The effect of longer-term plant sterol and stanol consumption on apolipoprotein CII and apolipoprotein CIII concentrations in humans

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Abstract

Background: Elevated fasting and postprandial serum levels of triacylglycerol (TAG) are a risk factor for cardiovascular disease (CVD). The dietary components plant sterols and stanols can be used as strategy against elevated TAG levels. In a recent study it was found that longer-term plant stanol consumption resulted in a higher postprandial TAG response compared to sterol consumption after a second meal. The explanation for this observed difference remained unclear. Possibly the clearance of TAG from the blood might be affected differently. Specialized particles are responsible for the transportation of TAG in the blood, called the lipoproteins. These particles contain apolipoproteins CII (apoCII) and CIII (apoCIII) which have an activating and inhibiting effect on the enzyme lipoprotein lipase (LPL) responsible for the clearance of TAG from the blood.

Objective: The objective was to examine if postprandial apoCII and apoCIII responses could explain observed differences in postprandial TAG response after longer-term sterol and stanol consumption.

Methods: Healthy subjects (n=42) participated in a randomized control trial consisting of three intervention periods during which the participants consumed either control margarine, or margarine enriched with plant sterols or plant stanols. Blood was sampled under fasting conditions and after the consumption of breakfast and lunch. ApoCII and apoCIII concentrations in serum were measured by an immunoturbimetric immunoassay.

Results: Both iAUC and $iAUC_{min}$ of postprandial apoCII concentrations were similar between all three conditions (p=0.965 and p=0.563 respectively). Postprandial iAUC and $iAUC_{min}$ of apoCIII concentrations also showed no difference between conditions (p=0.342 and p=0.955 respectively). In the control group, the iAUC of postprandial apoCIII concentrations was higher after the second meal compared to the first meal. (p=0.037).

Conclusion: Postprandial apoCII and apoCIII responses were not affected by longer-term plant sterol and stanol consumption, therefore changes in concentrations of these two apolipoproteins can be ruled out as explanation for the observed differences in TAG response.

Keywords

Apolipoprotein CII, apolipoprotein CIII, plant sterols, plant stanols, triacylglycerol.

Introduction

Cardiovascular disease (CVD) is the leading cause of death in Europe, with mortality rates of approximately 4 million per year (1). A risk factor for the development of CVD is dyslipidemia, consisting of multiple components. Two of these components are hypercholesteremia, characterised by elevated levels of LDL-cholesterol (LDL-C) and hypertriacylglycerolemia, characterised by elevated levels of triacylglycerol (TAG) (2,3). Reducing these levels is an important objective in controlling CVD. Dietary components can be used as a strategy against dyslipidemia. Plant sterols and stanols are bioactive components with a structure similar to cholesterol apart from an extra ethyl or methyl group. These components are present in nuts, seeds, vegetable oil, cereal and beans. Numerous studies have investigated and confirmed the LDL-C lowering effect of functional foods enriched with plant sterols and stanols (4-7). Recently, attention is drawn to plant sterols and stanols with regard to hypertriacylglycerolemia. Evidence for a lowering effect on fasting TAG levels is emerging (2,5). Since postprandial TAG levels might be a better indicator of CVD than fasting TAG levels, it is of interest to examine the potential TAG lowering effect of plant sterols and stanols in the postprandial state as well. To do so, Baumgartner *et al.* conducted a study where healthy volunteers consumed plant sterols or stanols over a period of 4 weeks. Stanol consumption resulted in a higher postprandial TAG response compared to the sterol consumption after a second meal was given (Baumgartner *et al.* unpublished data). The reason for this is unclear. For the transport of TAG in the blood there are specialised particles called the lipoproteins. Dietary fat in the form of TAG is packaged into chylomicrons, this is known as the exogenous pathway of lipoprotein metabolism. The endogenous pathway involves VLDL particles secreted by the liver (8). It is hypothesized that the clearance of TAG from the blood might be affected differently after longer-term sterol and stanol consumption. Postprandial TAG clearance is largely dependent upon the enzyme lipoprotein lipase (LPL), responsible for hydrolysing TAG (9). The apolipoproteins CII (apoCII) and CII (apoCIII) are of importance in TAG clearance with their roles in the activity of LPL. ApoCII is an essential activator of LPL and apoCIII has an

inhibiting effect (8).These apolipoproteins are constituents of lipoprotein particles namely, chylomicrons, VLDL and HDL (10). Due to their role on TAG metabolism, apoCII and apoCIII plasma concentrations have been linked to CVD as risk markers. The atherogenic role of apoCIII has been recognised for over a decade (11), whereas evidence for elevated apoCII concentrations as risk marker for CVD is limited (12). LPL activity can be estimated from absolute serum concentrations and ratio of the two. ApoCII and apoCIII concentrations will be analysed in order to answer the research question: "Can postprandial apoCII and apoCIII concentrations explain the difference in postprandial TAG response between plant sterol and stanols after longer-term consumption?"

