Systems diagnosis in chronic disease: prediction and evaluation

Fu, J.

Chapter 6

Systems response profiles to two Rehmanniae Radix formulae in metabolic syndrome patients

Based on

Junzeng Fu, Herman A. van Wietmarschen, Jan van der Greef, Yan Schroën, and Mei Wang

Systems response profiles to two Rehmanniae Radix formulae in metabolic syndrome patients

World Journal of Traditional Chinese Medicine, in press

Abstract

Objective To explore the effects of two Rehmanniae Radix formulae in patients with metabolic syndrome (MetS), a randomized controlled study was conducted.

Methods MetS patients were randomly assigned to receive either a classic Rehmanniae Six Formula (R6, or 'Liu Wei Di Huang Wan') or a novel multi-herbal Rehmanniae Radix containing formula SUB889 for 8 weeks. Western medicine related clinical parameters, Chinese medicine defined symptoms and syndromes as well as metabolomic profiles were evaluated at different time points.

Results R6 (n = 20) and SUB889 (n = 20) showed similar effects on MetS regarding the improvement of clinical parameters (waist circumference, body mass index, LDL-cholesterol, systolic blood pressure) and Qi/Yin deficiency ($p < 0.05$). Decreased levels of cholesteryl esters, phosphatidylcholines, triglycerides and sphingomyelins were found in the R6 group, while SUB889 formula resulted in increased levels of tricarboxylic acid cycle and glucose metabolism intermediates (malate, fumarate and pyruvate).

Conclusions R6 and SUB889 have similar effects on the treatment of MetS by improving Chinese medicine and Western medicine defined clinical outcomes. R6 is more effective in improving lipid profiles compared to SUB889. The exact mechanisms of the two formulae on MetS remain to be elucidated.

Introduction

Metabolic syndrome (MetS) is defined as a combination of central obesity, hyperglycemia, dyslipidemia and hypertension [1]. It is widely accepted that MetS is associated with an increased risk of cardiovascular disease and diabetes [2]. MetS, as defined by Western diagnostics, has no corresponding syndrome described in Chinese medicine [3,4].

Chinese medicine (CM) is a descriptive medicine developed by the accumulation of empirical experience over thousands of years. In patients suffering from one disease defined by Western medicine (WM), a variety of CM syndromes may be observed [5,6]. Yin deficiency and Qi deficiency are two of the most generic CM syndromes. Syndrome differentiation is an essential diagnostic part of CM: a patient is diagnosed by observation, listening, questioning and pulse feeling. Diagnosis is followed by providing a CM therapy that corresponds to the syndrome, effecting multiple targets in the human body [7].

In contrast, WM is designed to work in a 'one disease-one target' mode. Often single biomarkers are used for diagnosis and monitoring disease progression (for example: fasting glucose level, blood pressure, cholesterol level) [8]. Techniques such as metabolomics are developed for biomarker discovery in complex diseases which are not properly characterized by single biomarkers. Metabolomics is the systematic analysis of metabolites in body fluids, providing a comprehensive view of metabolic changes. Those patterns of change are potential biomarkers/biosignatures which can be used to optimize disease diagnosis and treatment [9]. By unifying CM, WM and metabolomics technologies, different levels of organization within the body (metabolites, pathways, overarching processes, organ function, and even mental and emotional functioning) can be measured and integrated as individual system response profiles. Based on the response profiles, a better understanding of how patients respond to treatments can be obtained, and guidance for personalized treatment can be provided.

