

## Original Article

# HDL subfraction changes with a low-fat, plant-based Complete Health Improvement Program (CHIP)

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**Background and Objectives:** Low HDL concentrations are considered an important risk factor for cardiovascular disease. Interventions promoting a low-fat, plant-based eating pattern appear to reduce CVD risk while paradoxically also reducing HDL concentrations. Recent studies show HDL to comprise a range of subfractions, but the role these play in ameliorating the risk of CVD is unclear. The purpose of this study was to characterise changes in HDL subfractions in participants where HDL decreased following the CHIP intervention which promotes a low-fat, plant-based diet, with physical activity. **Methods and Study Design:** Individuals (n=22; mean age=55.4±16.3 years; 45.5% men, 54.5% women) participating in a CHIP intervention were assessed at baseline and 30 days for changes in BMI, blood pressure, lipid profile, (including large-, intermediate- and small-HDL subfractions) and fasting glucose. **Results:** HDL significantly decreased (10.6%,  $p<0.001$ ) together with BMI (2.5%,  $p=0.028$ ), systolic blood pressure (7.1%,  $p=0.005$ ), total cholesterol (9.5%,  $p=0.002$ ), LDL (11.2%,  $p=0.007$ ) and fasting glucose (8.2%,  $p=0.028$ ). Triglycerides (TG) did not significantly change. Physical activity (22.7%,  $p=0.016$ ) and consumption of whole plant-foods (13.9%,  $p=0.003$ ) significantly increased, while non-plant (energy and animal) foods decreased (43.1%,  $p=0.009$ ). Large-, intermediate- and small-HDL decreased (-10.0%,  $p=0.003$ ; -8.3%,  $p=0.013$  and 22%,  $p=0.005$ , respectively). **Conclusions:** This paper discusses specific changes in HDL subfractions when overall-HDL decreases as a response to low fat, whole-food, plant-based eating and exercise. Additional research is required to elucidate the reasons through which behavioural therapies remodel the HDL particle and how this impacts the functional properties of HDL and CVD risk.

**Key Words:** HDL subfractions, CHIP, diet, CVD risk, behaviour

## INTRODUCTION

Population studies have shown an inverse association between HDL concentrations and CVD.<sup>1</sup> Consequentially, the National Cholesterol Education Program has advocated increasing HDL concentrations as an important strategy for the primary prevention of CVD.<sup>2</sup> The consistently strong inverse association between low HDL concentrations and the risk of cardiovascular events observed in epidemiological studies have traditionally been explained by its role in reverse cholesterol transport (RCT) from peripheral tissue to liver, also known as cholesterol efflux.<sup>3</sup> In addition, HDL protects LDL from oxidation, and has anti-atherogenic properties, mediated by various anti-inflammatory, anti-apoptotic, anti-thrombotic, vasodilatory and anti-infection mechanisms.<sup>4</sup>

Despite these documented anti-atherogenic properties, there is conflicting evidence that questions the simple direct relationship between HDL concentrations and risk of cardiovascular events. For example, many individuals who suffer coronary atherosclerotic events have normal or even elevated HDL concentrations.<sup>5</sup> Furthermore, when HDL concentrations are raised pharmacologically, they do not always correlate with reduced risk of coronary heart disease (CHD).<sup>6</sup> Other epidemiological studies

have shown that individuals who consume a low fat, plant-based diet are at lower risk of CVD and type 2 diabetes mellitus, despite having lowered HDL concentrations.<sup>7,8</sup> Patients placed on a behaviour change intervention, that incorporated this dietary regime, showed improvement in measured coronary artery percent diameter stenosis and symptomatic angina, despite reductions in HDL concentrations.<sup>9</sup>

The value of pharmacologically increasing HDL concentrations alone has been further questioned as the diverse functions of HDL have become better understood.<sup>5,10</sup> Recent studies have shown HDL to be more complicated in both structure and function than first thought. Fractionation by ultracentrifugation has shown that human HDL can be largely separated into two major

