised controlled design, predefined eligibility criteria, a pilot feasibility study, the same accredited laboratory at baseline and follow-up, and retention of 61 of 62 randomised

participants. The dietary assessment combined a researcher-assisted FFQ with a portion atlas, a 24-h dietary recall and a 72-h diary, and integrating this with season-specific milk PL composition provided participant-level estimates of total PL, PC, PE, and SM exposure; within the literature reviewed, no previous Latvian study had combined these data in this way. Finally, the interpretative framework was deliberately cautious: habitual exposure was distinguished from intervention allocation, baseline concentrations were included in the models, and compositional findings were not translated into efficacy claims, so that the absence of a lipid-lowering effect at a realistic dietary dose is itself an informative result.

Several limitations should be considered when interpreting the findings. First, the compositional analysis was based on one processing batch per season from a single dairy and cannot represent the variability of dairy products on the Latvian market; animal feeding, breed, lactation stage and farm management were not controlled, so the observed differences describe the analysed product set rather than national reference values. Second, the seasonal dimension rests on two independent cohorts, winter and spring, rather than on repeated measurements of the same women over a full year, which limits the separation of seasonal effects from differences between participants. Third, dietary PL intake estimates depend on FFQ accuracy; researcher assistance, the portion atlas, and comparison with the 24-h recall and the 72-h diary reduced but did not eliminate misreporting and misclassification, particularly for dairy products with similar names or fat concentration. Fourth, PL exposure from most dairy categories was estimated through milk-equivalent conversion rather than direct measurement, because PL analysis was not available for all products reported in the FFQ; the estimates are therefore approximations of habitual exposure, with possible misclassification for processed products such as curd, cheese, butter and cream. Fifth, the FFQ described habitual intake over the preceding 12 months, whereas the intervention lasted 28 days, so the exposure estimate reflects the dietary background rather than intake during the intervention period.

Sixth, blinding was not feasible for a food intervention, and the control group received no placebo, so awareness of allocation may have influenced behaviour or compliance. Seventh, the sample size limited statistical power for subgroup, seasonal, and interaction analyses and for detecting modest lipid changes. Eighth, the 28-day duration may detect early changes but not longer-term metabolic adaptation, sustained lipid changes or effects on body composition and cardiometabolic risk. Ninth, participants continued their usual lifestyle under free-living conditions, so residual confounding by diet, physical activity, alcohol, body weight change or medication use remains possible. Body mass index was not included as a covariate in the regression models, because it did not differ between the groups at baseline; a sensitivity analysis with BMI was not performed. Finally, the integrated analyses combining product composition, FFQ-derived intake and plasma lipids are exposure-related associations and not proof that milk PL caused the observed differences.

Despite these limitations, the thesis provides the first data on the concentration and class distribution of PL in raw milk and in its processing products (skimmed milk, cream, butter and buttermilk) obtained from the same batch of Latvian milk in two seasons, characterises one commercially available cultured buttermilk as a dietary source of milk PL, and provides clinically relevant information on the plasma lipid profile of perimenopausal women with moderately elevated LDL-C concentrations. Future studies should include a larger sample and a longer intervention, repeated seasonal sampling across the year, direct PL quantification of a wider range of dairy products from several producers, assessment of menopausal stage by hormonal markers, and a broader set of cardiometabolic outcomes.

CONCLUSIONS

1. The polar lipid concentration pattern was consistent with processing-related redistribution of milk fat globule membrane material, indicating that total fat concentration alone does not adequately characterise the polar lipid concentration in milk and dairy products.
2. Seasonal variation in the concentrations of quantified polar lipids was product-specific; the product × season interaction was significant for total quantified polar lipids ($p = 0.021$), phosphatidylcholine ($p < 0.001$), phosphatidylethanolamine ($p = 0.001$) and sphingomyelin ($p = 0.010$).
3. The commercially available standardised cultured buttermilk used in the intervention contained 27.89-32.52 mg 100 g^{-1} of total quantified polar lipids, and the daily intervention portion of 250 mL supplied 70-81 mg of polar lipids, representing up to one third of participants' habitual milk polar lipid intake.
4. Daily consumption of 250 mL of cultured buttermilk for 28 days did not yield statistically significant within-group changes in total cholesterol, low-density lipoprotein cholesterol, high-density lipoprotein cholesterol, non-high-density lipoprotein cholesterol, remnant cholesterol or triglyceride concentrations in the intervention group. The study did not demonstrate a lipid-lowering effect under the conditions investigated.
5. Total dairy product intake, expressed as milk equivalent, did not differ between winter and spring, whereas estimated habitual milk polar lipid exposure was significantly higher in winter than in spring (total polar lipids $p = 0.041$, Hedges' $g = 0.62$; phosphatidylethanolamine $p = 0.024$, Hedges' $g = 0.70$; sphingomyelin $p = 0.006$, Hedges' $g = 0.89$).
6. Habitual milk polar lipid exposure depends on dairy product selection and season-specific product composition, not on total dairy intake alone.
7. Higher estimated habitual milk polar lipid exposure was associated with higher post-intervention concentrations of high-density lipoprotein cholesterol ($\beta = 0.059$, $p = 0.004$) and triglycerides ($\beta = 0.067$, $p = 0.014$), but not with the primary outcome, low-density lipoprotein cholesterol.
8. The hypothesis was partially supported. Differences in polar lipid concentration and class distribution were observed among the studied milk and dairy products and between the analysed winter and spring batches. The 28-day cultured buttermilk intervention did not demonstrate a lipid-lowering effect. Associations between estimated habitual milk polar lipid exposure and selected plasma lipid parameters were exploratory and do not establish a causal effect of polar lipid intake.

