Quinoa seed lowers serum triglycerides in overweight and obese subjects - a dose-response randomized controlled clinical trial.

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Running title: Quinoa lowers serum triglycerides

Abbreviations

APOC-III, Apolipoprotein C-III
CHD, Coronary heart disease
CVD, Cardiovascular diseases
LPL, lipoprotein lipase
MetS, Metabolic Syndrome
T2DM, Type 2 diabetes mellitus

Clinical Trial Registry number and website: The trial was registered with the Australian New Zealand Clinical Trials Registry (ANZCTR http://www.anzctr.org.au/) as UTN U1111-1175-470.
Abstract

Background: Quinoa (Chenopodium quinoa) is a pseudocereal originally cultivated in the Andean region. The popularity of its seeds has increased in recent years due to the claims of health benefits and superfood qualities. Studies to date on health benefits of quinoa have been restricted to animal models and the results provide weak to moderate evidence to support improved plasma lipid profile. Clinical trials in humans to examine the claims of health benefits of quinoa are limited to few prospective studies and one randomised trial carried out in postmenopausal women. No studies have been conducted in the general population.

Objective: The objective of this randomized clinical trial was to investigate the effect of different quinoa doses (25 g and 50 g per day) on body composition, serum lipids and hormones and nutrient intake in overweight and obese humans.

Design: This was a dose-response randomized controlled, single-blinded, trial with a parallel design (one control and two treatment groups) that compared the effect of 25 g, 50 g in 50 overweight and obese subjects over 12 wk intervention time.

Results: Body composition, total cholesterol, LDL, HDL and nutrient intake were not significantly altered by quinoa consumption (P>0.05). Serum triglyceride concentration was reduced significantly in the 50 g quinoa group from 1.14 mmol/L to 0.72 mmol/L at 12 weeks (P<0.05). The prevalence of metabolic syndrome was also reduced in this group by 70%. No significant change in triglycerides were observed in the control and 25 g quinoa groups. Prevalence of metabolic syndrome was reduced by 40 % (from n = 7 at baseline to n = 4 at 12 weeks) in the 25 g group.

Conclusions: Consumption of 50 g/d of quinoa lowers serum triglyceride in overweight and obese subjects and reduces the prevalence of the metabolic syndrome. The trial was
registered with the Australian New Zealand Clinical Trials (ANZCTR http://www.anzctr.org.au/) as UTN U1111-1175-470.

Introduction

Quinoa (Chenopodium quinoa) is a pseudocereal originally cultivated in the Andean region between Bolivia and Peru. In recent years, the popularity of quinoa seeds has increased due to claims of health benefits and superfood properties. Research has shown that quinoa seeds have an improved macronutrient profile (1), including gluten free characteristics, a particularly beneficial essential amino acid ratio (2) and a superior phytochemical composition (3) compared with other cereals and grains.

In spite of the seeds’ composition and properties, scientific evidence supporting health claims such as weight loss, antidiabetic effects and appetite suppression in in vivo models are limited and the evidence is restricted to a few animal studies. Among this body of evidence, it can be observed that a possible health benefit of quinoa consumption may be linked to a potential lipid-lowering effect (4-6). Human clinical studies have been limited to prospective studies on the effect of cereal bars containg quinoa on cardiovascular disease markers and immunological responses in a cohort of celiac patients after consumption of quinoa flakes (7, 8); both studies showed changes in total cholesterol and changes in triglycerides. In addition, LDL cholesterol changes were reported in the study using cereal bars (8). Furthermore, a randomized controlled trial was carried out in postmenopausal women analyzing the effect of quinoa consumption on lipid profiles and oxidative stress markers (9). The results of this trial were in line with previous data reporting favourable changes in total cholesterol, LDL and triglycerides.
Obesity has reached epidemic proportions according to WHO in 2016, whereby an approximate number of 1.9 billion adults were overweight and 600 million of these were considered obese (10). A cluster of metabolic factors including abdominal fat measured as waist circumference, reduced high-density lipoprotein (HDL), increased triglycerides, augmented fasting plasma glucose and elevated blood pressure have been termed as the Metabolic Syndrome (MetS) (11, 12) and MetS has been linked with an increased risk of chronic disease states such as obesity, coronary heart disease (CHD) and type II diabetes mellitus (T2DM). MetS has a substantive impact on the economy reflected in the uses of health care resources and the decrease in productivity. In the USA the estimated annual cost for 2011 was $320.1 billion for CVD and stroke, with $195.6 billion associated with direct costs and $124.5 billion linked to loss of productivity due to premature deaths (13). Addressing the components of the MetS will lead to an increase in health and a potential reduction of costs to the economy.

To the author's knowledge, no previous studies have been conducted analyzing the effect of quinoa consumption in people who are overweight and obese in regards to MetS risk factors and health benefits. The aim of this research was to investigate a dose-response effect of quinoa seeds consumption in a randomized controlled trial design on body composition, circulating lipids, hormones and nutrient intake in overweight and obese humans.

