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Original article

Effects of a low-fat dietary regimen enriched with soy in children affected with heterozygous familial hypercholesterolemia

Oliver Helk [a], Kurt Widhalm [b, *]

[a] Division of Medicine III, Department of Nephrology and Dialysis, Medical University of Vienna, Austria
[b] Austrian Academic Institute for Clinical Nutrition, Austria

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SUMMARY

Introduction: Familial hypercholesterolemia (FH) is an inheritable, autosomal dominant disorder leading to pathologically increased levels of low-density-lipoprotein cholesterol (LDL-C). Dietary treatment remains an important tool in the management of affected children even after the decision for the initiation of pharmacotherapy is made. However, little evidence is available regarding the optimal dietary regimen for the treatment of children affected with FH.

Methods: We present results from a randomized controlled trial in paediatric patients affected with heterozygous FH, assessing the effect of a soy-enriched fat modified diet (soy group) compared to fat modified diet (Control group) alone on LDL-C over a period of 13 weeks. Furthermore, we monitored isoflavone levels in plasma and urine as markers of adherence to the dietary treatments.

Results: LDL-C decrease was statistically significantly greater in the soy group compared to the control group at week 7 (Control group 176.3 ± 27.8 mg/dl, soy group 154.7 ± 29.2 mg/dl, $p = 0.038$), and showed a trend towards significant at week 13 (Control group 179.9 ± 41.8 mg/dl, soy group 155.0 ± 30.2 mg/dl, $p = 0.089$). Relative LDL-C decrease correlated significantly with the following plasma isoflavone concentrations measured in week 7: daidzein ($p < 0.004$, $r = 0.576$) and genistein ($p < 0.017$, $r = 0.490$).

Conclusions: We provide evidence from a small randomized-controlled trial for the effectiveness and safety of a dietary treatment with soy in paediatric patients affected with heterozygous FH. The decrease in LDL-C was highly correlated with isoflavone levels, further highlighting a direct effect of soy ingestion. This study was registered under ClinicalTrials.gov Identifier No. NCT03563547.

1. Introduction

Familial hypercholesterolemia (FH) is an inheritable, autosomal dominant disorder leading to pathologically increased levels of low-density-lipoprotein cholesterol (LDL-C). With estimated prevalences of 1/500 to 1/200, FH can be considered one of the most common monogenetic disorders [1,2]. FH exists in both hetero- and homozygous forms (HeFH or HoFH) with LDL-C plasma concentrations typically ranging from 200–600 mg/dL (5–10 mmol/L) in patients affected with HeFH and >600 mg/dL (15.5 mmol/L) in subjects suffering from HoFH [2,3]. In children an LDL-C >190 mg/dL, or an LDL-C >160 mg/dL with a positive family history of premature coronary heart disease and/or high baseline cholesterol in one parent make the phenotypic diagnosis [3]. FH is most commonly caused by mutations in one or more of three different genes: (i) the LDL receptor (LDLR), (ii) apolipoprotein B (ApoB), and (iii) pro-protein convertase subtilisin/kexin 9 (PCSK9), with varying clinical phenotype [4]. As a direct result of its early manifestation in childhood as a life-long risk factor for premature cardiovascular disease (CVD), it is of special importance for family practitioners and paediatricians to be aware of FH [3]. In the pre-statin era CVD usually occurred in patients affected with HeFH in their fourth-to fifth decade [5,6].

While the immediate initialization of cholesterol-lowering medication in combination with life-style treatment is strongly endorsed in adults, the current guidelines from the European Atherosclerosis Society only recommend the consideration of pharmacotherapy in children affected with HeFH starting at the age of 8–10 [7]. Evidence based dietary treatment is therefore of special importance in patients below 8 years of age. If the decision for medication is made, statins are first-line therapy in both adults and children [8]. However, dietary- and life-style treatment remains an

* Corresponding author. Alserstrasse 14/4a, 1090, Vienna, Austria.
E-mail address: k.widhalm@gmx.at (K. Widhalm).

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List of abbreviations

ALT	Alanine-Aminotransferase
ApoA1	Apolipoprotein A1
ApoB100	Apolipoprotein B100
AST	Aspartate-Aminotransferase
CVD	Cardiovascular disease
FH	Familial hypercholesterolemia
FM	Fat-modified
GGT	Gamma-Glutamyl-Transferase
HDL-C	High density lipoprotein cholesterol
HeFH	heterozygous familial hypercholesterolemia
HoFH	homozygous familial hypercholesterolemia
LDL-C	Low density lipoprotein cholesterol
Lp(a)	Lipoprotein (a)
ODMA	O-Desmethylangolensin
OH-ODMA	6′-Hydroxy-O-desmethylangolensin
TC	Total cholesterol
TG	Triglycerides
TSH	Thyroid stimulation hormone
T3	Triiodothyronine
T4	Thyroxine
VLDL-C	Very low density lipoprotein cholesterol

important tool in the management of affected children and adherence to a healthy diet is of utmost importance in HeFH patients even after the decision for the initiation of pharmacotherapy was made [9]. While the beneficial effects of diets low in saturated fat and cholesterol are scientifically well established in adults, only little data is available on the effects of such dietary regimens on children affected with FH [10,11]. Furthermore, several studies have indicated that the currently recommended dietary regimens for the treatment of HeFH may be optimized by adding sources of plant sterols to the diet [12].

