

A study to evaluate chalcureb® a standardized powder derived from the sap of the *Angelica keiskei* (Ashitaba) on markers of health in adults with metabolic syndrome

Abstract

Introduction: Ashitaba, also known as *Angelica keiskei*, is a plant found to contain a class of physiologically active flavonoids known as chalcones, that have shown anti-obesity and anti-diabetes effects.

Objective: The goal of this pilot study was to evaluate the effects of Ashitaba, also known as *Angelica keiskei* (ChalCurb®), an ingredient derived from the ashitaba sap on aspects of metabolic health in 60 adults, 30 men 30 women with aspects of metabolic syndrome.

Methods: Subjects were randomly assigned to take either a 220mg capsule of supplement of Ashitaba, also known as *Angelica keiskei* (ChalCurb®) once a day with dinner, or to take a placebo for 12 weeks. BMI, waist circumference, HgA1c were done at screening to ensure subjects qualifications for the study. Quality of Life (QoL), visceral fat, lipids, HgA1c, body composition, and ghrelin were assessed at baseline and end of study. Gender comparison was done

Results: Change in visceral fat was not different between the groups. There was a moderate impact for men, not women, on ChalCurb® compared to placebo [baseline score $11.0 \pm 0.4 \text{ cm}^2$; Day 56 $10.2 \pm 0.4 \text{ cm}^2$ ($p=0.080$ with an effect size value of 0.49) and Day 84 score of $10.4 \pm 0.5 \text{ cm}^2$ ($p=0.069$ with effect size of 0.50)]. There were no overall or gender effects on body composition. There were no significant changes in total cholesterol, HDL or LDL. Small changes were observed in triglycerides in the ChalCurb® group. There was no impact on mood states, fatigue or vigor. Glucose control was not different between the groups. Ghrelin was positively impacted by the ChalCurb® intervention for both genders. (ChalCurb® Baseline 525.6 ± 44.4 to 491.3 ± 45.5 pg/dl vs. Placebo 457.1 ± 46.2 to 515.7 ± 55.0 pg/dl; $p=0.062$ with an effect size of 0.06 (ANCOVA)).

Conclusion: This study demonstrated that ChalCurb® may have a positive impact for those with metabolic syndrome. ChalCurb® may reduce visceral fat in men and lower ghrelin in both genders. Further research is warranted.

Keywords: ashitaba, metabolic syndrome, body composition, mood states, visceral fat

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Introduction

There is a growing trend for the use of medicinal plants for a variety of health concerns. One prevalent health concern is obesity. This is perhaps for good reason; in the United States from 2011 to 2012, nearly two-thirds of adults were obese or overweight.¹ Both pharmaceutical and behavioral interventions have had a limited success rate, suggesting that prevention may be the ideal approach. Ashitaba, also known as *Angelica keiskei*, is a plant found to contain a class of physiologically active flavonoids known as chalcones, which are abundantly found in the sap and to a lesser extent in the leaf and roots.²⁻⁷ Of the more than 20 chalcones that are found in ashitaba sap, two are 4-hydroxyderricin (4-HD) and xanthoangelol (XA).²⁻⁵ Specifically, *in vitro* and *in vivo* studies have demonstrated the anti-obesity and anti-diabetic actions of ashitaba and its associated active chalcones.³ In a recent study, it was found that administration of isolated 4-HD from ashitaba in hyperglycemic KK-Ay mice resulted in modest suppression of elevated blood glucose levels without adverse side effects.⁵ Compared to the complete suppression of the development of diabetes via pioglitazone administration, daily consumption of

ashitaba has been considered beneficial to hyperglycemic individuals not undergoing drug treatment for diabetes.⁵

Additionally, phenolic properties of compounds have been linked to vascular relaxation and antioxidant activity.^{8,9} For example, methanol leaf extracts from *F. deltoidea* have been found to correlate with antioxidant activity and enhance insulin-stimulated glucose uptake *in vitro* studies.⁹⁻¹¹ During an 8-week intervention testing the effects of *E. guineensis* and *F. deltoidea* leaf extract in adults with pre-diabetes, it was found that *F. deltoidea* significantly decreased both total and LDL cholesterol concentrations and *E. guineensis* significantly lowered waist circumference.⁹ Since 4-HD and XA contain phenolic properties, ashitaba is a hopeful, natural candidate for the prevention of diabetes in individuals with metabolic syndrome.