**Methods**

**Design**

A dose-response randomized controlled, single-blinded, trial that utilized a parallel design including one control and two treatment groups was undertaken. Researchers assessing study outcomes were blinded from participant interventions and codes.
Participants

Overweight and obese adults were recruited via advertisements at La Trobe University using flyers, social media and emails after ethical approval was obtained. Participants were included if they were aged between 18 - 65 years old and had a body mass index (BMI) greater than 25kg/m². Exclusion criteria were pregnancy, participants diagnosed with diabetes and heart diseases and participants taking blood lipid-lowering medication. Participants gave written consent prior to the commencement of the study after being briefed about study procedures and expectations.

Food Samples

White organic quinoa seeds were purchased from an Australian based company (Quilla Foods Pty) dedicated to the importation of Bolivian quinoa. Quinoa bags were carefully weighed and packed into transparent sachets of either 25 g or 50 g and packaged in a box of 42 sachets which constituted 6 weeks supply to each participant in the treatment groups.

Randomisation

Participants were randomly allocated into one of three treatment groups (control, 25 g and 50 g quinoa seed per day). This randomization was carried out following a block randomization protocol stratified by gender using a computer number generated program retrieved from the website random.org. Allocation sequences and assignment of participants’ interventions were carried out by an external researcher prior to the appointments.

Study Protocol

Participants attended appointments at La Trobe University Bundoora Campus at baseline, 6 wk and 12 wk of the intervention period. Participants allocated to the 25g and 50g quinoa
groups were given a quinoa supply for 6 weeks at baseline and again at their 6 wk appointment and were advised to consume one quinoa sachet per day. Participants were instructed on cooking methods for quinoa consumption and recipes to incorporate quinoa were given to participants that requested them. Participants were not instructed on how and when to consume the quinoa and were advised to retain their normal lifestyle and diet throughout the study. Participants were given a calendar to self-report quinoa consumption for compliance analysis. Participants in the control arm were advised to continue their normal routine avoiding the consumption of quinoa meals during the study period.

Study Outcomes

Primary outcomes included lipid profile and secondary outcomes such as body composition and dietary intake were measured at baseline, 6 wk and 12 wk. Hormones were also measured at baseline and 12 wk.

Anthropometric measures

Anthropometric measures such as body weight and height were recorded using a stadiometer (Wedderburn ®) and waist to hip ratio using a measuring tape according to WHO criteria (14). Body composition was analyzed using a dual energy x-ray absorptiometry (DEXA) scan (Discovery QDR series - Hologic Inc.) calibrated daily prior to measurement.

Serum lipids, hormones and glucose.

Fasting blood samples were collected in an 8.5 ml Serum Separation Tube (SST). After 30 min of incubation time at room temperature, blood was spun at 1300 x g for 10 min and the separated serum was stored at -80 C until analysis. Lipid profile (total cholesterol, HDL, LDL and triglycerides) and glucose concentrations were measured using enzymatic assays in a chemistry analyzer (Indiko, Thermo Scientific) as per manufacturer’s protocol.
Adiponectin, leptin, insulin and C-peptide were determined by multiplex ELISA on a Bio-Plex 200 system (Bio-Rad Laboratories Pty., Ltd) following manufacturer’s instructions. Acquired fluorescence data were analyzed by the Bio-Plex Manager software version 6.1 (Bio-Rad Laboratories Pty., Ltd).

**Dietary Intake**

Participants recorded their food intake using a 3-day food diary. They were provided with templates and were advised to record intake for three days following the first appointment and for three days prior to the second (6 wk) and final (12 wk) appointments. Data was analyzed using FoodWorks version 8 (Xyris, Australia).

**Compliance**

Participants were advised to complete a compliance checklist calendar every time they consumed a quinoa sachet. In addition, leftover sachets were returned at the following appointment.

**Prevalence of Metabolic Syndrome (MetS)**

Analysis of the abnormalities including in the MetS were reported using the criteria stated in the Third report of the expert panel on detection, evaluation and treatment of high blood cholesterol in Adults (Adult treatment panel III or ATPIII) (12) including, waist circumference (males > 102 cm, females > 88 cm), circulating triglycerides ($\geq$ 1.7 mmol/L), circulating HDL (males $< 1.04$ mmol/L, females $< 1.3$ mmol/L) and fasting glucose ($\geq$ 5.6 mmol/L).

**Statistical analysis**
Data was analyzed for normality using Kolmogorov-Smirnov test. Baseline characteristics were assessed by one-way ANOVA for parametric data and Kruskal-Wallis test for non-parametric data. Results are presented as mean ± SEM, except for lipid profile and hormones that are reported as median (IQR). Treatment differences were adjusted for baseline values and analyzed using a linear mixed model with fixed factors (baseline of the variable, treatment and time and interaction between treatment and time) and a subject-specific as a random effect. Non-normally distributed variables such as adiponectin, glucose, HDL and triglycerides were log-transformed in order to fit the model. When significant interactions were observed, pairwise comparisons with Bonferroni were carried out. Subgroup analysis was performed by a linear mixed model with the above considerations. Prevalence of metabolic syndrome (MetS) was analysed using one-way ANOVA for baseline values and repeated measures ANOVA for between and within the group comparison. All data was analyzed by SPSS Inc. software (Version 24.0). The level of significance was chosen as 5%.

The study was approved by La Trobe University Human Ethics Committee (HEC 14 – 065) and registered with the Australian New Zealand Clinical Trials Registry (ANZCTR http://www.anzctr.org.au/) with number UTN U1111-1175-470.