Specifically, the ingestion of soy is reported to exhibit beneficial effects on CVD by lowering LDL-C and thus could provide a therapeutic option through dietary means for reducing the risk of possible long-term adverse effects of FH [13]. Interestingly, it appears that only whole soy has LDL-C reducing properties, whereas the isolated soy isoflavones daidzein and genistein do not [14–16]. This may, in part, be explained by a displacement effect where foods rich in soy protein replace animal products which are generally higher in saturated fat content, thus leading to a more favourable nutritional profile [17]. Similar findings regarding the effectiveness of isolated isoflavones were reported from a small trial with hypercholesterolemic subjects in paediatric age [15]. However, a meta-analysis by Zhou et al. found that the LDL-C reducing effect of soy ingestion is greater when soy foods high in isoflavones are consumed [18]. Furthermore, a more recent study demonstrated that isoflavones from soy extract improved arterial stiffness in equol producers [19]. In conclusion, the exact role of soy isoflavones in regard to cardiovascular risk reduction remains elusive.

It is important to point out that there are reports on possible adverse effects of soy-enriched diets on the thyroid gland [20]. Nonetheless, administering soy is generally considered safe even in very young children [21].

Several studies have suggested the measurement of isoflavones in urine and/or plasma as a method of assessing adherence to soy consumption [22]. Daidzein and genistein, due to their characteristic of occurring naturally in soy-beans, have been identified as the most promising parameters for adherence to soy-enriched diet in adults as they were shown to correlate with soy-intake [23,24]. Furthermore, they have been validated as markers of adherence to dietary treatment in adults affected with familial hypercholesterolemia [25].

Based on these previous findings we hypothesize that the LDL-C lowering effect of a fat-modified diet could be further increased by the addition of soy-protein in children affected with HeFH.

2. Materials and methods

This study is a prospective randomized controlled trial.

2.1. Study protocol

The enrolled subjects were recruited from the outpatient clinic for disorders of metabolism of the Medical University of Vienna. Subjects were either allocated into a group treated with a dietary regimen high in unsaturated fats, low in saturated fats and enriched with soy-protein ("soy group") or a group treated with a diet high in unsaturated fats and low in saturated fats ("control group") alone at random. All subjects had been instructed to adhere to a fat-modified diet as described below prior to enrolment into the study as part of their routine treatment. Prior to being considered for inclusion in the study all subjects had to undergo nutritional protocoling using 24 h dietary recall protocols, which were completed by their legal guardians, for 7 days. This was done to confirm adherence to the routine dietary treatment. Furthermore, all patients had been screened for metabolic diseases other than FH as part of their initial routine assessment in our specialised centre.

Subjects in both groups had been instructed to achieve specific daily maximum intakes in total fat (≤30% of total energy intake), saturated fatty acids (≤10% of total fat intake) and cholesterol (≤300 mg). Furthermore the participating families had been trained to replace as many visible fat sources as possible with rapeseed oil due to its favorable composition of polyunsaturated fatty acids [26].

Subjects allocated to the soy group and their families were additionally instructed to consume at least 0.25 g of soy protein per kg bodyweight per day and were provided with recipes and practical advice on how to achieve this goal. Example provided: a child with a bodyweight of 30 kg would have to consume the equivalent of approx. 50 g of Tofu per day to meet the treatment target. This dosage had been reported as effective for LDL-C reduction in children in a previous, non-controlled study [10].

Further appointments with an experienced dietitian were made after enrolment for both treatment groups, totalling 7 training sessions (60 min each) over the course of the first 7 weeks of the trial. This was done to identify possible issues with the practical implementation of especially the soy-enriched diet.

Patients were asked to provide weekly urine samples and blood was drawn immediately after enrolment, week 7 and week 13, respectively. The urine- and plasma samples were immediately frozen and later analysed for their isoflavone content. Isoflavone levels were assessed at baseline to verify that the respective patients did not consume soy products prior to the trial. All enrolled subjects had to participate for 13 weeks or were excluded from the statistical evaluation (per protocol analysis). If relevant levels of isoflavones were detected in either urine or plasma of subjects in the Control-group the subjects were excluded from any further statistical evaluation.

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The study was approved by the Ethics Committee of the Medical University of Vienna. Informed consent from all participating subjects and their legal guardians was obtained prior to their enrolment in the study. Our research was conducted in accordance with the latest Declaration of Helsinki. This study was registered under ClinicalTrials.gov Identifier No. NCT03563547.

2.1.1. Inclusion criteria

Informed consent of both the patient and their legal guardian, age of 4–14, diagnosis of definite FH using the Simon Broome criteria [27], body weight within normal age-specific percentile range (Kromeyer-Hauschild et al.) [28], the proven ability and willingness to adhere to a fat-modified diet.

2.1.2. Exclusion criteria

Metabolic or genetic disorders other than FH, current infectious disease, current or history of cancerous disease, current treatment with lipid lowering drugs, failure to complete all trial-related assessments, reported habit of consuming soy products or adherence to a non-standard diet (e.g. vegetarianism) prior to enrolment into the study.

2.2. Laboratory methods

2.2.1. Isoflavones

In the plasma samples, the following soy isoflavones and metabolites were analyzed with GC/MS after enzymatic hydrolysis as aglycones as described in Rüfer et al., 2008 [29]: Daidzein, genistein, dihydrodaidzein, dihydrogenistein, glycitein, equol, 3′-hydroxydaidzein and *O*-desmethylangolensin (ODMA). In urine samples, the following isoflavones were assessed without enzymatic hydrolysis of the conjugative metabolites using a previously described UHPLC-MS/MS method [30]: Daidzein, genistein and their conjugative glucuronide, sulfate and sulfoglucuronide metabolites as well as the microbial degradation products dihydrodaidzein, dihydrogenistein, ODMA, 6-hydroxy- *O*-desmethylangolensin (OH-ODMA) and equol only as unconjugated aglycones.