Material and methods

Subjects

The subjects (n=42) were aged between 18 and 70 years old with a BMI in the range of 20-30 kg/m^2. Seventeen men and 25 women participated. The requirements for participation were no active CVD, usage of lipid lowering medication or medically prescribed diet and consumption of plant sterol or stanol enriched products.

Diet and design

The design of the study was a randomized placebo-controlled cross over trial. Participants were asked to consume 20 grams of test margarine per day, divided over 2 meals, during 3 intervention periods of 4 weeks. In between, a wash out period of equal duration was scheduled. The test margarine contained either 3 grams of plant sterol, 3 grams of plant stanol or none of these plant derived components. After every intervention period the participants were invited to the University after an overnight fast. Subsequent to drawing a fasting blood sample, the subjects were given a 500mL high fat milkshake containing 750 kcal of which 59.6 energy percentage (en %) of fat, 6.3 en% of protein and 35.2 en% of carbohydrate. Blood sampling continued every 2 hours over a period of 8 hours in total. Halfway the postprandial period, a second milkshake was given.

ApoCII and apoCIII analysis

To determine apoCII and apoCIII concentrations in the serum of the subjects, an immunoturbimetric assay was performed. With this method, apoCII and apoCIII reacted with antiserum to form an insoluble complex which could be measured turbidimetrically at 340 nm. Assay buffer and antibody reagent were provided by Randox Labaratories, UK and the Horiba ABX pentra 400 analyzer was used for measurement.

Calculations and statistical analysis

To compare postprandial responses between the three conditions the incremental area under the curve, ignoring the area beneath fasting conditions (iAUC) and incremental area under the curve, ignoring the area beneath the lowest concentration ($iAUC_{min}$) were calculated using the trapezoid rule. Univariate ANOVA and Bonferroni's post hoc test were performed (IBM SPSS statistics 21) to test for differences in iAUC between the three conditions. The first and second meal responses were compared with a two sided paired T-test. P value < 0.05 was considered to be statistically significant.

Results

ApoCII

Fasting and postprandial apoCII concentrations are shown in table 1. The fasting apoCII levels were comparable between the control, sterol and stanol condition (p=0.117). Changes in apoCII concentration from baseline and from the second shake onwards are provided in table 1 as well, although these changes were not statistically tested. For the evaluation of the total postprandial response, iAUC and $iAUC_{min}$ are provided in table 2. Both iAUC and $iAUC_{min}$ of the entire postprandial period were similar between all three conditions (p=0.965 and p=0.563 respectively). Because the difference in TAG response between the sterol and stanol condition was observed after the lunch shake (Baumgartner et al. unpublished results), the iAUCs after the first and second meal separately are given in table 2 as well. The iAUC did not differ between conditions after the first shake (p=0.771) and after the second shake (p=0.899). The $iAUC_{min}$ did not differ either after the first (p=0.443) and second meal (p=0.778) between conditions (table 2).

ApoCIII

Fasting and postprandial apoCIII concentrations are shown in table 3. The fasting apoCIII levels were comparable between the sterol, stanol and control condition (p=0.09). Changes in apoCIII concentration from baseline and from the second shake onwards are provided in table 3 as well, although these changes were not statistically tested. For the evaluation of the total postprandial response, iAUC and $iAUC_{min}$ are provided in table 4. Both iAUC and $iAUC_{min}$ of the entire postprandial period were similar between all three conditions (p=0.0342 and p=0.955 respectively). Because the difference in TAG response between the sterol and stanol condition was observed after the lunch shake (Baumgartner et al. unpublished results), the iAUCs after the first and second meal separately are given in table 4 as well. The iAUC did not differ between conditions after the first shake (p=0.518)

and after the second shake (p=0.611). The $iAUC_{min}$ did not differ either after the first (p=0.473) and second meal (p=0.934) between conditions (table 4).

