

Bovine Peptic Casein Hydrolysate Ameliorates Cardiovascular Risk Factors in a Model of ApoE-deficient Mice but not Overweight, Mildly Hypercholesterolaemic Men

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ABSTRACT

Associations have been shown between consumption of bovine dairy and decreased prevalence of metabolic related disorders. Milk peptides may promote both angiotensin-I- converting enzyme (ACE) inhibition for blood pressure (BP) lowering and insulin action for better glycaemic control. Less is known of other metabolic parameters. The aim of this study was to investigate effects of dairy peptic casein hydrolysate (CH) on markers of cardiovascular disease (CVD) risk in (1) an apolipoproteinE (ApoE) - deficient mouse model of high-fat fed hypercholesterolaemia, and, (2) a clinical study of moderate overweight and hypercholesterolaemia. In Trial 1, ApoE-deficient mice were supplemented with high dose CH (~1g/kg body weight) in a randomised, 9-wk, parallel design intervention, and blood and tissue samples harvested. In Trial 2, 24 mildly hypercholesterolaemic men were supplemented with lower dose CH (~0.1g/kg body weight, 10g/day, 3-wks) and matched whey protein control (WP, 10g/day, 3-wks) in a randomised, 9-wk, cross-over design intervention. Diets were separated by a 3-wk washout. Fasting blood and urine samples were collected, and blood pressure (BP) measured weekly. Clinical trial registration number, ACTRN 12611001013954. In ApoE-deficient mice, administration of CH significantly inhibited circulating total cholesterol concentrations by 37% (TC, P<0.01) and decreased aorta atherosclerotic lesion score by 25% (P<0.01). In the clinical study there were no significant differential effects of CH supplementation on CV markers, including serum lipids (TC, LDL-C, HDL-C, triglyceride), glucose and BP. Whilst high dose bovine peptic CH attenuated CVD risk in a murine ApoE deficient model of aggressive hypercholesterolaemia, no evidence of amelioration of risk by supplementation with a lower dose of CH in an overweight population of mildly hypercholesterolaemic men was found.

Key words: Casein hydrolysate, ApoE deficient mice, Overweight, Hyperlipidaemic, Men, Serum cholesterol.

INTRODUCTION

There is a wealth of evidence to show that dietary intervention can modify disease risk, and longterm health outcomes. Obesity, adverse

metabolic health, type 2 diabetes (T2D) and cardiovascular (CV) risk have long been associated with energy dense, higher fat diets, whilst conversely there is growing evidence in support of higher protein diets for weight loss¹ and amelioration

of metabolic risk. The positive associations between cow's milk and metabolic health that have been shown in observational studies may well be driven by dairy protein^{2, 3, 4-7} likely to be acting through a favourable impact on body weight and body composition, a consequence of enhanced satiety and promotion of lean mass⁸, as well as through direct metabolic effects such as dairy hypotensive angiotensin-I- converting enzyme (ACE) inhibitory peptides⁹⁻¹¹ and promotion of insulin secretion¹². Inhibition of dipeptidyl peptidase-IV (DPP-IV) activity¹³, a mechanism utilised in the pharmaceutical incretin-based therapies for control of hyperglycaemia and T2D¹⁴, may underlie the insulin effects. These insulinotropic effects also have the potential to alter lipaemia since insulin inhibits hormone-sensitive lipase and release of free fatty acids (FFA)¹⁵ and there are several studies which have shown milk peptides to decrease serum levels of triacylglycerol^{8, 16, 17}. Less is known of possible hypocholesterolaemic effects of milk proteins, particularly casein^{18, 19} although some positive findings have been reported for whey protein fractions^{16, 20, 21, 22}, recently leading to the development of lactostatin (Ile-Ile-Ala-Glu-Lys), a bioactive peptide derived from α -lactoglobulin in cow's milk with reported hypocholesterolemic activity higher than the phytosterol and pharmaceutical *b*-sitosterol,^{23, 24}. Certainly other dietary proteins, in particular soybean protein, have long been shown to favourably alter levels of circulating lipids²⁵⁻²⁷, although the hypocholesterolaemic effect size has more recently been questioned²⁸. Experimental studies using hydrolysed soy protein have shown stimulation of low-density lipoprotein receptor (LDL-R) transcription to contribute to the mechanism²⁹.

