




BMJ Open Effects of milk protein concentrate supplementation on metabolic parameters, adipocytokines and body composition in obese women under weight-loss diet: study protocol for a randomised controlled trial

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To cite: Haidari F, Elahikhah M, Shariful Islam SM, *et al.* Effects of milk protein concentrate supplementation on metabolic parameters, adipocytokines and body composition in obese women under weight-loss diet: study protocol for a randomised controlled trial. *BMJ Open* 2022;**12**:e064727. doi:10.1136/bmjopen-2022-064727

► Prepublication history for this paper is available online. To view these files, please visit the journal online (<http://dx.doi.org/10.1136/bmjopen-2022-064727>).

Received 18 May 2022

Accepted 08 September 2022



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ABSTRACT

Introduction Obesity impairs metabolic function and increases the risk of cardiovascular disease and type 2 diabetes mellitus. Evidence suggests that high-protein diets help to increase weight loss and protect against weight gain. Milk protein concentrate (MPC) is a dairy product with a high protein content with a ratio of casein and whey protein similar to skim milk. This trial aims to evaluate the effect of MPC supplementation in obese women under a weight-loss diet.

Methods and analysis We will conduct a 2-month open-label, parallel-group, randomised controlled trial to determine the effect of MPC supplementation on levels of glycaemic and lipid profile, leptin, adiponectin, appetite, waist circumference, body mass index and body composition in 44 premenopausal obese women on a weight-loss diet.

Ethics and dissemination This protocol, approved by the Medical Ethics Committee of Ahvaz University of Medical Sciences, is in accordance with the Declaration of Helsinki (approval number: IR.AJUMS.REC.1399.795). The trial results will be published in peer-reviewed journals.

Trial registration number Iranian Registry of Clinical Trials (IRCT20201223049804N1).

INTRODUCTION

Obesity is defined as the excessive accumulation of fat in the body (body mass index (BMI) $\geq 30 \text{ kg/m}^2$) and is associated with dysregulation of glucose and lipoprotein metabolism.¹ In particular, it has been shown that visceral fat, as an independent factor, impairs metabolic function and increases the risk of cardiovascular disease and type 2 diabetes mellitus.² It is reported that additional production of proinflammatory cytokines by macrophages in visceral adipose tissue leads to insulin resistance.³ Inflammatory cytokines produced by adipose tissue prevent the binding of insulin

STRENGTHS AND LIMITATIONS OF THIS STUDY

- ⇒ We will conduct a 2-month open-label, parallel-group, randomised controlled trial to determine the effect of 30-gram milk protein concentrate supplementation on levels of glycaemic and lipid profile, leptin, adiponectin, appetite, waist circumference, body mass index and body composition in 44 premenopausal obese women on a weight-loss diet.
- ⇒ A limitation of this study is that other adipokines affecting energy homeostasis in the body and incretin hormones will not be measured due to restrictions in funding.
- ⇒ Another limitation of this study is that all of the subjects will only be female.

to its receptors, thereby induce insulin resistance in target tissues.⁴ According to the WHO in 2016, more than 1.9 billion adults were overweight and 650 million were obese (13% of the world's adult population).⁵ Also, the prevalence of obesity in adult female and male Iranians was reported at 29.3% and 13.6%, respectively.⁶ Probably due to differences in sex hormones, unknown molecular mechanisms and genetics, women have a greater risk of obesity compared with men.⁷

Adipose tissue is an important metabolic organ that is crucial for insulin sensitivity and energy homeostasis in the body.⁸ This tissue produces two hormones leptin and adiponectin called adipocytokines which influence lipid and glucose metabolism. According to studies with large populations, the ratio of leptin to adiponectin is an indicator of insulin sensitivity in the body.^{9 10} Leptin regulates appetite, energy expenditure, and food intake by binding to