Therefore, the goal of this exploratory study was to evaluate the effects of Ashitaba, also known as *Angelica keiskei* (ChalCurb®), an ingredient derived from the ashitaba sap that is standardized to 3% 4-HD and 5% XA, on visceral fat, body composition, lipids, energy levels and blood sugar control in adults with metabolic syndrome.

Materials and methods

In a randomized double-blind, placebo-controlled manner, 60 adults were enrolled in this pilot trial. The sixty adults were comprised of 30 men and 30 women, so that both study groups would end up having 15 men and 15 women, for a total of 30 subjects per group. Subjects were randomly assigned to take either a 220mg capsule of Ashitaba, also known as *Angelica keiskei* (ChalCurb®) once a day with dinner, or to take a placebo for 12 weeks. The major study inclusion criteria included the following:

- a. Male or Female subjects, aged 35–70 years, nonsmoker.
- b. Subject exhibited indicators of metabolic syndrome according to the following criteria:
- c. Abdominal (central) obesity: waist circumference ≥ 40 inches (102cm) for males and ≥ 35 inches (88cm) for females.
- d. Body Mass Index (BMI) of 25.0–40.0 kg/m² (inclusive)
- e. In addition, one of the following indicators of metabolic syndrome:
- f. Raised blood pressure (BP): systolic BP > 120 and < 160 or diastolic BP > 80 and < 90 mmHg;
- g. Triglyceride (TG) level > 150 mg/dl;
- h. High-density lipoprotein (HDL) level < 40 mg/dl for men and < 50 mg/dl for women;
- i. Fasting glucose ≥ 100 mg/dL (5.6 mmol/L);
- j. HgA1c 5.7 to 7.0%.
- k. Female subjects were required to agree to use one of the following medically acceptable contraceptive methods from the Screening visit through the end of the study, unless otherwise stated. Acceptable methods include: abstinence, same sex-partner, double barrier (condom, diaphragm, or cervical cap with spermicidal foam, gel, or cream); intrauterine device with or without hormones in place or hormonal contraception (oral, injectable, implantable, transdermal, or vaginal) used consecutively for at least 3 months prior to Screening visit; vasectomized partner or bilateral insertion of Essure® implants for at least 6 months prior to the Screening visit; bilateral tubal ligation, hysterectomy, bilateral oophorectomy, or postmenopausal status with amenorrhea (no menses) for at least 1 year prior to the Screening visit.

Study visit flow

After signing an Informed Consent, subjects underwent an in-person screening visit (to determine if the individual met the study inclusion/exclusion criteria) followed by the Randomization visit and two additional follow-up Study visits, including the End of Study visit.

The Screening visit included review and signing of the Informed Consent, medical and surgical history questionnaire, medical and supplement usage check, gender, age, ethnicity, tobacco (smoking) history, anthropometrics (height, weight, BMI), waist circumference measurement (standardized technique), laboratory tests (including metabolic panel, CBC with differential, lipids, and HgBA1c. If subject met the screening inclusion/exclusion criteria, they were randomized at the next study visit.

The Randomization visit allowed for baseline testing, along with administering quality of life (QoL) questionnaires. Follow up Study Visits at Day 56 and Day 84 (End of Study) allowed for monitoring of compliance, monitoring of adverse events, concomitant medication review, repeat testing for visceral fat, body composition, lipids, HgBA1c and ghrelin, while also allowing for repeat QoL testing.