SECINĀJUMI

1. Polāro lipīdu saturu piena produktos nosaka piena pārstrādes procesu ietekmēta tauku lodīšu membrānas stabilitāte, apstiprinot, ka kopējais tauku saturs nevar pietiekami raksturot polāro lipīdu saturu pienā un piena produktos.
2. Kvantitatīvi noteikto polāro lipīdu satura sezonālās izmaiņas ir atkarīgas no produkta veida; produkta × sezonas mijiedarbība ir statistiski nozīmīga kopējiem kvantitatīvi noteiktajiem polārajiem lipīdiem ($p = 0.021$), fosfatidilholīnam ($p < 0.001$), fosfatidiletanolamīnam ($p = 0.001$) un sfingomielīnam ($p = 0.010$).
3. Intervencē izmantotās mazumtirdzniecībā iegādājamās raudzētās paniņas saturēja 27.89-32.52 mg 100 g^{-1} kopējo polāro lipīdu, nodrošinot intervences porcijā (250 mL) 70-81 mg polāro lipīdu dienā jeb vienu trešdaļu no piena polāro lipīdu daudzuma uzturā.
4. Raudzētu paniņu lietošana 28 dienas intervences grupai neapstiprināja statistiski nozīmīgas kopējā holesterīna, zema blīvuma lipoproteīnu holesterīna, augsta blīvuma lipoproteīnu holesterīna, kopējā aterogēno lipoproteīnu holesterīna, remnanta holesterīna vai triglicerīdu koncentrācijas izmaiņas grupas ietvaros. Pētītajos apstākļos lipīdu līmeni pazeminoša iedarbība netika pierādīta.
5. Kopējais piena produktu patēriņš, izteikts piena ekvivalentā, ziemā un pavasarī neatšķīrās, savukārt aprēķinātais piena polāro lipīdu daudzums uzturā ziemā bija būtiski lielāks nekā pavasarī (kopējie polārie lipīdi $p = 0.041$, Hedžesa $g = 0.62$; fosfatidiletanolamīns $p = 0.024$, Hedžesa $g = 0.70$; sfingomielīns $p = 0.006$, Hedžesa $g = 0.89$).
6. Piena polāro lipīdu daudzums uzturā ir atkarīgs no piena produktu izvēles un sezonai raksturīgā produktu sastāva, nevis no kopējā piena produktu patēriņa.
7. Lielāks piena polāro lipīdu daudzums uzturā bija saistīts ar augstāku augsta blīvuma lipoproteīnu holesterīna ($\beta = 0.059$, $p = 0.004$) un triglicerīdu ($\beta = 0.067$, $p = 0.014$) koncentrāciju pēc intervences, bet ne ar primāro iznākuma rādītāju - zema blīvuma lipoproteīnu holesterīna koncentrāciju.
8. Pētījuma hipotēze ir apstiprināta daļēji. Pētītajos piena un piena produktos analizētajās ziemas un pavasara sezonās konstatētas polāro lipīdu satura un sastāva relatīvā sadalījuma atšķirības. 28 dienu raudzētu paniņu intervence lipīdu līmeni pazeminošu iedarbību nepierādīja. Saistības starp aprēķināto piena polāro lipīdu daudzumu uzturā un atsevišķiem plazmas lipīdu rādītājiem bija izpētes rakstura un neapstiprināja polāro lipīdu uzņemšanas cēloņsakarīgu ietekmi.

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ANNEXES / *PIELIKUMI*

Annex I / *Pielikums I*

Table A1.1 / *A1.1. tabula*

MRM transitions of polar lipid standards used for LC-MS analysis / *Ar LC-MS analizēto polāro lipīdu standartu MRM pārejas*

Compound / *Savienojums*	Molecular formula / *Molekulformula*	Ionisation mode / *Jonizācijas režīms*	Precursor ion, m/z / *Prekursora jons, m/z*	Product ion, m/z / *Produkta jons, m/z*	Collision energy, V / *Sadursmes enerģija, V*	Retention time, min / *Aiztures laiks, min*	Comment / *Piezīme*
SM(d18:1/16:0)	$C_{39}H_{79}N_2O_6P$	$[M+H]^+$	704.4	184.1	-27.0	14.21	Sphingomyelin / *Sfingomielīns*
SM(d18:1/18:0)	$C_{41}H_{83}N_2O_6P$	$[M+H]^+$	731.7	184.1	-29.0	14.70	Sphingomyelin / *Sfingomielīns*
PE(16:0/18:2)	$C_{39}H_{74}NO_8P$	$[M+H]^+$	716.6	575.5	-25.0	14.69	Phosphatidylethanolamine / *Fosfatidiletanolamīns*
PE(16:0/18:1)	$C_{39}H_{76}NO_8P$	$[M+H]^+$	718.5	577.5	-24.0	14.96	Phosphatidylethanolamine / *Fosfatidiletanolamīns*
PC(16:0/18:1)	$C_{42}H_{82}NO_8P$	$[M+H]^+$	760.5	184.2	-32.0	15.76	Phosphatidylcholine / *Fosfatidilholīns*
PC(18:1/18:1)	$C_{44}H_{84}NO_8P$	$[M+H]^+$	786.5	184.2	-33.0	15.97	Phosphatidylcholine / *Fosfatidilholīns*

Note. $[M+H]^+$ denotes the protonated molecular ion. Retention times and collision energies refer to the analytical conditions used in this study. / *Piezīme. $[M+H]^+$ apzīmē protonētu molekulāro jonu. Aiztures laiki un sadursmes enerģijas attiecas uz šajā pētījumā izmantotajiem analīzes apstākļiem.*

Table A1.2 / *A1.2. tabula*

Calibration parameters, limits of detection and quantification for polar lipid standards analysed by LC-MS / *Ar LC-MS analizēto polāro lipīdu standartu kalibrēšanas parametri, noteikšanas un kvantificēšanas robežas*

Compound / *Savienojums*	Calibration equation / *Kalibrēšanas vienādojums*	R^2	LOD (μg mL^{-1})	LOQ (μg mL^{-1})
SM(d18:1/16:0)	y = 80121483.4x - 670044.226	0.9995311	0.02214	0.06710
SM(d18:1/18:0)	y = 10736146.5x - 53926.229	0.9996376	0.01947	0.05899
PE(16:0/18:2)	y = 768284.3x - 2733.262	0.9988669	0.03156	0.09565
PE(16:0/18:1)	y = 1385670.6x - 11218.443	0.9986552	0.03439	0.10421
PC(16:0/18:1)	y = 82945737.7x - 7050.456	0.9998506	0.01042	0.03156
PC(18:1/18:1)	y = 23312914.0x + 24327.569	0.9997450	0.01361	0.04123

Note. In the calibration equations, x is standard concentration (μg mL^{-1}) and y is instrument response. LOD and LOQ denote the limits of detection and quantification: LOD = 3.3σ/S and LOQ = 10σ/S, where S is the slope and σ is the standard error of the intercept multiplied by √12. Regression was unweighted, with a freely estimated intercept. / *Piezīme. Kalibrēšanas vienādojumos x ir standarta koncentrācija (μg mL^{-1}), bet y - instrumenta atbildes signāls. LOD un LOQ apzīmē noteikšanas un kvantificēšanas robežas: LOD = 3,3σ/S un LOQ = 10σ/S, kur S ir taisnes slīpums, bet σ - brīvā locekļa standartkļūda, reizināta ar √12. Izmantota regresija bez svēršanas ar brīvi aprēķinātu brīvo locekli.*