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subfractions, HDL2 (large HDL) and HDL3 (small HDL), with further subpopulations existing within these subfractions.<sup>4</sup> These subpopulations exhibit substantial differences in their array of lipids (lipidome) and proteins (proteome) resulting in variations in size, density, structure and composition, as well as metabolic and functional roles.<sup>11</sup> These particles undergo continuous remodelling through interactions with various enzymes, lipid transfer proteins and cell surface proteins.<sup>11</sup>

There is currently no consensus as to the clinical benefits of the various HDL subfractions. Several large-scale epidemiologic studies have investigated the risk of CHD when HDL was separated by size. In some studies, the smaller, denser HDL3 particles are associated with favourable atheroprotective functions and clinical outcomes, including protection from CHD.<sup>11-13</sup> In others, the lighter large HDL particles appear to be linked to antiatherogenic functions,<sup>14</sup> and are inversely associated with hypertension,<sup>15</sup> CHD and atherosclerosis.<sup>16,17</sup> Furthermore, Asztalos et al,<sup>18</sup> suggested that the large HDL subfraction is inversely associated with disease burden, while the role of small HDL is unclear, proposing that some particles in the subpopulation are atheroprotective, and others are positively associated with CVD.

While the positive association of diet modification (e.g. low calorie, low fat, vegetable rich) with reduced cardiovascular risk is well documented, the relationship of this dietary change to changes in HDL subfractions has not been previously investigated. Recently we reported that when individuals underwent the CHIP intervention, which incorporates a low-fat, plant-based diet, average HDL concentrations decreased despite improvements in all other measured markers of cardiovascular risk including blood pressure, BMI, total cholesterol (TC), LDL, TG and fasting plasma glucose (FPG).<sup>19</sup> The purpose of this study was to therefore characterise the changes in HDL subfractions in individuals participating in the CHIP intervention, where HDL decreased.

## METHODS

### Participants

This study, without a reference group, evaluated the pre- to post-biometric changes of 30 individuals (mean age  $56.4 \pm 15.1$ ; 40% men, 60% women) who self-selected to participate in a CHIP intervention conducted in a community center in New South Wales, Australia. Following the intervention, HDL was found to decrease in 22 individuals (mean age =  $55.4 \pm 16.3$  years; 45.5% men, 54.5% women), and increase in eight individuals (mean age  $59.3 \pm 11.6$ , 25% men, 75% women). The difference in age was not statistically significant ( $p=0.542$ ). There were no inclusion/exclusion criteria other than the participant being able to pay a \$AUD399 program cost. Participants were invited to attend the intervention through word of mouth invitation, local media avenues and advertising through local health care providers. Consent for the study was obtained from Avondale College of Higher Education Human Research Ethics Committee (Approval No. 20:10:07). As the purpose of this study was to explore changes in HDL subfractions among participants of the CHIP intervention, only those individuals whose HDL decreased were included in the analysis.

### Intervention

The CHIP intervention is a well-published intervention shown to reduce selected risk factors associated with chronic disease.<sup>20</sup> Volunteers who had previously undertaken an eight-hour training course facilitated the intervention. The intervention involved 12 group sessions over 4 weeks, conducted in a community hall. Each session was approximately 1.5 hours in duration and involved viewing a pre-recorded lecture presented by a health expert, cooking demonstrations and interactive group activities. The intervention had a nutrition focus, but the content of the program also addressed physical activity (advocating at least 30 minutes or 10,000 steps as measured by pedometers supplied to each participant) and elements from the positive psychology literature such as stress management and emotional wellbeing.

The eating pattern prescribed in the program was low-fat by the standards of national dietary guidelines. This was achieved by encouraging participants to move towards a whole food, plant-based diet ad libitum, with emphasis on the consumption of whole-grains, legumes, fresh fruits and vegetables. This diet was recommended in order to achieve a daily target of fewer than 20% of calories from fat and less than 10 teaspoons of added sugar, one teaspoon of salt (87 mmol of sodium) and 129  $\mu$ mol of cholesterol. Participants were also encouraged to consume 2-2.5 L of water daily.

### Outcomes

Before participating in the CHIP intervention (baseline) and then again at 30 days (post-intervention), participants' height, weight, and blood pressure were taken. In addition, fasting (12-hour) blood samples were collected by trained phlebotomists and analysed for TC, LDL, HDL, HDL subfractions, TG and FPG concentrations.