Herbal medicine is one of the most commonly administered CM therapies due to its ease of use, low costs and good compliance. An herbal formula consisting of multiple herbal constituents can be considered as a holistic and synergetic medicine. Rehmannia Six Formula (R6 or 'Liu Wei Di Huang Wan') has been used in clinical practice for thousands of years in China and is now widely used as a combination treatment for metabolic disorder related diseases including diabetes, hypertension, and hyperlipidemia [10–12]. R6 is made from a concentrated water extract of six herbs: Rehmanniae Radix Praeparata, Dioscoreae Rhizoma, Corni, Poria, Alismatis Rhizoma and Moutan Cortex in a proportion of 8:4:4:3:3:3 [13]. According to Chinese medicine principles, R6 is known to promote the health of patients with Yin deficiency by nourishing 'Kidney-liver Yin-deficiency'. Based on our previous study, R6

can improve the lipid profiles of MetS patients. Furthermore, the CM syndrome Yin and Qi deficiency were found to be improved by using the herbal formula over 8 weeks, and a specific sequence of events in the timing of those effects was discovered—Yin deficiency was found to be improved first after which Qi deficiency was improved [13]. There are no other studies reporting the use of R6 as a treatment for MetS, but there is a randomized controlled trial which reported using R6 to treat chronic kidney disease induced by MetS [14]. R6 is also reported as effective when used in combination with Western drugs to treat type 2 diabetes or hypertension [10,11]. In addition, Huang et al. reported reduction of kidney failure risk among type 2 diabetic patients by taking R6 [15].

Based on CM guidelines and traditional practices, a novel formula SUB889 was designed for MetS subjects with Qi and Yin deficiency, but more focused on motivating 'Yang' without damaging 'Yin', an effect that is lacking in R6. SUB889 is a multi-component formula containing nine herbs: Rehmanniae Radix, Polygoni Multiflori Radix, Cistanches Herba, Lycii Fructus, Polygonati Rhizoma, Ganoderma, Acanthopanacis Senticosi Radix, Atractylodis Rhizoma, Ephedrae Herba in a proportion of 12:6:6:5:5:5:5:3:1.

Even though the two Rehmanniae Radix formulae, R6 and SUB889, have different herbal compositions, both formulae contain Rehmanniae Radix as the 'emperor herb'. In CM theory an emperor herb is the principle herb in a formula, which plays a major role in the treatment effect. However, in the preparation of R6, Rehmanniae Radix is processed by repeated steaming and drying, which is named Rehmanniae Radix Preparata (Shu Di Huang); in the preparation of SUB889 dried rehmannia root is used without any processing (Sheng Di Huang). The processing procedure changes the properties of Rehmanniae Radix from 'releasing the Heat' to 'tonifying Yin' [16]. Besides that, the chemical composition is also changed by the processing procedure. Irodoids and oligosaccharides were reported to decrease while monosaccharides increase after processing [17,18]. Dried Rehmanniae Radix was chosen instead of Rehmanniae Radix Preparata in SUB889 to focus on the blood-cooling and nourishing Yin, activating the pulse and eliminating stasis, to replenish the Kidney in a reasonable way. Except for Rehmanniae Radix, there is no overlap of herbs between R6 and SUB889. The ingenuity in SUB889 is that the combination of all herbs can nourish Yin and motivate Yang at the same time. The roles of other herbs of SUB889 were explained in the Supplementary Method 'Roles of herbs in SUB889 except for Rehmanniae Radix'.

In order to compare the effects of the R6 and SUB889 formulae, MetS patients were randomized into two groups and subjected to an 8-week treatment with either R6 or SUB889. During this period, metabolite profiles, clinical parameters, CM syndromes and related

symptoms were measured at several time points up to 8 weeks after treatment. The measurements were analysed to evaluate treatment effects at different levels of organization within human body. The aim of the study is to combine all levels of data to explore the similarities and differences between MetS patients' response profiles to the two Rehmanniae Radix formulae. Furthermore, the response profiles will not only provide a better understanding of the treatment mechanisms involved using CM multi-herbal formulae (e.g. R6 and SUB889), but also improve our understanding of the biology of MetS.

Patients and Methods

The study design and subjects have been described by Wietmarschen et al. for MetS patients with R6 treatment [13], but also described here since it is essential for understanding the analysis presented in this paper.

