Serum CathepsinD in pregnancy: Relation with metabolic and inflammatory markers and effects of fish oils and probiotics

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Abstract *Background and aims:* Elevated circulating levels of CathepsinD (CatD) have been linked to metabolic deviations including liver inflammation. We investigated 1) whether supplementation with probiotics and/or fish oil affects CatD and 2) whether the CatD concentration would associate with gestational diabetes (GDM), low-grade inflammation, lipid metabolism, body fat % and dietary composition.

Methods and results: Overweight/obese pregnant women (n = 438) were randomized into fish oil + placebo, probiotics + placebo, fish oil + probiotics or placebo + placebo groups. Fish oil contained 1.9 g docosahexaenoic acid and 0.22 g eicosapentaenoic acid and probiotics were *Lacticaseibacillus rhamnosus* HN001 (formerly *Lactobacillus rhamnosus* HN001) and *Bifidobacterium animalis* ssp. *lactis* 420, 10^{10} colony-forming units each). Serum CatD levels were analysed by ELISA, GlycA and lipid metabolites by NMR, high sensitive C-reactive protein (hsCRP) by immunoassay, and intakes of energy yielding nutrients and n-3 and n-6 fatty acids from food diaries at both early and late pregnancy. GDM was diagnosed by OGTT. CatD concentrations did not differ between the intervention groups or by GDM status. Multivariable linear models revealed that body fat % and GlycA affected CatD differently in healthy women and those with GDM.

Conclusion: The serum CatD concentration of pregnant women was not modified by this dietary intervention. Serum CatD was influenced by two parameters, body fat and low grade inflammation, which were dependent on the woman's GDM status.

1. Introduction

Overweight and obesity during pregnancy predispose the mother to metabolic disturbances which might translate into gestational diabetes (GDM). Regarding the long-term consequences, women with GDM have a seven-fold risk for developing type 2 diabetes [1] and a more than doubled risk for suffering from nonalcoholic fatty liver disease (NAFLD) after pregnancy [2]. NAFLD is a spectrum of hepatic conditions ranging from nonalcoholic fatty liver (NAFL) to nonalcoholic steatohepatitis (NASH), which may further progress to liver fibrosis, cirrhosis and hepatocellular carcinoma.

Recently, serum cathepsinD (CatD) concentrations have been proposed as a new marker for hepatic inflammation [3]. In biopsy proven patients with NASH, increased circulating CatD concentrations were observed, and further CatD levels correlated directly with serum ALT concentrations, the commonly used serum marker for steatohepatitis [4]. CatD belongs to the cathepsin family; it is an aspartate peptidase having a role in protein turnover but it may also be involved in liver fibrogenesis [5]. CatD may also have a specific role in pregnancy; in animal studies, CatD has been shown to participate in the placentation process [6,7]. In humans, CatD is expressed in placenta [8] and reduced circulating CatD concentrations have been found in patients with preeclampsia [9]. So far, only one study has investigated CatD concentrations during pregnancy in humans, demonstrating an increase in its concentration from the first to the third trimester in healthy pregnant women [10]. These authors speculated that this increase might be related to the rising concentration of estrogen throughout the course of the pregnancy. These preliminary findings raise the question of whether there are changes in CatD concentrations in overweight and obese pregnant women as well as in those with GDM as both groups have a higher risk for developing metabolic inflammation and NAFLD.

It is not known whether it is possible to modify CatD concentrations by diet, either in non-pregnant or pregnant conditions, a topic that deserves further study. Intakes of n-3 fatty acids from fish oil have shown benefits on NAFLD by decreasing the levels of serum liver enzymes, such as ALT and GGT as well as on liver fat and steatosis scores in patients with NAFLD [11]. Moreover, the intake of probiotics conferred benefits on ALT, AST and GGT in NAFLD patients [12] and decreased AST, GGT and the fatty liver index, as analysed by Shear Wave Elastography, in type 2 diabetes patients with NAFLD [13]. Interestingly, in type 2 diabetic patients with NAFLD, a combination of probiotics and n-3 fatty acids from flax and wheat germ (250 mg of each) for 8 weeks decreased the fatty liver index when compared to placebo [14]. The effects on CatD concentrations of consumption of fish oil and probiotics, either separately or in combination, have not been studied so far.