At baseline and again at 30 days, participants were also asked to complete a personal behaviour questionnaire with self-reported diet and physical activity, to assess compliance to the principles advocated by the CHIP intervention. Regarding physical activity, participants were asked to indicate how many times per week they performed at least 30 minutes of light, moderate or strenuous activities. Similarly, with diet, participants were asked to indicate how many serves of 21 different foods were consumed per week or per day (whichever was more appropriate) on average in the preceding 2 weeks. The foods included to measure dietary compliance to the CHIP principles included whole grain cereals, processed cereals, meat, fish, eggs, nuts/seeds, dairy, dairy alternatives, legumes, potatoes, other vegetables, vegetable soup, salads, fruit, sweet snacks/desserts, fast/take away foods, fruit juice, caffeinated drinks, soft drinks/cordials, alcohol and water.

### HDL fractionation

The Quantimetrix Lipoprint System™ HDL Subfractions Kit (Redondo Beach, CA; Catalog No. 48-9002) was used to separate and measure HDL cholesterol subfractions, using the 4-30% gradient polyacrylamide gel tube electrophoresis method. This method was able to resolve up to ten subfractions of HDL, which were grouped into three categories: Large HDL subfractions 1-

3, Intermediate HDL subfractions 4-7 and Small HDL subfractions 8-10, relative to particle size.

### Data analysis

The data were analysed using IBM<sup>TM</sup> Statistics (version 19) and expressed as mean  $\pm$  standard deviation (SD). Personal behaviour was assessed by average weekly self-reported physical activity performed for at least 30 minutes and average dietary intake, including alcohol consumed over the last 2 weeks, through categorical frequency questionnaires. Smoking status was assessed by the questions relating to never smoking, past smoking (including years since quitting) and current smoking (including average cigarettes smoked per day). Physical activity was measured by converting the three categorical variables of light, moderate and strenuous physical activity into separate continuous variables and then summing these to give a weekly frequency physical activity performed for at least 30 minutes. For dietary intake the categorical variables of 19 separate foods and drinks were converted to continuous variables by determining the midpoint of the range for each category and then summing the frequency of intake to create three separate scales: 1. plant foods (wholegrain cereals, nuts, dairy alternatives, legumes, potatoes, other vegetables, salad, vegetable soup and fruit), 2. energy foods (processed cereals, sweets, fast food, juice, soft drinks, caffeinated drinks) and 3. animal foods (meat, fish, eggs, dairy). A continuous variable for alcohol was also created by the method used for the FFQ described above. The extent of changes (baseline to post-intervention) in the biometric risk factors and behavioural factors was assessed using paired t-tests. The relationships between 30-day and change in HDL subfractions, were separately explored with the other biometrics, diet and physical activity using ANCOVA. The changes in each of the HDL subfractions were explored as these self-control for variation in baseline and 30-day HDL subfractions. Three ANCOVA models (one analysis for each HDL subfraction) - controlling for age, gender, relevant baseline HDL subfraction, as well as change in physical activity, diet scales, BMI and lipids were then conducted. As FPG was highly correlated with BMI ( $r=0.827$ ,  $p<0.001$ ) and TC was highly correlated with LDL ( $r=0.955$ ,  $p<0.001$ ), FPG and TC were not added to the regression model. In order to explore the direction of change in the relationships found between the separate HDL subfractions, and other lipid biometrics, diet and physical activity, these were characterised by whether the HDL subfraction increased or decreased and then examined using Pearson product-moment correlation coefficient. For all analyses, results were considered significant at  $p<0.05$ .

## RESULTS

### Cohort demographics

Ten men and 12 women commenced and completed the 30-day intervention. There was no significant difference in the age of male and female participants ( $53.1\pm 19.1$  versus  $57.3\pm 14.2$ ,  $p=0.565$ ). Of these 22 participants, 17 had never smoked, while five were former smokers (range in years since quitting: 4-43 years). Twenty one of the participants never consumed any type of alcohol, with

the remaining participant reduced their consumption from 5 drinks per week at baseline to one per week at 30 days.