Subjects

Human subjects were recruited from the Chengdu No. 1 People's Hospital in China. Subjects with MetS (waist circumference for men ≥ 90 cm, for women ≥ 8 0 cm; systolic blood pressure ≥ 130 mmHg or diastolic blood pressure ≥ 85 mmHg) and diagnosed as Qi and Yin deficiency were recruited. Subjects with a diagnosis of another disease or using medication were excluded. This study followed guidelines of the Declaration of Helsinki and Tokyo for humans, and was approved by the Ethics Committee of Chengdu No.1 People's Hospital. A doctor explained the details of the study to the subjects and ensured written informed consent of each subject.

Study design

The study was designed as a randomized controlled study in which two groups received an herbal formula for treating MetS, either R6 or SUB889 for 8 weeks. Qi /Yin deficiency and their related symptoms were recorded by one out of four CM doctors at the start of the study (T0), after 2 (T2), 4 (T4), 6 (T6) and 8 (T8) weeks of treatment. Fasting blood samples were collected and used to perform routine clinical chemistry (T0, T4, T8) and metabolic profiling (T0, T8).

Treatment

The R6 used in this study was an industrial and standardized product (batch number 4071161), manufactured by Beijing Tong Ren Tang Group Co., Ltd (Beijing, China). Daily intake of R6 was equivalent to active ingredients extracted from 9 g herbal mixture. The SUB889 was produced and tested for this study by Chengdu Jinhanfang Science and

Technology Service Co., Ltd (Chengdu, China). Daily intake of SUB889 was equivalent to active ingredients extracted from 12g herbal mixture (within the safety range of CM practice). In total 3 batches of SUB889 were produced for quality control, and one batch was used for the clinical study to minimize variation in the medication (batch number 050103). The procedure for producing SUB889 is described in the Supplementary Method section. Quality is controlled according to the Pharmacopeia of P. C. China 2010. No other Chinese herbal medication or Western medicine was allowed during this period.

Western medicine diagnosis: clinical parameters

Fasting blood samples were obtained for the measurement of glucose, insulin, triglycerides (TGs), total cholesterol (T-C), high-density lipoprotein cholesterol (HDL-C), low-density lipoprotein cholesterol (LDL-C), glycated hemoglobin (HbA1c) and creatinine at T0, T4 and T8. Age and gender was documented. Weight, waist circumference, body mass index (BMI), systolic blood pressure (SBP) and diastolic blood pressure (DBP) were measured at T0, T2, T4, T6 and T8. All clinical measurements were conducted by the Chengdu No.1 People's hospital.

Chinese medicine diagnosis: syndrome differentiation

The Qi/Yin deficiency syndromes were diagnosed and corresponding symptoms were recorded by trained CM doctors. The typical symptoms of Yin deficiency are 'heat sensation in the chest, palms and soles', 'hectic fever and night sweat', 'head giddiness', 'tinnitus', 'insomnia and dreamful', 'dry mouth and pharynx', 'constipation', 'thin tongue shape', 'red tongue substance' (i.e. the color of tongue body), 'yellow tongue coating' and 'weak pulse'; Qi deficiency is characterized by the symptoms 'fatigue', 'spontaneous sweating', 'taciturn', 'weak joints and waist', 'head giddiness', 'tongue shape normal or enlarged', 'pale-red tongue substance' and 'thready and weak pulse'(i.e. soft, feels like a sewing thread, weak, without strength but not scattered by pressure) [19,20]. Symptoms were measured as ordinal or nominal variables, and Qi- and Yin-deficiency scores were calculated by summing the related symptom scores. The calculation rules are described in Supplementary Table S1.

Metabolic profiling

Plasma samples collected at T0 and T8 were measured by the Netherlands Organization for Applied Scientific Research (TNO) Zeist in the Netherlands. Extra plasma of the subjects was pooled and used to create quality control (QC) samples.