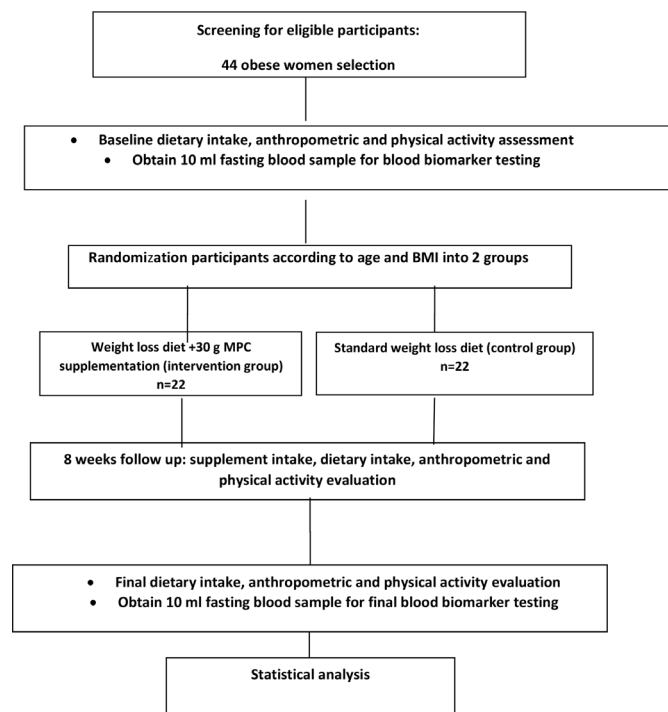


Figure 1 Protocol flow diagram. BMI, body mass index; MPC, milk protein concentrate.

its receptors in the hypothalamus and helps to control weight.¹¹ In obese individuals, leptin levels increase and lead to the development of obesity and insulin resistance.¹² Conversely, adiponectin acts as an insulin sensitizer and improves lipid profile by increasing tissue fat oxidation. Nevertheless, decreased levels of adiponectin have been reported in obese individuals.¹³

Obesity is a complex disease caused by the interaction of genetics, epigenetics, economic, physiological, social and environmental factors (lifestyle, diet and physical activity).^{14 15} It has been shown that the models of diet-induced obesity, due to their similarity to human obesity, are often used in metabolic studies.¹⁶ Fat-rich diets play a role in the induction of obesity, impaired glucose homeostasis as well as insulin resistance and hyperlipidaemia.¹⁷ Studies have shown that regular intake of dietary protein, especially dairy protein, may have beneficial effects on weight control and metabolic syndrome.^{18 19}

Complete dairy protein or milk protein concentrate (MPC) is a relatively new dairy product with high protein content (70% protein) that is made by combining three methods including diafiltration, ultrafiltration and spray-drying from pasteurised skim milk (31% protein). MPC contains both casein and whey protein, and the ratio of these two proteins is similar to skim milk (4:1 or 80:20). This ingredient is used in dairy products such as cheeses and yoghurts.²⁰ A study in obese rats showed that MPC reduced body weight and fat accumulation more than casein and whey protein alone. This may be due to the longer digestion process and greater satiety effects than the other two proteins.¹⁸ Also, MPC, as a source of protein with a high biological value that contains all kinds

of essential and non-essential amino acids, can prevent muscle wasting.²¹ Another study reported that intraduodenal infusion of MPC significantly improved the effects of sitagliptin including glycaemic and short-term food intake suppression. The results of this study confirm the hypothesis that the consumption of dairy protein may be useful as a complementary therapy to enhance the glycaemic and food intake suppressive effects of GLP-1-based pharmacotherapies.²² Furthermore, a low-energy diet helps to improve systemic dysmetabolism by inducing weight loss,²³ has positive effects on adiponectin concentration²⁴ and is associated with decreased serum levels of leptin in obese individuals.²⁵

To the best of our knowledge, there is no study to investigate the effects of MPC along with a weight-loss diet in obese women. Therefore, the present study aims to investigate if supplementation of the 30-gram MPC in adjunct with a weight-loss diet will be useful in improving metabolic parameters, serum levels of adipocytokines and body composition in obese women.

METHODS AND ANALYSIS

Study design

We will conduct an 8-week open-label, parallel-group, randomised controlled trial with a superiority framework. The proposed clinical trial will be conducted at the Nutritional Research Center, Department of Nutrition, Ahvaz Jundishapur University of Medical Sciences for 8 weeks to assess the efficacy of the 30mg MPC in adjunct with weight-loss diet in obese women. Recruitment for this study and data collection began in April 2022 and are expected to end in September 2022 (figures 1 and 2).