Results

Participants

One hundred and fifty-one participants expressed interest in the study. After screening, 131 met the inclusion criteria and were deemed eligible to participate. Sixty-seven eligible participants declined involvement and 64 participants were randomized. Nine participants discontinued the study after the baseline appointment (two in the control group, three in the 25 g group and four in the 50 g group) and five after the 6 wk appointment (one in control,
three in 25 g and one in 50 g group). Fifty participants successfully completed the 12 wk intervention period. Figure 1 shows an overview of recruitment, randomization process and further analysis of data. Seventy-one percent were women (n=53) and the study group was aged between 20 - 64 years (mean ± SEM: 37.96 ± 1.43). According to the WHO criteria for obesity classification (10) 42% (n=31) were considered overweight (BMI > 25 kg/m²), 28% (n=21) Obese type I (BMI between 30-34.9 kg/m²), 15% (n=11) Obese type II (BMI between 35 – 39.9 kg/m²) and 15% (n=11) Obese type III (BMI > 40 kg/m²). There were no significant differences between groups at baseline (control and interventions) (Table 1).

Participants were randomized from February 2015 to May 2016, follow up period ended August 2016.

Effect of Quinoa on Anthropometric Measures and Body Composition

No significant differences were observed for body composition measurements after the 6 and 12 wk intervention time period between and within treatment groups. Table 2 describes these findings. In the control group, body weight varied from a 3.6% decrease (-2.92 kg) at 6wk to 4.1% increase (3.19 kg) at 12 wk. In both intervention groups, changes in body weight were reported as 0.5% increase (0.43 kg) at 6 wk whereas a 1.5% (-1.32 kg) and 1.7% (-1.43 kg) decrease at 12 wk in 25 and 50 g respectively, was observed. There were no differences in body fat and total lean percentage. There was a decrease in waist circumference in the control group of 2.8 cm at 6 wk but an increase of 3.3 cm at 12 wk. In the 25 g group waist circumference increase 0.6 cm at 6 wk and decreased by 1 cm at 12 wk whereas in the 50 g group there was a reduction of 0.2 cm at 6 wk and 1.3 cm at 12 wk.

Serum lipids, hormones and glucose

The interaction effects between lipids, lipoproteins and fasting glucose with quinoa intake is reported in Table 3. No significant effect was observed for total cholesterol, HDL, LDL and
fasting glucose between and within groups at 6 and 12 wk of quinoa consumption. However, a significant difference was noted for triglycerides in the between groups analysis. A pairwise comparison adjusted with a Bonferroni correction revealed that triglycerides were significantly lower in the 50 g group compared to the control group (0.72 mmol/L and 1.25 mmol/L respectively, \( p=0.022 \)). Furthermore, within-group comparison showed that the 50 g group had significantly lower triglyceride at the 12 wk time point compared to baseline (1.14 mmol and 0.72 mmol/L for baseline and 12 wk respectively, \( p=0.001 \)). No significant difference was observed at 6 wk compared to baseline. These results indicate that there is a dose-response effect with daily consumption of 50 g of quinoa reducing serum triglycerides by approximately 28.1% after 6 wk of diet intervention and by 36.8% after 12 wk.

Triglyceride changes in the 25 g group were not significant at either 6 and 12 wk, although it showed a slight reduction when compared to baseline values (1.36 mmol/L baseline, 1.33 at 6 wk and 1.27 mmol/L at 12 wk).

Further analysis revealed that 15.8% of all participants in the study had triglycerides exceeding optimum concentrations of 1.7 mmol/L, the cut off according to the ATPIII, MetS criteria (12). Analysis of the quinoa effect on normo and hypertriglyceridemic groups showed a significant change (\( p=0.048 \)) in participants with normal triglycerides consuming 50 g of quinoa per day compared to control at 12 wk (Figure 2). Reductions were also observed within 50 g group from 0.86 mmol/L at baseline to 0.76 mmol/L at 6 wk and 0.70 mmol/L at 12 wk respectively, albeit non-significant (\( p = 0.336 \)). This represents a reduction of 12.7% and 18.4% at 6 and 12 wk, respectively. No significant differences were observed in triglycerides in participants consuming 25 g of quinoa. In the hypertriglyceridemic group, a significant decrease was observed at 12 wk (\( p= 0.001 \)) in participants consuming 50 g of quinoa. Triglycerides were reduced by 30.4% (from 2.83 mmol/L at baseline to 1.97 mmol/L).
at 6 wk and 58% (from 2.83 mmol/L baseline to 1.18 mmol/L) at 12 wk. Participants consuming 25 g had a reduction of 18.5% (2.7 mmol/L at baseline to 2.20 mmol/L) at 6 wk and 23.7% (from 2.7 mmol/L at baseline to 2.06 mmol/L) at 12 wk. No statistically significance was observed in the between group comparison. Of note triglyceride concentrations were normalized to within healthy range (< 1.7 mmol/L) in the hypertriglyceridemic group consuming 50 g Quinoa.