Table 1. Fasting and postprandial apoCII concentrations after a high-fat breakfast shake and high-fat lunch shake.

	Control			Sterol			Stanol		
Time (hours)	Absolute value	Change from T0	Change from T4	Absolute value	Change from T0	Change from T4	Absolute value	Change from T0	Change from T4
0	3.68 (1.50)	–	–	3.46 (1.39)	–	–	3.54 (1.44)	0	–
2	3.79 (1.45)	0.11 (0.39)	–	3.45 (1.42)	−0.01 (0.33)	–	3.63 (1.49)	0.09 (0.17)	–
4	3.51 (1.64)	−0.17 (0.76)	–	3.39 (1.47)	−0.07 (0.31)	–	3.55 (1.52)	0.01 (0.39)	–
6	3.52 (1.53)	−0.16 (0.33)	0.01 (0.70)	3.26 (1.48)	−0.20 (0.41)	−0.13 (0.25)	3.35 (1.60)	−0.19 (0.33)	−0.20 (0.38)
8	3.50 (1.52)	−0.18 (0.46)	−0.01 (0.70)	3.18 (1.51)	−0.28 (0.61)	−0.21 (0.52)	3.19 (1.61)	−0.35 (0.49)	−0.36 (0.44)

Values are means (SD) and are expressed as mg x min/dL

Table 2. Incremental areas under the curve of apoCII concentrations after high-fat breakfast and lunch shake over the total period and separated into the period after the first and second shake.

	Control	Sterol	Stanol	P value
Total				
iAUC	38(65)	37 (67)	35 (63)	0.965
$iAUC_{min}$	149(148)	180 (176)	171 (81)	0.563
Meal 1				
iAUC	20 (29)	16 (22)	20 (29)	0.771
$iAUC_{min}$	49 (78)	43 (33)	43 (30)	0.443
Meal 2				
iAUC	11(45)	9(28)	8 (20)	0.889
$iAUC_{min}$	44 (47)	50 (76)	53 (62)	0.778

Values are means (SD) and are expressed as mg x min/dL

Table 3. Fasting and postprandial apoCIII concentrations after a high-fat breakfast shake and high-fat lunch shake.

	Control			Sterol			Stanol		
Time (hours)	Absolute value	Change from T0	Change from T4	Absolute value	Change from T0	Change from T4	Absolute value	Change from T0	Change from T4
0	10.35 (2.81)	0	–	9.87 (2.93)	–	–	9.95 (2.78)	0	–
2	10.30 (2.71)	−0.05 (0.56)	–	9.87 (2.72)	0 (0.64)	–	10.06 (2.86)	0.11 (0.44)	–
4	9.75 (2.73)	−0.60 (1.47)	–	9.56 (3.00)	−0.31 (0.70)	–	9.79 (3.01)	−0.16 (1.51)	–
6	10.56 (2.86)	0.21 (0.77)	0.81 (1.45)	9.90 (3.05)	0.03 (0.94)	0.34 (0.81)	10.22 (3.17)	0.27 (0.88)	0.43 (1.64)
8	10.04 (2.92)	−0.31 (0.93)	0.29 (1.42)	9.61 (2.98)	−0.26 (1.03)	0.05 (0.97)	9.63 (2.96)	−0.32 (0.87)	−0.16 (1.60)

Values are means (SD) and are expressed as mg/dL.

Table 4. Incremental areas under the curve of apoCIII concentrations after high-fat breakfast and lunch shake over the total period and separated into the period after the first and second shake.

	Control	Sterol	Stanol	P value
Total				
iAUC	89 (162)	98 (132)	127 (200)	0.342
$iAUC_{min}$	362 (815)	344(247)	343 (306)	0.955
Meal 1				
iAUC	31 (54)	36 (53)	45 (87)	0.518
$iAUC_{min}$	150 (410)	110 (77)	126 (126)	0.473
Meal 2				
iAUC	115 (81)	81 (92)	103 (81)	0.611
$iAUC_{min}$	147 (239)	138 (131)	151(108)	0.934

Values are means (SD) and expressed as mg x min/dL

Ratio apoCIII/apoCII

Fasting and postprandial apoCIII/apoCII ratio's are shown in table 5. The fasting ratio's were comparable between the sterol, stanol and control condition (p=0.346). Changes in ratio from baseline and from the second shake onwards are provided in table 5 as well, although these changes were not statistically tested.

