Lentil-based diets attenuate hypertension and large-artery remodelling in spontaneously hypertensive rats

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Abstract
Hypertension is a major risk factor for CVD, the leading cause of mortality worldwide. The prevalence of hypertension is expected to continue increasing, and current pharmacological treatments cannot alleviate all the associated problems. Pulse crops have been touted as a general health food and are now being studied for their possible effects on several disease states including hypertension, obesity and diabetes. In the present study, 15-week-old spontaneously hypertensive rats (SHR) were fed diets containing 30 % w/w beans, peas, lentils, chickpeas, or mixed pulses or a pulse-free control diet for 4 weeks. Normotensive Wistar–Kyoto (WKY) rats were placed on a control diet. Pulse wave velocity (PWV) was measured weekly, while blood pressure (BP) was measured at baseline and week 4. Fasting serum obtained in week 4 of the study was analysed for circulating lipids. A histological analysis was carried out on aortic sections to determine vascular geometry. Of all the pulse varieties studied, lentils were found to be able to attenuate the rise in BP in the SHR model ($P<0.05$). Lentils were able to decrease the media:lumen ratio and media width of the aorta. The total cholesterol (TC), LDL-cholesterol (LDL-C) and HDL-cholesterol levels of rats fed the pulse-based diets were found to be lower when compared with those of the WKY rat and SHR controls ($P<0.05$). Although all pulses reduced circulating TC and LDL-C levels in the SHR, only lentils significantly reduced the rise in BP and large-artery remodelling in the SHR, but had no effect on PWV. These results indicate that the effects of lentils on arterial remodelling and BP in the SHR are independent of circulating LDL-C levels.

Key words: Media:lumen ratio; Blood pressure; Aortic remodelling; Non-soya legumes; Spontaneously hypertensive rats

CVD is the leading cause of mortality worldwide(1). Hypertension is a major risk factor for future CVD, and the incidence of hypertension is expected to continue increasing(2). Already 20–30 % of North Americans have been diagnosed as hypertensive(2,3) and as many or more can be classified as prehypertensive(3).

The most diagnosed form of hypertension, accounting for ≥90 % of diagnoses, is essential hypertension(3,4). Essential hypertension, also known as primary hypertension, is high blood pressure (BP) with no known aetiology. The reduction of BP, especially through lifestyle and dietary changes, reduces the risk of CVD, stroke and kidney failure(3). Essential hypertension is inherently difficult to treat using targeted therapies such as those offered by pharmaceutical intervention. Pharmaceuticals, such as angiotensin-converting enzyme inhibitors or β-blockers, which target specific signalling pathways, can alleviate BP with continued dosing, but cessation of treatment usually results in a return to dysregulated BP levels. Additionally, the effectiveness of drug intervention in those people with prehypertension – estimated at over 30 % of the population – is unclear(5). In fact, the seventh report on hypertension by the Joint National Committee suggests that prehypertensive people manage their disease through lifestyle changes, namely diet and exercise, and not through pharmaceutical use(6). Furthermore, people on drugs are often able to reduce medication doses with improved dietary habits(3).

Dietary Approaches to Stop Hypertension diet intake is one such approach for attenuating hypertension in the at-risk or affected population(5,7). Similar diets have been suggested by the American Heart Association and the Public Health Agency of Canada(2,3). Focusing on vegetables, fruits, nuts and low-fat dairy products and avoiding foods high in Na, processed foods and red meat have been shown to be beneficial.
in patients with hypertension(8). Increasing vegetable intake is especially prudent, as an estimated 50% of adults and 70% of children in Canada do not consume the daily recommended amounts of fruits and vegetables(9). Additionally, legumes are underconsumed by individuals on a typical Western diet, with only about one in eight people consuming pulses on a daily basis(10).