### Biometrics

Significant mean reductions were recorded in six of the eight biometric risk factors (including HDL) at 30 days, with the exception of diastolic blood pressure (DBP) (almost reached significance) and TG (Table 1). Participants with the highest initial concentrations of HDL ( $\geq 1.3$  mmol/L versus  $<1.3$  mmol/L) experienced the greatest decreases in HDL in the 30 days [ $0.21\pm 0.13$  mmol/L (11.3%,  $n=12$ ) versus  $0.10\pm 0.08$  mmol/L (9.1%,  $n=10$ );  $p=0.035$ ].

### HDL and its subfractions

Intermediate HDL comprised the greatest concentration of subfractions at baseline and 30 days, with small HDL the lowest concentration (Table 2). All three HDL subfractions decreased over the 30 day intervention, with the greatest relative decrease in small HDL (-22.7%) and the smallest decrease in intermediate HDL (-8.3%) (Table 2).

### Relationships between 30-day HDL subfractions, biometrics and behavioural factors

All participants completed the 30-day CHIP intervention. Mean physical activity of at least 30 minutes at a time increased by more than 20% over the 30 days (Table 1). The majority of participants (87%) made 17 of 21 (80%) changes towards the recommendations of the CHIP intervention to increase plant foods and decrease animal and energy foods. Overall frequency of consumption of plant foods increased about 14%, while animal and energy foods decreased by more than 40% over the 30 days (Table 1).

As there were no participants who were current smokers and all former smokers had quit at least 4 years prior to the study, the ANCOVA model was not adjusted for smoking. Nor was it adjusted for alcohol consumption as there was only one drinker who reduced their consumption from five drinks per week to one drink. After adjusting for age, sex, BMI, TG, LDL, 30-day plant foods, animal foods and energy foods, and baseline concentrations of the respective HDL subfraction, the baseline subfraction measure was the largest predictor of the corresponding 30 day intermediate and large subfraction measure. For small HDL, the baseline HDL subfraction, was the largest predictor of 30-day small HDL after age (Table 3). In addition to baseline concentrations, 30-day TG was an inverse predictor of 30-day large HDL, while 30-day animal foods was a positive predictor and 30-day energy foods an inverse predictor of 30-day small HDL (Table 3).

### Relationships between change in HDL subfractions, biometrics and behavioural factors

For small HDL, age and baseline small HDL were found to be inverse predictors of change (Figure 1, Table 4). For intermediate HDL, change in LDL directly predicted change in this subfraction, while change in plant foods was an inverse predictor (Figure 1, Table 4). For large HDL, change in TG was an inverse predictor and change

**Table 1.** Mean changes in selected risk factors

Biometric	Participants (n)	Baseline		30 day		Mean change	95% confidence interval	% change	t statistic	p value
		Mean	SD	Mean	SD					
BMI (kg/m <sup>2</sup> )	22	29.8	9.95	29.1	9.41	-0.74	-1.39, -0.09	-2.5	-2.36	0.028
SBP (mmHg)	22	137	31.9	127.3	22.5	-9.68	-16.0, -3.35	-7.1	-3.18	0.005
DBP (mmHg)	22	77.1	11.8	72.8	7.52	-4.27	-8.75, 0.20	-5.5	-1.99	0.060
TC (mmol/L)	22	4.94	1.07	4.47	0.95	-0.47	-0.74, -0.20	-9.5	-3.59	0.002
LDL (mmol/L)	22	2.84	0.92	2.52	0.76	-0.32	-0.54, -0.10	-11.2	-3.01	0.007
HDL (mmol/L)	22	1.51	0.55	1.35	0.50	-0.16	-0.21, -0.11	-10.6	-6.24	<0.001
TG (mmol/L)	22	1.29	0.63	1.32	0.69	0.02	-0.17, 0.21	1.6	0.23	0.820
FPG (mmol/L)	22	5.69	1.53	5.22	1.11	-0.46	-0.87, -0.05	-8.2	-2.36	0.028
Physical activity	22	8.34	3.20	10.23	3.87	1.89	0.39, 3.38	22.7	2.26	0.016
Plant foods	22	44.3	17.9	50.4	14.2	6.14	0.54, 11.74	13.9	2.28	0.003
Non-plant†	22	11.9	9.04	6.75	8.27	-5.11	-8.84, -1.39	-43.1	-2.86	0.009

BMI: Body mass index; SBP: Systolic blood pressure; DBP: Diastolic blood pressure, TC: Total cholesterol; LDL; Low-density cholesterol; HDL: High-density cholesterol, TG: Triglycerides; FPG: Fasting plasma glucose

†Non-plant refers to animal and energy foods combined.