2.2.2. Further parameters

Further assessed laboratory parameters include LDL-C, HDL-C, Very Low Density Lipoprotein Cholesterol (VLDL), TC, Triglycerides (TG), Apolipoprotein A1 (ApoA1), Apolipoprotein B100 (ApoB100) and Lipoprotein (a) (Lp(a)) as well as Creatinine, Thyroid stimulation hormone (TSH), Triiodothyronine (T_3) and Thyroxine (T_4) as safety parameters as well as Aspartate-Aminotransferase (AST), Alanine-Aminotransferase (ALT) and Gamma-Glutamyl-Transferase (GGT). All non-isoflavone related measurements were performed in the centralized clinical laboratory of the Vienna General Hospital. TC and TG levels were measured enzymatically by using test kits from Boehringer Roche GmbH (Mannheim, Germany): CHOD-PAP and GPO-PAP (enzymatic calorimetric methods). Aspartate Amino Acetyl Transferase (ASAT), Alanine Amino Transferase (ALT) and Gamma-Glutamyltransferase (γ - GT), Creatinine, Urea and Platelets were determined before and after 5 months of treatment. Levels of ASAT, ALT, γ - GT, and creatinine determined enzymatically by test kit of Vitros chemistry. ApoA1, ApoB100 and Lp(a) were measured by nephelometry with test kits from Roche Diagnostics. LDL-C was calculated using the formula postulated by Friedewald et al. [31].

2.3. Statistical analysis

All statistical analyses were performed with IBM SPSS Statistics for Windows, Version 24.0. Armonk, NY: IBM Corp. All medians are reported with 25th and 75th Quantiles. Means are reported with standard deviations.

2.3.1. Sample size calculation

The sample size calculation was performed using a precision based model. We retrospectively analyzed LDL-C concentrations of 10 randomly selected children suffering form HeFH who had previously been treated in our outpatient clinic with a fat-modified diet. Mean LDL-C in this sample was 166 mg/dl ± 13 mg/dl. In order to detect an expected difference in LDL-C concentrations of 10% between groups [22] (corresponding to 16 mg/dl) with a statistical power of 90% a sample size of 15 subjects per group is required (alpha = 0.05). We therefore aimed to include 30 patients in our study with an estimated dropout rate of 10%. To reach equal patient numbers in both groups, the required sample size was rounded up to 34 patients.

2.3.2. Evaluation of clinical parameters

Our main hypothesis was that LDL-C can be significantly lowered by a fat modified diet enriched with soy-protein compared to a control group undergoing. Thus, we compared LDL-C between groups over time using a two-way mixed ANOVA followed by Tukey's post hoc-test. Possible effects on other lipoproteins (HDL-C, VLDL-C, TC) were evaluated in the same manner.

2.3.3. Evaluation of plasma isoflavones

The concentrations of plasma isoflavones (daidzein, genistein, glycitein, equol and ODMA) of each time point were correlated with LDL-decrease in percent in the respective time frame by calculating Spearman's Rho.

2.3.4. Evaluation of urinary isoflavones

First, the median, creatinine adjusted concentrations of daidzein and genistein between week 1 and 7 and week 8 and 13 were summarized with those of their respective metabolites. In detail daidzein was summarized with dihydrodaidzein, daidzein-4′-glucuronide, daidzein-7-glucuronide, daidzein-4′-sulfate, daidzein-7-sulfate, daidzein-7,4′-disulfate, daidzein-7-glucuronide-4′-sulfate. Genistein levels were summarized with its respective metabolites in the same manner. The resulting values (Dai + Met and Gen + Met) were then correlated with LDL decrease in percent in the respective time frame.

3. Results

A total of thirty-four (34) patients had initially been enrolled into the study. Four (4) of these subjects failed to participate for the full duration of the study and were thus excluded. Four (4) more subjects were excluded from the final statistical evaluation due to incomplete data due to scheduling problems (2) and drastic changes in dietary habits compared to baseline (2). Of the remaining 26 subjects, 13 had been allocated to the soy- and the control group each (Fig. 1). Age and sex distribution across groups is provided in Fig. 2. Baseline characteristics are provided in Table 1. Mean Energy- and macronutrient intake were not different between groups at baseline (Table 2). Data are mean ± standard error, unless otherwise stated. As the data on LDL-C had initially been skewed a square root transformation was applied. Furthermore, the assumption of sphericity was violated for VLDL-C and Lp(a), therefore a Greenhouse-Geisser correction was applied.

3.1. Lipids and lipoproteins

Mean serum lipid and lipoprotein concentrations at baseline, week 7 and week 13 are provided in Table 1. A statistically significant interaction between group and time on LDL-C ($p < 0.002$,