Casein is the predominant protein in bovine milk accounting for ~80% of the total protein content of whole milk. Screening conducted within our laboratory identified hydrolysed casein as a putative bioactive protein fraction with positive effects on blood lipids. In order to assess possible cardioprotective effects, the aim of this study was to firstly administer oral peptic casein hydrolysate (CH) in an ApoE knockout mouse model of human atherosclerosis³⁰⁻³⁶. If CH was found to be efficacious in this extreme murine phenotype of exaggerated lipid abnormalities, the second aim

was to conduct a clinical study to investigate the effects of peptic CH on adverse metabolic health in overweight men identified with increased CV risk through mild hypercholesterolaemia, known to be characteristic of large numbers of adults globally³⁷.

MATERIALS AND METHODS

ApoE-deficient mouse model

The apolipoprotein E knockout (ApoE^{-/-}) atherosclerotic mouse model, previously studied in our laboratory³², was used to investigate the effects of bovine CH on serum and tissue markers of CVD risk. ApoE^{-/-} mice have decreased circulating levels of serum ApoE and exhibit exaggerated lipid abnormalities and atherosclerosis even on a low-fat, low-cholesterol diet. 3-wk old female C57BL/6 mice were bred and housed in the Animal Resource Unit, Faculty of Medical and Health Sciences, University of Auckland, Auckland, New Zealand. The mice were kept in an air-conditioned room with controlled humidity, temperature, and a 12 hour light: dark cycle. All experiments were conducted under a protocol approved by the Animal Ethics Committee at the University of Auckland.

Diets

After weaning at 3-wks of age, 12 mice were maintained on conventional lab chow (Harlan Teklad Global 2018, 24 en% protein, 18 en% fat, 58 en% CHO, 13 kJ/g) for a 3-wk period. One group of 6 animals was then randomised to a Harlan Teklad TD88137-based high-fat diet (15 en% protein, 42 en% fat; 43 en% CHO, 19 kJ/g; 0.2% w/w cholesterol) used to induce atherosclerosis for a further 9-wks, whilst the second group of 6 animals was randomised to the TD88137-based high-fat diet containing ~1g/kg body weight/day CH [10g/kg, ~1% w/w of total diet; ~5% w/w of total protein]. The total protein content of the diet was maintained constant between the TD88137 diets, hence CH was substituted for protein (=casein). The Harlan Teklad Global 2018 lab chow was purchased from Harlan Teklad (Indianapolis, IN, US). The TD88137-based high-fat control and +CH diets were produced locally by Plant and Food Research Ltd (Palmerston North, New Zealand). The composition of the rodent diets are shown in Table 1. To produce the CH supplement, a 5% total solids solution of mineral acid casein was prepared by dissolving

bovine casein in sterile water. This was then heated to 37°C (pH 3.8-4.0), and mixed for 1 hour in a Cowles dissolver (MorehouseCowles, Chino, CA, US) at an agitator speed setting of 1460 rpm. The pH was then adjusted to 3.0 using ~2mL of 10% HCl and remixed for a further 1 hour in the Cowles dissolver. Pepsin was then dissolved using water and the reaction was initiated by adding the peptic solution to the mineral acid casein. An enzyme substrate ratio of 0.05:1 on a weight to weight basis was used. Over the following 4.5 hours, the pH was checked at 30 minute intervals and maintained at pH 3.0 by adding 20% HCl as required. The pre-dissolved pepsin was then added and the solution was re-adjusted to pH 3.0. The reaction was allowed to continue for the next 14.5 hours. At 20 hours the enzyme was inactivated by adjusting the pH to 7.5 with 20% NaOH. The final product was evaporated to 20% total solids using a thin film evaporator and spray dried, and was expected to be fully soluble. CH replaced a proportion of the casein fraction of the diet, which was the sole protein component, by direct substitution.