**Table 2.** Mean changes in HDL subfractions (mg/dL)

HDL particle size	Participants (n)	Baseline		30 day		Mean change	95% confidence interval	% change	t statistic	p value
		Mean	SD	Mean	SD					
Large	22	19.5	12.4	17.5	12.8	-1.95	-3.18, -0.73	-10.0	-3.31	0.003
Intermediate	22	31.3	8.45	28.7	7.19	-2.59	-3.91, -1.27	-8.3	-4.09	0.001
Small	22	7.82	2.91	6.05	2.79	-1.77	-2.93, -0.61	-22.7	-3.18	0.005

**Table 3.** Statistically significant demographic, behavioural and biometric associates of 30-day HDL subfractions

HDL subfraction	Associates†	F	p	B (95% CI)	$\eta^2$ (%)‡
30-day Large HDL	Baseline large HDL	510	<0.001	0.977 (0.886, 1.07)	96.4
	30-day TG	10.8	0.004	-2.53 (-4.15, -0.916)	36.2
30-day Intermediate HDL	Baseline intermediate HDL	152	<0.001	0.801 (0.666, 0.936)	88.4
30-day Small HDL	Age	21.0	<0.001	-0.105 (-0.154, -0.057)	55.2
	Baseline small HDL	14.8	0.001	0.468 (0.208, 0.728)	45.8
	30-day energy foods	4.81	0.043	-0.216 (-0.424, -0.008)	22.0
	30-day animal foods	7.84	0.012	0.440 (0.109, 0.772)	31.6

†Covariates in ANCOVA models - Age, sex, BMI, baseline concentration of the relevant subfraction, the energy foods, animal foods and plant foods scales, and 30-day BMI, LDL and TG.

‡ $\eta^2$  partial eta square.

in physical activity a direct predictor of change in this subfraction (Figure 1, Table 4).

We then explored the lipid and behaviour relationships found for intermediate and large HDL by whether these subfractions increased or decreased. For participants where large HDL decreased there was a strong inverse correlation with TG ( $r=-0.564$ ,  $p=0.029$ ,  $n=15$ ), but this was not found for participants where large HDL increased ( $r=-0.001$ ,  $p=0.999$ ,  $n=7$ ). We also found a strong, but not significant correlation with physical activity among participants where large HDL increased ( $r=0.684$ ,  $p=0.090$ ,  $n=7$ ), but not where large decreased ( $r=-0.116$ ,  $p=0.680$ ,  $n=15$ ). For participants where intermediate HDL decreased or increased, no significant relationships were found with change in LDL ( $r=0.247$ ,  $p=0.357$ ,  $n=16$  and  $r=0.133$ ,  $p=0.802$ ,  $n=6$ , respectively). However, a strong positive correlation was found between increases in intermediate HDL and plant foods ( $r=0.862$ ,  $p=0.027$ ,  $n=6$ ), but not with decreases in this HDL subfraction ( $r=-0.077$ ,  $p=0.775$ ,  $n=16$ ).