Aims and study hypotheses

The primary aim of the current trial is to examine the effect of 8 weeks of MPC supplementation on levels of leptin, adiponectin, waist circumference (WC), BMI and body composition in obese women on a weight-loss diet. The secondary aim is to determine the effect of 8 weeks of MPC supplementation on glycaemic and lipid profile, insulin and appetite. It is hypothesised that 8-week supplementation of the MPC in adjunct with a weight-loss diet will improve measures of investigation.

Participants

Forty-four premenopausal obese women aged 18 years or more referred to the diet clinic in the city of Ahvaz meeting the inclusion criteria will be recruited in this trial after obtaining their informed written consent. The following inclusion criteria will be applied: aged 18 years and older; BMI range of 30–40 kg/m²; absence of menopause; absence of lactation and pregnancy; absence of food allergies; not having eating disorders, particularly binge eating disorder or bulimia; not having cancer, hepatic, renal, thyroid and gastrointestinal disorders; no surgery for weight loss; no weight loss over the past 6 months; not taking any herbal medicine and

TIMEPOINT**	STUDY PERIOD							
	Enrolment	Allocation	Post-allocation					Close-out
	$-t_1$	0	Week 1	Week 3	Week 6	Week 8	etc.	t_x
ENROLMENT:								
Eligibility screen	X							
Informed consent	X							
Randomization		X						
Allocation		X						
INTERVENTIONS:								
[Intervention A]			←————→					
[Intervention B]			←————→					
ASSESSMENTS:								
Demographic variables	X	X						
primary outcome variables: levels of adiponectin and leptin, and the anthropometric status.			X	X	X	X	etc.	X
Secondary outcome variables: fasting plasma insulin and glucose levels, serum levels of lipid profiles (total cholesterol, LDL-c, HDL-c, and triglyceride), homeostasis model assessment of insulin resistance (HOMA-IR), and appetite status.			X	X	X	X	etc.	X

Figure 2 Template of recommended content for the schedule of enrolment, interventions and assessments. HDL-c, high-density lipoprotein cholesterol; LDL-c, low-density lipoprotein cholesterol.

drug that reduces appetite and weight (such as caraway extract, celery extracts, metformin, orlistat, etc) and vitamin–mineral supplements. The subjects with any of the following criteria will be excluded: become pregnant during the study, unwilling to continue, changes in diet during the study period and no consumption of powders exceeding 10% of total administered powders.

Patient and public involvement

Patients were not involved in the development and design of the study protocol. The public was not involved. Study results will not be disseminated to participants specifically. However, if participants are interested in the results of the research, they will receive any information, the manuscript and published research on this topic in the future.

Sample size

The required sample size is calculated based on data from a previous human study by Faghieh *et al*,²⁶ which assessed the effects of cow milk consumption in adjunct with a weight-loss diet on weight and fat loss. A mean difference in weight of 1.6 kg between the two groups

is aimed to be detected for a specified α of 0.05 and a study power of 80%. Based on the proposed formula for parallel clinical trials,²⁷ we reached a sample size of 20 participants in each group. Assuming a possible drop-out rate of 10%, 22 patients will be enrolled in each group.

Randomisation

The subjects will be randomly stratified according to age and BMI using a permuted block randomisation procedure by Random Allocation Software. The ratio of intended numbers of participants in each of the matched groups will be 1:1. They will be assigned to one of the two study groups (figure 1):

1. Standard weight loss group (n=22) (control group).
2. MPC supplementation weight loss group (n=22) (intervention group).

A member of the research team who is not involved in assessing the outcome of the study will be responsible for generating the allocation sequence and will allocate participants to the sequence.