Pulse crops, among the first cultivated crops(11,12), are leguminous plants that are grown for reasons other than their oil(13,14). Pulses are protein-dense, high-fibre foods that contain variable levels of phytochemicals and antioxidants depending on the genus, species, and year and region of growth(15–21). In *in vitro* experiments, extracts prepared from pulses have been shown to affect several of the signalling pathways associated with hypertension and vascular remodelling, including the renin–angiotensin–aldosterone system through angiotensin-converting enzyme inhibition(16,22–24). The consumption of pulse crops has been shown to decrease the risk of heart attack, CHD and overall CVD in human subjects(25,26). However, no studies have been carried out to determine whether a specific pulse variety is more effective in vivo.

The spontaneously hypertensive rat (SHR) is the most common animal model of human essential hypertension(27,28). Before the onset of hypertension, SHR experience marked arterial stiffness and arterial remodelling due to aberrant vascular smooth muscle cell growth and/or remodelling(29–31). Following atherosclerosis, arterial stiffness is the best predictor of CVD and mortality(32). Therefore, in the present study, we examined the effects of pulse-based diets on hypertension, arterial stiffness and vascular remodelling in SHR.

**Materials and methods**

**Animals and diet**

For the present study, 15-week-old male SHR and Wistar–Kyoto (WKY) rats were obtained from Charles River. The rats were housed individually under controlled laboratory conditions (12 h light–12 h dark cycle and 40–60% humidity). The rats were acclimatised for a minimum of 12 d using an American Institute of Nutrition 93 growth diet(33) (henceforth: 'control') diet and then fed the experimental diets for 4 weeks. Previous studies have shown that 4-week intervention with diets and/or concentrated plant extracts is sufficient for attenuating BP in SHR(34–36).

Pulses were soaked overnight (except lentils), cooked and then freeze-dried. Freeze-dried pulses were ground using a Retsch ZM 200 mill (Retsch) at 1200 rpm and passed through a 0·5 mm screen before incorporation into the diets. The cooked and freeze-dried pulse powder was added to the diets at 30% w/w (Table 1). This level has been used previously in diets with no adverse effects on the growth of rats(13).

**Table 1. Diet formulations**

<table>
<thead>
<tr>
<th>Ingredients* (g/kg)</th>
<th>AIN-93G control</th>
<th>Bean</th>
<th>Pea</th>
<th>Lentil</th>
<th>Chickpea</th>
<th>Mixed pulse</th>
</tr>
</thead>
<tbody>
<tr>
<td>Casein</td>
<td>200</td>
<td>113</td>
<td>113</td>
<td>113</td>
<td>113</td>
<td>113</td>
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<tr>
<td>Maize starch</td>
<td>397</td>
<td>229·5</td>
<td>229·5</td>
<td>229·5</td>
<td>229·5</td>
<td>229·5</td>
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<tr>
<td>Maltodextrin</td>
<td>132</td>
<td>132</td>
<td>132</td>
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<tr>
<td>Sucrose</td>
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<tr>
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<td>5</td>
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<tr>
<td>L-Cys</td>
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<td>3</td>
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<tr>
<td>Choline bitartrate</td>
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<td>2.5</td>
<td>2.5</td>
<td>2.5</td>
<td>2.5</td>
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<tr>
<td>Mineral mix AIN-93G MX</td>
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<td>35</td>
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<tr>
<td>Vitamin mix AIN-93 VX</td>
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<tr>
<td>Soyabean oil (with 0·02% tert-butylhydroquinone)</td>
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<tr>
<td>Pulse powder†</td>
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<td>Black bean</td>
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<td>75</td>
<td>–</td>
<td>–</td>
<td>–</td>
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<td>White navy bean</td>
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<td>Red kidney bean</td>
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<td>75</td>
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<td>–</td>
<td>–</td>
<td>18·75</td>
</tr>
<tr>
<td>Green pea</td>
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<td>–</td>
<td>150</td>
<td>–</td>
<td>–</td>
<td>37·5</td>
</tr>
<tr>
<td>Yellow pea</td>
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<td>–</td>
<td>150</td>
<td>–</td>
<td>–</td>
<td>37·5</td>
</tr>
<tr>
<td>Red lentil</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>150</td>
<td>–</td>
<td>37·5</td>
</tr>
<tr>
<td>Green lentil</td>
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<td>–</td>
<td>150</td>
<td>–</td>
<td>37·5</td>
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<tr>
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<table>
<thead>
<tr>
<th>Proximate analysis (%)</th>
<th>Moisture</th>
<th>DM</th>
<th>Crude protein</th>
<th>Crude fibre</th>
<th>Fat</th>
<th>Ash</th>
</tr>
</thead>
<tbody>
<tr>
<td>AIN-93G</td>
<td>6·6</td>
<td>93·4</td>
<td>18·6</td>
<td>1·4</td>
<td>6·8</td>
<td>3·1</td>
</tr>
<tr>
<td>* Ingredients ordered from Dyets, Inc.</td>
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<td>† Pulse powders were prepared as described in the Materials and methods section.</td>
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isofibrous and isoenergetic by appropriate adjustments of casein, maize starch and cellulose content. Samples of each diet were sent to Central Testing Labs Limited for proximate analysis (Table 1). SHR were assigned to one of the following six groups (n 8 per group): control (SHR-Ctrl); bean (SHR-B; white navy, black, pinto and red kidney); pea (SHR-P; green and yellow); lentil (SHR-L; red and green); chickpea (SHR-C); mixed pulse (SHR-M; combination of beans, peas, lentils and chickpeas). Normotensive WKY rats were fed the control diet (WKY). The protocol was approved by the University of Manitoba Protocol Management and Review Committee in accordance with proper animal care and experimentation as outlined by the Canadian Council on Animal Care.