We have previously shown that women with GDM display a distinct serum metabolomic profile and higher concentrations of GlycA, a novel marker for low-grade inflammation, when compared to women without GDM [15]. This may have consequences on how these women with the higher metabolic burden respond to dietary treatments. Indeed, in our previous study with the same population, the impact of probiotics and fish oil on insulin-like growth factor binding-protein-1 levels, was influenced by their GDM status [16]. Based on these findings, we hypothesized that the GDM status could also modify the potential impact of diet and dietary supplements on the hepatic manifestations related to the observed metabolic disturbances, as evaluated via changes in CatD concentrations.

Therefore, the aim was to examine 1) whether supplementation with probiotics and fish oil separately or in combination, would exert any effect on CatD concentrations during pregnancy and 2) whether serum CatD concentrations associate with GDM, low-grade inflammation, lipid metabolism, body fat % and dietary composition.

2. Methods

2.1. Research design and methods

This study is a further analysis from the data of a double-blind, placebo-controlled randomized trial evaluating the effects of fish oil and/or probiotic dietary supplements on maternal and child health, specifically maternal glycaemic control and allergy in the child. The women were recruited to participate in the trial in the Turku University Hospital and University of Turku in Finland between October 2013 and July 2017 (ClinicalTrials.gov, NCT01922791). The Ethics Committee of the Hospital District of Southwest Finland approved the study protocol and all participants provided written informed consents before the start of the study. A total of 439 pregnant women from southwest Finland were included in the clinical trial with the following inclusion criteria: self-reported pre-pregnancy BMI ≥ 25 kg/m^2 and the absence of other chronic diseases, but allergy or asthma were allowed. One participant with familial hypercholesterolemia was excluded from the analyses. The study has been described in more detail previously [17].

The characteristics of the women, i.e. family history of diabetes, education and smoking habits were asked by questionnaires filled in during the study visits. Prepregnancy BMI was calculated by dividing self-reported weight in kilograms, obtained from women's welfare clinic records, by height measured with a wall stadiometer to the nearest 0.1 cm in early pregnancy.

Before the start of the intervention, the women were randomized into four groups: fish oil + placebo (i.e., placebo for probiotics), probiotics + placebo (i.e., placebo for fish oil), fish oil + probiotics, or placebo + placebo (placebo for probiotics and placebo for fish oil). Probiotic supplements contained *Lacticaseibacillus rhamnosus* HN001 (formerly *Lactobacillus rhamnosus* HN001) (ATCC SD5675; DuPont, Niebüll, Germany) and *Bifidobacterium animalis* ssp. Lactis 420 (DSM 22089; DuPont), 10^{10} colony-forming units per capsule. Fish oil supplement capsules (Croda Europe Ltd., Leek, U.K) consisted 2.4 g of n-3 fatty acids, 1.9 g docosahexaenoic acid (22:6 n-3) DHA and 0.22 g eicosapentaenoic acid (20:5 n-3) EPA and 0.28 g other n-3 fatty acids, such as docosapentaenoic acid (DPA). The placebo capsules for probiotics contained microcrystalline cellulose and the placebo capsules for the fish oil contained medium-chain fatty acids (capric acid C8 54.6%, caprylic acid C10 40.3%). Compliance with the consumption of capsules was assessed by a phone call at mean 28 weeks of gestation, by interview at the late pregnancy study visit, and by counting the numbers of consumed fish oil capsules, i.e., subtracting the capsules returned to the study unit from the total provided by a random sample of 62 women (14% of participants). Good compliance (taking study capsules ≥5 days/week) was reported by 88.4% of the women (NS between the four intervention groups). The compliance calculated from the returned fish oil capsules indicated that a mean of 91.8% (SD 15.9) of the capsules had been consumed [17]. Also, lipid metabolites reflecting the intake of fish oil (n-3 fatty acids) were clearly separated according to the intervention groups in principal component analysis [18].