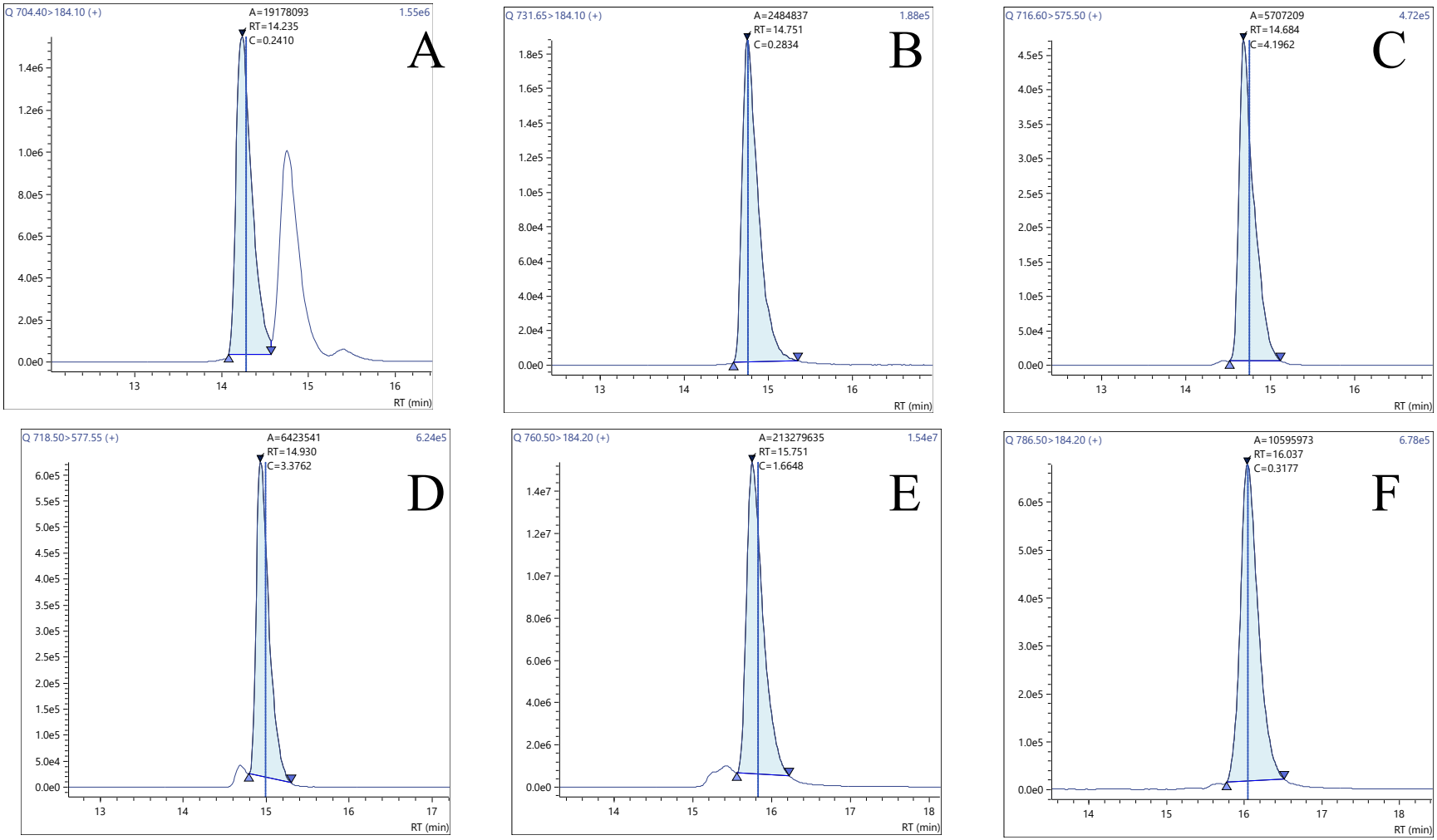


Fig. A1.2. **Extracted ion chromatograms of selected polar lipid compounds detected in the buttermilk by LC-MS** / *A1.2. att.* ***Ar LC-MS metodi paniņu noteikto atlasīto polāro lipīdu savienojumu ekstrahēto jonu hromatogrammas***

Note. Panels A-F correspond to SM(d18:1/16:0), SM(d18:1/18:0), PE(16:0/18:2), PE(16:0/18:1), PC(16:0/18:1) and PC(18:1/18:1), respectively. GlcCer(d18:2/16:0) was not detected in the analysed buttermilk sample. / *Piezīme. Paneļi A-F attiecīgi atbilst SM(d18:1/16:0), SM(d18:1/18:0), PE(16:0/18:2), PE(16:0/18:1), PC(16:0/18:1) un PC(18:1/18:1). GlcCer(d18:2/16:0) analizētajā paniņu paraugā netika konstatēts.*

Approval from the Ethics Committee of Rīga Stradiņš University / *Rīgas Stradiņa universitātes Ētikas komitejas lēmums*

Annex
Veidlapa Nr. E-9(3)
APSTIPRINĀTA
ar Rīgas Stradiņa universitātes rektora
2018. gada 26. septembra rīkojumu Nr. 5-1/238/2018

Rīgas Stradiņa universitātes
Pētījumu ētikas komitejas
LĒMUMS
Rīgā

23.11.2022 2-PĒK-4/513/2022

	Komitejas sastāvs	Kvalifikācija	Nodarbošanās
1	Profesors Jānis Vētra	Dr.habil. med.	Morfoloģijas katedra
2	Asoc. Prof. Zanda Daneberga	Dr.med.	OI Molekulārās ģenētikas laboratorijas vadītāja
3	Asoc. Prof. Anita Vētra	Dr.med.	Rehabilitācijas katedras vadītāja
4	Profesore Ingrīda Čēma	Dr.habil. med.	Mutes medicīnas katedras vadītāja
5	Docente Anna Junga	Dr.med.	Morfoloģijas laboratorijas vadītāja
6	Vadošā pētniece, docente Karina Palkova	Ph.D.	Advokāte, Doktora studiju programmas vadītāja
7	Marina Siņkovska		Datu drošības un pārvaldības nodaļas vadītāja

Pieteikuma iesniedzējs/i: **Svetlana Aleksejeva**

Pētījuma / pētnieciskā darba nosaukums: Paniņu funkcionālo īpašību novērtējums

Pētījumu ētikas komitejas sēdes datums: 27.10.2022.