Intervention

All subjects will follow a hypocaloric diet of 800 kcal below estimated energy requirements, which will be designed by a trained dietitian. Energy needs will be estimated by the Mifflin-St Jeor equation.²⁸ In the control group, the percentage of macronutrients will be 55%, 30%, and 15% for carbohydrates, fat, and protein, respectively.²⁹ For the intervention group, an isocaloric weight-loss diet with MPC supplements will be prescribed. Each of the intervention group participants will receive 30-gram MPC powders daily lasting 8 weeks. MPC powders will be supplied by Pegah Dairy Industries Co, Tehran, Iran. The MPC powders will be provided in a sachet form. Each sachet will contain 30-gram MPC (105 kcal, 0.4g of lipid, 6g of carbohydrate and 20 g of protein; 20% whey protein and 80% casein). Considering the calorie of each MPC sachet (105 kcal), 905 calories below estimated energy needs will be regarded for the intervention group. Participants in the intervention group will be instructed to add one sachet to 250mL cold water and consume it immediately, every morning on an empty stomach.³⁰ To check compliance, subjects will be requested to record the time and date of powder intake. Moreover, to ensure that the intervention group participants regularly consume the powders, they will be contacted every 3 days by a dietitian, or if it was not possible to call them, they would be followed through SMS. To create a variety in the diet while maintaining the general principles of diet, all subjects will be given a dietary exchange list and a diet according to their food habits.³¹ The study subjects will be asked not to change their dietary habits and physical activity during the study (8 weeks).

The assigned study intervention may need to be modified or discontinued by trial investigators for various reasons, including gastrointestinal upset, allergic reactions and withdrawal of participant consent. We will call the patients weekly and ask them about any adverse events occurring following MPC consumption. If the reported adverse events are correlated with MPC consumption, participants will be asked to stop taking MPC supplements, and also, they will be immediately referred to a specialist for therapy.

Outcomes

The primary outcomes consist of levels of adiponectin, leptin, body composition, WC and BMI. Secondary outcomes will be fasting plasma insulin and glucose levels, serum levels of lipid profiles (total cholesterol, low-density lipoprotein cholesterol (LDL-c), high-density lipoprotein cholesterol (HDL-c) and triglyceride), Homeostasis Model Assessment of Insulin Resistance (HOMA-IR) and appetite status. All these factors will be measured at baseline and end of the study. We would manage multiplicity issue about multiple primary outcomes using Bonferroni correction.

Assessment of dietary intake, anthropometric parameters and physical activity

A sociodemographic questionnaire will be recorded at the beginning of the study. Dietary intake will be evaluated

by 3 days 24 hours' recall questionnaires (2 weekdays and 1 weekend day) at the beginning, middle and end of the study. Total energy and macronutrient intake will be calculated by Nut IV (the Hearst Corporation, San Bruno, California, USA). A trained dietitian will evaluate anthropometric variables such as WC, body weight, height and BMI after overnight fasting with minimum clothing. At the baseline and the end of the study, WC will be measured in a standing position using a tape with an accuracy of 1.0cm above the iliac crest, just below the lowest rib margin and at the end of normal expiration. Body weight will be measured with the accuracy of 100 g using a Seca scale at the baseline and end of the study. Stature will be measured in a relaxed position by a Seca stadiometer with an accuracy of 0.5 cm. BMI will be calculated as body weight (kg) divided by the square of height (m), at the beginning and end of the study. The direct segmental multifrequency bioelectrical impedance method (Inbody 270, Biospace, Korea) will be used to calculate body composition including total body fat and fat-free mass percentage.

To evaluate the physical activity levels, the International Physical Activity Questionnaire (IPAQ) will be used at the baseline and the end of the trial via interviewing, and the results will be expressed as metabolic equivalent hours per week. The Persian translation of the short form IPAQ has been validated by Dashti *et al* (Cronbach's $\alpha=0.7$ and test-retest reliability coefficient=0.9).³²

Assessment of appetite

The Council on Nutrition Appetite Questionnaire, which was adopted by Wilson *et al*,³³ will be used for measuring appetite. This questionnaire contains eight single-domain items, scales of each item ranged from 1 to 5. Thus, the total score ranges from 8 to 40 points. A score of less than 28 is a cause for concern. The validity of this questionnaire has been investigated in Iran (Cronbach's $\alpha=0.77$).³⁴