Anthropometrics and morphology

Waist circumference was measured by standard method^{12,13} as follows:

- I. Stand and place a tape measure around the person's middle, just above their hipbones.
- II. Make sure tape is horizontal around the waist.
- III. Keep the tape snug around the waist, but not compressing the skin.
- IV. Measure the waist just after person breathes out.
- V. Take a minimum of two measurements; if first two measurements are the same, no need for further measurement. If measurements differ by > 1 cm or $\frac{1}{2}$ inch, redo and use the average of the three measurements for the final measurement.

Visceral body fat measurement

Visceral fat was measured by Ultrasound (Accusons2000 with 4C1 transducer, Siemens, Erlangen, Germany), this includes measure of fat surrounding intra-abdominal organs via ultrasound at Randomization visit (within -14 days of visit), follow-up visit (Day 56) and EOS visit (Day 84). Visceral Fat Measurement - Measurements of visceral fat thickness was performed with the patient positioned in a supine position, and the convex 3–4 MHz transducer cross-sectionally placed on the midline, 1cm above the umbilical scar, during the expiratory phase, without pressure on the abdomen in order not to distort the measurement. Measurement was made utilizing an Accuson s2000 with 4C1 transducer. Visceral fat thickness corresponds to the distance in centimeters between the posterior surface of the linea alba and the plane of the posterior aortic wall. It is convenient to observe that the linea alba is many times thick, allowing the distinction between the anterior and posterior surfaces, or many times thin, showing up on the images as a trace. In the latter case, the trace is considered as the anterior and posterior surfaces for the purpose of measurement. Generally, there is accumulation of extraperitoneal fat on the midline, right under the linea alba, showing up as hypoechoic and ellipsoid image; for the purposes of measurement, such fat is included in the visceral fat thickness. The aorta is usually located at left from the midline, and once its image is identified, a horizontal line is drawn, passing through its posterior wall up to the midline. At the intersection of such a line with the line from the linea alba (first caliper) the second caliper is positioned to measure the visceral fat thickness. The measurement calculation and determination was made by a board-certified radiologist and expressed in cm².^{14,15}

Body composition

This included measurement of percent body fat, fat mass and fat-free mass in pounds via ultrasound at Randomization visit and End of Study visit (Day 84). The purpose was to learn if the intervention had any effects on overall body composition over the course of the study. Body composition was measured by standardized Ultrasound technique (Intelamatrix BodyMetric Pro, Brentwood, CA. <http://>

intelamatrix.com/FullSite/Science-Validation.html). Validated ultrasound technology was utilized for body composition analysis. The handheld ultrasound (2D, <3MHz transducer) has been validated for measuring body composition in a wide variety of populations. Body composition was determined by utilizing the seven points for body composition analysis as outlined by Jackson-Pollack. In addition, with the site-specific known fat mass (in mm) and fat-free mass (in mm), overall body composition was determined. The subject remained standing during all measurements.¹⁶⁻¹⁸

Profile of mood states (POMS)

Measures present mood/energy state by a list of adjectives. POMS measures six dimensions of affect or mood, including tension-anxiety, depression-dejection, anger-hostility, vigor-activity, fatigue-inertia, and confusion-bewilderment. This study used the vigor-activity and fatigue-inertia subsets as efficacy endpoints. Subjects were asked to use a 5-point Likert scale (0=not at all, 1=a little, 2=moderately, 3=quite a bit, 4=extremely), to review a list of feelings and rank the degree to which they are experiencing each feeling. For the vigor-activity subset, the higher the score, the greater the vigor-activity. For the fatigue-inertia subset, the lower the score, the less the fatigue-inertia.¹⁹⁻²¹

Safety and exploratory efficacy laboratory tests

Metabolic - Glucose, HgBA1c, Lipids (Total Cholesterol, HDL, LDL and Triglycerides) were measured. Overall blood sugar control was measured by change in HgBA1c. In addition, to the aforementioned tests, ghrelin levels were also measured at Randomization and End of Study.