Blood and tissue analyses

At baseline (day 1) and at 9-wk follow-up, animals were fasted overnight and blood collected by tail bleeding into heparinized Drummond capillary tubes (Drummond Scientific Company, Broomall, PA USA), sealed and centrifuged to obtain plasma. Total cholesterol (TC) was measured using a Lipotrend-C meter (Boehringer Mannheim, Germany) and Lipotrend cholesterol strips (Roche Diagnostics, Basel, Switzerland). Tissue samples from the thoracic aorta were collected and processed for en-face lesion analysis. The thoracic aorta was selected as the anatomic site of investigation since lesion development in either the distal abdominal or the caudal aorta was not observed in these 15-wk old mice. The dissected aorta was fixed with 4% paraformaldehyde in PBS (pH 7.4), and the proximal part of the aorta up to the aortic root was isolated and cleaned of adventitial fats and connective tissue. The aorta was cut longitudinally, opened and pinned onto a black silicone jelly plate. The lesions were clearly visible without staining, and were analyzed using two methods: (i) in the first method unstained aorta were scored based on a linear ranking system from a minimum of zero (0) for no lesion development to

a maximum of 4.0 for severe lesion development, ie where greater than 60% of the total area of the dissected thoracic aorta was covered by the lesion; (ii) in the second method images of the thoracic aorta were recorded and analyzed with Image J software (National Institute of Health, US)³⁸ in order to calculate the total lesion area. Lesion area was then expressed as a percentage of the total luminal surface area, as described by Kauser *et al.*³⁹.

Clinical study

Participants

Participants for the clinical study were recruited at the Human Nutrition Unit in Auckland, New Zealand via newspaper and poster advertisement and via e-mail circulation. Inclusion criteria were male gender, aged 18-70 yrs, with increased risk of CVD identified by mildly raised LDL-C (>3.0 mmol/L). Exclusion criteria included significant lipid, hypertension and metabolic disorders, including diabetes mellitus, which required clinical intervention or pharmaceutical treatment, and/or previous CV events. This study obtained ethical approval from the Northern X Regional Ethics Committee, Auckland, New Zealand., and all participants gave written informed consent to participate. The trial was registered with the Australian New Zealand Clinical Trials Register ACTRN 12611001013954.

Protocol

This was a 2 treatment, single blind, randomised, cross-over study. A total of 24 mildly hypercholesterolaemic men consumed beverages containing CH or WP for 2 periods of 3-wks, shown in diet intervention studies to be of sufficient duration to detect clinically significant changes in lipid profile^{40,41}. Each diet treatment was separated by a 3-wk washout. Participants came to the research clinic for a screening visit where written informed consent was obtained and medical history, demographics and anthropometry recorded. A fasting blood sample was drawn to review serum biochemistry. During each 3-wk treatment period eligible participants attended the clinic on 6 occasions. Visits included a baseline (day 0) visit, for completion of registration and randomisation procedures, and 5 follow-up visits on day 1, day 7, day 14, day 21, and day 22. At each visit participant compliance, body weight and blood pressure were

recorded, and a urine and a fasting blood sample collected. Adverse events and concomitant medications were also recorded. Two blood samples were collected at baseline (d0, d1) and at the end of the intervention (d21, 22) to minimise the within-subject variability at these critical time points. Samples were stored at -80°C until later batch analyses of blood lipids and glucose. Urine samples were analysed for uric acid and creatinine as markers of poor metabolic health.

Diets

Participants were given beverages containing 10g of CH or 10g of WP daily, which they consumed as part of their diet. Based on the ApoE rodent study where high doses of bovine milk proteins were administered in order to show a significant amelioration of hypercholesterolaemia, the intent of the clinical study was to deliver a high but tolerable daily dose of CH to the participants. The CH was prepared using the same methods as the rodent study, described in full above, where mineral acid casein at 5% total solids in RO water was hydrolyzed with pepsin at pH 3.0 and 37°C for 19 hours. An enzyme substrate ratio of 0.05:1 on a weight to weight basis was used. The reaction was stopped by adjusting pH to 7.5 and the resultant hydrolysate was evaporated and spray dried. The hydrolysate was given to the participants dissolved into a 500mL grapefruit drink. The method of delivery for the 2 supplements was developed in-house and designed to match for flavour and bitterness of taste as closely as possible. Masking the intensely bitter flavour of protein hydrolysates is a significant issue in clinical studies, since only µg quantities of protein hydrolysates such as CH can be consumed in the absence of flavour masking. Prior to commencement of the clinical trial, we conducted a pilot study which demonstrated that natural bitter taste of fresh grapefruit juice entirely masked the bitterness of the CH supplement (results not shown). CH is relatively insoluble in water and required a long period of mixing (>30 minutes) in order to ensure solubility within the 500mL water volume prior to administration to the participant.