The gas chromatography/mass spectrometry (GC/MS) platform measures a large variety of metabolite classes important for the biological functioning of humans, such as amino acids, fatty acids and sugars. Briefly, 100 µL of plasma was spiked with an internal standard (ISTD) mixture and extracted by methanol, followed by centrifugation and transferring the supernatant. After evaporating the supernatant to dryness, a two-step derivatization procedure was applied to the samples. The derivatized extracts were analyzed with an Agilent 6890N gas chromatograph equipped with an Agilent 5973 mass selective detector. Chromatographic separations were performed on a DB5-MS capillary column (30 m × 250 µm i.d., 0.25-µm film thickness; J&W Scientific, Folson, USA), using helium as the carrier gas at a flow rate of 1.7 mL/min. The raw data were pre-processed using Chemstation G1701CA (Agilent, version D.01.02). The method used is described in detail by Koek et al [21].

The liquid chromatography/mass spectrometry (LC/MS) platform targets fatty acids, phosphatidylcholines, triglycerides and other lipid classes. In brief, 10 µL of plasma was deproteinized by adding isopropanol containing an ISTD mix. This lipid profiling was conducted using TSQ Quantum Discovery Triple Quad mass spectrometer equipped with a Surveyor MS HPLC system. Chromatographic separations were performed with Alltech Prosphere C4 300Å column (150 × 3.2mm i.d., 5 µm; Alltech, Lexington, USA) in combination with a Symmetry 300 C4 guard column (10 × 2.1mm i.d., 3.5 µm; Waters, Milford, USA). The raw data were pre-processed using LCQuan (Thermo Fisher Scientific, version 2). The method used is described in detail by van Wietmarschen et al [13] and Draisma et al [22].

For each metabolite, the peak area was corrected by the appropriate internal standard (ISTD), and response ratios ($\text{Area}_{\text{analyte}}/\text{Area}_{\text{ISTD}}$) were then used in the subsequent analysis. An in-house developed algorithm was applied to compensate and correct for instrumental drift during the measurements. For each identified metabolite the standard deviation of its peak areas of the individual QC samples was computed relative to the average peak area over all QC samples (relative standard deviation, RSD) [22]. The RSDs of the metabolites in the QC samples to assess the quality of the measurements of the targeted metabolites in each analytical platform. Subject samples were measured in duplicate and the average value was calculated for each metabolite. Log transformation (generalized logarithm transformation) and auto scaling (mean-centered and divided by the standard deviation of each variable) were performed to normalize the metabolomics data before statistical analysis.

Statistical analysis

The aim of the data analysis was to discover similarities and differences of subjects' response profiles between R6 and SUB889 therapy. Those similarities and differences are related to the various types of data—clinical parameters, CM syndromes and related symptoms and metabolite profiles. Statistical calculations were performed with MetaboAnalyst 3.0 (available at http://www.metaboanalyst.ca/) [23] and IBM SPSS Statistics for Windows, Version 23. The level of statistical significance was set at $p < 0.05$.

Within group, paired t-tests were used to identify which metabolites/clinical parameters were significantly changed after 8-week treatment. Change ratios of metabolites and clinical parameters (level of metabolite or clinical parameter$_{T8}$ / level of metabolite or clinical parameter$_{T0}$) were calculated. Between groups, the independent t-test was used to identify which ratios were significantly different. To further determine the intergroup differences in the metabolite profiles, a hierarchical cluster analysis was performed and a heatmap was constructed by MetaboAnalyst to visualize changes of metabolite ratios.

To compare symptom changes after treatment, Marginal Homogeneity tests were applied to tongue and pulse related symptoms and Wilcoxon signed ranks tests were applied to other symptoms; to compare the symptom differences between groups, a Mann-Whitney U-test and a Chi-square test was performed to ordinal or nominal variables separately [24,25]. For each subject, Qi-/Yin-deficiency scores were calculated by summing related symptom scores every two weeks. These summed scores are considered as Qi-/Yin-deficiency states.