Circulating hormones were not significantly affected by quinoa consumption. Adiponectin increased in the control group by 18% and by 21% in the 25 g quinoa group and 6% in the 50 g group, during the intervention period. Leptin and C-peptide reduced by 21% in the control group and increased by 11% in the 25 g group. In addition, leptin decreased by 3% whereas C-peptide increased in the 50 g group. Insulin concentration remained stable in the control and 25 g group and had a decrease in the 50 g group (14%) Results are reported in Table 3.

Prevalence of MetS

The prevalence of the MetS as defined by the ATP III criteria in the treatment groups is reported in Figure 3. According to the criteria for MetS, 24% (n = 12) of total participants had three or more risk factors including waist circumference (men > 102 cm and women > 88 cm), reduced HDL (men < 1.04 mmol/L and women < 1.3 mmol/L), high triglycerides ($\geq$ 1.7 mmol/L) and higher fasting blood glucose ($\geq$ 5.6 mmol/L) at baseline. No changes in the prevalence were observed at 6 wk for control and 50 g group, whereas the 25 g group showed a reduction of 14% in the prevalence at this time point. At the completion of the study, there was a decrease in the occurrence of MetS in the 25 g group of 41% (n=3 compared to n=7 at baseline) and in the 50 g by 70% (n=2 compared to n=3 at baseline). On the contrary, the prevalence of MetS in the control group increased by 6.8%. Reduction in the prevalence of MetS was as a result of the increase in HDL and reduction in triglycerides concentration. No
changes were observed in waist circumference and fasting glucose among the groups. Mean differences between and within groups reported no statistically significant differences

**Dietary Intake**

Quinoa consumption did not significantly alter nutrient intake during the intervention time. **Table 4** summarises these results. There was an increase in reported energy intake in the control group of 359 kJ at 6 wk and a reduction of 252 kJ at 12 wk. In the 25 g group there was a reduction 364 kJ at 6 wk and a further increase of 385 kJ at 12 wk. Conversely, the 50 g group showed no difference at 6 wk followed by a decrease 585 kJ at 12 wk. There was no reported trends in the changes of macronutrients among all treatment groups. In the control group protein and fibre intake increased whereas total fat decreased at both 6 and 12 wk, carbohydrates increased at 6 wk but decreased at 12 wk. For the 25 g group there was an increase in protein and carbohydrates intake and a reduction in total fat at 6 and 12 wk, dietary fibre intake increased at 6wk but decreased at 12 wk. Finally, in 50 g group there was a decrease in total fat and dietary fibre and an increase in carbohydrates at 6 and 12 wk, whereas protein intake reported an increase at 6 wk but a reduction at 12 wk. in protein and carbohydrate intake at 6 wk but a decrease in 12 wk.

**Compliance**

According to self-reported calendars, participants in the 25 g group show compliance to prescribed quinoa consumption of over 90.0% at 6 wk and 89.6% at 12 wk, whereas participants in the 50 g group had a compliance of over 90.7% at 6 wk and 82.3% at 12 wk.

**Discussion**

The aim of this dose-response randomized controlled study was to assess the relationship of quinoa consumption on anthropometric, biochemical and dietary data in subjects who are
overweight or obese. There was no effect of quinoa on anthropometric or body composition, circulating hormones, total cholesterol, HDL, LDL cholesterol and glucose. No effect was also observed for dietary intake data. However, our results show that consumption of 50 g/day of quinoa seeds for 12 weeks reduces serum triglycerides in overweight and obese adults.

Triglycerides have been extensively linked with cardiovascular diseases as an independent risk marker. Early evidence showed that in individuals surviving cardiovascular events serum triglycerides are augmented compared with healthy controls even after controlling for other lipoproteins such as LDL (15, 16). Some authors also argue that the relative risk of a cardiovascular event may increase around 32% in men and 14% of women for each mmol/L increase in circulating triglycerides (17). Furthermore, recent evidence suggest that triglycerides are not only a risk marker but also has an active pathogenic role in the development of cardiovascular events. Mutations in the intermediates of lipids metabolism lead to changes in cardiovascular risk. Modifications in proteins such as apolipoprotein C-III (APOC3) (18, 19) results in a reduction in the risk of coronary artery calcification, Conversely, alterations in enzymes like lipoprotein lipase (LPL) (20) causes increase in coronary heart disease. This confirms that the reduction in triglycerides achieved in this study may potentially reduce the cardiovascular disease risk.

Circulating triglycerides are the result of a complex network involving the synthesis in the liver, absorption of chylomicrons by the intestine, peripheral lipolysis mediated by the action of lipoprotein lipase (LPL) and hepatic clearance of the remnants molecules. Early evidence showed that in obese subjects (21) hypertriglyceridemia is the consequence of the increase in hepatic VLDL production due to an increased flux of free fatty acids (FFA) from adipose
tissue (22), this overproduction causes a further impairment in the LPL activity in adipose
tissue and muscle increasing, thus, the circulating triglycerides concentrations.

A reduction of 36% after consumption of 50g quinoa for 12 wk observed in our study is
greater than previous reports of a 16% reduction in healthy subjects consuming 19.5g quinoa
for 4 wk (8) and a 4% reduction in overweight postmenopausal women consuming 25g
quinoa for 4 wk (9). The mechanism by which quinoa consumption results in a reduction of
triglycerides are not fully understood. A reduction in intestinal dietary fat absorption
observed through the increase in lipid content in the faeces was reported in rodents fed a diet
containing quinoa protein extract and a quinoa extract enriched with 20
hydroxyecdysteroid(4, 23). A possible underlying mechanism for this quinoa effect may be
based on bile acids activity. In an in vitro essay it was demonstrated that quinoa proteins have
a higher bile acid binding capacity affecting absorption of lipids (4). Bile acid emulsification
of fats constitute an essential part in intestinal lipid absorption.