Statistical power and analysis

An *a priori* power analysis was performed, using the data from a previous 3-wk dietary intervention trial in hyperlipidaemic men conducted

within our laboratory, in order to provide estimates of variance components. The analysis used a similar study design to the current CH trial, where repeat blood samples were collected at baseline prior to start of treatment, at weekly intervals, and at the final follow-up visit (averaging the two baseline values, d0/d1, and the last two values). The analysis concluded that a sample size of n=24 had the power to detect an effect size of 10% of the baseline mean LDL-C concentration as significant. Data analysis in the clinical study was performed on the 24 participants, who completed both arms of this cross-over intervention. Outcome variables including the primary outcome LDL-C were analysed using repeated measures ANOVA, where effects of treatment, time and the interaction between treatment*time were investigated. Baseline data was collected by repeat sampling over 2 days (d0/d1) prior to the start of the intervention and were combined and expressed as a mean value. Repeat sampling time-points at the end of the intervention (d21/d22) however were treated as individual days and were not combined. Descriptive statistics are presented as mean, SD. Efficacy data is presented as mean, SEM. The level of statistical significance was set at P<0.05.

RESULTS

ApoE^{-/-} knockout mouse model

As expected, 6-wk old ApoE^{-/-} mice fed the TD88137-based high-fat control diet for a period of 9-wks developed multiple lipid plaque lesions within the lumen of the thoracic aorta, as illustrated in Figure 1. The mean atherosclerotic lesion score in this group of animals was 3.7 which, based on a visual scale for which the maximum score was 4.0, represented a high % coverage of the total luminal surface by aortic lesion. Supplementation with ~1% w/w CH over a period of 9-wks both significantly inhibited the circulating concentrations of TC by 37% (P<0.01, Figure 2A) and also significantly decreased the lesion score by 25% (p<0.01, Figure 2B) when compared to the control high-fat fed animals. Lesion area, expressed as a percentage of the total luminal surface, also tended to decrease in the CH fed animals (Figure 2C), but this change did not reach statistical significance. Conversely, the CH supplemented diet had no effect on bodyweight over the 9-wk period, nor was there

evidence of any significant change in either spleen or liver weight (all, $P > 0.05$, data not shown).

Clinical Study

Twenty five male participants were randomised into this cross-over study. One

participant completed only the first arm of the study due to relocation overseas, and was replaced within the randomisation scheme. Hence, in total, 24 participants completed the 2 periods of supplementation with CH and WP, with a minimum washout period between each treatments of 3-wks

Table 1: Composition of diets for the ApoE-deficient mouse model and the clinical study of mild hypercholesterolaemia

Diet	ED kJ/g	Protein g	CH g	WP g	Protein En %	Fat En %	CHO En %
ApoE-deficient mouse study							
Harlan Teklad Global 2018, post weaning	13	186 ^{\$}	0	0	24	18	58
TD88137-based high-fat diet, control	19	173 [#]	0	0	15	42	43
TD88137-based high-fat diet + CH	19	173 [#]	10 [#]	0	15	42	43
Clinical study							
CH supplement, 10g/day	17	10	10 [*]	0	100	0	0
WP supplement, 10g/day	17	10	0	10 [*]	100	0	0