2.2. Blood sampling and analyses

Blood samples were drawn from the antecubital vein after a minimum of 10-h' fasting at baseline (early pregnancy visit; mean 13.8 (SD 2.1) weeks of gestation) and at the late pregnancy visit (35.2 (0.9)) (Table 1). Serum was prepared within 30 min after the blood was collected. Freshly prepared serum was used to analyze a recognized marker of low-grade inflammation, hsCRP, by an automated colorimetric immunoassay on a Dade Behring Dimension RXL autoanalyzer (Siemens Healthcare, Camberly, Surrey, UK) in an accredited Turku University Hospital Laboratory according to their quality control system. The rest of the serum samples were frozen in aliquots at −80 °C until analysed for CatD, another marker of low-grade inflammation, GlycA, and lipidomics. Serum CatD concentrations were determined by an enzyme-linked immunosorbent assay (USCN Life Science, Wuhan, China). Baseline and late pregnancy samples from one subject were always analysed on the same plate. GlycA is a composite nuclear magnetic resonance (NMR) biomarker of systemic inflammation. GlycA contains N-acetyl sugar groups originating from multiple acute phase circulating glycoproteins: α1-acid glycoprotein, haptoglobin, α1-antitrypsin, α1-antichymotrypsin and transferrin [19]. GlycA was quantified from serum samples using a commercial high-throughput proton NMR, metabolomics platform (Brainshake Ltd, Helsinki, Finland) as previously described [20]. The lipidomics were analysed using the same NMR-metabolomics platform and serum cholesterol, VLDL-cholesterol, LDL-cholesterol, HDL-cholesterol as well as serum triglycerides (TG), VLDL-TG, LDL-TG and HDL-TG were correlated with CatD.

2.3. Clinical measures, dietary intake and physical activity

GDM was diagnosed on the basis of a 2-h 75-g oral glucose tolerance test (OGTT) if one or more values were at or above the threshold level: 0 h ≥ 5.3, 1 h ≥ 10.0, and 2 h ≥ 8.6 mmol/L, according to the Finnish Current Care Guidelines. An OGTT was offered by maternal welfare clinics to all women between 24 and 28 weeks of gestation and to high-risk women also at 12−16 weeks of gestation (BMI ≥35 kg/m², previous GDM, glucosuria, polycystic ovarian syndrome, or a family risk of diabetes). GDM was diagnosed in 119 women (30%), while 278 women (70%) did not develop GDM (results from 41 women not available due to drop-out or their refusal to undergo the OGTT; they were excluded when analyzing the impact of GDM status on CatD concentrations).

The proportion of body fat was measured by air displacement plethysmography (the Bod Pod system, COSMED, Inc., Concord, CA, USA).

Participants recorded 3-day food diaries in the week before the study visit according to given instructions. Mean daily intakes of energy and energy yielding nutrients (i.e. fat, SFA, MUFA, PUFA, n-3 PUFA, n-6 PUFA, carbohydrates and protein as a proportion of energy intake), were calculated using computerized software, Aivo diet 2.0.2.3 (Aivo, Turku, Finland), which uses the Food and Nutrient Database of the National Institute for Health and Welfare (www.fineli.fi).

The intensity, frequency and duration of the habitual leisure-time physical activity during the preceding week were asked in the questionnaire. The metabolic equivalent index for leisure-time physical activity (MET-index) was calculated from the product of intensity x frequency x duration of activity (MET h/wk) as described earlier [21].

2.4. Statistical analyses

There were no a priori data for the effects of probiotics or fish oil on serum CatD concentrations during pregnancy when the clinical trial was initiated, nor information on changes in CatD concentrations that would be clinically

Table 1 Clinical characteristics and dietary intake of all the pregnant women and those in the four intervention groups (n = 438).