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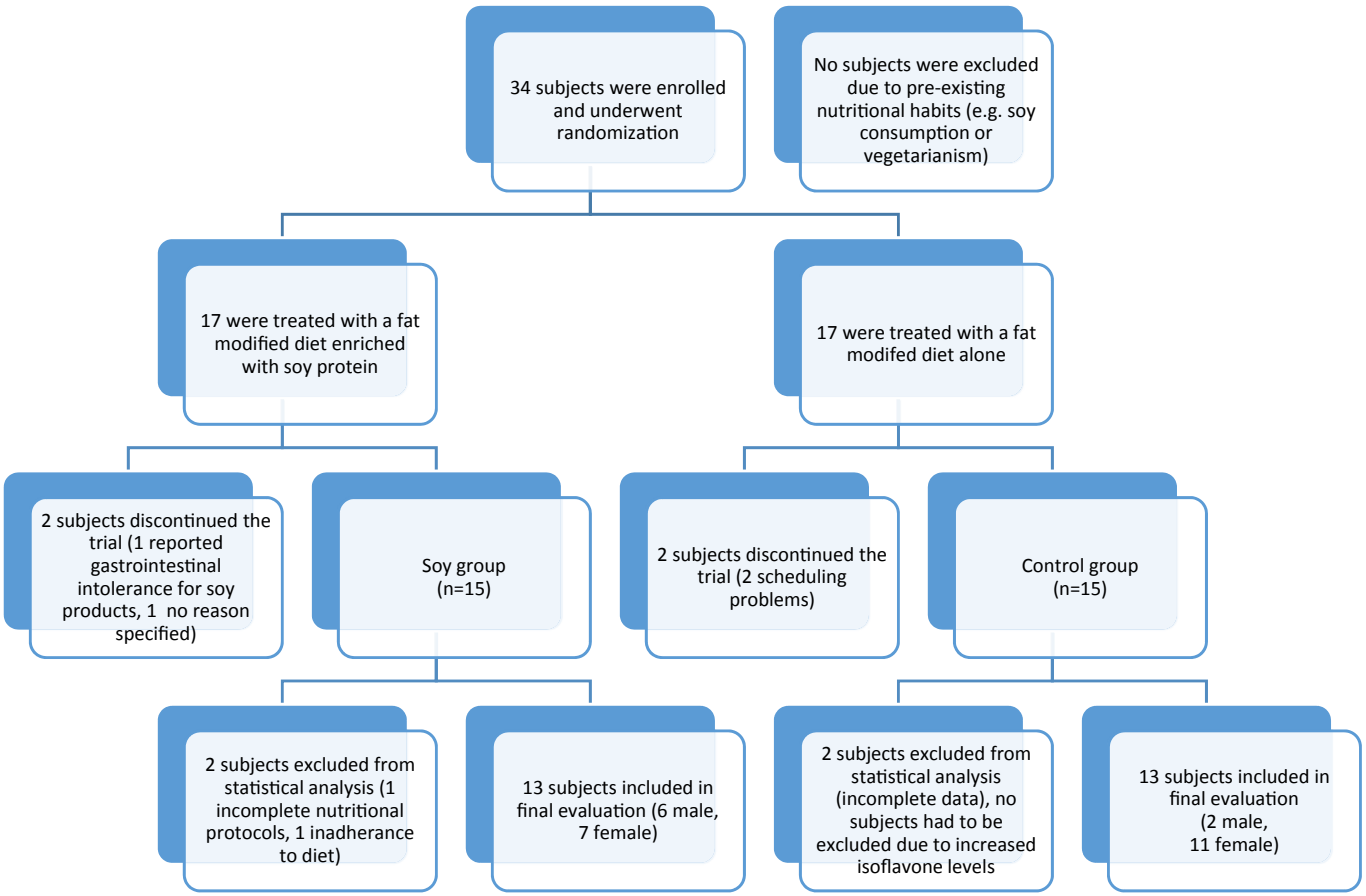

Fig. 1. Number of included subjects and drop-outs.

partial $\eta^2 = 0.253$) was detected. Testing for main effect for group revealed that LDL-C was statistically significantly greater in the control group compared to the soy group at week 7 ($p = 0.038$, control group 176.3 ± 27.8 mg/dl, soy group 154.7 ± 29.2 mg/dl) and showed a trend towards significant at week 13 ($p = 0.089$, control group 179.9 ± 41.8 mg/dl, soy group 155.0 ± 30.2 mg/dl). No statistically significant differences were found at baseline ($p = 0.803$) Table 3.

Furthermore, there was a statistically significant interaction between group and time on TC ($p = 0.015$, $\eta2 = 0.161$). Tukey's post hoc testing showed no significant differences between groups at baseline ($p = 0.424$), week 7 ($p = 0.211$) or week 13 ($p = 0.312$).

There was no statistically significant interaction between group and time on HDL-C concentration ($p = 0.961$, partial $\eta2 = 0.002$), TG ($p = 0.240$, partial $\eta2 = 0.117$), VLDL-C ($p = 0.276$, $\eta2 = 0.052$) or Lp(a) ($p = 0.378$, $\eta2 = 0.034$).

3.2. Isoflavones

No relevant concentrations of isoflavones were detected in the control group at any timepoint.

3.2.1. Plasma isoflavones

As the data on plasma isoflavone concentrations was not normally distributed, Spearman's correlation coefficient was calculated. LDL-C decrease in percent from week 1 to week 7 correlated significantly with the following plasma isoflavone concentrations measured in week 7: daidzein ($p < 0.004$, $r = 0.576$) and genistein ($p < 0.017$, $r = 0.490$) (Fig. 3). Relative LDL-C decrease did not correlate significantly with glycitein or ODMA. Furthermore, a significant correlation was found for genistein at week 13 and LDL-C decrease between week 1 and week 13 in percent. No significant correlations were found for daidzein, ODMA and glycitein values from week 13.

Detectable concentrations of equol were found in one subject in week 7 and in another subject in week 13. Due to these findings, no correlations were performed for equol.

3.3. Urine isoflavones

Dai + met and gen + met correlated significantly with LDL-C decrease between week 1 and 7 ($p = 0.006$, $r = 0.523$ and $p = 0.01$, $r = 0.493$, respectively). There was no significant correlation between these parameters between week 8 and 13 (dai + met: $p = 0.085$, $r = 0.330$, gen + met: $p = 0.190$, $r = 0.284$).