Table 5. Fasting and postprandial apoCIII/apoCII ratio after high-fat breakfast and lunch shake.

	Control			Sterol			Stanol		
Time (hours)	Absolute value	Change from T0	Change from T4	Absolute value	Change from T0	Change from T4	Absolute value	Change from T0	Change from T4
0	3.56 (0.68)	–	–	3.30 (0.92)	–	–	3.52 (0.82)	–	–
2	3.38 (0.94)	−0.18 (0.70)	–	3.42 (1.16)	0.12 (0.91)	–	3.86 (1.40)	0.34 (0.15)	–
4	3.64 (1.26)	0.08 (0.38)	–	4.48 (1.71)	1.18 (0.27)	–	3.29 (0.89)	−0.23 (0.20)	–
6	2.32 (0.42)	−1.24 (0.36)	−0.37 (0.32)	2.53 (0.66)	−0.77 (0.39)	−1.95 (0.25)	3.21 (0.73)	−0.31 (0.42)	−0.08 (0.32)
8	2.30 (0.37)	−1.26 (0.53)	−0.20 (0.52)	2.65 (0.26)	−0.65 (0.63)	−1.83 (0.48)	2.72 (0.47)	−0.80 (0.84)	−0.57 (0.76)

Values are means (SD)

First and second meal response

To compare the apoCIII and apoCII responses after the first and second meal, the total repsonse was split in two in the control condition. The iAUC of postprandial apoCIII concentrations gave values of 54 ± 150 mg x min/dL after the first meal, and 115 ± 247 mg x min/dL after the second meal. The iAUC of apoCIII was higher after the second meal (p=0.037). The iAUC of apoCII after the first meal was 20± 29 mg x min/dL and 11 ± 45 mg x min/dL after the second meal. These values were comparable (p=0.298)

Discussion/Conclusion

For this thesis, the aim was to evaluate the postprandial effects of plant sterols and stanol esters on apoCII and apoCIII responses. To do so, the subjects received a first meal in the morning and a second meal at lunch. It was shown that iAUCs during the 8-hour test period for apoCIII and apoCIII were similar between the control, sterol and stanol conditions as were the iAUCs of the period after the first and second meal. The same was true for the fasting levels of apoCII, apoCIII and the apoCIII/apoCII ratio. The iAUC for apoCIII after the second meal was higher compared to the iAUC after the first meal.

The measured fasting apoCIII levels are within the range 4.4-10.6 mg/dL found by the Malati et al. (13). Fasting apoCII concentrations are also within the range 1.0-6.2 mg/dL as determined by this research group. The mean baseline apoCIII concentrations found in this study are also in agreement with the levels found by Barr et al. (8). The fasting apoCII concentrations in this study seem lower compared to their findings. This could be due to the fact that their study population consisted of only 5 male subjects whereas

in this study both men women participated. Men have higher apoCII levels compared to women (14). When comparing the fasting apoCIII and apoCII values with the reference values determined by Sakubayashi et al., apoCII was within the range of 1.6±4.2 mg/dL (14). ApoCIII however, exceeded the upper limit of 5.5-9.5 mg/dL (14). An explanation for this difference can be the fact that in the study of Sakubayashi et al. a Japanese study population was used, whereas in this study Caucasian individuals participated. Possibly, an ethnical difference in plasma apoCII and apoCIII concentration exists. Indeed African American youths have lower fasting apoCIII concentrations compared to Caucasian youths (15). This study compared apoCII and apoCIII responses between the three conditions. With the activating effect of apoCII and the inhibiting function of apoCIII on the LPL activity, the ratio of the two may have be a better measure to estimate effects on postprandial LPL activity and thereby TAG hydrolysis. Due to the limited duration of the internship, only the fasting apoCIII/apoCII ratio could be compared between the conditions, not the postprandial apoCIII/apoCII ratios. One limitations of this study was the fact that it was performed in a healthy population. Participants originated from a previous study were the effect of plant sterol and stanol consumption was examined on fasting oxyphytosterol concentrations (16). To investigate the effect of plant sterols and stanols on postprandial TAG responses, it might have been better to select subjects with high baseline TAG values. The difference in TAG responses between plant sterol and stanols after the second meal was caused by the highest age group (Baumgartner et al. unpublished data). Possibly, this subgroup was underrepresented for the detection of a difference in apoCII and apoCIII responses.