	All	Fish oil + Placebo (n = 110)	Probiotics + Placebo (n = 109)	Fish oil + Probiotics (n = 109)	Placebo + Placebo (n = 110)	p-value
Age, years	30.6 ± 4.6	30.4 ± 4.8	30.8 ± 4.8	30.8 ± 4.6	30.4 ± 4.1	0.820*
Prepregnancy BMI, kg/m^2	29.7 ± 4.2	30.0 ± 4.2	29.9 ± 4.7	29.3 ± 3.9	29.7 ± 4.2	0.594*
Overweight, % (n)	60.7% (266/438)	62 (56.4)	70 (64.2)	68 (62.4)	66 (60.0)	0.663†
Obese, % (n)	39.3% (172/438)	48 (43.6)	39 (35.8)	41 (37.6)	44 (40.0)	0.663†
Primipara, % (n)	47.9% (210/438)	53 (48.2)	52 (47.7)	52 (47.7)	53 (48.2)	1.000†
Diagnosed with GDM, % (n)¶	30.0% (119/397)	31 (30.4)	32 (31.1)	30 (31.3)	26 (27.1)	0.914†
Smoked before pregnancy, % (n) ≠	21.9% (86/393)	17 (17.0)	27 (28.1)	14 (14.1)	28 (28.6)	0.022‡
Smoked during pregnancy, % (n) #	4.9% (19/391)	2 (2.0)	6 (6.3)	5 (5.1)	6 (6.1)	0.437‡
Higher education (college or university), % (n) §	61.1 (239/391)	66 (66.0)	59 (62.8)	56 (56.6)	58 (59.2)	0.546†
MET-index (h/wk, early pregnancy)&	4.8 [9.0]	4.8 [10.0]	7.5 [9.5]	4.8 [9.8]	7.5 [9.0]	0.524**
Diet (% of energy) $						
Fat	35.4 ± 6.6	35.5 ± 6.8	35.2 ± 6.8	34.8 ± 6.5	35.8 ± 6.6	0.693*
PUFA	5.6 ± 1.6	5.8 ± 1.9	5.6 ± 1.6	5.6 ± 1.4	5.6 ± 1.4	0.629
N-3 fatty acids	1.6 ± 0.4	1.7 ± 0.5	1.6 ± 0.4	1.6 ± 0.5	1.6 ± 0.4	0.599*
N-6 fatty acids	4.4 ± 1.3	4.5 ± 1.5	4.3 ± 1.2	4.3 ± 1.1	4.3 ± 1.1	0.559*
MUFA	12.2 ± 2.8	12.3 ± 2.8	12.1 ± 2.7	11.9 ± 2.7	12.5 ± 2.9	0.368*
SFA	12.9 ± 3.1	12.8 ± 3.3.	12.9 ± 3.2	12.7 ± 3.0	13.1 ± 3.1	0.758*
Protein	16.7 ± 3.4	16.5 ± 4.0	16.5 ± 3.3	16.9 ± 3.0	16.8 ± 3.3	0.834*
Carbohydrates	45.5 ± 6	45.5 ± 6.8	45.7 ± 7.0	45.8 ± 6.7	44.9 ± 5.6	0.761*

Results are expressed as mean ± SD, median [IQR] or in numbers and (in percentages). *One-way ANOVA. †x2 test. ‡Fisher exact test. **Kruskal-Wallis test. ˙N = 102 in fish oil + placebo, 103 in probiotics + placebo and 96 in fish oil + probiotics and placebo + placebo groups, ≠ N = 100 in fish oil + placebo, 96 in probiotics + placebo, 99 in fish oil + probiotics and 98 placebo + placebo groups, #N = 100 in fish oil + placebo, 95 in probiotics + placebo and 98 in fish oil + probiotics and placebo + placebo groups. §N = 100 in fish oil + placebo, 94 in probiotics + placebo and 99 in fish oil + probiotics and 98 in placebo + placebo groups. & N = 108 in fish oil + placebo, 109 in probiotics + placebo and 108 in fish oil + probiotics and 109 in placebo + placebo groups. $ N = 105 in fish oil + placebo, 106 in probiotics + placebo and 106 in fish oil + probiotics and 104 in placebo + placebo groups.

meaningful. Therefore we calculated the retrospective sample size by using effect size. The sample size was estimated to be 100 women per groups to detect an effect size of 0.4 (i.e. mean difference 40, SD 100) on CatD concentrations between groups with 80% power and the two-tailed 5% level of significance. The differences in clinical characteristics and dietary intake between intervention groups were compared with one-way ANOVA, Kruskal-Wallis test, chi-square test or Fisher exact test, as appropriate. The impact of the GDM status and intervention on the CatD concentration was evaluated by 2-way ANOVA, including the main effects of GDM status and intervention, and GDM status × intervention interaction effect. One-way ANOVA with Tukey's HSD post-hoc test was used to compare the differences in variables between tertiles of CatD, GlycA and hsCRP. Paired sample t-test was applied when the changes in CatD levels from early to late pregnancy were analysed. Pearson's correlation was utilized when analyzing the correlation between the CatD concentration and other variables. A multivariable linear model was performed to evaluate whether GlycA and body fat % were independently associated with CatD with the model being adjusted for the dietary intervention. A linear model was utilized to evaluate whether prepregnancy BMI modified the association between early pregnancy CatD or GlycA tertiles and the change in CatD level from early to late pregnancy. Smoking before pregnancy differed between the intervention groups but was not related to CatD levels (p < 0.39), and thus the analyses were not adjusted with the smoking status. Statistical analyses were performed using IBM SPSS Statistics for Window, version 26 (IBM Corp., Amonk, NY). P values less than 0.05 were considered statistically significant, except in correlations analyses where the significance was set at p value less than 0.01. All statistical tests were two-sided.