ApoE mouse study: Harlan Teklad 2018 diet was provided to all animals for 3-wks post weaning, after which they were randomised to TD88137 control or TD88137 + casein hydrolysate, CH; ED, energy density; WP, whey protein; CHO, carbohydrate; en%, percentage of energy; + CH, supplemented with ~1g/kg body weight/day CH [10g/kg, ~1% w/w, of total diet; ~5% w/w of total protein]; \$, g/kg; #, g/kg, casein as sole protein source; Clinical study: CH and WP supplements, *10g/day, in addition to protein content of regular home diet

Table 2: Baseline characteristics of the 24 overweight male participants who completed both arms of the intervention

	Mean	SD	Range
Age (y)	41.5	11.3	18-63
Body weight (kg)	89.8	13.9	65.9-112.3
BMI (kg/m ²)	28.1*	3.6	22.4-34.6
Waist circumference (cm)	94.7*	10.8	76-114
SBP (mmHg)	133*	13	106-160
DBP (mmHg)	78	9	59-93
Total cholesterol (mmol/L)	6.3*	1.2	4.6-9.8
LDL-C (mmol/L)	4.2*	0.9	3.1-5.7
HDL-C (mmol/L)	1.2	0.2	0.9-1.6
TAG (mmol/L)	2.1*	1.3	0.6-5.4
TC:HDL-C ratio	5.4*	1.0	3.5-7.3
Glucose (mmol/L)	5.0	0.5	4.5-6.9
Urine uric acid (mmol/L)	3.4	1.3	1.5-6.5
Urine creatinine (mmol/L)	13.9	5.2	6.6-24.8

BMI, body mass index; SBP, systolic blood pressure; DBP, diastolic blood pressure; LDL-C, low density lipoprotein cholesterol; HDL-C, high density lipoprotein cholesterol; TAG, triacylglycerol. *above normal range as defined by: BMI < 25 kg/m²; Waist circumference < 94 cm; SBP/DBP < 130/85 mmHg; Total cholesterol < 5.0 mmol/L; LDL-C < 3.4 mmol/L; TAG < 1.7 mmol/L; TC:HDL-C ratio < 4.5

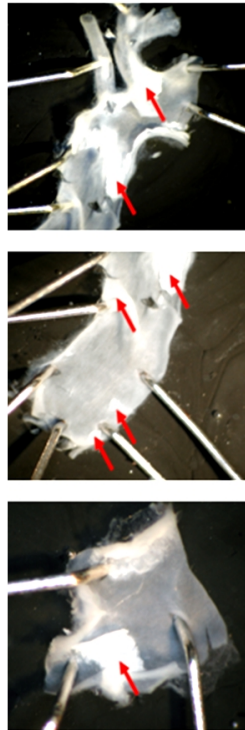


Fig. 1: Lipid lesions in the thoracic aorta of ApoE^{-/-} mice. 6-wk old ApoE-deficient mice fed the TD88137-based control diet for 9-wks developed sites in their thoracic aorta that were extensively coated with lipid (bright white areas marked by arrows)

when they returned to their regular unsupplemented diet. A cohort of 12 participants were randomly allocated to CH in phase 1 and then crossed over to WP in phase 2, and vice versa for the alternate cohort of 12 participants. Baseline characteristics for the 24 participants who completed the intervention are shown in Table 2. The group were middle aged, overweight males with some evidence of central obesity as demonstrated through mean waist circumference above the normal range. Mean, SD age was 41.5, 11.3 years, mean body mass index (BMI) was 28.1, 3.6 kg/m², mean waist circumference was 94.7, 10.8 cm, and mean systolic blood pressure (SBP)/diastolic blood pressure (DBP) was 133/78, 9 mmHg. Analysis of blood biochemistry showed the group to have normal fasting glucose and confirmed the presence of mild hyperlipidaemia, based on TC (6.3, 1.2 mmol/L), calculated LDL-C (4.2, 0.9 mmol/L), triglyceride (TAG, 2., 1.3 mmol/L) and TC:HDL ratio (5.4, 1.0).