3.4. Safety parameters and side effects

No pathologically increased or decreased levels of AST, ALT, GGT, TSH, T_3 or T_4 were detected at any point in time. One subject discontinued the study due to gastric discomfort after consumption of soy-products. No other side effects were reported or detected.

4. Discussion

We provide evidence from a randomized controlled trial that a diet enriched with soy may be of added benefit in the dietary treatment in paediatric patients affected with HeFH. Side effects

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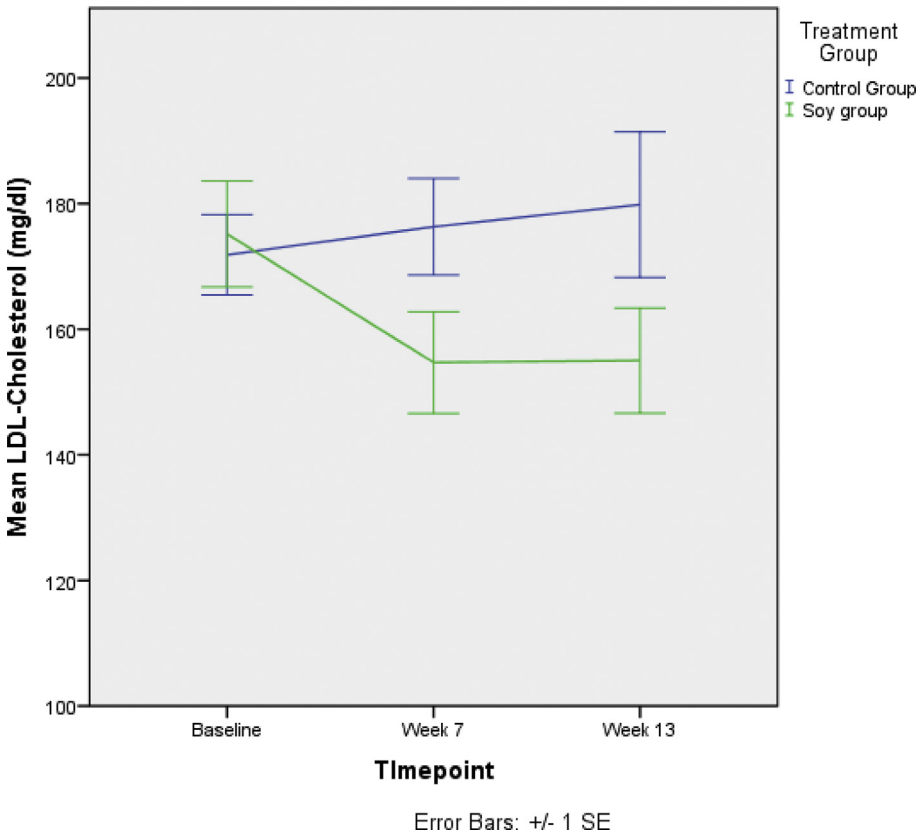

Fig. 2. Age and sex distribution across groups.

Table 1
Baseline characteristics; comparisons between groups were made with *t*-tests.

	Soy group (n = 13)	Control group (n = 13)	P value
Age (yrs)	9.46 (±4.05)	8.0 (±3.46)	0.564
Height (cm)	135.98 (±22.74)	128.05 (±22.74)	0.292
Weight (kg)	32.78 (±14.72)	27.97 (±22.39)	0.557
BMI	16.77 (±2.95)	15.75 (±3.15)	0.635
Sex (m/f)	7/6	2/11	

Table 2
Energy- and macronutrient intake between groups at baseline. Values for macronutrients represent the relative amount to total energy intake.

	Soy group (n = 13)	Control group (n = 13)	P value
Energy intake (kcal)	1487.03 (±475.29)	1503.22 (±502.83)	0.899
%E from Carbohydrates	52.58 (±5.33)	51.92 (±7.31)	0.783
%E from Sugars	17.36 (±5.42)	16.99 (±7.23)	0.759
%E from Protein	14.07 (±3.21)	15.21 (±5.02)	0.679
%E from Fat	33.2 (±5.61)	34.1 (±6.9)	0.872
%E from saturated Fat	7.21 (±1.91)	7.51 (±2.04)	0.614

were limited to slight gastric discomfort after the consumption of soy-products in one subject, who thus chose to discontinue the trial. In none of the enrolled patients a clinically significant change in thyroid hormones or parameters of liver function could be detected. Overall, the diet was well tolerated and well accepted.

We found a statistically significant decrease in LDL-C of approximately 10% from baseline in the soy group. These findings are in line with results from similar studies in adult patients [22,32] and are also in line with those from a previous, non-controlled longitudinal study [10]. A slight decrease in HDL-C occurred in both groups, which was not significant. However, it was minimal and can possibly be explained by random effects. We also detected an effect of the diet on TC, however, no significant changes between groups were detected in the post-hoc tests after adjusting for multiple comparisons. In line with our hypothesis is thus seems, that any reduction in TC through a soy enriched diet may be more variable and less pronounced when compared to the effects on LDL-C. It remains possible that age or unwanted changes in lifestyle and nutrition occurring in the subjects after the soy enriched diet was newly introduced affected our findings, however, the reduction in LDL-C correlated with both urine and plasma isoflavone concentrations.