Pētījuma protokols: Izskatot augstāk minētā pētījuma pieteikuma materiālus, t.sk., protokolu, ir redzams, ka pētījuma mērķi - "Novērtēt paniņu polāro lipīdu ietekmi uz asins plazmas lipīdiem.", ir paredzēt sasniegt, iesaistot dalībniekus, bez kāda apdraudējuma veselībai, drošībai un dzīvībai, klīniskā pētījumā (veicot asins paraugu ņemšanu un izdarot atbilstošas analīzes, pārbaudes, mērījumus), veicot iegūto datu apstrādi un analīzi, kā arī publiskojot iegūtos rezultātus. Personu (dalībnieku) informēta brīvprātīga piekrišana piedalīties, iegūto personu datu apstrāde un aizsardzība, to pielietošana, glabāšana, anonimitāte un konfidencialitāte ir nodrošināta. Līdz ar to pieteikums atbilst pētījuma ētikas prasībām.

Komitejas lēmums: Piekrist pētījuma īstenošanai

Komitejas priekšsēdētājs Jānis Vētra Tituls: Dr.habil. med., profesors.

ŠIS DOKUMENTS IR ELEKTRONISKI PARAKSTĪTS AR DROŠU ELEKTRONISKO PARAKSTU UN SATUR LAIKA ZĪMOGU

K. Ķauķe
Tālrunis: 26691306

Informed consent of the participant / *Dalībnieka informētā piekrišana*

Latvijas Biozinātņu un tehnoloģiju universitāte

We invite you to participate in a study on the analysis of the polar lipid composition of buttermilk and their effect on the blood lipid profile

Objectives of the study:

1. to analyse the factors influencing the composition and content of polar lipids in milk;
2. to determine polar lipids in milk and its processed products;
3. to assess the effect of buttermilk polar lipids on plasma lipids.

Background to the study:

Hormonal changes in a woman's life cycle affect her lipid profile. The transition from childbearing age to menopause occurs most frequently between 45-55 years when low-density lipoprotein cholesterol (LDL-C) levels increase by an average of 2 mg/dL (0.11 mmol/L) per year. At the same time, ageing is associated with an increase in total cholesterol (TC) and triglycerides (TG) and a decrease in high-density lipoprotein cholesterol (HDL-C). These changes in the lipid profile increase the risk of cardiovascular disease (CVDs), which is the leading cause of death in Europe and Latvia. Buttermilk is a by-product of milk processing, produced by butter-making, and rich in polar lipids. Animal studies have shown that sphingolipids lower cholesterol, but in vivo studies are lacking. Consumption of buttermilk may reduce plasma cholesterol concentrations by inhibiting its bioavailability in the intestine.

Benefits of the study:

You will have the opportunity to gain insight into your diet's nutrient supply and get a blood lipid profile. At a societal level, the potential benefit of your participation in the study will be identifying the role of buttermilk intake in CVDs prevention.

Study risks:

The study involves collecting venous blood samples for the following parameters: TC, LDL-C, HDL-C, TG, alanine aminotransferase (ALT) and glucose. Venous puncture is one of the most common invasive medical procedures medical staff perform in the laboratory or its branches. The most common risks associated with venipuncture during blood sampling are haematoma and pain at the puncture site. Much less common risks are infection, bleeding from the puncture site, hypotension, loss of consciousness and fainting, phlebitis, nerve damage, haemoconcentration, arterial puncture, petechiae, allergic reaction to disinfectant solution, latex, fear and phobia of needle stick, blood, swelling at the puncture site, thrombosis.

Your participation:

Contact details in case of questions or queries:
Svetlana Aleksejeva,s.aleksejeva@gmail.com , tel. 29422183

Your participation in the study involves completing a questionnaire, food diaries, and blood tests and possibly consuming buttermilk regularly during the study.

Right to refuse or terminate participation in the study:
Participation in the study is voluntary. You may withdraw from the study at any time.

Inclusion criteria:

- reside in Latvia;
- women;
- perimenopause symptoms (with vasomotor symptoms (hot flushes, night sweats) and infrequent periods);
- 45-55 years;
- moderately elevated LDL-C (3.01-4.12 mmol L^{-1}) within the last 6 months;
- stable body weight (+/- 2 kg) in the previous 6 months.

Study exclusion criteria:

- noncompliance with the inclusion criteria;
- history of primary hyperlipidemia;
- CVDs (ischemic heart disease, myocardial infarction, stroke, history of chronic heart failure, angina pectoris, arrhythmia);
- endocrine diseases (type 1 or type 2 diabetes mellitus, thyroid pathology (hypothyroidism, hyperthyroidism), adrenal disease (Cushing's syndrome, secondary renal failure));
- liver diseases (non-alcoholic fatty liver disease (NATAS), alcoholic fatty liver disease (ATAS), hepatitis, cirrhosis, chronic liver failure); chronic kidney disease; history of gastrointestinal surgery;
- hormonal therapy;
- immunosuppressive therapy;
- pharmacological treatment of hyperlipidemia or hypertension therapy (drugs, food supplements).

Study design:
Before starting the study, a researcher will contact you, who will explain the research and ask you to sign an informed consent form. You can only participate in the study after signing the informed consent form.
Before starting the study, you will be asked to fill in a questionnaire with the researcher with general questions on demographics, dietary habits, smoking, drinking and physical activity habits, and health status. In addition, you will be asked to complete a 24-hour diet diary with the researcher, in which you will need to record everything you have eaten for the previous day.
At the start of the study, you will be asked to complete a food diary, which asks you to record everything you eat over 72 hours, and a food frequency questionnaire.
Data from the 24-hour diet diary, 72-hour diet diary and food frequency questionnaire will be used to analyse your dietary data. The questionnaires will be anonymised, and you will be given

Contact details in case of questions or queries:
Svetlana Aleksejeva,s.aleksejeva@gmail.com , tel. 29422183

a unique code, which will be indicated on the informed consent form, the study questionnaire, and the diet diary.
At the start of the study, you must have blood tests to determine your TC, LDL-C, HDL-C, TG, ALT and glucose. Blood sample collection will be possible at an accredited laboratory, which will be contracted, and locations and possible collection times will be communicated to you by the researcher.
After the blood test, your participation in the study can begin, which will last for 28 days. If you are in the control group, you will not need additional dietary changes during this period. However, participants in the intervention group will be required to consume the recommended amount of buttermilk the researchers provided daily for 28 days. You will be told more about the product at your first appointment.
You will automatically receive reminders to take your buttermilk if you are in the intervention group.
Throughout the study, you can contact the researcher with any questions.
At the end of the study, you will need a repeat blood test to determine the following parameters: TC, LDL-C, HDL-C, TG, ALT, and glucose. The blood sample will be collected from an accredited laboratory, which will be contracted. The researcher will communicate locations and possible times to you.