Results and discussion

Subjects

In total 67 subjects were recruited in the study and randomly assigned to receive R6 or SUB889 treatment. Seven included subjects dropped out at various time points of the trial. In total 60 subjects completed the study of which 29 were in the R6 group, and the other 31 subjects in the SUB889 group. Metabolomics analyses were performed on 20 samples from each group due to budget constraints, therefore all of the following analyses were performed on these 40 subjects. Independent student t-tests and Chi-square tests were applied to test for significant differences between two treatment group, as illustrated in Table 1 (R6 vs. SUB889, T0), there were no significant differences in clinical characteristics between the two groups at baseline (0 week).

Changes of clinical parameters

The results of the comparisons between the R6 and SUB889 groups as well as within each of the treatment groups are shown in Table 1. SBP and Central obesity related parameters—waist circumference and BMI decreased significantly after 8-week treatment in both groups. The body weight declined (67.1 ± 12.4 kg vs. 65.0 ±11.9 kg, p = 0.001) in the SUB889 group while in the R6 group the decrease was not significant (63.5 ± 5.7 kg vs. 62.7 ± 5.7 kg, p = 0.055). It seems that SUB889 has more effect on losing weight. In both groups, SBP and LDL-C decreased significantly. The decrease of T-C (5.4 ± 0.8 mmol/L vs. 4.6 ± 1.0 mmol/L, p = 0.003) and the increase of insulin level (9 ± 5 μU/mL vs. 10 ± 5 μU/mL, p = 0.038) were only found in R6 group. Therefore, R6 may improve the lipid profiles through affecting the insulin level in the human body. The change ratios of clinical parameters between the groups were similar (Table 1, R6 vs. SUB889). All the clinical parameter changes over time are shown as bar plots in Supplementary Fig S1.

Table 1. T-tests of clinical parameters within and between R6 (n=20) and SUB889 (n=20) group

	R6 (n=20)			**SUB889 (n=20)**			**p value[b]**
	T0	**T8**	**p value[a]**	**T0**	**T8**	**p value[a]**	
Age (years)	49±10		-	50±12		-	-
Gender (male/female)	5/15		-	8/12		-	-
Body weight (kg)	63.5±5.7	62.7±5.7	0.055	67.1±12.4	65.0±11.9	0.001	0.066
Waist circumference (cm)	88.9±6.2	87.6±5.7	0.001	91.0±7.3	88.6±6.6	0.001	0.168
Body mass index (kg/m^2)	24.9±2.3	24.5±2.2	0.005	25.7±4.3	25.0±4.1	<0.001	0.120
Plasma glucose (mmol/L)	5.4±0.7	5.5±0.8	0.93	5.6±0.8	5.5±0.7	0.803	0.815
Plasma insulin (μU/mL)	9±5	10±5	0.038	9±7	8±6	0.435	0.071
Total cholesterol (mmol/L)	5.4±0.8	4.6±1.0	0.003	4.9±0.8	4.7±1.0	0.203	0.126
Triglyceride (mmol/L)	3.1±2.7	2.4±1.7	0.053	2.5±1.4	2.2±1.3	0.218	0.585
LDL-cholesterol (mmol/L)	3.4±0.8	2.9±0.8	0.024	3.2±0.8	2.9±0.9	0.009	0.452
HDL-cholesterol (mmol/L)	1.4±0.3	1.3±0.3	0.654	1.3±0.3	1.3±0.3	0.488	0.389
HbA1c (%)	5.7±0.7	5.7±0.4	0.216	5.4±1.3	5.3±1.3	0.824	0.637
Systolic blood pressure (mmHg)	135±4	126±7	<0.001	138±8	127±11	0.001	0.593
Diastolic blood pressure (mmHg)	75±6	74±6	0.56	76±8	74±7	0.417	0.782
Creatinine (μmol/L)	64±19	63±18	0.877	70±15	69±13	0.785	0.919

[a] Paired t-tests were applied to compare the clinical parameters between 0 week and 8 weeks within group;

[b] Independent t-tests were applied to compare the change ratios of clinical parameters between R6 and SUB889.