A previous study by Weghuber et al. [10] demonstrated beneficial effects of soy ingestion on lipoprotein profile using a higher amount of soy, i.e. 0.5 gof soy protein equivalent per kg bodyweight per day. However, in the study at hand were able to demonstrate an effect of soy ingestion on LDL-C levels at a lower recommended intake.

There was no significant change in LDL-C from week 7 to week 13, while isoflavone concentrations in both urine and plasma decreased. Although isoflavone concentrations in both plasma and urine showed very strong correlation with LDL-decrease in week 7, correlations grew weaker as the trial progressed. This may be interpreted as further evidence for a decline in adherence to the diet and coincided with the discontinuation of weekly appointments with our dietitian. Morimoto et al. reported from their trial in adults that isoflavones become less reliable as a parameter of soy intake if smaller quantities of soy are ingested [23]. We further hypothesize that these findings reflect a lower intake of soy that was sufficient to maintain decreased LDL-C levels.

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Table 3
Mean lipoprotein levels between groups at baseline, week 7 and week in mg/dl. P values represent the interaction term between time and group in a repeated measures ANOVA. Asterisks highlight significant differences in Bonferroni post-hoc tests between groups and within timepoints (* < 0.05).

	Soy group (n = 13)	Control group (n = 13)	P value (Interaction term)
	Mean (±Std dev)	Mean (±Std dev)	
LDL-C week 1	175.2 (±30.5)	171.9 (±23.1)	0.002*
LDL-C week 7	154.7 (±29.2) *	176.3 (±27.8) *	
LDL-C week 13	155.0 (±30.2)	179.9 (±41.8)	
Total C week 1	262 (±32.6)	248.5 (±39.9)	0.015*
Total C week 7	242.3 (±37.5)	259.2 (±35.9)	
Total C week 13	242.5 (±37.2)	254.3 (±39.9)	
HDL-C week 1	64.2 (±15.6)	61.1 (±13.1)	0.961
HDL-C week 7	64 (±10.8)	61.5 (±11.5)	
HDL-C week 13	62.9 (±9.3)	58.5 (±12.6)	
TG week 1	82.7 (±39.1)	96.1 (±51.1)	0.240
TG week 7	65.5 (±14.3)	94.6 (±36.3)	
TG week 13	71.9 (±23.4)	80.00 (±16.7)	
VLDL week 1	16.6 (±7.8)	19.1 (±10.3)	0.276
VLDL week 7	13.08 (±2.9)	18.9 (±7.4)	
VLDL week 13	14.5 (±4.6)	15.9 (±3.4)	
Lp(a) week 1	30.15 (±25.6)	64.2 (±70.3)	0.378
Lp(a) week 7	29.7 (±25.7)	68.0 (±76.8)	
Lp(a) week 13	29.5 (±28.7)	59.3 (±59.3)	
ApoA1 week 1	134.5 (±18.4)	139.8 (±17.1)	0.602
ApoA1 week 7	138.1 (±12.8)	138.0 (±14.6)	
ApoA1 week 13	135.6 (±12.8)	136.7 (±14.6)	
ApoB week 1	120 (±14.9)	126.8 (±15.4)	0.635
ApoB week 7	112.5 (±20.2)	124.2 (±20.6)	
ApoB week 13	112.5 (±18.8)	122.5 (±21.46)	

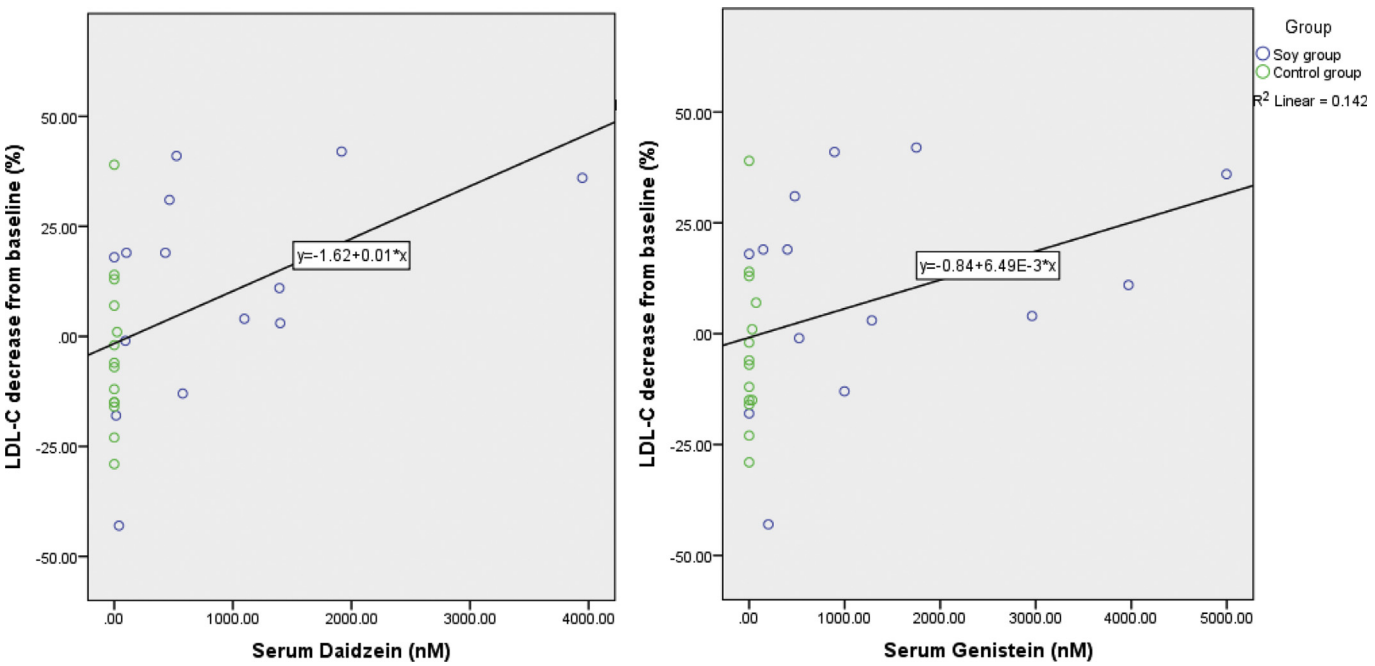

Fig. 3. Plasma LDL-C concentrations between groups.