Chemistry - Included BUN, Creatinine, AST, ALT, ALP, total protein, albumin, globulin, GGT, total bilirubin, calcium, chloride, CO₂, sodium, potassium. A screening serum pregnancy test was also performed on female subjects.

Hematology - Included RBC, WBC, Hgb, Hct, MCV, MCH, MCHC, RDW, Platelets, MPV and differential.

Blood sampling and handling

Blood samples were collected for the Screening visit and End of Study (Day 84) visit, including the exploratory/lipid, chemistry and hematology panels. If the subject had blood test results from screening deemed unacceptable for participation by the Investigator, he/she was not enrolled. The time and date of collection of each blood sample was recorded. After the blood samples were drawn into the vacutainer tubes, they will be processed in accordance with site SOPs to ensure sample integrity. Samples were transported and processed by the research site's local laboratory staff. Blood samples were also collected at the Randomization visit and the end of study for Ghrelin. Ghrelin levels were analyzed by Keystone Bioanalytical Labs (Contract Bioanalytical Laboratory, Keystone, PA). Ghrelin samples collected in BD P800 tubes, #366421 (8mL) and #366420 (2mL). After the blood sample was drawn, the tube was gently inverted 8 to 10 times to allow the sample to mix with the anticoagulant. Samples were kept on ice until processed. Centrifuge at approximately 1100–1300RCF for 10 and 20 minutes respectively, for the 2 and 8mL tubes, at approximately 4°C. The plasma was transferred into two appropriately labeled polypropylene screw cap tubes using a disposable pipette. Samples were equally split. Labels contained protocol/study number, subject number, study day and nominal time.

The time from blood collection to centrifugation was no more than 30minutes.

Plasma samples were placed in a storage freezer at -70°C+10°C or on dry ice within 60minutes of blood collection. Samples remained in the freezer until they were ready to be shipped to the bioanalytical laboratory for analysis.

All non-ghrelin blood tests were conducted and analyzed by the research site's local laboratory (Mercy Hospital Laboratory, Springfield, MO). Ghrelin was analyzed by a contract laboratory, Keystone Bioanalytical Labs (Keystone, PA). All blood tests were conducted under standard conditions following standard laboratory practices.

Statistical approaches

Pharmacodynamic analysis

The main efficacy analysis was conducted on a Per-Protocol (PP) basis, using the per protocol population. This study was considered a pilot exploratory study.

The main efficacy assessments involved tests of ChalCurb® (Investigational Product)-vs-Placebo (between-product) endpoints, and tests of within-product endpoints. Changes and differences were considered statistically significantly indicative of efficacy if the p-value for the comparison was less than 0.05. As this is a pilot exploratory study, within group changes are of interest.

A directional hypothesis was tested to compare between group effects between Product and Placebo via a 1-sided p-value for the Student's t-test. Using a 1-sided hypothesis, when appropriate, allow for greater power in the test for seeing significance that was truly in the data. The directional hypothesis was used for following types of Student's t-tests:

- (a) When Product was hypothesized to be greater in value than Placebo.
- (b) When Product was hypothesized to be lesser in value than Placebo

Prior to reporting the findings of the Student t-test, a check of the variances between the product and placebo groups was performed via LeVene's test. If the results of the LeVene's test were significant (p<0.05), indicating unequal variance (heterogeneity), SPSS provided adjusted degrees of freedom and significance value that corrected for the unequal variances. The adjusted value was used for reporting when heterogeneity was present.

Differences in measurement over time between the product and placebo groups were also investigated via analysis of covariance (ANCOVA) tests. The ANCOVA analyses included covariates of (a) BMI at screening and (b) initial measurement of the outcome. Gender was also included in the ANCOVA analyses as a control variable because gender is considered an effect modifier, as males and females gain and loses weight differently.