Other approaches, such as increased dietary fiber intake has been associated with
improvement of lipid profile in a number of randomized clinical trials (24-26). Although
human studies are inconclusive, some authors argue that dietary fiber may have an important
role in hepatic cholesterol synthesis linked to bile acids regulation (27). Albeit quinoa has an
enhance content of soluble and insoluble fiber than other cereals and grains (28, 29), our
results do not support these findings.

Triglycerides reduction in our study is comparable with the reduction evidenced in
pharmacological therapy employing nicotinic acid 40 % (30), fibrates 35% (31) and statins
20% (32). In addition, consumption of 3.4 g/day of omega-3 fatty acids may reduce
triglycerides by 27% in healthy subjects with mild hypertriglyceridemia (1.7 mmol/L and 5.6
mmol/L)(33). Each of these treatments has a very different mechanism of action and cannot be compared with the possible quinoa seeds intake triglyceride lowering mechanism.

Quinoa consumption has increased steadily over the recent years. In 2011 consumption of quinoa in Boliva was reported to be around 1.11 Kg/capita/year and in 2012 it escalated to 2.37 Kg/capita/year. In contrast, in non-producer countries such as Canada and Australia quinoa was not consumed in reported quantities in 2011 and it reached just 227 g/per capita/year in 2014 in Canada and 81 g/capita/year in Australia. Therefore the reduction of triglycerides observed in our study would require a significantly higher amount of quinoa consumption compared to the current data. However, quinoa is a suitable replacement for grains consumption whose intake was in the range of 112 to 175 g per day for the Australian population in 2011-12 (34). In addition to providing a higher amount of nutrients such as protein and micronutrients, quinoa may have an important role in the reduction of risk of cardiovascular diseases.

Incorporating quinoa to the diet did not alter the overall nutrient intake of participants. There was a positive change with respect to a reduction of the prevalence of the MetS due to the postive changes in HDL cholesterol and circulating triglycerides. Although the numbers were small, making small substitutions to diet quality without concomitant weight loss can improve the metabolic profile of overweight and obese participants.

Study limitations

This study revealed a diminution of circulating triglycerides when quinoa seeds are consumed as part of the daily diet. However, further investigation must be conducted to determine the role of the quinoa seeds intake in participants with a high circulating triglycerides due to the small sample size which may not reflect this particular subgroup properties. In addition, a
compliance biomarker should be established in order to have an independent measure of quinoa consumption.

**Conclusions**

In our study, we demonstrate that consumption of 50 g/day of quinoa seeds for 12 weeks reduces serum triglycerides and therefore the prevalence of MetS in overweight and obese people. Further studies are needed to elucidate a clear mechanism of action by which quinoa seeds lower circulating triglyceride concentration.

**Acknowledgments**

We thank all our research participants for their interest and dedication to this project.

The author’s responsibilities were as follows DNP, JR and MJ design the project; DNP conducted the clinical trial; DNP carried out laboratory experiments in this manuscript; DNP and MJ performed the statistical analysis; DNP, MJ, AT and JR wrote the manuscript.
References


33. Skulas-Ray, A.C., P.M. Kris-Etherton, W.S. Harris, J.P. Vanden Heuvel, P.R. Wagner, and S.G. West. Dose-response effects of omega-3 fatty acids on triglycerides, inflammation, and

Table 1

Baseline characteristics of overweight and obese participants in control and treatment groups