Considering these findings, it is important to find healthy, soy products especially palatable to children to make the implementation of the diet easier. Our results also highlight the need for continuous dietary counselling in these patients.

Several weaknesses of the study at hand need to be pointed out. Our study population was recruited from patients that were already undergoing treatment in our specialised centre. While this lead to high comparability regarding pre-existing dietary habits between subjects, it reduces the applicability of our findings to a less highly selected population. Furthermore, while the mean age and age distribution of both groups was comparable there was a distinct sex-imbalance between the treatment groups. It is therefore possible that some of our findings may be caused by sex-specific effects. We are also unable to draw conclusions whether soy treatment may be particularly effective in a certain age sub-group as our study was underpowered to detect such differences.

5. Conclusion

We provide evidence from a small randomized-controlled trial for the effectiveness and safety of a dietary treatment with soy in paediatric patients affected with HeFH. Our results demonstrate that isoflavones from both urine and plasma may serve as indicators of treatment success regarding LDL-C reduction and thus

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make the hypothesis that soy ingestion directly leads to a more favourable lipoprotein profile more plausible. Correlation of Dai + Met and Gen + Met with LDL-C decrease was superior to daidzein or genistein alone. Further trials with larger case numbers will be required to confirm our findings, while dose-response studies between soy intake and isoflavone concentrations will provide further insight regarding the potential role of isoflavones as markers of adherence to diet. Furthermore, questions remain regarding the ability of paediatric patients to adhere to this form of diet for a prolonged amount of time.

Individual contributions of authors

Oliver Helk (OH): Responsible for writing of the article, analysis of the data.

Kurt Widhalm (KW): Responsible for study design, formulation of research questions, clinical treatment and diagnosis of patients as well as final content.

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All authors (OH, KW) have read and approved the manuscript.

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Declaration of interest

No financial or other conflict of interest could be detected for any of the contributing authors.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.clnesp.2019.09.009.

References

[1] Liyanage KE, Burnett JR, Hooper AJ, van Bockxmeer FM. Familial hypercholesterolemia: epidemiology, Neolithic origins and modern geographic distribution. Crit Rev Clin Lab Sci 2011;48:1–18.

[2] Benn M, Watts GF, Tybjaerg-Hansen A, Nordestgaard BG. Familial hypercholesterolemia in the Danish general population: prevalence, coronary artery disease, and cholesterol-lowering medication. J Clin Endocrinol Metab 2012;97:3956–64.

[3] Wiegman A, Gidding SS, Watts GF, Chapman MJ, Ginsberg HN, Cuchel M, et al. Familial hypercholesterolaemia in children and adolescents: gaining decades of life by optimizing detection and treatment. Eur Heart J 2015;36:2425–37.

[4] Vogt A. The genetics of familial hypercholesterolemia and emerging therapies. Appl Clin Genet 2015;8:27–36.

[5] Mabuchi H, Miyamoto S, Ueda K, Oota M, Takegoshi T, Wakasugi T, et al. Causes of death in patients with familial hypercholesterolemia. Atherosclerosis 1986;61:1–6.

[6] Hovingh GK, Davidson MH, Kastelein JJ, O'Connor AM. Diagnosis and treatment of familial hypercholesterolaemia. Eur Heart J 2013;34:962–71.

[7] Catapano AL, Reiner Z, De Backer G, Graham I, Taskinen MR, Wiklund O, et al. ESC/EAS guidelines for the management of dyslipidaemias the task force for the management of dyslipidaemias of the European society of cardiology (ESC) and the European Atherosclerosis society (EAS). Atherosclerosis 2011;217:3–46.

[8] Cuchel M, Bruckert E, Ginsberg HN, Raal FJ, Santos RD, Hegele RA, et al. Homozygous familial hypercholesterolaemia: new insights and guidance for clinicians to improve detection and clinical management. A position paper from the Consensus Panel on Familial Hypercholesterolaemia of the European Atherosclerosis Society. Eur Heart J 2014;35:2146–57.

[9] Descamps OS, Tenoutasse S, Stephenne X, Gies I, Beauloye V, Lebrethon MC, et al. Management of familial hypercholesterolemia in children and young adults: consensus paper developed by a panel of lipidologists, cardiologists, paediatricians, nutritionists, gastroenterologists, general practitioners and a patient organization. Atherosclerosis 2011;218:272–80.

[10] Weghuber D, Widhalm K. Effect of 3-month treatment of children and adolescents with familial and polygenic hypercholesterolaemia with a soya-substituted diet. Br J Nutr 2008;99:281–6.

[11] Laurin D, Jacques H, Moorjani S, Steinke FH, Gagne C, Brun D, et al. Effects of a soy-protein beverage on plasma lipoproteins in children with familial hypercholesterolemia. Am J Clin Nutr 1991;54:98–103.