Ethical considerations and data protection:
The permission of the Ethics Committee of Riga Stradiņš University will be obtained before the start of the study.
The research will follow the guidelines of the Declaration of Helsinki and the Convention for the Protection of Human Rights and Dignity of Human Beings in Biology and Medicine, as well as other laws and regulations, taking precautions to avoid harm to human subjects. Your privacy rights during the study will be safeguarded following the General Data Protection Regulation (Regulation No 2016/679 of the European Parliament and the Council) and the Law on the Processing of Personal Data of the Republic of Latvia.
The study's data will be kept entirely confidential and accessible only to the closed research team associated with it. Data processing and storage will be carried out according to the requirements of the legislation on the protection of natural persons in the processing of personal data. Throughout the study, data from identifiable individuals will be processed as necessary to unambiguously identify dietary questionnaire data and laboratory analysis results.

Study researchers:
The research will be carried out by Mg. sc. sal. Svetlana Aleksejeva Dr. sc. ing, prof. Inga Ciproviča and Dr. med., asoc. prof. Laila Meija.

I have read the information above. I have been briefed on the purpose and process of the study. I have had the opportunity to ask questions and have received answers. I have been informed that I can withdraw from the study at any time and that this will not affect my future medical care. I agree to participate in the study voluntarily.

Contact details in case of questions or queries:
Svetlana Aleksejeva,s.aleksejeva@gmail.com , tel. 29422183

The remainder of the consent form (pages 1-4) remains with the participant.

I have read the information above. I have been briefed on the purpose and process of the study. I have had the opportunity to ask questions and have received answers. I have been informed that I can withdraw from the study at any time and that this will not affect my future medical care. I agree to participate in the study voluntarily.
By signing below, you confirm that you have read and understood the above information and agree to participate in the study to assess the functional properties of buttermilk.

Name, surname, signature of the participant:

_____
Name, surname

____.____._____
Date (dd.mm.yyyy)

Receipt of results
If you would like to receive the results of your dietary analysis, please provide your email address:

_____
e-mail (in block capitals)

Receiving reminders
If you will be part of a study group, please provide your telephone number to receive reminders about the product:

_____
phone number

To receive reminders, please tick your preferred app (you can tick more than one app):

☐ WhatsApp ☐ Telegram ☐ Text message

Researcher's name, signature:____*Svetlana Aleksejeva*_____ _____
Name, surname

____.____._____
Date (dd.mm.yyyy)

Contact details in case of questions or queries:
Svetlana Aleksejeva,s.aleksejeva@gmail.com , tel. 29422183

By signing below, you confirm that you have read and understood the above information and agree to participate in the study to assess the functional properties of buttermilk.

Name, surname, and signature of the participant:

_____
Name, surname

____.____._____
Date (dd.mm.yyyy)

Researcher's name, signature:____*Svetlana Aleksejeva*_____
Name, surname

____.____._____
Date (dd.mm.yyyy)

This part of the consent form (page 5) should be handed over to the researcher.

4

Contact details in case of questions or queries:
Svetlana Aleksejeva,s.aleksejeva@gmail.com , tel. 29422183

Baseline questionnaire / *Pamata anketa*

THE QUESTIONNAIRE

Participant ID: _____

I. General information

1.1. Age: _____ (whole years)

1.2. Weight: _____ (kg) **1.3. Height:** _____ (cm)

1. 4. Waist circumference: _____ (cm) **1.5. Hip circumference:** _____ (cm)

1.6. Your region of residence:

☐ Riga ☐ Pierīga ☐ Zemgale

☐ Vidzeme ☐ Kurzeme ☐ Latgale

1.7. Your educational qualifications (indicate the highest level attained):

☐ basic education ☐ general secondary ☐ secondary special

☐ incomplete higher education ☐ highest

1.8. Your occupation:

☐ full-time paid employee ☐ part-time paid employee ☐ self-employed

☐ unemployed ☐ housewife

1.9. Your marital status:

☐ unmarried ☐ married ☐ divorced

☐ in a civil marriage ☐ widow

1.10. Children under the age of 18 living in the family:

☐ no children ☐ one ☐ two

☐ three ☐ four or more

II. Lifestyle and health

2.1. In the last 6 months, have you taken any medication or supplements?

☐ no ☐ yes

2.1.1. If you answered NO to question 2.1, please go to question 2.2; if YES, please specify which:

☐ for digestive disorders ☐ antibiotics ☐ against insomnia

☐ against pain, fever, inflammation ☐ against convulsions ☐ hormonal medications

☐ against high blood pressure ☐ hormonal contraception ☐ hyperlipidaemic drugs

☐ medications to lower blood glucose levels ☐ vitamins, food supplements

☐ other: _____

2.1.2. Please indicate the names of taken medications:

_____

2.2. Do you usually do light physical activity every day?
For example, light walking, moving around without a car, climbing stairs, and house cleaning.

☐ yes ☐ no ☐ more often yes ☐ more often no

2.3. How many minutes a week do you usually do moderate-intensity physical activity?
For example, brisk walking, swimming, cycling, gardening

☐ up to 150 minutes ☐ 150-300 minutes ☐ more than 300 minutes

2.4. How many minutes per week do you usually do high-intensity aerobic exercise?
For example, jogging, hard gardening (shovelling snow, digging up the garden), hiking in mountainous terrain

☐ up to 75 minutes ☐ 75-150 minutes ☐ more than 150 minutes

2.5. How many minutes per week do you usually do muscle-strengthening exercises for the main muscle groups?
For example, lifting weights, resistance exercises (pull-ups, push-ups, squats), and yoga.