At baseline there was no significant difference between CH and WP treatments for any of the measured serum lipids or plasma glucose (mean d0 + d1, all, P>0.05). There was no evidence that CH significantly altered any of TC, LDL-C, HDL-C, or TAG (Figure 3) when compared with WP over the 3-wk intervention period (treatment*time, all, P>0.05). There was also no significant differential

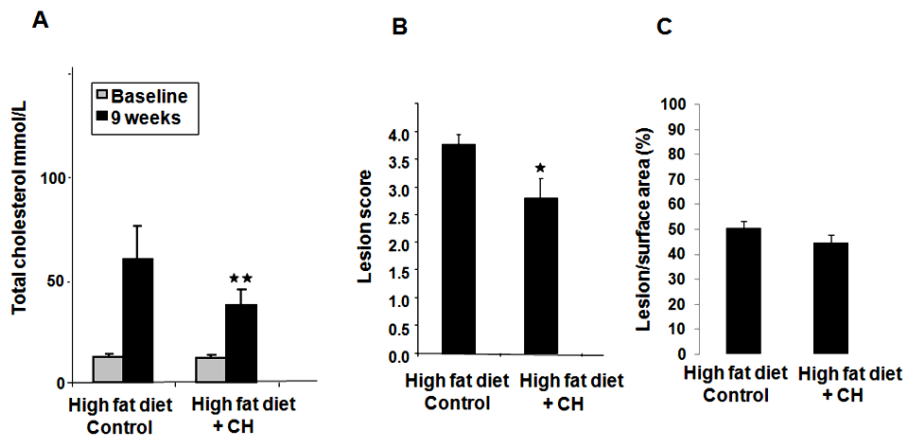


Fig. 2: Effect of a casein hydrolysate (CH) supplemented diet on markers of cardiovascular risk in the ApoE^{-/-} mouse. 6-wk old ApoE-deficient mice were fed a high-fat control diet control or a high-fat diet supplemented with ~1% CH, for 9-wks. Serum total cholesterol was measured (panel A); the size and number of lipid plaques in the aorta were measured to give a lesion score, 0-4.0 (panel B); and the lesion cover as a percentage of total luminal surface was calculated (panel C). Baseline refers to serum total cholesterol levels at day 1 (ie. start of high-fat feeding). *P<0.05, **P<0.01, treatment effect

change in circulating concentrations of plasma glucose between the 2 diets (treatment*time, $P>0.05$). A non-significant decrease from baseline was observed in both SBP and DBP over the 3-wk

intervention period on both treatments, but there was no differential effect of CH relative to WP control (treatment*time, $P>0.05$). There was also no evidence that CH significantly altered urinary excretion of urate or creatinine (data not shown).