3. Results

3.1. Characteristics of the study participants

The participating women (n = 438) were overweight (60%) or obese (40%), the majority being well educated and expecting their first child, with no differences in the clinical characteristics between the intervention groups, except in smoking before pregnancy (Table 1). Also, no differences in the dietary intake between the intervention groups, including n-3 PUFA intake, were seen either at early (the baseline) (Table 1) or late pregnancy (data not shown).

3.2. CatD concentrations in relation to intervention and maternal GDM

We evaluated whether the CatD concentrations during pregnancy were affected by the type of dietary

Table 2 The effects of the intervention group and gestational diabetes (GDM) on the CathepsinD (Cat D, ng/mL) concentrations.

	n	Cat D in early pregnancy	p-value*	n	Cat D in late pregnancy	p-value*
Group effect			0.558			0.357
Fish oil + placebo	100	244.7 (14.2)		89	206.9 (15.2)	
Probiotics + placebo	103	238.9 (14.0)		91	186.7 (15.2)	
Fish oil + probiotics	94	216.8 (14.7)		87	207.2 (15.5)	
Placebo + placebo	94	232.5 (15.2)		85	226.6 (16.2)	
GDM effect			0.434			0.692
Women with GDM	118	238.9 (12.2)		99	203.8 (13.2)	
Women without GDM	273	227.5 (8.0)		253	209.9 (8.2)	
GDM × group interaction effect			0.367			0.614

*2-way ANOVA. Values are adjusted means (standard errors) from ANOVA model.

intervention or maternal GDM status (Table 2). The CatD concentrations did not differ among the intervention groups or by GDM status at early or late pregnancy. In addition, the GDM × group interaction was non-significant.

The CatD concentrations decreased from early to late pregnancy in the fish oil group (-37.8 ± 121.5; p = 0.004) and in the probiotic group (-41.5 ± 105.6; p < 0.001), but not in the combined intervention group (-13.4 ± 161.9; P = 0.434) or in the placebo group (-1.4 ± 125.9; p = 0.901).

3.3. CatD concentrations over pregnancy and associations with GDM and maternal characteristics

Overall CatD concentrations decreased from early (232.7 ± 130.7 ng/ml) to late pregnancy (207.6 ± 129.9 ng/ml) i.e. a difference of 23.7 ± 126.1 ng/ml (p < 0.001). Interestingly, the higher the CatD value in early pregnancy, the more it decreased during the pregnancy (Fig. 1, Supplemental Table 1). Nevertheless in 36.7% of the women, the CatD concentration actually increased. The development of CatD concentrations during pregnancy was not influenced by the value of the prepregnancy BMI (CatD × BMI interaction effect, p = 0.895).

Interestingly, during pregnancy, the CatD concentration decreased significantly in those women with GDM (-42.7 ± 135.3 ng/ml; p = 0.002) but not in the women without this condition (-14.4 ± 122.6 ng/ml; p = 0.065). Nonetheless, the proportion of women in whom the CatD concentration declined was similar in both women with GDM (70.4%) and those without GDM (60.1%, p = 0.074, Pearson Chi-square).

When investigating in more detail the relationships between CatD concentrations and maternal characteristics (BMI, body fat percentage, dietary composition, low-grade inflammatory markers, lipid metabolites and MET index), distinct relationships were observed according to the GDM status with regard to body fat % and low grade inflammation marker GlycA. The more traditional low-grade inflammation marker i.e. hsCRP, as well as values of BMI, dietary composition, lipid metabolites and MET-index did not correlate with the CatD concentration (all, p > 0.05). A positive association between early pregnancy CatD and body fat % was observed in the women with GDM (r = 0.264, p = 0.004), but not in those without GDM (r = -0.067, p = 0.27). Positive associations were detected between GlycA and CatD concentrations at early pregnancy in all women (r = 0.162, p = 0.001) and women with GDM (r = 0.242, p = 0.008) but not in healthy women (r = 0.111, p = 0.067).