Blood pressure measurements

BP measurements, using tail-cuff plethysmography, were taken at baseline and the week before the completion of the study. The rats were acclimatised to the BP apparatus the day before the measurements were taken. The rats were assessed six at a time with five acclimatisation cycles and ten measurement cycles using the CODA™ system (Kent Scientific).

Pulse wave velocity measurements

Pulse wave velocity (PWV) in the femoral artery was measured weekly using a 10 MHz electrocardiogram (ECG)-triggered Doppler probe (Indus Instruments). The rats were anaesthetised using isoflurane, and three measurements per rat were taken per session. Triplicate measurements had an average %CV<7.

Fasting serum analysis

In week 4 of the study, the rats were subjected to a 12 h overnight fast. A saphenous blood sample was collected from the fasted rats, allowed to clot on ice for 1 h, and then spun for 15 min at 1000 g. Fasting serum was separated into aliquots and stored at −80°C until analysed with a Cobas C111 autoanlyser (Roche Diagnostics GmbH). Serum was analysed for total cholesterol (TC), LDL-cholesterol (LDL-C), HDL-cholesterol, TAG, glucose, urica and creatinine levels.

Tissue weights

The rats were killed by sodium pentobarbital overdosing. The peri-renal, mesenteric and epididymal fat pads, along with the livers and hearts, were excised and weighed. The hearts were further dissected to isolate the left ventricle (LV), which was weighed separately. At the time of the completion of the study, a section of the descending aorta was placed into an optimal cutting temperature medium, then frozen in a dry-ice and ethanol bath, and stored at −80°C.

Aortic histology

A cryotome was used to section the aorta into 5 μm sections, three per slide. The slides were kept at −80°C until stained. The slides were fixed in paraformaldehyde for 8 min, washed with 1 X PBS (137 mM-NaCl, 3 mM-KCl, 10 mM-Na2HPO4 and 1.5 mM-KH2PO4) for 10 min and hydrated in double-distilled water for 15 min. Elastin Stain Kit (no. HT25A-1KT, lot no. 120M4344; Sigma Aldrich) was used according to the manufacturer’s protocol to differentiate between elastin (black), muscle (yellow) and collagen (red). Differentiation in working ferric chloride was done for 3 min, followed by Van Gieson’s staining for 90 s to obtain the best contrast. The slides were mounted using VectaMount, and images were captured on an Evos® microscope (AMG) using 4 X and 20 X objectives. ImagePro Plus (Media Cybernetics) was used to measure the lumens circumference and outer-media circumference of the sections. Calculations to determine lumen diameter, media thickness, medial:lumen ratio, media cross-sectional area and external diameter were carried out. On average, two images of non-consecutive aortic sections were analysed per rat.