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>Control</th>
<th>25 g/day</th>
<th>50 g/day</th>
<th>P value</th>
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<tbody>
<tr>
<td><strong>Participants</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Gender</td>
<td>Males</td>
<td>Females</td>
<td>Males</td>
<td>Females</td>
</tr>
<tr>
<td>n (%)</td>
<td>5</td>
<td>11</td>
<td>7</td>
<td>9</td>
</tr>
<tr>
<td><strong>Age (years)</strong></td>
<td>38</td>
<td>35</td>
<td>40</td>
<td></td>
</tr>
<tr>
<td><strong>BMI (Kg/m²)</strong></td>
<td>29.91</td>
<td>32.84</td>
<td>31.49</td>
<td></td>
</tr>
<tr>
<td><strong>Body Composition</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Body Weight (Kg)</td>
<td>81.28 ± 4.03</td>
<td>89.91 ± 4.31</td>
<td>85.85 ± 2.80</td>
<td>0.286</td>
</tr>
<tr>
<td>Body Fat (%)</td>
<td>40.21 ± 2.0</td>
<td>41.41 ± 1.73</td>
<td>41.25 ± 1.72</td>
<td>0.894</td>
</tr>
<tr>
<td>Total lean (%)</td>
<td>61.22 ± 2.36</td>
<td>59.79 ± 2.14</td>
<td>58.75 ± 1.72</td>
<td>0.862</td>
</tr>
<tr>
<td>BMC (Kg)</td>
<td>2.42 ± 0.12</td>
<td>2.46 ± 0.10</td>
<td>2.48 ± 0.09</td>
<td>0.927</td>
</tr>
<tr>
<td>Waist Circumference (cm)</td>
<td>102.97 ± 3.19</td>
<td>109.32 ± 3.64</td>
<td>106.63 ± 2.07</td>
<td>0.268</td>
</tr>
<tr>
<td><strong>Lipid Profile and Glucose (mmol/L)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total Cholesterol</td>
<td>4.69 (3.49 - 5.38)</td>
<td>4.45 (3.32 - 5.49)</td>
<td>4.36 (3.51 - 5.58)</td>
<td>0.729</td>
</tr>
<tr>
<td>LDL</td>
<td>3.93 (2.90 - 4.45)</td>
<td>3.90 (3.10 - 4.69)</td>
<td>3.25 (2.87 - 3.53)</td>
<td>0.221</td>
</tr>
<tr>
<td>HDL</td>
<td>1.20 (0.80 - 1.43)</td>
<td>1.04 (0.89 - 1.23)</td>
<td>1.28 (0.79 - 1.56)</td>
<td>0.749</td>
</tr>
<tr>
<td>Triglycerides</td>
<td>1.07 (0.84 - 1.21)</td>
<td>1.36 (0.90 - 2.38)</td>
<td>1.14 (0.611 - 1.73)</td>
<td>0.345</td>
</tr>
<tr>
<td>Fasting glucose</td>
<td>4.71 (4.58 - 5.07)</td>
<td>4.85 (4.58 - 6.63)</td>
<td>4.65 (4.19 - 5.09)</td>
<td>0.481</td>
</tr>
<tr>
<td><strong>Hormones</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Adiponectin (µg/ml)</td>
<td>15.33 (9.74 - 27.10)</td>
<td>10.73 (4.83 - 15.27)</td>
<td>13.91 (10.28 - 21.50)</td>
<td>0.176</td>
</tr>
<tr>
<td>Leptin (ng/ml)</td>
<td>11.08 (3.85 - 17.18)</td>
<td>10.86 (5.52 - 15.82)</td>
<td>6.64 (3.45 – 20.4)</td>
<td>0.947</td>
</tr>
<tr>
<td>C-Peptide (ng/ml)</td>
<td>1.00 (0.71 - 1.12)</td>
<td>1.04 (0.88 - 1.72)</td>
<td>0.99 (0.84 - 1.43)</td>
<td>0.398</td>
</tr>
<tr>
<td>Insulin (mIU/L)</td>
<td>11.31 (4.97 - 16.69)</td>
<td>11.60 (3.21 - 15.89)</td>
<td>11.46 (7.65 - 19.34)</td>
<td>0.551</td>
</tr>
<tr>
<td><strong>Nutrient Intake</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Energy Intake (kJ)</td>
<td>8066.71 ± 691.32</td>
<td>7908.55 ± 774.10</td>
<td>7618.25 ± 595.96</td>
<td>0.885</td>
</tr>
<tr>
<td>Protein (g)</td>
<td>87.68 ± 11.14</td>
<td>85.37 ± 13.38</td>
<td>84.53 ± 6.13</td>
<td>0.972</td>
</tr>
<tr>
<td>Total Fat (g)</td>
<td>75.86 ± 7.54</td>
<td>73.65 ± 8.07</td>
<td>79.64 ± 7.38</td>
<td>0.851</td>
</tr>
<tr>
<td>Carbohydrates (g)</td>
<td>201.96 ± 16.92</td>
<td>193.52 ± 14.59</td>
<td>167.23 ± 17.25</td>
<td>0.296</td>
</tr>
<tr>
<td>Dietary Fibre (g)</td>
<td>23.60 ± 2.42</td>
<td>24.20 ± 3.85</td>
<td>22.52 ± 2.26</td>
<td>0.908</td>
</tr>
<tr>
<td>KJ from Protein (%)</td>
<td>18.30 ± 1.32</td>
<td>17.57 ± 1.27</td>
<td>19.51 ± 1.27</td>
<td>0.559</td>
</tr>
<tr>
<td>KJ from Lipids (%)</td>
<td>34.80 ± 1.72</td>
<td>34.40 ± 1.51</td>
<td>38.54 ± 1.81</td>
<td>0.171</td>
</tr>
<tr>
<td>KJ from Carbohydrates (%)</td>
<td>41.64 ± 1.21</td>
<td>42.07 ± 2.15</td>
<td>39.64 ± 1.53</td>
<td>0.610</td>
</tr>
<tr>
<td>KJ from Fat (%)</td>
<td>2.46 ± 0.27</td>
<td>2.43 ± 0.20</td>
<td>2.53 ± 0.22</td>
<td>0.948</td>
</tr>
<tr>
<td>KJ from others (%)</td>
<td>0.94 ± 0.16</td>
<td>1.23 ± 0.20</td>
<td>1.04 ± 0.17</td>
<td>0.531</td>
</tr>
</tbody>
</table>

1 Refers to one-way ANOVA for body composition and nutrient intake and Kruskal-Wallis for lipid profile, glucose and hormones
2, 5-6 Data is expressed as mean ± SEM
3-4 Data is expressed as median (IQR)

BMC, bone mineral density; HDL, high-density lipoprotein; LDL, low-density lipoprotein
Table 2

Effect of Quinoa on body Composition (mean ± SEM) in overweight and obese participants at 6 and 12 wk intervention.