Multivariable linear models were examined for all women and also separately for women with and without GDM to evaluate whether GlycA and body fat %, i.e. the variables found statistically significant in the correlation analyses, independently affected the CatD concentration and the dietary intervention was included in the models. In the model with all women, GlycA (β = 204.9, 95% CI 78.9 to 330.9, p = 0.001) was associated with CatD concentrations at early pregnancy, but body fat % was not (p = 0.720). In healthy women, the association with CatD was significant for GlycA (β = 187.3, 95% CI 27.9 to 346.8, p = 0.021), but body fat % was not (p = 0.073). In women with GDM, GlycA was not associated (p = 0.125), but body fat % (β = 5.15, 95% CI 0.23 to 10.06, p = 0.040) was associated with CatD. When all the women were tested at late pregnancy, the CatD level was not associated with either GlycA or body fat % (p = 0.331 and p = 0.202, respectively). In healthy women, the CatD level was not associated with that of GlycA (p = 0.789), but was associated with body fat % (β = -3.47, 95% CI -6.68 to -0.27, p = 0.034). In GDM women, the CatD concentration was not associated with either GlycA (p = 0.334) or body fat % (p = 0.194).

3.4. CatD concentrations and inflammation markers

We undertook a more detailed examination of the relationship between CatD concentrations and low-grade inflammation by categorizing the women into tertiles either according to their GlycA concentrations (Supplemental Table 2) or to their hsCRP concentrations at baseline (Supplemental Table 3). A decline in CatD concentrations from early to late pregnancy was evident between the GlycA tertiles (P = 0.009). The smallest decrease in the CatD level during pregnancy was associated with the lowest GlycA tertile and the largest decrease in CatD was associated with the highest GlycA tertile
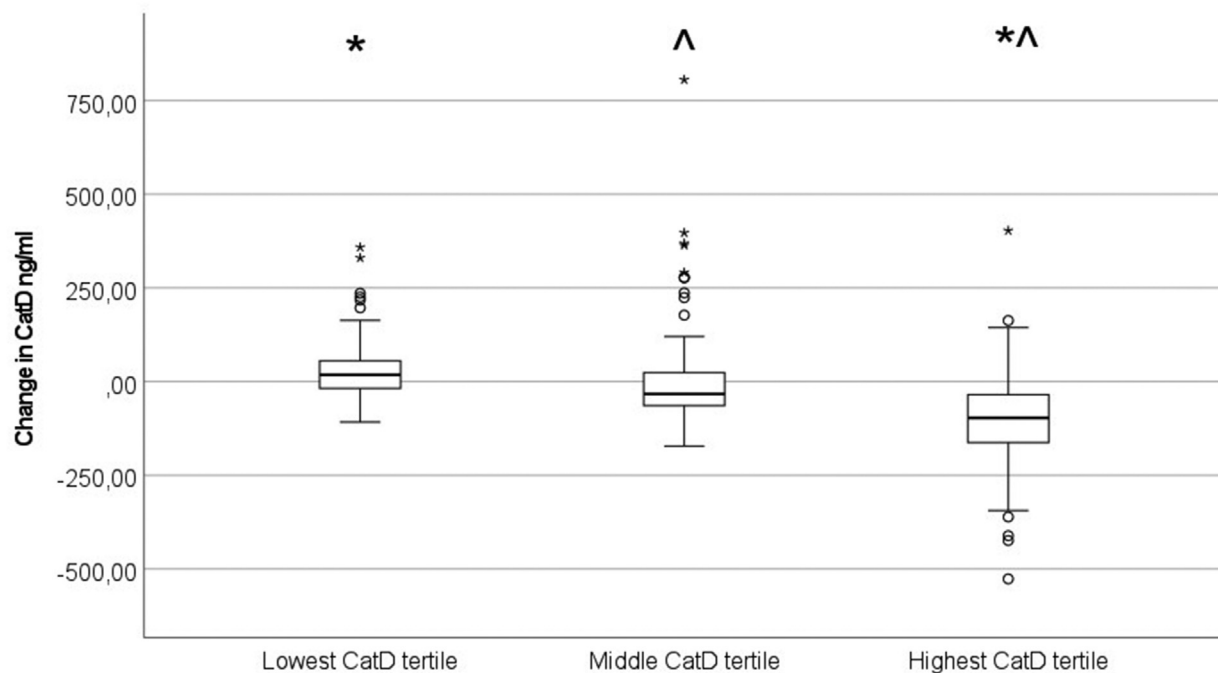
Figure 1 Change in the cathepsinD concentration in the cathepsin tertiles at early pregnancy in all women in the trial. *ˆ denotes a statistically significant difference between the tertiles (1-way ANOVA, Tukey corrected P-values).