Statistical analysis

Data were analysed using a repeated-measures ANOVA for BP and PWV and a one-way ANOVA for endpoint data using the Statistical Analysis Systems software package 9.2 (SAS). A post hoc Duncan’s multiple range test was used to determine significance at the P<0.05 level. When necessary, data were log-transformed to achieve normality and homogeneity. The data are reported as means with their standard errors.

Results

Body and tissue weights

At baseline, there were no differences between any of the groups with respect to body weight (BW). At 4 weeks, the SHR groups weighed 8.2% less than the WKY rat group; however, the pulse-based diets did not alter BW (Table 2). The WKY group had higher peri-renal, mesenteric and epididymal fat pad weights than all the SHR groups when normalised by BW. The SHR-B group had lower peri-renal and epididymal fat pad weights than the SHR-Ctrl group. The fat pad weights of all the other SHR groups were between those of the SHR-Ctrl and SHR-B groups and were not significantly different from each other. There were no differences between any of the groups with respect to liver weight.

Blood pressure

At baseline and week 4, the WKY group had significantly lower mean arterial pressure, systolic BP and diastolic BP than all the SHR groups (Fig. 1(A)–(C)). At week 4, the SHR-L group had lower mean arterial pressure than the SHR-Ctrl (144 (SE 8) v. 171 (SE 7) mmHg) and SHR-M (144 (SE 8) v. 177 (SE 8) mmHg) groups, with that of all the other SHR groups falling between the two extremes (Fig. 1(A)). The SHR-L group had lower systolic BP than the SHR-Ctrl group (174 (SE 6) v. 201 (SE 6) mmHg) (Fig. 1(B)) and lower diastolic BP than the SHR-M group (131 (SE 8) v. 163 (SE 8) mmHg) (Fig. 1(C)).
Pulse wave velocity
At baseline, the SHR groups had a higher PWV than the WKY rat group, indicative of stiffer arteries. At weeks 1–4, the WKY group had a significantly lower PWV than all the SHR groups, with no significant changes due to diet being observed (Fig. 1(d)).

Cardiovascular remodelling
All the SHR groups had higher heart weight (6%), heart weight:BW ratio (15%), LV weight (10%) and LV weight:BW ratio (15%) than the WKY group (Table 2). No significant changes were observed for any of the dietary interventions.

There were no differences between any of the groups with respect to lumen diameter or external diameter (data not shown). The WKY group had a smaller media width than all the SHR groups (Fig. 2(a)). The WKY group had a lower media:lumen ratio than all the SHR groups, except the SHR-L group. The SHR-L group had a significantly lower media:lumen ratio than the SHR-B and SHR-C groups (5.22 (SE 0.14), 5.75 (SE 0.11) and 5.75 (SE 0.15), respectively), and the ratios of all the other SHR groups fell in between the values of these groups. The SHR-L group had the smallest media width among all the SHR groups, although it was not significantly different from that of the SHR-Ctrl group (Fig. 2(b)). In Fig. 2(c), representative aortic sections after staining are shown.

Serum analysis
The WKY group had significantly higher fasting TC, LDL-C, HDL-cholesterol, TAG and glucose levels than all the SHR groups (Fig. 3(a)–(e)). The SHR-Ctrl group had significantly higher fasting TC, LDL-C and HDL-cholesterol levels than all the SHR groups on the pulse-containing diets (Fig. 3(a)–(c)). There were no differences among the SHR groups with respect to TAG or glucose levels. The WKY group had lower serum urea levels than all the SHR groups, with no dietary differences being observed (Fig. 3(f)). There were no significant differences between any of the groups with respect to serum creatinine levels (data not shown).