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>Control</th>
<th>25 g/day</th>
<th>50 g/day</th>
<th>P value treatment(^1)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>6 weeks</td>
<td>12 weeks</td>
<td>6 weeks</td>
<td>12 weeks</td>
</tr>
<tr>
<td>Body Composition (Kg)(^2)</td>
<td>28.45 ± 0.72</td>
<td>29.85 ± 1.19</td>
<td>33.45 ± 1.38</td>
<td>32.81 ± 1.43</td>
</tr>
<tr>
<td>BMI</td>
<td>78.43 ± 2.80</td>
<td>81.62 ± 4.31</td>
<td>90.34 ± 4.19</td>
<td>89.02 ± 4.24</td>
</tr>
<tr>
<td>Body Weight</td>
<td>39.47 ± 2.0</td>
<td>40.29 ± 1.99</td>
<td>41.28 ± 1.73</td>
<td>41.41 ± 1.93</td>
</tr>
<tr>
<td>Body Fat (%)</td>
<td>61.86 ± 2.29</td>
<td>61.09 ± 2.31</td>
<td>59.85 ± 1.98</td>
<td>59.74 ± 2.15</td>
</tr>
<tr>
<td>Total lean (%)</td>
<td>1.87 ± 0.11</td>
<td>2.42 ± 0.13</td>
<td>1.95 ± 0.09</td>
<td>2.44 ± 0.11</td>
</tr>
<tr>
<td>BMC</td>
<td>100.15 ± 3.62</td>
<td>103.26 ± 3.62</td>
<td>109.88 ± 3.61</td>
<td>108.86 ± 3.54</td>
</tr>
</tbody>
</table>

\(^1\) Indicate the main effect of treatment observed between groups (baseline adjusted based on linear mixed model)

\(^2\) Data are expressed as mean ± SEM

BMC, bone mineral density
Table 3

Effect of Quinoa on fasting biochemical measures (median (IQR)) in overweight and obese participants in control and treatment groups at 6 and 12 wk intervention

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>Control</th>
<th>25 g/day</th>
<th>50 g/day</th>
<th>P value&lt;sup&gt;1&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>6 weeks</td>
<td>12 weeks</td>
<td>6 weeks</td>
<td>12 weeks</td>
</tr>
<tr>
<td>Lipid Profile and Glucose (mmol/L)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total Cholesterol&lt;sup&gt;2&lt;/sup&gt;</td>
<td>4.65 (3.70 – 5.56)</td>
<td>4.71 (4.03 – 5.35)</td>
<td>4.69 (3.48 – 5.43)</td>
<td>4.27 (3.30 – 4.88)</td>
</tr>
<tr>
<td>LDL</td>
<td>3.73 (3.06 – 4.86)</td>
<td>3.58 (2.81 – 4.70)</td>
<td>3.78 (2.93 – 4.46)</td>
<td>4.15 (3.62 – 4.61)</td>
</tr>
<tr>
<td>HDL</td>
<td>1.41 (1.10 – 1.48)</td>
<td>1.20 (1.08 – 1.62)</td>
<td>1.05 (0.89 – 1.55)</td>
<td>1.02 (0.93 – 1.37)</td>
</tr>
<tr>
<td>Triglycerides</td>
<td>1.22 (0.64 – 1.34)</td>
<td>1.25 (1.11 – 1.33)</td>
<td>1.33 (0.85 – 2.07)</td>
<td>1.27 (0.92 – 1.80)</td>
</tr>
<tr>
<td>Fasting glucose</td>
<td>4.72 (4.54 – 5.07)</td>
<td>4.96 (4.39 – 5.20)</td>
<td>4.85 (4.61 – 5.32)</td>
<td>5.04 (4.98 – 5.56)</td>
</tr>
<tr>
<td>Hormones&lt;sup&gt;3&lt;/sup&gt;</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Adiponectin µg/ml</td>
<td>NM</td>
<td></td>
<td>18.15 (11.80 - 27.01)</td>
<td>13.08 ( 5.49 – 22.89)</td>
</tr>
<tr>
<td>Leptin (ng/ml)</td>
<td>8.84 (3.77 – 15.57)</td>
<td></td>
<td>11.06 (5.65 – 17.68)</td>
<td>11.06 (5.65 – 17.68)</td>
</tr>
<tr>
<td>C-Peptide (ng/ml)</td>
<td>0.79 (0.69 - 1.35)</td>
<td></td>
<td>1.16 (0.74 - 1.58)</td>
<td>1.16 (0.74 - 1.58)</td>
</tr>
<tr>
<td>Insulin (mIU/L)</td>
<td>12.72 (5.15-22.68)</td>
<td></td>
<td>11.75 (6.20-15.57)</td>
<td>11.75 (6.20-15.57)</td>
</tr>
</tbody>
</table>

<sup>1</sup>Indicate the main effect of treatment, observed between groups (baseline adjusted based on linear mixed model)

<sup>2,3</sup>Data are expressed as median (IQR)