☐ up to 2 times a week ☐ 2 times a week ☐ more than 2 times a week

2.6. Do you smoke?
Cigarettes, electronic cigarettes (number of cigarettes per day corresponds to the number of times per day you smoke)

no, I've never smoked
I don't smoke now, but I used to
yes, but not every day
yes, daily 1-5 cigarettes per day
yes, at least 6-10 cigarettes every day
yes, between 11 and one pack per day
yes, more than one pack per day

II. Eating habits

3.1. What is your average daily number of meals (excluding snacks)?

less than 2 2-3 4-5 more than 5

3.2. What is your average number of snacks per day?

no 1 2 3 more than 4

3.3. Please indicate whether you have made any of the following changes to what you eat or drink in the last 6 months.

I haven't changed anything smaller portions larger portions less fruit and vegetables
more fruit and vegetables less meat more meat less sugar
more sugar less alcohol more alcohol less drinking water
more drinking water less salt more salt less fish
more fish less sour milk products more sour milk products
intermittent fasting

3.4. Do you exclude any foods from your daily diet (for health or other reasons):

no fish seafood olas
cow's milk lactose vegetables fruits, berries
dried fruits potatoes nuts, seeds sour milk products
cereals and their products gluten (wheat, barley, rye) poultry meat (chicken, turkey, duck, goose)

☐ animal meat (cattle, pigs, sheep, goats, rabbits)
☐ other: _____

3.5. How often do you drink alcoholic beverages?

☐ never ☐ once a month or less ☐ 2-4 times a month

☐ once per week ☐ 2-3 times a week ☐ 4 times a week and more

3.5.1. If you answered NEVER to question 3.5, please go to question 4.1; if YES, please specify:
How much alcohol do you tend to consume on a typical drinking occasion?
One serving of alcohol:
beer 500ml - 1.6 servings; cocktail, cider 300ml - 1 serving; wine 150ml (glass) - 1.2 servings; spirits 200ml - 5 servings

☐ 1 or 2 servings ☐ 3 or 4 servings ☐ 5 or 6 servings

☐ 7 or 8 servings ☐ 10 servings or more

III. History of diseases

4.1 Have you ever been diagnosed with any of the following diseases?

Disease	No	Yes
Hypertension (high blood pressure)		
Diseases of the digestive system (stomach ulcers, gastritis, gastrooesophageal reflux disease, irritable bowel syndrome)		
Other chronic diseases (if YES, please specify which)		

Other chronic diseases:

_____

_____

_____

24-hour dietary recall form / *24 stundu atcerēšanās anketa*

24-HOUR RECALL QUESTIONNAIRE FOR ONE DAY

What to write down?

- All foods and drinks that are eaten or drunk.
- Be as specific as possible about the type of food, e.g. coarse bread, bran bread.
- For ready-to-eat products, give the name and manufacturer where possible
- Indicate whether seeds, bran, or grains have been added to bread.
- Indicate whether porridge is quick-cooking or wholemeal. Also, indicate whether it is cooked in milk or water and whether butter, sugar, or jam is added.
- Don't forget to mention nuts and dried fruit.
- For dairy products, indicate the % fat content, e.g. 2.5% milk, 12% cream; for cheese, indicate the name, e.g. Dutch.
- For soups, indicate the meat used and the main ingredients, e.g. pea soup with chicken ham and potatoes, and whether and how much cream is added.
- For meat or fish dishes, indicate the type of meat or fish, the type of minced meat product, and the fat in which the dish is cooked (e.g., chicken fillet fried in sunflower oil, boiled tongue with mayonnaise). If the meat is fried with flour, this should also be recorded.
- For flour and cottage cheese dishes, specify ingredients if possible (e.g. crepes - wheat flour, milk, egg.
- Indicate the ingredients and type of fat used in salads (e.g., tomato salad with olive oil).
- Indicate all beverages, whether sugar, honey, or milk, are added to tea and coffee and how much is added.
- Please also indicate the type and amount of alcohol consumed.

How do I specify the portion size? It is most accurate to use a kitchen scale and weigh the products. If scales are unavailable, then commonly used units such as teaspoons, dessert spoons, tablespoons, and cups can be used. It is difficult to determine the weight of food outside the home, but it can be ascertained by looking at the menu or the description or by measuring 'by eye'. The 'Photo Atlas of Food and Food Portions' can also be used (*to be used only during the study*).

Example:

Meal /time	**Food /ingredients /preparation /**	**Manufacturer of the product (if known)**	**Quantity**
Breakfast 07:30 *Time*	Cooked oat porridge with banana: ● Oatmeal, wholemeal ● Milk 2,5% ● Water ● Banana ● oil/butter/boil Black coffee (ground coffee beans) sugar/milk/honey etc.	 ● Herkules ● Tukuma piens ● tap ● unknown ● Tukuma piens ● Merrild	 ● 4 Tablespoons ● ½ glass ● ½ glass ● one medium-sized ● 5 g ● 150ml

Annex VI / *Pielikums VI*

Table A6.1 / *A6.1. tabula*

Food categories and examples of products included in FFQ / *FFQ kategorijas un pārtikas patēriņa biežuma aptaujā iekļauto produktu piemēri*

Category / *Kategorija*	Examples of products / *Produktu piemēri*
Grains and grain-based products / *Graudaugi un graudaugu produkti*	Pasta, rice, buckwheat, breakfast porridges, barley, pearl barley, millet, amaranth, quinoa, sweetened breakfast cereals, and muesli / *Makaroni, rīsi, griķi, brokastu putras, mieži, grūbas, prosa, amarants, kvinoja, saldinātas brokastu pārslas un musli*
Bread / *Maize*	Rye bread, white bread, seed bread, crispbread, and rusks / *Rudzu maize, baltmaize, sēklu maize, galetes un sausiņi*
Potatoes / *Kartupeļi*	Boiled potatoes, fried potatoes, French fries, and potato pancakes / *Vārīti kartupeļi, cepti kartupeļi, frī kartupeļi un kartupeļu pankūkas*
Meat and poultry / *Gaļa un mājputnu gaļa*	Pork, beef, veal, lamb, mutton, game meat (venison, wild boar, and elk), rabbit meat, and poultry / *Cūkgaļa, liellopu gaļa, teļa gaļa, jēra gaļa, aitas gaļa, medījumu gaļa (brieža, mežacūkas un aļņa gaļa), truša gaļa un mājputnu gaļa*
Meat offal and bacon / *Gaļas subprodukti un bekons*	Liver, tongue, lungs, heart, stomach, salted bacon, and smoked bacon / *Aknas, mēle, plaušas, sirds, kuņģis, sālīts bekons un kūpināts bekons*
Processed meat products / *Gaļas izstrādājumi*	Canned meat, sausages, frankfurters, jamón or prosciutto, and smoked meat / *Gaļas konservi, desas, cīsiņi, hamons vai prošuto un kūpināta gaļa*
Eggs / *Olas*	Hen eggs and quail eggs / *Vistu olas un paipalu olas*
Fish / *Zivis*	Freshwater fish and marine fish / *Saldūdens zivis un jūras zivis*
Fish and seafood products / *Zivju un jūras produktu izstrādājumi*	Sprats, fish roe, fish fingers, crab sticks, seafood, and cod liver / *Šprotes, zivju ikri, zivju pirkstiņi, krabju nūjiņas, jūras produkti un mencu aknas*