Discussion
Pulse crops and their phytochemical isolates have been widely studied in vitro for their effects on vascular remodelling and oxidative stress, and in vivo for their anti-nutritional components and effects on serum lipid profiles. However, to the best of our knowledge, a study testing the anti-hypertrophic and BP-lowering effects in vivo or one determining which pulse variety has the greatest effect is yet to be carried out. We found that a mixture of red and green lentils had the greatest effect on BP and large-artery remodelling in SHR in the present study. The consumption of pulses at 30% w/w in the diets had no adverse effects during the study period. The pulse-based diet-fed SHR gained weight at a similar rate to the SHR control group throughout the study period.

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and there were no significant differences in BW at the completion of the study. Similar results have been reported previously for Sprague–Dawley rats fed diets containing 30% w/w pulses. In the present study, the SHR consumed approximately 26% of daily energy derived from pulses. With regard to human consumption, Mitchell et al. have reported that the highest quartile of pulse consumers in the USA ate 277 g of pulses per d and had a daily energy intake of 11820kJ; this represents approximately 16% of daily energy derived from pulses based on 686 kJ/100 g of boiled chickpeas. A dose study is necessary to determine whether smaller quantities of lentils (e.g. 15% w/w in a rodent diet) would also be effective for BP reduction and large-artery remodelling in SHR. Higher doses could be achieved in human diets by developing food products containing dehydrated pulse powders, as boiled and drained lentils contain approximately 70% water.

The consumption of pulses has been shown to have a modest hypotensive effect in human subjects. However, in the studies, the subjects were normotensive at baseline, and while consumption of pulses was indicated, the particular pulse variety was not important and it is, therefore, impossible to determine which pulse variety/varieties provided the benefits. In the present study, among all the pulse-based diets tested, the lentil-based diet was found to be able to attenuate the rise in BP experienced by the SHR model to the greatest extent. The lentil-based diet-fed rats experienced a 1 mmHg rise in systolic BP compared with the 26 mmHg rise experienced by the SHR control group. With respect to diastolic BP, the lentil-based diet-fed group exhibited an increase of 10 mmHg compared with that of 30 mmHg observed in the control group. This is a step towards supporting anti-hypertensive claims for lentils based on in vitro data. Pulses contain phenolic compounds exhibiting antioxidant effects when assayed in vitro, but they may also act as selective inhibitors of angiotensin-converting enzyme, endothelin-1 and matrix metalloproteinases. These effects could explain the reduced BP and remodelling observed in the

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**Fig. 1.** (A) Mean arterial pressure, (B) systolic blood pressure and (C) diastolic blood pressure at baseline and week 4. (D) Pulse wave velocity at baseline and weeks 1–4. Mean values with unlike letters were significantly different (P < 0.05). WKY, Wistar–Kyoto (WKY) rats; SHR, spontaneously hypertensive rat (SHR) controls; SHR-bean group; SHR-pea group; SHR-lentil group; SHR-chickpea group; SHR-mixed group.
pulse-based diet-fed SHR, as angiotensin II activity and oxidative stress are among the major driving forces behind both these events\(^{(45,46)}\).

The fact that there was no change in arterial stiffness, as indicated by PWV measurements, is puzzling, given that PWV measurements have been shown to be closely associated with BP parameters (systolic BP, mean arterial pressure, diastolic BP and pulse pressure) in rats\(^{(47,48)}\) and human subjects\(^{(49,50)}\). The study carried out by Cosson \textit{et al.}\(^{(47)}\) involved simultaneous, invasive measurements of PWV and BP in SHR and WKY rats and generated significant correlations between PWV and BW, heart rate and mean blood (arterial) pressure. Similar correlations have been shown in human subjects when BP and PWV parameters were obtained simultaneously\(^{(49,50)}\). In the present study, BP and PWV measurements were not taken simultaneously; PWV was measured in anaesthetised rats and BP was measured through a non-invasive procedure in conscious rats. As a result, it was not possible to normalise PWV data against BP data. BP fluctuations during PWV measurements might explain the reason for not observing potential, small reductions in PWV in the present study, but this cannot be determined due to the non-simultaneous nature of the data collection process. It is also possible that there were no changes to detect, although lentils did attenuate aortic remodelling, which also contributes to PWV.