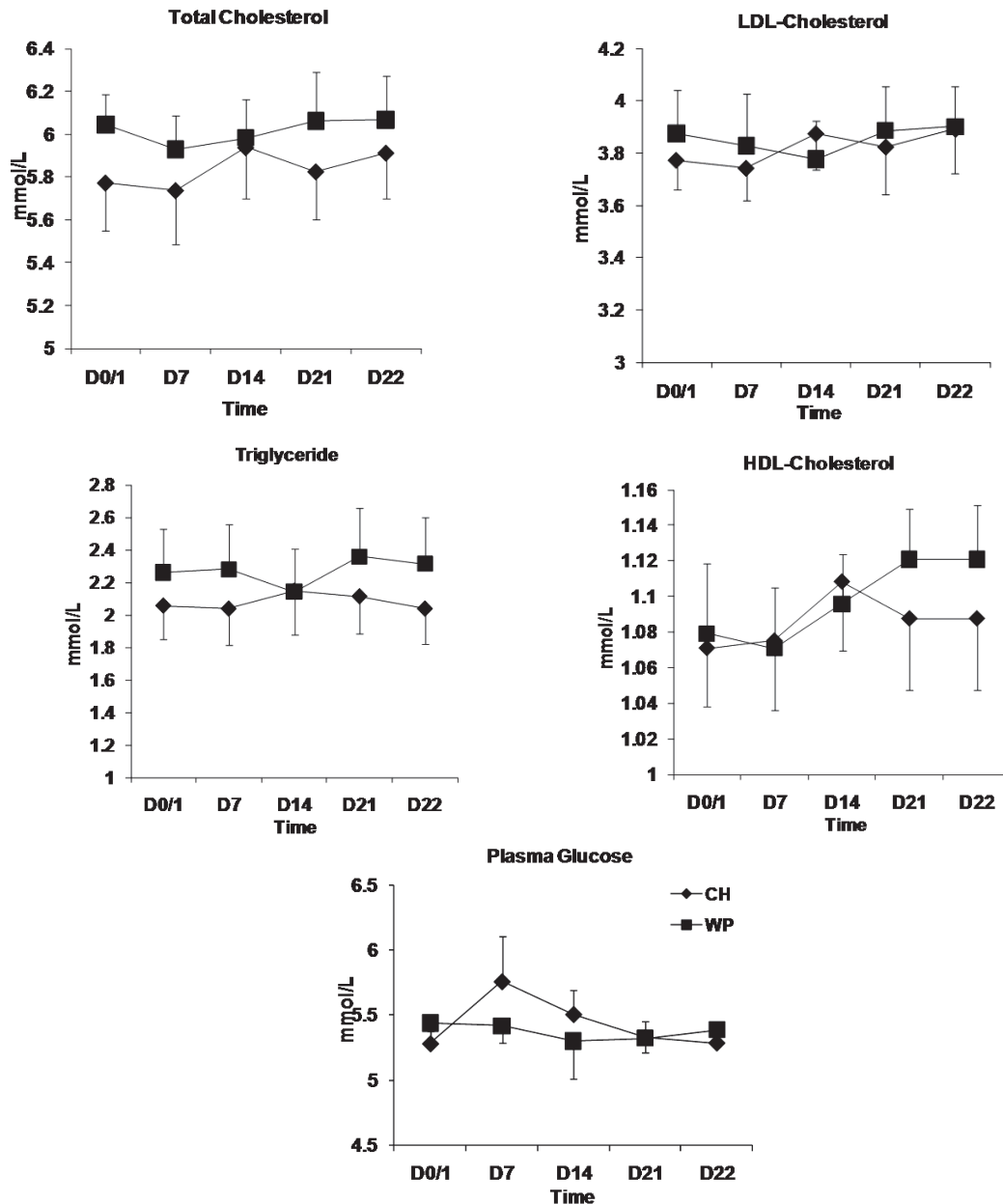


Fig. 3: Effect of casein hydrolysate (CH, Δ %) and whey protein (WP, \square %) supplementation on serum lipid and glucose profiles in a group of men identified with mild hypercholesterolemia, based on raised LDL-C. Participants were supplemented for a 3-wk period on 2 separate occasions, during a 9-wk randomised cross-over study. No significant difference (treatment*time, $P>0.05$, ns) for any of the measured variables between treatments. D 0/1 (mean of samples collected on days 0/1, pre-intervention baseline) ; D, day

DISCUSSION

In this study of ApoE^{-/-} deficient mice we showed that a ~1% CH-supplemented diet fed for a period of 9-wks significantly ameliorated the spontaneous increase in plasma total cholesterol which is the characteristic phenotype of this rodent model of hyper-cholesterolaemia. The effect was large, with the 37% suppression of total cholesterol similar to that shown to be achieved through statin therapies⁴², and this led us to conduct a clinical study the aims of which were to identify protective effects of CH at a lower, palatable dose in a representative adult population with early stage, moderate hypercholesterolaemia. In addition to the suppression of total cholesterol concentration, the ApoE rodent model also showed a significant decrease in atherosclerotic lesion score within the thoracic aorta of CH-fed animals. It is important to note that dietary casein is not known to protect against atherosclerosis in rodents, as compared with other proteins, in particular soy which is widely regarded as protective in experimental^{29, 43} and clinical²⁵ studies, raising the possibility that substitution of casein with CH serves only to decrease possible pro-atherogenic activity of dietary casein⁴⁴. Unlike typical soy-based mouse chows, a casein-based purified diet contains no phytoestrogens or other phytochemicals known to influence atherosclerosis and lipoprotein metabolism in various rodent models^{43, 45-51}, and hence has been considered to provide a neutral 'reagent' for such studies. Given that in our current trial CH replaced just 5% (w/w) of the total casein content of the diet, it is unlikely that such a small decrease in dietary protein can explain the large suppression in total cholesterol. We propose a more probable scenario is that casein digested with pepsin may give rise to caseinopeptides that are further resistant to digestion, and thus may display anti-atherogenic activity. Similar effects are known with soybean protein hydrolysate where it has been proposed that soy peptides stimulate LDL-R transcription in the liver and decrease circulating cholesterol concentrations²⁹.