Table A6.1 continuation / *A6.1. tabulas turpinājums*

Category / *Kategorija*	Examples of products / *Produktu piemēri*
Milk and dairy products / *Piens un piena produkti*	Milk, yoghurt, kefir, buttermilk, ayran, sour cream, curd, cheese, and other dairy products / *Piens, jogurts, kefīrs, paniņas, airāns, skābais krējums, biezpiens, siers un citi piena produkti*
Vegetable oils, plant- and animal-based fats, and dressings / *Augu eļļas, augu un dzīvnieku izcelsmes taukvielas un mērces*	Margarine, butter, olive oil, vegetable oils, seed oils, soy sauce, spicy sauces, white salad dressings, mayonnaise, tomato sauces, ketchup, and similar products / *Margarīns, sviests, olīveļļa, augu eļļas, sēklu eļļas, sojas mērce, asās mērces, baltās salātu mērces, majonēze, tomātu mērces, kečups un līdzīgi produkti*
Plant-based meat substitutes / *Augu izcelsmes gaļas aizstājēji*	Tofu, tempeh, and soy meat / *Tofu, tempehs un sojas gaļas aizstājēji*
Plant-based dairy substitutes / *Augu izcelsmes piena produktu aizstājēji*	Plant-based milk alternatives and plant-based cheese alternatives / *Augu izcelsmes piena alternatīvas un augu izcelsmes siera alternatīvas*
Vegetables / *Dārzeņi*	Fresh, cooked, fermented, and canned vegetables / *Svaigi, termiski apstrādāti, fermentēti un konservēti dārzeņi*
Fruits and berries / *Augļi un ogas*	Fresh, canned, and dried fruits and berries / *Svaigi, konservēti un žāvēti augļi un ogas*
Mushrooms / *Sēnes*	Wild and cultivated edible mushrooms / *Savvaļas un kultivētās ēdamās sēnes*
Legumes / *Pākšaugi*	Green beans, peas, lentils, and chickpeas / *Zaļās pupiņas, zirņi, lēcas un turku zirņi*
Nuts and seeds / *Rieksti un sēklas*	Walnuts, hazelnuts, peanuts, almonds, cashews, nut or seed butter or paste, flaxseeds, chia seeds, sesame seeds, hemp seeds, sunflower seeds, pumpkin seeds, and similar products / *Valrieksti, lazdu rieksti, zemesrieksti, mandeles, Indijas rieksti, riekstu vai sēklu sviests vai pasta, linsēklas, čia sēklas, sezama sēklas, kaņepju sēklas, saulespuķu sēklas, ķirbju sēklas un līdzīgi produkti*

Table A6.1 continuation / *A6.1. tabulas turpinājums*

Category / *Kategorija*	Examples of products / *Produktu piemēri*
Sweets / *Saldumi*	Biscuits, pastries, muffins, waffles, cakes, chocolate, halva, kozinaki, sherbet, cereal bars, confectionery, marshmallows, marmalade, fruit jelly, sorbet, ice cream, sweet popcorn, corn puffs, and breadsticks / *Cepumi, konditorejas izstrādājumi, mafini, vafeles, kūkas, šokolāde, halva, kozinaki, šerbets, graudaugu batoniņi, konfektes, zefīrs, marmelāde, augļu želeja, sorbets, saldējums, saldais popkorns, kukurūzas nūjiņas un maizes standziņas*
Sugars and related foods / *Cukurs un saistītie pārtikas produkti*	Jam, preserves, chocolate spread, sugar substitutes, sugar, syrups (agave, maple, and similar syrups), and honey / *Ievārījums, konservēti augļu produkti, šokolādes krēms, cukura aizstājēji, cukurs, sīrupi (agaves, kļavu un līdzīgi sīrupi) un medus*
Fast food and salty snacks / *Ātrās uzkodas un sāļās uzkodas*	Dumplings, meat pie, vegetarian pie, sausage roll, hot dog, cheburek, pizza, burger, tortilla, kebab, salted popcorn, crackers, and crisps / *Pelmeņi, gaļas pīrāgs, veģetārais pīrāgs, desiņa mīklā, hotdogs, čebureks, pica, burgers, tortilja, kebabs, sāļais popkorns, krekeri un čipsi*
Lemonades and energy drinks / *Limonādes un enerģijas dzērieni*	Lemonades, kvass, malt beverages, and energy drinks / *Limonādes, kvass, iesala dzērieni un enerģijas dzērieni*
Juices / *Sulas*	100 % fruit juice, fruit juice drinks, fruit nectars, and vegetable and root vegetable juices / *100 % augļu sula, augļu sulas dzērieni, augļu nektāri, dārzeņu un sakņu dārzeņu sulas*
Alcoholic beverages / *Alkoholiskie dzērieni*	Wine, sparkling wine or Prosecco, beer, sweetened alcoholic beverages, alcoholic beverages without added sugar, and non-alcoholic alternatives (wine, sparkling wine, vermouth, gin, and similar beverages) / *Vīns, dzirkstošais vīns vai Prosecco, alus, saldināti alkoholiskie dzērieni, alkoholiskie dzērieni bez pievienota cukura un bezalkoholiskās alternatīvas (vīns, dzirkstošais vīns, vermuts, džins un līdzīgi dzērieni)*