(-1.4 ± 136.9 ng/ml versus -51.7 ± 119.6, p = 0.006, bottom vs top tertiles) (Supplemental Table 2). This finding was not modified by inclusion of the prepregnancy BMI value (GlycA × BMI interaction effect, p = 0.921). Associations of hsCRP and GlyA tertiles with CathepsinD were not modified by the intervention groups or by the GDM (all interaction effects, p > 0.05). The impact of early pregnancy GlycA on the differences in the CatD concentration during pregnancy was further confirmed when analyzing the women who had exhibited either a decrease or an increase in their CatD levels; those with a decrease had a higher GlycA value in early pregnancy compared to those with an increase (1.23 ± 0.11 vs 1.18 ± 0.10 mmol/l, P < 0.001). With regard to hsCRP, no statistically significant difference was detected in these changes among the hsCRP tertiles, but in late pregnancy, CatD levels were higher in the lowest hsCRP tertile (228.2 ± 142.1 ng/ml) when compared to the middle tertile (183.5 ± 112.7 ng/ml) (p = 0.021) (Supplemental Table 3). In relation to the declines or elevations of CatD concentrations, higher hsCRP concentrations in early pregnancy were detected in women who displayed a decrease in CatD levels when compared to those in whom the values increased (7.0 ± 5.29 vs 5.88 ± 3.95 mg/l, p = 0.025).

4. Discussion

Serum CatD concentrations decreased over time from early to late pregnancy in this high-risk group of overweight and obese pregnant women. Women who had higher CatD concentrations in early pregnancy, exhibited the largest decrease in their CatD concentrations during pregnancy. Furthermore, also a higher low-grade inflammatory status at baseline was associated with a larger decrease in their CatD concentrations. The dietary intervention exerted no impact on the CatD concentrations.

The observed decrease in serum CatD concentrations from early to late pregnancy is in contrast to an earlier study [10] in which CatD concentrations increased from gestational weeks 11–13 to 36–40. In that previous smaller study (n = 76), the women were apparently healthy (based on the fact that neither prepregnancy BMI nor the possible incidence of GDM was reported), whilst we investigated a group of overweight and obese women at risk for suffering metabolic aberrations, in fact about every fifth woman did indeed develop GDM. Interestingly, those women who had higher early pregnancy CatD and GlycA concentrations displayed a greater decrease in CatD. It is however noteworthy that our study had a dietary intervention and an inspection of the group of healthy women in the placebo + placebo group (n = 62) revealed no change in the CatD concentration over the course of pregnancy.

No differences in the CatD concentration among the four intervention groups were seen, and the intervention effect was not modified by the GDM status of the women. We have also reported earlier that the dietary intervention exerted no impact on the incidence of GDM [17]. Interestingly, a decrease in the CatD level during pregnancy was seen in the probiotic and in the fish oil groups, but not in the combination or placebo groups. It may be that probiotics and fish oil are able to influence the pregnancy-