Mean differences over time for the study outcomes were investigated within each of the two groups of Product and Placebo. The within-groups differences were tested as the mean change from initial measurement to each subsequent time point by the paired samples t-test or by the non-parametric Wilcoxon test if the data distribution of the outcome variable deviated substantially from normal.

Effect Size determination

The following formulas were used to compute effect sizes for the independent samples t-tests, paired t-tests, and ANCOVA analyses of this study.

Cohen's d measures the difference between product and placebo in terms of standard deviation units. This allows for comparisons of effects across all tests.

Thresholds for the effect sizes of effect using Cohen's d with an Independent T test as:

- Small=.20
- Moderate=.50
- Large=.80

Effect size calculation for when using paired sample Student T tests

Standard paired sample Student T tests thresholds for the sizes of effect using Cohen's d as:

- Small=.01
- Moderate=.06
- Large=.14

Effect size calculations for when ANCOVA analysis was utilized:

Standard ANCOVA thresholds for the sizes of effect using Cohen's d as:

- Small=.02
- Moderate=.13
- Large=.26

Statistical software utilized for this study included SPSS v22 and R v3.2. All inferential tests were set at a 95% level of significance ($p < .05$). SPSS v22 was used to perform the descriptive and inferential tests. R v3.4 and the ggplot2 package were used to generate graphs of the analysis findings.

Results

Baseline characteristics

One subject (Subject #129, placebo group, male) did not complete all visits. The subject dropped from the study after Visit 2. Thus, 60 subjects were included in the safety analyses, but only N=59 subjects were included in the efficacy evaluations (n=29 male subjects and n=30 female subjects). The average age of all study-enrolled participants was 48.0 ± 1.3 years with a height of 68.0 ± 0.5 inches and body weight of 233.4 ± 5.4 pounds, this included a mean body mass index value of $35.2 \pm 0.5 \text{ kg/m}^2$. The 30 subjects randomized to the Intervention group had a baseline Visceral fat score of $9.5 \pm 0.4 \text{ cm}$, Placebo group $9.7 \pm 0.5 \text{ cm}$, Intervention group had a percent body fat score of $30.9 \pm 1.1\%$, while the Placebo group baseline percent body fat was $30.9 \pm 1.4\%$. Mean glucose for all enrolled was $88.5 \pm 2.0 \text{ mg/dl}$. Total cholesterol for the Intervention group was $209.4 \pm 6.4 \text{ mg/dl}$, while the Placebo was $203.8 \pm 7.3 \text{ mg/dl}$. The Intervention group has a baseline Triglyceride value of $185.1 \pm 17.1 \text{ mg/dl}$, with Placebo $144.6 \pm 12.8 \text{ mg/dl}$ (significant baseline difference between groups $p=0.03$).

Visceral fat

There was no significant difference in visceral fat between the treatment and placebo groups for both male and female subjects over time when examining the data per the whole group. However, since the study included 15 males and 15 females per group, gender analysis was of interest. Males in the Intervention group at baseline had a visceral fat measurement of $11.0 \pm 0.4 \text{ cm}^2$ which by Day 56 (month two) was largely reduced to $10.2 \pm 0.4 \text{ cm}^2$ ($p=0.080$ with an effect size value of 0.49) and at Day 84 remained largely reduced at $10.4 \pm 0.5 \text{ cm}^2$ ($p=0.069$ with effect size of 0.50). The change for those males on placebo was non significant at these same time-points. Females on the Intervention Product or placebo did not achieve statistical significant or large effect size changes over the 84 days of the study.

Body composition

There were no significant overall changes in percent body fat throughout the study in either test group. Neither gender experienced a significant change in percent body fat over the course of the study. The same non-significant changes for actual fat mass were observed by group and by gender. Overall, the Intervention product and placebo groups also had no significant impacts on fat free mass over the course of the study.