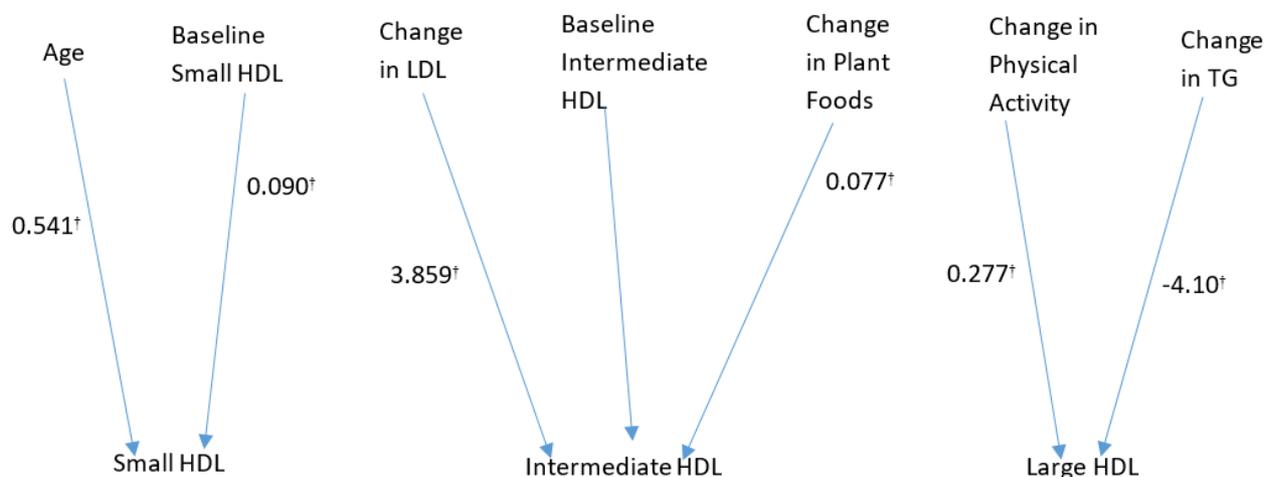
## DISCUSSION

This study confirms our previous findings that when individuals move towards a low fat, plant-based diet with physical activity, HDL concentrations of the majority tend to decrease while all other measures of cardiovascular risk improve, except for TG.<sup>19</sup> These findings are also supported by other epidemiological and clinical studies.<sup>7,8</sup> However, it is not clear why TG did not change in this

study. A meta-analysis of personal behaviour interventions incorporating low fat-high carbohydrate diets, also found an overall increase in TG concentrations.<sup>21</sup> It was suggested that weight loss mobilises energy stored as fat (triglycerides) into the bloodstream, which appear to normalise over time.<sup>22</sup> Furthermore, carbohydrate intake is associated with increased TG.<sup>23</sup>

When we explored the effects of the CHIP intervention on HDL subfractions, we found that among individuals where HDL decreased, intermediate HDL was the most abundant at baseline and post-intervention, with small HDL the least abundant. This is supported by the findings of Sabaka et al.<sup>24</sup> However, other studies, including the Diabetes Prevention Program (DPP) have shown that small HDL is the most abundant subfraction compared to large HDL by a ratio of three or more to one.<sup>13,25,26</sup> In the present study, the concentration of large to small HDL particles at 30-days was almost three to one, the reverse found in the DPP program. We also found that all three HDL subfractions decreased at 30 days, with the decrease in small HDL being about fivefold greater than the decrease in large or intermediate HDL. However, DPP found a 5% decrease in small HDL and together with the PREDIMED (Prevention with Mediterranean diet) study, reported increases in large HDL (17-24%) following their programs.<sup>25,27</sup>

Traditionally, the Mediterranean diet is high in unprocessed plant foods (grains, vegetables, fruits, legumes, nuts/seeds and extra virgin olive oil), moderate in



**Figure 1.** Observed determinants (as reported in Table 4) of change in HDL sub-fractions at 30 days. †β - Parameter estimate.

**Table 4.** Statistically significant demographic, behavioural and biometric associates of change in HDL subfractions

HDL subfraction	Associates <sup>†</sup>	F	p	B (95% CI)	η <sup>2</sup> (%) <sup>‡</sup>
Change in Large HDL	Change in TG	15.5	0.001	-4.10 (-6.28, -1.92)	44.9
	Change in physical activity	4.44	0.049	0.277 (0.002, 0.552)	18.9
Change in Intermediate HDL	Change in LDL	18.6	<0.001	3.59 (1.84, 5.34)	50.8
	Baseline intermediate HDL	25.6	<0.001	-0.255 (-0.360, -0.149)	58.7
	Change in plant foods	5.07	0.037	-0.077 (-0.149, -0.005)	22.0
Change in Small HDL	Age	13.0	0.002	-0.090 (-0.143, -0.038)	40.6
	Baseline small HDL	14.8	0.01	-0.541 (-0.836, -0.246)	43.7

<sup>†</sup>Covariates in ANCOVA models - Age, sex, BMI, baseline concentration of the relevant subfraction, the energy foods, animal foods and plant foods scales, and 30-day BMI, LDL and TG.