Assessment of biochemistry variables

At baseline and end of the study, 10mL of venous blood samples (in regular tubes) will be collected after 10–12 hours of overnight fasting. Fasting blood glucose and lipid profile (total cholesterol, LDL-c, HDL-c and triglyceride) will be evaluated by the enzymatic method with kits from Pars-Azmoon (Tehran, Iran). Insulin levels will be measured by chemiluminescent immunoassay. HOMA-IR will be calculated by the following formula: fasting glucose (mg/dL)×fasting insulin (μ U/mL)/405. ELISA kits will be used to determine serum leptin and adiponectin levels. All data will be entered electronically at the participating site where the data originated. Original study forms will be entered and kept on file at the participating site. Participant files are to be stored in numerical order and stored in a secure and accessible place and manner. Participant files will be maintained in storage for a period of 3 years after completion of the study. All principal investigators will be given access to the cleaned data sets.

Statistical analysis

Data analysts will be blinded after the assignment to interventions. All statistical analyses will be performed using the IBM SPSS Statistics software (V.23). The normality of the variables will be confirmed using the Kolmogorov-Smirnov test. A X^2 test will be applied to compare the categorical data between treatment groups at the baseline. Independent sample t-test and Mann-Whitney test will be used to compare parametric continuous and non-parametric data between the groups, respectively. Paired sample t-test or Wilcoxon signed-rank test will be applied to compare data within the groups. Post-intervention differences in responses between intervention and control groups will be tested by analysis of covariance with the use of the baseline measurements of the outcome variables as covariates. The per cent change of each variable will also be computed by the formula $[(E-B)/B \times 100]$, where E is the end of intervention values and B is the baseline values. The intention-to-treat method will be applied for data analysis, which considers all participants in the trial and ignores anything that happens after randomisation such as misallocation and non-compliance. A p value of less than 0.05 will be considered to be statistically significant.

Ethics and dissemination

This protocol, approved by the Medical Ethics Committee of Ahvaz University of Medical Sciences, is in accordance with the Declaration of Helsinki (approval number: IR.AJUMS.REC.1399.795). The monitors from the Medical Ethics Committee of Ahvaz University of Medical Sciences will discuss the protocol in detail and identify and clarify any areas of weakness. Written informed consent will be obtained from participants before participation in the research project by researchers. All participant information will be stored in locked file cabinets in areas with limited access. This investigation was registered on the Iranian Registry of Clinical Trials (IRCT20201223049804N1).

DISCUSSION

Epidemiological studies show that the intake of milk and dairy products is inversely associated with a lower risk of metabolic disorders and cardiovascular diseases.²⁹ Differences in the digestion kinetics of whey and casein proteins facilitate the stimulation of gastric hormones that delay gastric emptying, thus increasing feelings of fullness and attenuation of food particle breakdown and release in the small intestine. Whey and casein proteins differentially affect postprandial blood glucose and satiety mechanisms, with relevance to type 2 diabetes and obesity.³⁰ It seems that complete dairy consumption improves body composition and insulin sensitivity to a greater extent than whey or casein alone. In a diet-induced obese rat model, administration of complete dairy protein reduced weight gain and body fat mass accumulation more so than intake of whey or casein alone.¹⁸ The intrainestinal presence of specific bioactive components, whole proteins,

and select amino acids found within MPC is linked with insulin and gut peptide secretions, as well as suppression of food intake.^{35–37}

This is the first study that investigates the effect of supplementation of the MPC in adjunct with a weight-loss diet on improving metabolic parameters, serum levels of adipocytokines and body composition in obese women. Results of this trial will help us understand the effectiveness of MPC along with a weight-loss diet to improve metabolic parameters, serum levels of adipocytokines, appetite and body composition compared with controlled participants. In addition, the results of this trial will help obese women to reduce weight and improve their cardiometabolic health.

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Acknowledgements We thank Ahvaz Jundishapur University of Medical Sciences for funding this trial.

Contributors FH and VA designed the research protocol. FH, VA and ME were involved in the set-up of the original intervention study and follow-up study. FH, ME, MM, HS, SMSI and VA were involved in the writing of the article and carefully reviewed the article. FH acted as the guarantor.