Lipids

Overall, there were no between groups or within group changes observed in total cholesterol over the course of the study. Baseline triglyceride levels were significantly different at baseline ($p=0.033$) with higher levels in the Intervention group. By Day 84, per ANCOVA analysis there was a significant increase in triglycerides in the ChalCurb® group that was also of small effect (Intervention Group baseline 185.1 ± 17.1 to end of study $201.1 \pm 14.7 \text{ mg/dl}$ vs. Placebo baseline 144.6 ± 12.8 to end of study $145.4 \pm 11.2 \text{ mg/dl}$ with ANCOVA $p=0.036$; Effect Size=0.8. There were no significant group or gender effects on HDL. There were also no group or gender effects for LDL over the course of the study.

Mood states (vigor, fatigue)

Profile of Mood States (POMS) measurements for Vigor indicated no difference between the groups or genders over the course of the study. The POMS inertia subset (fatigue) was not significantly different between the two groups. There were also no significant differences between the genders for POMS-inertia/fatigue rating.

Glucose control

Fasting glucose levels were not comparatively significantly impacted by the Intervention or Placebo over the course of the study (89.6 ± 3.7 to $105.2 \pm 5.3 \text{ mg/dl}$ vs. 87.9 ± 1.5 to $101.5 \pm 3.7 \text{ mg/dl}$; $p=0.788$). There was no gender effects observed. There were no significant group differences for HgBA1c over the 84 days of the study. The Intervention group had a baseline percentage of $5.4 \pm 0.1\%$ and end of study value of $5.7 \pm 0.2\%$, while the Placebo group had a baseline of $5.4 \pm 0.1\%$ and end of study value of $5.6 \pm 0.1\%$ (comparative p value of 0.450). There was also no gender effects observed on HgBA1c.

Ghrelin

There was a large effect size observed for the comparative changes in Ghrelin between the groups, this tended to also be statistically significant over the study period (Investigational Product

Baseline 525.6 ± 44.4 to 491.3 ± 45.5 pg/dl vs. Placebo 457.1 ± 46.2 to 515.7 ± 55.0 pg/dl; $p=0.062$ with an effect size of 0.06 (ANCOVA). There was no gender effects noted.

Discussion

In a prior published study that assessed the effect of ChalCurb® on body weight and visceral fat in adults with metabolic syndrome and overweight adults, no significant difference between the Treatment and Placebo groups was found. However, a significant reduction of visceral fat and body weight in the treatment group after 8 weeks of supplementation was observed from within-group analysis.²² The potential differences observed in body composition responses between men and women may provide an explanation for observed responses to ChalCurb® in this study. As previously mentioned, Ashitaba, also known as *Angelica keiskei* (ChalCurb®) contains 4-HD and XA, which are two chalcones that have been associated with suppression of blood glucose levels, possibly through insulin-stimulated glucose uptake.^{5,9-11} In the current study, a gender effect was reported whereby men experienced a reduction in visceral fat, whereas women did not. In terms of body composition, men tend to have more central fat distribution, whereas women tend to have more peripheral fat distribution.^{23,24} Since the peripheral fat distribution in women is associated with improved insulin sensitivity, overweight men tend to have greater insulin resistance than overweight women.²⁵ As a result, it is expected that women should respond better than men when given supplementation of ChalCurb®. However in this study no impacts on percent body fat and overall body composition were noted in the groups or by gender.

This study also found no group effects on lipids (Total cholesterol, HDL, LDL). It was believed that if an intervention reduced visceral fat and also had an impact on body composition, it too might positively impact lipid levels in adults with metabolic syndrome. However, no such finding occurred, possibly due to the duration of the treatment or due to the lack of subjects exhibiting metabolic syndrome. It appears that triglyceride levels were elevated at the end of study and the elevation from the start of the study is worthy of further exploration. This study did not control for exercise or food intake. Since food intake may also have an effect on metabolic response between different genders, future studies should address these issues. Specifically, men have been shown to display higher postprandial triglyceride levels even when visceral adipose tissue is accounted for.²³ Surprisingly, for male subjects in the active group, triglyceride levels significantly decreased from baseline to EOS. Although we conclude that supplementation of ChalCurb® has a more positive effect on men than women with metabolic syndrome, greater sample size and other gender-specific parameters must be included in future studies. Consequently, gender-specific dosages of ChalCurb® should be established to maximize its effect on individual needs. Thus, a comparative pharmacological analysis of the factors that influence visceral fat, body composition, blood lipids, energy levels and blood sugar control, is recommended in order to determine the ideal gender-specific dosages of ChalCurb®.