Rodent models are typically not as susceptible to atheromatous plaque development as are human arteries\(^{(51)}\), but rodent arteries still undergo remodelling in a manner similar to human arteries despite their morphological differences. The lentil-based diet was able to attenuate aortic remodelling to the greatest extent among all the pulse-based diets, leading to the smallest media:lumen ratio in all the SHR groups, which was not significantly different from that of the normotensive control group. The media:lumen ratio has been shown to increase as a result of hypertension, diabetes and arterial stiffness\(^{(52)}\). Although obtaining these data requires an invasive approach, the media:lumen ratio is the best marker of vascular remodelling and has been proposed as a possible indicator of hypertension treatment effectiveness\(^{(52)}\).

Despite the significant changes in arterial remodelling observed in the aorta on consumption of the lentil-based diet, there was no effect on heart or LV weights as a result of consuming any pulse-containing diet. LV remodelling is a common morphological response to chronic hypertension, with a more muscular wall required to pump blood into the higher-pressure environment in the arteries\(^{(53)}\). The absence of a dietary response in this regard could be attributed to individual variability, time or duration of the intervention.
It is possible that the 4-week duration was not long enough to observe changes in LV remodelling or that remodelling in the rats at this age was past the point of reversal by diet alone.

All the pulse-based diets were able to reduce serum TC and LDL-C levels in SHR compared with the control diet. Similar results with respect to cholesterol levels have been observed in human interventions, with an 8% reduction in TC levels when 150 g of pulses were consumed per day. This is probably due to the high fibre content of the pulses. However, according to proximate analysis, the pea-based diet was the only diet containing more amounts of crude fibre than the control diet. The fibre content of the control diet is mainly attributable to cellulose, an insoluble fibre. The pulse-based diets had some amounts of cellulose, but also had a mixture

Fig. 3. Fasting serum (A) total cholesterol (TC), (B) LDL-cholesterol (LDL-C), (C) HDL-cholesterol (HDL-C), (D) TAG, (E) glucose and (F) urea levels. a,b,c Mean values with unlike letters were significantly different (P<0.05). WKY, Wistar–Kyoto; SHR, spontaneously hypertensive rats.
of soluble and insoluble fibres derived from the pulses themselves. The addition of soluble fibre could be the reason for the decreased serum cholesterol levels observed in the pulse-based diet-fed rats, as soluble fibre has been reported to be associated with lower circulating cholesterol levels (54). Pulse crops also contain anti-nutritional compounds – such as saponins – that have been reported to be associated with hypocholesterolaemia (14–16,22). This is especially important because serum LDL-C is a well-researched risk factor for atherogenesis and future CVD in humans and reducing circulating LDL-C level is considered to be an important clinical outcome for treatment (55). Whether the observed changes were mediated through antioxidant (16,18,56), anti-remodelling (57) and/or angiotensin-converting enzyme inhibitory effects remains to be determined (23,24). However, the fact that LDL-C levels were decreased by all the diets without a parallel improvement in vascular function clearly indicates that cholesterol is not the target through which lentils are able to positively affect blood vessel remodelling.

Conclusions

A diet rich in pulses should be part of a healthy, well-balanced lifestyle. All pulses were able to decrease the levels of circulating cholesterol, a known risk factor for CVD; however, only lentils were able to affect the pathways involved in vascular remodelling and hypertension. Since the mechanism by which these changes are mediated has yet to be determined, additional studies to determine whether lentils can affect resistance-artery remodelling, endothelial dysfunction and the mechanism of action for these changes are warranted.

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