HDL, high-density lipoprotein; LDL, low-density lipoprotein NM, value not measured
Table 4 Effect of Quinoa on nutrient intake (mean ± SEM) outcomes of overweight and obese participants in control and treatment groups at 6 and 12 wk intervention

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>Control 25 g/day</th>
<th>Control 50 g/day</th>
<th>25 g/day P value</th>
<th>50 g/day P value</th>
<th>P value treatment</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>6 weeks</td>
<td>12 weeks</td>
<td>6 weeks</td>
<td>12 weeks</td>
<td></td>
</tr>
<tr>
<td>Energy (kJ)</td>
<td>8425.26 ± 670.43</td>
<td>8173.67 ± 577.70</td>
<td>7543.71 ± 657.56</td>
<td>7929.18 ± 541.45</td>
<td>7638.19 ± 414.77</td>
</tr>
<tr>
<td>Protein (g)</td>
<td>90.68 ± 12.08</td>
<td>95.39 ± 11.52</td>
<td>95.21 ± 13.11</td>
<td>92.30 ± 11.31</td>
<td>90.35 ± 8.06</td>
</tr>
<tr>
<td>Total Fat (g)</td>
<td>72.72 ± 8.44</td>
<td>74.24 ± 8.22</td>
<td>64.48 ± 6.27</td>
<td>69.31 ± 10.92</td>
<td>70.45 ± 6.39</td>
</tr>
<tr>
<td>Carbohydrates (g)</td>
<td>225.04 ± 17.43</td>
<td>199.70 ± 9.08</td>
<td>194.41 ± 19.04</td>
<td>198.87 ± 13.67</td>
<td>187.78 ± 13.13</td>
</tr>
<tr>
<td>Dietary Fibre (g)</td>
<td>24.93 ± 2.23</td>
<td>24.92 ± 2.23</td>
<td>20.27 ± 2.34</td>
<td>24.86 ± 2.78</td>
<td>23.76 ± 2.19</td>
</tr>
<tr>
<td>kJ from Protein (%)</td>
<td>17.71 ± 1.17</td>
<td>19.47 ± 1.55</td>
<td>21.17 ± 1.78</td>
<td>20.04 ± 2.31</td>
<td>19.98 ± 1.55</td>
</tr>
<tr>
<td>kJ from Total fat</td>
<td>31.05 ± 2.29</td>
<td>33.02 ± 2.12</td>
<td>32.12 ± 1.83</td>
<td>31.29 ± 3.13</td>
<td>33.87 ± 2.16</td>
</tr>
<tr>
<td>kJ from Carbohydrates (%)</td>
<td>45.26 ± 2.35</td>
<td>41.34 ± 2.06</td>
<td>42.31 ± 2.24</td>
<td>41.98 ± 2.57</td>
<td>40.65 ± 1.69</td>
</tr>
<tr>
<td>kJ from Fibre (%)</td>
<td>2.59 ± 0.37</td>
<td>2.53 ± 0.33</td>
<td>2.18 ± 0.24</td>
<td>2.52 ± 0.24</td>
<td>2.52 ± 0.24</td>
</tr>
<tr>
<td>kJ from others (%)</td>
<td>1.24 ± 0.37</td>
<td>1.20 ± 0.31</td>
<td>1.30 ± 0.26</td>
<td>1.24 ± 0.31</td>
<td>0.87 ± 0.13</td>
</tr>
</tbody>
</table>

1 Indicate the main effect of treatment, observed between groups (baseline adjusted based on the linear mixed model)
Figure Titles and legends

**Figure 1.** Participants flow-chart CONSORT guidelines

**Figure 2.** Mean changes in subgroup triglycerides concentration. (A) normal triglycerides (<1.7 mmol/L) participants. (B) high triglycerides (≥ 1.7 mmol/L) participants. Values were calculated as the difference between 6 wk, 12 wk and baseline and they were compared using a linear mixed-effect model with time as within subject factor and intervention group as a between-subject factor. Data is presented as mean ± SEM obtained *denoted significant changes during 12-week intervention, P < 0.05.

**Figure 3.** The prevalence of MetS in participants from control and treatment groups across intervention period, number of participants is specified on each bar.
Total Enquiries = 151

- fail to meet inclusion criteria = 20
- eligible Participants = 131
- Declined to participate = 67
- Randomised = 64

Control = 19
- Discontinue Intervention
  - Allocation to control group instead of treatment = 2
  - Loss follow up = 1
  - Complete = 16
    - Analysis
      - Lipid Profile = 16
      - Glucose = 16
      - Hormones = 15-12
      - Body composition = 16

25 gr/day = 22
- Discontinue Intervention
  - Dislike Intervention = 2
  - Time Constraints = 1
  - Loss follow up = 3
  - Complete = 16
    - Analysis
      - Lipid Profile = 16
      - Glucose = 16
      - Hormones = 15-9
      - Body composition = 16

50 g/day = 23
- Discontinue Intervention
  - Dislike Intervention = 1
  - Time Constraints = 1
  - Loss follow up = 3
  - Complete = 18
    - Analysis
      - Lipid Profile = 18
      - Glucose = 18
      - Hormones = 15-7
      - Body composition = 18
Total prevalence of MetS (%)