Funding This research is funded by Ahvaz Jundishapur University of Medical Sciences (grant number: NRC-9912).

Disclaimer This funding source has no role in the design of this study and will not have any role during its execution, analyses, interpretation of the data or decision to submit results.

Competing interests None declared.

Patient and public involvement Patients and/or the public were not involved in the design, or conduct, or reporting, or dissemination plans of this research.

Patient consent for publication Obtained.

Provenance and peer review Not commissioned; externally peer reviewed.

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REFERENCES

- Atawia RT, Bunch KL, Toque HA, *et al.* Mechanisms of obesity-induced metabolic and vascular dysfunctions. *Front Biosci* 2019;24:890.
- Takei R, Inoue T, Sonoda N, *et al.* Bilirubin reduces visceral obesity and insulin resistance by suppression of inflammatory cytokines. *PLoS One* 2019;14:e0223302.



- 3 Chawla A, Nguyen KD, Goh YPS. Macrophage-Mediated inflammation in metabolic disease. *Nat Rev Immunol* 2011;11:738–49.
- 4 Barazzoni R, Gortan Cappellari G, Ragni M, et al. Insulin resistance in obesity: an overview of fundamental alterations. *Eat Weight Disord* 2018;23:149–57.
- 5 Murtagh E, Collaboration NRF. *Worldwide trends in children's and adolescents' body mass index, underweight, overweight and obesity, in comparison with adults, from 1975 to 2016: A pooled analysis of 2,416 population-based measurement studies with 128.9 million participants Pre-published version*, 2017.
- 6 Feigin V, Collaborators GRFGlobal, GBD 2015 Risk Factors Collaborators. Global, regional, and national comparative risk assessment of 79 behavioural, environmental and occupational, and metabolic risks or clusters of risks, 1990–2015: a systematic analysis for the global burden of disease study 2015. *Lancet* 2016;388:1659–724.
- 7 Reilly JJ, El-Hamdouchi A, Diouf A, et al. Determining the worldwide prevalence of obesity. *The Lancet* 2018;391:1773–4.
- 8 Naowaboot J, Chung CH, Choi R. Rutin stimulates adipocyte differentiation and adiponectin secretion in 3T3-L1 adipocytes. *J Med Assoc Thai* 2015;98 Suppl 3:S1–6.
- 9 López-Jaramillo P, Gómez-Arbeláez D, López-López J, et al. The role of leptin/adiponectin ratio in metabolic syndrome and diabetes. *Horm Mol Biol Clin Investig* 2014;18:37–45.
- 10 Finucane FM, Luan J, Wareham NJ, et al. Correlation of the leptin:adiponectin ratio with measures of insulin resistance in non-diabetic individuals. *Diabetologia* 2009;52:2345–9.
- 11 Zulfania AK, Tahir Ghaffar AK, SURO MA. Correlation between serum leptin level and body mass index (BMI) in patients with type 2 diabetes mellitus. *JPMA* 2019;2020.
- 12 Ghadge AA, Khair AA. Leptin as a predictive marker for metabolic syndrome. *Cytokine* 2019;121:154735.
- 13 Martinez-Huenchullan SF, Tam CS, Ban LA, et al. Skeletal muscle adiponectin induction in obesity and exercise. *Metabolism* 2020;102:154008.
- 14 Jouret B, Ahluwalia N, Cristini C, et al. Factors associated with overweight in preschool-age children in southwestern France. *Am J Clin Nutr* 2007;85:1643–9.
- 15 Hruby A, Hu FB. The epidemiology of obesity: a big picture. *Pharmacoeconomics* 2015;33:673–89.
- 16 Lutz TA, Woods SC. Overview of animal models of obesity. *Curr Protoc Pharmacol* 2012;58:5.
- 17 Hariiri N, Thibault L. High-Fat diet-induced obesity in animal models. *Nutr Res Rev* 2010;23:270–99.
- 18 Eller LK, Reimer RA. Dairy protein attenuates weight gain in obese rats better than whey or casein alone. *Obesity* 2010;18:704–11.