It has also been suggested that the higher levels of estrogen in women result in greater insulin-sensitivity due to estrogen maintaining glucose homeostasis and substrate metabolism.^{23,26,27} With this in mind, we thought we might also see improvement or positive changes in overall glucose control (fasting glucose and HgBA1c). However, there were no observed differences between the groups over the course of the study, indicating no impact on acute or longer-term (16 weeks) glucose control within the confines of this study. Compared to

premenopausal women, postmenopausal women have approximately 2.6 times more visceral adipose tissue, which can be due in part by reduced in estrogen levels in postmenopausal women.^{23,28,29} In a study determining the effect 4HD and XA has on glucose homeostasis in skeletal muscle cells, it was determined that oral administration of both Ashitaba, ChalCurb® containing 150.6mg/g 4HD and 146.0mg/g XA suppressed acute hyperglycemia in oral glucose tolerance tests of mice, as explained by the translocation of glucose transporter (GLUT) 4.³⁰ In comparison, it may be interesting to investigate the effect these chalcones have on short-term glucose tolerance in humans. Perhaps, ChalCurb® has a greater effect on glucose tolerance after meals rather than over a longer period of time. That being said, short-term measurements could be beneficial in understanding the positive effects ChalCurb® has on individuals susceptible to glucose intolerance.

Ghrelin is often thought of as a hunger hormone. Measuring ghrelin levels is one way of looking at impacts of an intervention on hormones that surround hunger and perhaps eating and overall body weight. In this study, we found that the ChalCurb® intervention group experienced a meaningful reduction in ghrelin as compared to the placebo group. Reduced ghrelin levels are thought to correlate with reduced food intake, however as this was not a weight loss study or a satiety feeding study, ghrelin was examined in an exploratory manner to see if ChalCurb® would have any impact on such. Future studies should include more in-depth research looking at ChalCurb® and ghrelin. Lower ghrelin levels may help with weight control, which is important in helping to combat metabolic syndrome.

Given the variability in results obtained in this study, we seek a mechanistic understanding of how 4HD and XA have an effect on lipid accumulation. In a study, male stroke-prone spontaneously hypertensive rats (SHRSP) that were fed diets containing 0.07% 4-HD showed a reduction in VLDL levels without any effect on HDL, as well as significant decreases in relative liver weight and hepatic triglyceride content. Additionally, there were no significant differences in bodyweight or food intake between the two groups, signifying that 4-HD controls diet.³¹ As well, in a different study using 3T3-L1 adipocytes, it was found that 4HD and XA inhibited adipocyte differentiation through AMPK and mitogen-activated protein kinase pathways, which resulted in a down-expression of adipocyte transcription factors.³² In accordance, another study using an *in vitro* model of hepatic steatosis with human HeG2 cells found that 4HD and XA reduced lipid accumulation through activation of LKB1/AMPK pathways. The objective of this study was to focus on metabolic health, ChalCurb® supplementation may have a positive impact on metabolic health deserving further attention and research.

Conclusion

This study demonstrated that Ashitaba, also known as *Angelica keiskei* (ChalCurb®) may have a positive role and impact for those with metabolic syndrome.

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Conflicts of interest

Dr. Douglas Kalman is employed by QPS an independent research organization that conducted the study. Vincent Hackel is employed by JBLS, the company that sponsored the study.

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