Based on the obtained results, apoCII and apoCIII responses do not seem to be the explanation for the difference in TAG response after sterol and stanol consumption in this sample. Other factors exist that can influence TAG levels in the blood. Impaired insulin-signaling for instance, strongly correlates with circulating TAG levels (17). A difference in insulin response could provide an explanation. However this has been ruled out already by Baumgartner et al. (Baumgartner et al. unpublished data). Besides TAG hydrolysis from lipoprotein particles in the blood, TAG absorption, TAG synthesis-, or clearance of TAG-rich lipoprotein remnants could provide an explanation for the observed differences after sterol and stanol consumption. Food-derived TAG is first digested into fatty acids and monoacylglycerol before it can be absorbed in the enterocytes of the small intestine. Within the enterocytes, fatty acids and monoacylglycerol are re-esterified into TAG and packaged into chylomicron particles for transportation throughout the body (18). De novo lipogenesis is the process in the liver that contributes to TAG levels in the blood by converting non-lipid substrates, particularly glucose, to TAG (18). The formed TAG is secreted

into the bloodstream via VLDL particles. De novo lipogenesis and subsequently VLDL-TAG increased following meals (19). It is possible that the observed difference in TAG response arose from changes in this process. Lastly, lipolysis of TAG converts the chylomicron and VLDL particles into lipoprotein remnants (20). These remnants still contain some TAG and are taken up by the liver via the LDL-receptor related protein and LDL receptor (18). The sterol or stanol consumption may have affected this process differently resulting in a difference in TAG response. Regarding fasting apoCIII concentrations as risk marker for CVD (21) these finding suggest that longer-term plant sterol and stanol consumption are not a useful dietary strategy to reduce levels fasting apoCIII. It should be noted, however, that the study population was not optimal to determine this and it cannot be excluded to be true for individuals with high baseline levels of apoCIII.

This study is the first to report postprandial changes in apoCIII and apoCII concentrations after the consumption of two meals within 8 hours. Even though it was not the primary aim of this thesis, it was an interesting finding that the apoCIII response after the second meal was higher compared to the response after the first meal. Based on postprandial measurements performed by Barr et al. a decrease in apoCIII concentration was expected after a meal (8). Consistent with these observations a decrease after the first meal was found in this study. In contrast, after the second meal the apoCIII concentrations reached values above baseline level. Although no distinction between TAG in chylomicrons and VLDL was made, it has been documented that a rapid rise of chylomicrons into the circulation occurs one hour after the consumption of a second meal. Apparently a portion of dietary fat from the first meal is temporarily stored and rapidly released upon consuming a second meal. The second meal does not have to contain fat for this phenomenon to occur (22). Given that apoCIII is part of chylomicrons (9) it could explain the rise in apoCIII after the second meal. From the functional point of view of apoCIII, the rise in postprandial apoCIII levels after the second meal would mean LPL is inhibited, thereby reduced clearing TAG from the blood (23). Additionally to the inhibiting function on LPL, apoCIII interferes with apolipoprotein E-mediated receptor binding, delaying removal of remnant particles (24). Also, the AUC of apoCIII was recently correlated with de novo lipogenesis , resulting in the synthesis of VLDL-TAG (19). Based on its multiple roles in TAG metabolism, a rise in apoCIII would suggest an increase of TAG levels. This is consistent with the findings in the previous study where TAG response was higher after the second meal (Baumgartner et al. unpublished data).

To conclude, longer-term plant sterol and stanol consumption does not affect postprandial apoCII and apoCIII responses and can be ruled out as explanation for the observed differences in postprandial TAG response in this study sample. To be certain the iAUC and

$iAUC_{min}$ of the apoCIII/apoCII ratio instead of the apoCIII and apoCII responses separately should be determined and evaluated. The next step would be to investigate other possible explanations, for example TAG absorption, TAG synthesis, or clearance of TAG-rich lipoprotein remnants. It would be interesting to correlate the postprandial apoCIII/apoCII response with the postprandial TAG response to confirm the theoretical effect of the ratio on TAG clearance via LPL activity.

Role of the student

Claire Lemmens was an undergraduate student in BioMedical Sciences working under the supervision of Sabine Baumgartner. The topic was proposed by the supervisor. My task as bachelor student was to measure the apoCII and apoCIII concentrations in the blood samples of the participants in the laboratory together with a professional analyst, formulating the research question, data processing, formulating conclusions and writing a research paper.

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