- 19 Poddar KH, Hosig KW, Nickols-Richardson SM, et al. Low-Fat dairy intake and body weight and composition changes in college students. *J Am Diet Assoc* 2009;109:1433–8.
- 20 Zhang J, Liu D, Liu Y, et al. Effects of particle size and aging of milk protein concentrate on the biophysical properties of an intermediate-moisture model food system. *Food Biosci* 2020;37:100698.
- 21 Cava E, Yeat NC, Mittendorfer B. Preserving healthy muscle during weight loss. *Adv Nutr* 2017;8:511–9.
- 22 Olivos DR, McGrath LE, Turner CA, et al. Intraduodenal milk protein concentrate augments the glyceic and food intake suppressive effects of DPP-IV inhibition. *Am J Physiol Regul Integr Comp Physiol* 2014;306:R157–63.
- 23 Hansen TT, Hjorth MF, Sandby K, et al. Predictors of successful weight loss with relative maintenance of fat-free mass in individuals with overweight and obesity on an 8-week low-energy diet. *Br J Nutr* 2019;122:468–79.
- 24 Salehi-Abargouei A, Izadi V, Azadbakht L. The effect of low calorie diet on adiponectin concentration: a systematic review and meta-analysis. *Horm Metab Res* 2015;47:549–55.
- 25 Maffei M, Halaas J, Ravussin E, et al. Leptin levels in human and rodent: measurement of plasma leptin and ob RNA in obese and weight-reduced subjects. *Nat Med* 1995;1:1155–61.
- 26 Faghhih S, Abadi AR, Hedayati M, et al. Comparison of the effects of cows' milk, fortified soy milk, and calcium supplement on weight and fat loss in premenopausal overweight and obese women. *Nutr Metab Cardiovasc Dis* 2011;21:499–503.
- 27 Kirby A, Gebbski V, Keech AC. Determining the sample size in a clinical trial. *Med J Aust* 2002;177:256–7.
- 28 Mifflin MD, St Jeor ST, Hill LA, et al. A new predictive equation for resting energy expenditure in healthy individuals. *Am J Clin Nutr* 1990;51:241–7.
- 29 Haidari F, Aghamohammadi V, Mohammadshahi M, et al. Whey protein supplementation reducing fasting levels of anandamide and 2-AG without weight loss in pre-menopausal women with obesity on a weight-loss diet. *Trials* 2020;21:1–10.
- 30 Kung B, Anderson GH, Paré S, et al. Effect of milk protein intake and casein-to-whey ratio in breakfast meals on postprandial glucose, satiety ratings, and subsequent meal intake. *J Dairy Sci* 2018;101:8688–701.
- 31 Haidari F, Aghamohammadi V, Mohammadshahi M, et al. Effect of whey protein supplementation on levels of endocannabinoids and some of metabolic risk factors in obese women on a weight-loss diet: a study protocol for a randomized controlled trial. *Nutr J* 2017;16:70.
- 32 Dashti S, TT S, Esfehiani AJ. Effect of physical activity level on emotional status of Iranian women. *World Applied Sciences Journal* 2014;30:852–7.
- 33 Wilson M-MG, Thomas DR, Rubenstein LZ, et al. Appetite assessment: simple appetite questionnaire predicts weight loss in community-dwelling adults and nursing home residents. *Am J Clin Nutr* 2005;82:1074–81.
- 34 SHAFIEI SM. *Relationship between nutritional status and appetite among HIV patients on HAART*, 2014.
- 35 Diepvens K, Häberer D, Westerterp-Plantenga M. Different proteins and biopeptides differently affect satiety and anorexigenic/orexigenic hormones in healthy humans. *Int J Obes* 2008;32:510–8.
- 36 Hall WL, Millward DJ, Long SJ, et al. Casein and whey exert different effects on plasma amino acid profiles, gastrointestinal hormone secretion and appetite. *Br J Nutr* 2003;89:239–48.
- 37 Liddle RA, Green GM, Conrad CK, et al. Proteins but not amino acids, carbohydrates, or fats stimulate cholecystokinin secretion in the rat. *Am J Physiol Gastrointest Liver Physiol* 1986;251:G243–8.