[12] Malhotra A, Shafiq N, Arora A, Singh M, Kumar R, Malhotra S. Dietary interventions (plant sterols, stanols, omega-3 fatty acids, soy protein and dietary fibers) for familial hypercholesterolaemia. Cochrane Database Syst Rev 2014:Cd001918.

[13] Taku K, Umegaki K, Sato Y, Taki Y, Endoh K, Watanabe S. Soy isoflavones lower serum total and LDL cholesterol in humans: a meta-analysis of 11 randomized controlled trials. Am J Clin Nutr 2007;85:1148–56.

[14] Liu ZM, Ho SC, Chen YM, Ho S, To K, Tomlinson B, et al. Whole soy, but not purified daidzein, had a favorable effect on improvement of cardiovascular risks: a 6-month randomized, double-blind, and placebo-controlled trial in equol-producing postmenopausal women. Mol Nutr Food Res 2014;58:709–17.

[15] Zung A, Shachar S, Zadik Z, Kerem Z. Soy-derived isoflavones treatment in children with hypercholesterolemia: a pilot study. J Pediatr Endocrinol Metab : JPEM 2010;23:133–41.

[16] Widhalm K, Brazda G, Schneider B, Kohl S. Effect of soy protein diet versus standard low fat, low cholesterol diet on lipid and lipoprotein levels in children with familial or polygenic hypercholesterolemia. J Pediatr 1993;123:30–4.

[17] Jenkins DJ, Mirrahimi A, Srichaikul K, Berryman CE, Wang L, Carleton A, et al. Soy protein reduces serum cholesterol by both intrinsic and food displacement mechanisms. J Nutr 2010;140:2302s–11s.

[18] Zhuo XG, Melby MK, Watanabe S. Soy isoflavone intake lowers serum LDL cholesterol: a meta-analysis of 8 randomized controlled trials in humans. J Nutr 2004;134:2395–400.

[19] Hazim S, Curtis PJ, Schär MY, Ostertag LM, Kay CD, Minihane A-M, et al. Acute benefits of the microbial-derived isoflavone metabolite equol on arterial stiffness in men prospectively recruited according to equol producer phenotype: a double-blind randomized controlled trial. Am J Clin Nutr 2016;103:694–702.

[20] de Souza Dos Santos MC, Goncalves CF, Vaisman M, Ferreira AC, de Carvalho DP. Impact of flavonoids on thyroid function. Food Chem Toxicol – Int J Publ Br Ind Biol Res Assoc 2011;49:2495–502.

[21] Badger TM, Ronis MJ, Hakkak R, Rowlands JC, Korourian S. The health consequences of early soy consumption. J Nutr 2002;132:559s–65s.

[22] Bard JM, Paillard F, Lecerf JM. Effect of phytosterols/stanols on LDL concentration and other surrogate markers of cardiovascular risk. Diabetes Metab 2015;41:69–75.

[23] Morimoto Y, Beckford F, Franke AA, Maskarinec G. Urinary isoflavonoid excretion as a biomarker of dietary soy intake during two randomized soy trials. Asia Pac J Clin Nutr 2014;23:205–9.

[24] Jaceldo-Siegl K, Fraser GE, Chan J, Franke A, Sabate J. Validation of soy protein estimates from a food-frequency questionnaire with repeated 24-h recalls and isoflavonoid excretion in overnight urine in a Western population with a wide range of soy intakes. Am J Clin Nutr 2008;87:1422–7.

[25] Mateo-Gallego R, Baila-Rueda L, Mouratidou T, De Castro-Oros I, Bea AM, Perez-Calahorra S, et al. Serum plant sterols as surrogate markers of dietary compliance in familial dyslipidemias. Clin Nutr (Edinb Scotl) 2015;34:490–5.

[26] Kruse M, von Loeffelholz C, Hoffmann D, Pohlmann A, Seltmann AC, Osterhoff M, et al. Dietary rapeseed/canola-oil supplementation reduces serum lipids and liver enzymes and alters postprandial inflammatory responses in adipose tissue compared to olive-oil supplementation in obese men. Mol Nutr Food Res 2015;59:507–19.

[27] Marks D, Thorogood M, Neil HA, Humphries SE. A review on the diagnosis, natural history, and treatment of familial hypercholesterolaemia. Atherosclerosis 2003;168:1–14.

[28] Kromeyer-Hauschild K, Wabitsch M, Kunze D, Geller F, Geiß HC, Hesse V, et al. Perzentile für den Body-mass-Index für das Kindes- und Jugendalter unter Heranziehung verschiedener deutscher Stichproben. Monatsschrift Kinderheilkd 2001;149:807–18.

[29] Rufer CE, Bub A, Moseneder J, Winterhalter P, Sturtz M, Kulling SE. Pharmacokinetics of the soybean isoflavone daidzein in its aglycone and glucoside form: a randomized, double-blind, crossover study. Am J Clin Nutr 2008;87:1314–23.

[30] Soukup ST, Al-Maharik N, Botting N, Kulling SE. Quantification of soy isoflavones and their conjugative metabolites in plasma and urine: an automated and validated UHPLC-MS/MS method for use in large-scale studies. Anal Bioanal Chem 2014;406:6007–20.

[31] Friedewald WT, Levy RI, Fredrickson DS. Estimation of the concentration of low-density lipoprotein cholesterol in plasma, without use of the preparative ultracentrifuge. Clin Chem 1972;18:499–502.

[32] Wong JM, Kendall CW, Marchie A, Liu Z, Vidgen E, Holmes C, et al. Equol status and blood lipid profile in hyperlipidemia after consumption of diets containing soy foods. Am J Clin Nutr 2012;95:564–71.

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