Table A6.1 continuation / *A6.1. tabulas turpinājums*

Category / *Kategorija*	Examples of products / *Produktu piemēri*
Coffee and tea / *Kafija un tēja*	Coffee, cocoa, chocolate drinks, instant 3-in-1 coffee drinks, latte, cappuccino, black or green tea, and herbal tea / *Kafija, kakao, šokolādes dzērieni, šķīstošie kafijas dzērieni "3 vienā", latte, kapučīno, melnā vai zaļā tēja un zāļu tēja*
Water / *Ūdens*	Still water and flavoured carbonated mineral water / *Negāzēts ūdens un aromatizēts gāzēts minerālūdens*
Dietary supplements / *Uztura bagātinātāji*	B-group vitamins, omega-3 fatty acids, iron, magnesium, phospholipids, calcium, vitamin D, vitamin C, probiotics, fibre supplements, meal replacements, and similar products / *B grupas vitamīni, omega-3 taukskābes, dzelzs, magnijs, fosfolipīdi, kalcijs, D vitamīns, C vitamīns, probiotikas, šķiedrvielu uztura bagātinātāji, ēdienreižu aizstājēji un līdzīgi produkti*

Note. The categories follow the grouping used to calculate food-group intake in this study. / *Piezīme. Kategorijas atbilst šajā pētījumā izmantotajam pārtikas produktu grupējumam, pēc kura aprēķināts katras grupas patēriņš*

Table A7.1 / *A7.1. tabula*

Estimated average daily intake of food products and beverages, mean ± SD / *Aprēķinātais vidējais pārtikas produktu un dzērienu patēriņš dienā, vidējā vērtība ± SD*

Food products or beverages / *Pārtikas produkti vai dzērieni*	Total (n = 61), g day^{-1} / *Kopā (n = 61), g diena^{-1}*	Intervention group (n = 31), g day^{-1} / *Intervences grupa (n = 31), g diena^{-1}*	Control group (n = 30), g day^{-1} / *Kontroles grupa (n = 30), g diena^{-1}*	p-value / *p-vērtība*
Grains, grain-based products / *Graudaugi un graudaugu produkti*	74.55 ± 44.84	67.63 ± 41.39	81.71 ± 47.80	0.276
Bread / *Maize*	68.69 ± 48.64	58.69 ± 40.75	79.02 ± 54.40	0.147
Potatoes / *Kartupeļi*	44.86 ± 45.89	50.34 ± 58.54	39.20 ± 27.30	0.778
Meat, poultry / *Gaļa un mājputnu gaļa*	89.45 ± 70.94	89.88 ± 62.93	89.00 ± 79.47	0.569
Meat offal / *Gaļas subprodukti*	5.46 ± 5.61	6.46 ± 6.48	4.43 ± 4.42	0.233
Meat products / *Gaļas izstrādājumi*	14.99 ± 12.32	17.78 ± 13.94	12.10 ± 9.80	0.139
Eggs / *Olas*	51.41 ± 53.20	38.11 ± 29.26	65.16 ± 67.71	0.101
Fish / *Zivis*	38.67 ± 32.72	35.82 ± 25.84	41.61 ± 38.82	0.988
Fish products / *Zivju izstrādājumi*	6.23 ± 8.31	6.79 ± 10.40	5.65 ± 5.51	0.629
Milk, milk products / *Piens un piena produkti*	305.71 ± 185.57	298.56 ± 203.34	313.10 ± 168.40	0.282
Vegetable oils, plant- and animal-based fats, dressings / *Augu eļļas, augu un dzīvnieku valsts izcelsmes tauki un mērces*	27.43 ± 18.95	29.20 ± 22.14	25.60 ± 15.14	0.692
Substitutes for meat products, plant-based / *Augu valsts izcelsmes gaļas produktu aizstājēji*	1.42 ± 5.87	0.42 ± 1.71	2.45 ± 8.13	0.256

Table A7.1 continuation / *A7.1. tabulas turpinājums*

Food products or beverages / *Pārtikas produkti vai dzērieni*	Total (n = 61), g day^{-1} / *Kopā (n = 61), g diena^{-1}*	Intervention group (n = 31), g day^{-1} / *Intervences grupa (n = 31), g diena^{-1}*	Control group (n = 30), g day^{-1} / *Kontroles grupa (n = 30), g diena^{-1}*	p-value / *p-vērtība*
Dairy substitutes, plant-based / *Augu valsts izcelsmes piena produktu aizstājēji*	52.23 ± 140.18	37.01 ± 126.45	67.95 ± 153.67	0.187
Vegetables / *Dārzeņi*	203.62 ± 124.64	201.73 ± 103.53	205.57 ± 145.06	0.493
Fruits, berries / *Augļi un ogas*	232.85 ± 150.14	199.54 ± 98.96	267.27 ± 184.65	0.182
Mushrooms / *Sēnes*	4.93 ± 4.89	4.90 ± 4.75	4.97 ± 5.12	1.000
Legumes / *Pākšaugi*	17.80 ± 22.26	16.96 ± 14.57	18.68 ± 28.36	0.582
Nuts, seeds / *Rieksti un sēklas*	20.54 ± 17.04	19.14 ± 14.34	21.99 ± 19.58	0.880
Sweets / *Saldumi*	46.48 ± 36.79	38.65 ± 27.47	54.58 ± 43.42	0.108
Sugars and related foods / *Cukurs un saldvielas*	6.95 ± 7.93	5.53 ± 6.21	8.42 ± 9.25	0.197
Fast food, salty snacks / *Ātrās uzkodas un sāļās uzkodas*	29.00 ± 20.23	30.67 ± 24.32	27.28 ± 15.12	0.880
Soft drinks, energy drinks / *Limonādes un enerģijas dzērieni*	17.46 ± 23.50	20.17 ± 30.32	14.66 ± 13.23	0.686
Juices / *Sulas*	39.88 ± 47.18	33.89 ± 35.93	46.07 ± 56.50	0.273
Alcoholic beverages / *Alkoholiskie dzērieni*	45.04 ± 55.22	44.31 ± 53.77	45.79 ± 57.59	0.708
Coffee, tea / *Kafija un tēja*	741.57 ± 293.38	725.10 ± 331.63	758.58 ± 252.44	0.658
Water / *Ūdens*	1187.20±411.03	1195.58±418.35	1178.54±410.30	0.745

Note. Food categories are defined in Annex VI; corresponding medians and quartiles are reported in Table 3.13. / *Piezīme. Pārtikas produktu kategorijas definētas VI pielikumā; atbilstošās mediānas un kvartiles sniegtas 3.13. tabulā.*