We then proceeded to evaluate the effects of 3-wks supplementation with CH and WP in a group of overweight, mildly hyperlipidemic male participants, who otherwise had an unrestricted diet

and physical activity pattern. We found that after 2-wks of supplementation with both CH and WP treatments there was a trend for SBP and DBP to decrease relative to baseline levels, but the decrease was less than 5% with no evidence of a differential hypotensive effect of the CH beverage. This was surprising since previous clinical studies have reported antihypertensive effects of dairy-derived protein hydrolysates^{11, 52} including casein^{52, 53}, which *in vitro* evidence shows to contain ACE-inhibitory peptides^{54, 55}. Dairy-derived CH contains a number of hypotensive peptides including Val-Pro-Pro (VPP) and Ile-Pro-Pro (IPP)^{9, 56, 57} and the 'C12' peptide^{52, 53}, shown to be efficacious when given as encapsulated peptides and/or in tablet form to rats and humans for periods of weeks to months.

In addition, in contrast to the findings in the ApoE-deficient mouse model of hypercholesterolaemia where a CH-supplemented diet significantly decreased total cholesterol by 37%, there was no evidence of cholesterol lowering in our clinical study. CH did not alter the serum levels of total or LDL-cholesterol over a 3-wk period in this group of overweight, hypercholesterolaemic men. The trial was well powered and whilst GI effects may have differed between the rodent and clinical models, it is also possible that 10 g/d of CH given over a 3-wk period may be an insufficient dose given for insufficient duration. Whilst high for a clinical study, this dose was 10 fold lower than that shown to be effective in our ApoE model where CH was given for 9-wks. Body weight, BMI, and markers of blood chemistry were also not differentially affected by the 3-wk administration of CH. Of particular interest was the hypothesised effect of CH on fasting plasma glucose concentrations, since several of the participants in this study were identified as overweight with raised levels of fasting glucose, and commercial hydrolysates claiming improved glucose control in diabetic patients have previously appeared in the market place, including the extensively hydrolysed casein product InsuVital^{TM58}. However, no evidence of glucose lowering was found in our clinical study. No changes were also found in any urine parameters measured including urine uric acid levels, of interest due to the association with dyslipidaemias, and predictive of the risk of acute inflammatory arthritis, ie. gout. High

levels of uric acid, the final oxidation product of purine metabolism, are commonly associated with visceral obesity, insulin resistance, T2D and hypertension as well as dyslipidaemia. Notably, dairy has been associated with decreased risk of gout, postulated to act through inhibition of inflammatory response to monosodium urate crystals within the joint^{4,59}. In conclusion, data from the high-fat fed ApoE-deficient mouse model, an aggressive model of hypercholesterolaemia, showed that CH given at a high dose for a prolonged period of 9-wks significantly ameliorated circulating levels of total cholesterol by 37% and decreased atherosclerotic lesion score by 25%. However in a clinical model of moderate hypercholesterolaemia characterized by raised levels of LDL-C in overweight men, there was no evidence that a lower dose of CH given for 3-wks significantly improved lipid profile or the associated markers of adverse metabolic health measured in this study.

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Disclosures

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Statement of Authors Contribution to Manuscript

Y-KC: Management of the clinical trial intervention, participant screening, recruitment & registration, data entry, preparation of manuscript

ATM: Protocol design, human ethics approval, participant safety including adverse event monitoring, preparation of manuscript

RKK: Conduct of animal studies, data interpretation

GWK: Design, protocol, ethics approval and conduct of animal studies, data interpretation, preparation of manuscript

NH: Preparation of dairy protein fractions

LX: Data handling, preparation of manuscript

SDP: Protocol design, human ethics approval, trial oversight, data interpretation, preparation of manuscript, senior author

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