<sup>‡</sup>η<sup>2</sup> partial eta square.

fish/shellfish and wine and low in meat, dairy, eggs, animal fats and discretionary foods.<sup>28</sup> On the other hand, the CHIP diet is a whole food, plant-based diet, with emphasis on the consumption of whole-grains, legumes, fresh fruits and vegetables, consumed ad libitum. The DPP did not specify a diet type other than to instruct the participants to choose low calorie and fat substitutes at each concentration of the US Department of Agriculture Food Guide Pyramid, in order to achieve the weight reduction goal of 7% of initial body weight, while also incorporating 150 minutes per week of moderate physical activity.<sup>29</sup> The PREDIMED study was not able to explain which portion of the Mediterranean diet facilitated the increase in large HDL. In the PREDIMED study, total HDL increased 4%, while all other biometrics showed minimal decreases, except TG, which decreased 12% after one year.<sup>27</sup> In the DPP group, total HDL increased 3%, while all other biometrics showed greater decreases than that of the PREDIMED study after one year.<sup>25</sup> However, the decreases in biometrics in both these studies were not as great as were found in the present study, except for TG. It would appear the different dietary and personal behaviour patterns have differing effects on lipids and HDL subfractions.

The relationship becomes more complex when baseline HDL and changes in HDL following the CHIP intervention are considered, as decreases in HDL were greater when baseline HDL was higher. In terms of metabolic syndrome (MetS) risk factors, the participants in the present study started the program with two of the five classic markers for MetS, BMI and FPG; BP, HDL and TG were within the healthy range. By 30 days, FPG had normalised, reducing the number of MetS markers to one. Participants in the PREDIMED and DPP studies commenced their programs with four of the classic markers for MetS (BMI, BP, HDL and FPG versus BMI, HDL, TG and FPG (BP was not measured in DPP); respectively).<sup>25,27</sup> By the end of one year, all elevated risk factors remained elevated in both studies, except for TG in the DPP group, which normalised. Furthermore, baseline concentrations of LDL were higher in these studies than the present study (3.80 mmol/L, 3.21 mmol/L and 2.84 mmol/L; respectively). Exploring baseline and the changes in the various risk factors may help to explain the observed changes in HDL and its subfractions in the PREDIMED, DPP and the present study. Certainly, in the present study, baseline concentrations of some HDL subfractions were strong predictors of change in these subfractions.

Large HDL is processed or catabolised by direct uptake into the liver by scavenger receptor class B1; by undergoing lipolysis by lipases; or by exchange of its cholesteryl ester for TG from apoB-containing lipoproteins via cholesterol ester transfer protein (CETP) (apoB is then taken up by LDL receptors on the hepatocytes).<sup>12</sup> The transfer via CETP is believed to occur under conditions of elevated TG, resulting in HDL particles that are susceptible to hydrolysis by hepatic lipase and reduced plasma HDL concentrations. Consistent with this, we found an inverse association between TG and 30-day large HDL and changes in large HDL in this study.

In terms of antioxidant activity, one proposed role of small HDL is to protect LDL from oxidation.<sup>12</sup> LDL, in

particular oxidised LDL, is associated with CVD and CHD. Given that the dietary regime in the present study was principally low-fat and plant-based with an abundance of antioxidants, it is expected that LDL is less likely to be oxidised. Together with the low-fat intake, the requirement for small HDL for RCT would be lower as LDL is less likely to accumulate in arterial wall macrophages. Indeed, of all the HDL subfractions, we found the greatest decrease in small HDL. Furthermore, we found that LDL decreased to normal levels following the intervention, while LDL only marginally decreased in the DPP and PREDIMED studies, remaining elevated in both interventions (small HDL also only marginally decreased or remained steady in these studies). Whilst we did not find an association between small HDL and plant foods in the present study, we found a direct relationship between small HDL and animal foods. However, we did find a direct relationship between intermediate HDL and plant foods but the implications of this relationship are yet to be determined.