induced changes in CatD levels and also other metabolic and inflammatory changes that take place over the course of pregnancy. Both probiotics and fish oil have been previously demonstrated to modify other inflammatory markers such as TNF-alpha. In a pilot study in patients with NAFLD, consumption of synbiotics (7 probiotic strains and fructooligosaccharide and probiotic cultures) reduced serum hsCRP and TNF-alpha and TNF-kB [22], but according to a meta-analysis of patients with NAFLD, probiotics conferred no benefit in reducing the level of TNF-alpha [12]. With regard to the fish oil, supplementation of 4 g/day fish oil (182 mg of EPA and 129 mg of DHA/1g capsule) for 3 months has been reported to reduce the serum levels of TNF-alpha and leukotriene B4 in patients with NAFLD [23]. Somewhat unexpectedly, the combined intake of probiotics and fish oil in our study had no impact on the concentrations of CatD. The reason for this finding is not clear, one potential mechanism may be attributable to the interaction of these supplements, e.g. probiotics may incorporate exogenous n-3 fatty acids into the outer cell membrane or as cellular fatty acids, which may interfere with the functional potential of the probiotics [24]. Furthermore, we cannot exclude the potential impacts of the placebo for fish oil, the medium-chain saturated fatty acids, on the metabolism [25]. In our study, compliance to consume the intervention products is unlikely to explain the finding since the compliance, which we evaluated by interviews and capsule counting as well as serum metabolome analyses [18], was found to be good. In addition, no differences in the dietary intakes of n-3 fatty acids were seen between the intervention groups. Both probiotics and fish oils share similar, as well as distinct biological properties and one could expect synergistic effects as was shown in our recent study on serum metabolites [18] and another study investigating the potential of *Lactobacillus reuteri* and n-3 supplementation on DNA methylation [26] although this effect was not evident with regard to inflammatory markers or clinical benefits [16,17].

Several factors are believed to promote liver inflammation, e.g. dietary factors, gut microbiota and adipose tissue derived factors [27]. NAFLD has been associated with adiposity, insulin resistance and alterations in lipid metabolism. At odds with a previous study [4], we did not observe any correlation between the CatD level and serum total cholesterol or other lipid particles. In our study, CatD did seem to be associated with maternal low grade inflammation. This was evident as a greater decrease in CatD levels in women with a higher inflammatory status, as assessed by GlycA, in early pregnancy as well as the correlation between GlycA and CatD levels, but only in women with GDM. Nonetheless, according to the multivariable analyses that considered both GlycA and body fat percentage, an association was revealed between CatD and GlycA in women without GDM. GlycA is a novel marker for low grade inflammation and its levels are known to be elevated in many metabolic diseases [19,28], including liver diseases like NAFLD [29]. Both GlycA and hsCRP have been detected in association with fatty liver [30], and based on our findings, the GlycA level may also reflect liver inflammation, and this may also be observed in the CatD concentration. We have previously shown that in overweight and obese pregnant women, the GlycA level was superior to hsCRP in reflecting the metabolic status of these women, e.g. in the amounts of lipids associated with hepatic inflammation [31].

The strength of this trial is that the study population is a high-risk group for developing metabolic diseases and liver inflammation and even NAFLD. One limitation is that we have no information on the activity levels of transaminases, other liver enzymes and different inflammatory markers other than hsCRP and GlycA during pregnancy. Regarding the postpartum period, NAFLD has been detected in 24% of women experiencing a previous GDM [32]. These values have been confirmed in two publications; in the first report that women with a previous GDM are more likely to develop NAFLD i.e. in 38% of women with GDM as compared to 17% of women without GDM [33]; in the second, 14% of women with previous GDM as compared to 5.8% of women without GDM [34]. Further longitudinal studies are needed to clarify whether our high-risk study population will develop fatty liver/NAFLD and whether the CatD concentration in this population reflects their liver inflammatory status.

To conclude, we have demonstrated that the CatD concentrations declined during pregnancy, particularly in women with GDM. Serum CatD concentrations were influenced by some maternal characteristics i.e., body fat, and low grade inflammatory status, which were dependent on the GDM status. Finally, no impact of our intervention on CatD concentrations was observed. A decrease in CatD concentrations due to the consumption of either probiotics or fish oil supplements was seen, an observation that deserves further investigation.

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Author contributions

KL devised the original clinical study and directed the project. JP, KL and KM planned the study, LD performed the CatD analysis, JP and RSS supervised the CatD analyses. KM and TV conducted the statistical analyses, KM, JG, KL, ACEV, RS-S, TV and JP interpreted the results. KM, JG and KL wrote the paper. All authors read, commented, and

approved the final version of the paper. KL has the primary responsibility for the final content.

Declaration of competing interest

None.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.numecd.2022.02.011.

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