HDL particles may also differ between individuals with different personal behaviours. Alcohol is more strongly correlated with small HDL than large HDL.<sup>30</sup> In the present study, all participants either did not drink alcohol or significantly reduced consumption to less than one serve per week, which may also explain the decrease in small HDL. It is also well recognised that physical activity increases HDL.<sup>31</sup> HDL subfractions may also respond differently to physical activity. Campbell et al (2011), found that large HDL increased and small HDL decreased with continuous or intermittent exercise, being mediated through increases in lecithin cholesterol acyl transferase activity, involved in esterifying the cholesterol in the HDL particle, so that more can be taken up, thereby increasing its size.<sup>32</sup> In the present study, physical activity increased by more than 20% and a direct relationship was found between physical activity and large HDL.

Personal behaviour choice can be complex and interventions to address chronic disease risk factors are heterogeneous. Furthermore, the variety of techniques used to fractionate (and damage) lipid fractions, based on density, size and charge, produces different particle profiles.<sup>11</sup> In addition, HDL is complex, with greater variation in structure, protein composition and physiological function than LDL.<sup>12,33,34</sup> Given that disease states and behavioural factors can affect the remodelling of this family of particles, it is therefore not surprising that outcomes observed across studies create conflicting data on the role of HDL subpopulations on CHD, atherosclerosis and the metabolism of cholesterol.<sup>35</sup>

### **Strengths and Limitations**

The strengths of this study are that the overall 30-day biometric results are comparable to other studies of the CHIP intervention delivered by both health professionals and trained volunteers in the United States and Australasia,<sup>36-39</sup> as well as comparing favourably to other professionally delivered behavioural interventions.<sup>40-42</sup> This study is novel in that it presents changes in HDL subfractions that differ from other published studies and may be explained by the dietary regimen. Another strength of the study is that biometrics were not self-reported but meas-

ured by the same health professionals using the same equipment at baseline and 30 days.

A major limitation of this study is that a reference group was not included. Hence we are not able to determine whether the changes in HDL subfractions were due to the intervention or some other unrelated factor. Another limitation is that self-reported personal behaviours, such as dietary intake and physical activity, carry bias and therefore may have been inadequately measured in the study. Nevertheless, we were concerned with changes in behavioural measures. Given that the participants completed the same questionnaire pre- and post-intervention the reporting bias may have been reduced. Furthermore, the relatively small sample size resulted in many associations though strong, not reaching statistical significance. Further investigation on a larger cohort is warranted. A further limitation was the short follow-up time after which the benefits gained by both groups may have been lost or diminished, such as in the DPP and PREDIMED studies. A small New Zealand study found that 106 CHIP participants who returned for follow-up assessment, on average 4 years after completion of the intervention, were able to maintain improvements in most of their biometrics.<sup>43</sup>

### Conclusion

The literature supports an important role for HDL in ameliorating the risk of CVD, but the role of the various subfractions in this process is still unclear as HDL is a highly complex molecule, both structurally and functionally. The results of this study have provided some valuable insights. We found that HDL decreases as individuals move towards a low-fat, plant-based diet, with physical activity. However, our observation that the individual HDL subfractions (small, intermediate and large) decrease with a plant based diet and physical activity, and are dependent on baseline lipid concentrations, is novel. Furthermore, these HDL subfractions respond differentially to different behavioural factors.

Additional research is required to clarify the role played by disease processes and various behavioural therapies in modelling and re-modelling the proteome and lipidome of the HDL particle and to extend our knowledge of the functional properties of HDL and its various subfractions. This and the development of non-destructive standardised biochemical techniques to differentiate all the HDL subfractions will also aid in providing consistent information on the functional properties of particles and assist in developing therapies to support individuals with a range of chronic disease risk factors.

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### AUTHOR DISCLOSURES

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