Changes of symptoms and syndromes

According to CM, treatment effects can be observed as improvements of the corresponding CM syndrome. The operational definitions of the clinical diagnosis are not mutually exclusive, for example Qi and Yin deficiency contain overlapping symptoms (head giddiness, thready and weak pulse). Furthermore, a patient can show mixed syndromes, and syndromes can change during treatment. In this study, syndromes were represented by deficiency scores, and these scores were calculated by summing related symptom variables (Fig 1). The higher the deficiency score, the worse the patient is with regard to the corresponding syndrome. For each treatment time point the scores are plotted in Fig 2. It shows that both Rehmanniae Radix formulae reduced the deficiency scores after 8 weeks.

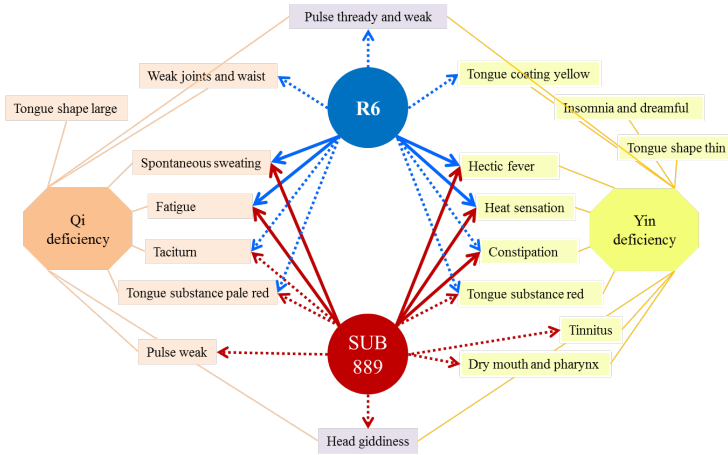


Figure 1. The treatment effects of R6 and SUB889 on CM symptoms related to Qi or Yin deficiency, comparing week 0 with week 8. Blue arrows indicate a symptom significantly improved with the R6 treatment (, $p < 0.01$; , $p < 0.05$); red arrows indicate a symptom significantly improved with SUB889 treatment (, $p < 0.01$; $p < 0.05$). Symptoms with light orange, light yellow or grey colors are related to Qi deficiency, Yin deficiency and both deficiencies respectively.

R6 is traditionally used to treat Kidney- and Liver-Yin deficiency, leading to a decrease of Yin-deficiency. However, in our study Qi-deficiency scores also decreased. This phenomenon may be explained by the observation that Qi- and Yin deficiency was present in all patients in the study, and moreover there was a significant correlation between these two deficiency scores (Pearson correlation coefficient = 0.362, $p < 0.001$). Furthermore, according to CM theory, Deficiency is the results of an imbalance in the human body, which can be restored with the use of herbal formulae [7]. As both Qi and Yin are essential components to maintain this balance, the reduction of Yin deficiency may cause the improvement of Qi deficiency. This can explain that the improvement of Qi-deficiency scores together with Yin-deficiency

scores during the R6 treatment. However, the mechanism behind it still needs to be further studied. Interestingly two different formulae demonstrated similar effects on CM syndrome deficiency scores—although the sequential events on improvement of symptoms were not exactly the same.

Mann-Whitney U-tests were applied to compare the R6 and SUB889 treatment in the deficiency scores, no significant differences were found at all the time points. Therefore, R6 and SUB889 have a similar effect over 8 weeks when considering the syndrome level. However, the decrease of the Yin deficiency score between T2 and T4 (Yin deficiency $score_{week\ 2}$ - Yin deficiency $score_{week\ 4}$) during SUB889 treatment was larger than that during R6 treatment ($p = 0.020$). Both Qi- and Yin-deficiency scores decreased after 8-week treatment ($p < 0.001$), which means R6 and SUB889 have significant effects in treating MetS based on CM diagnosis.

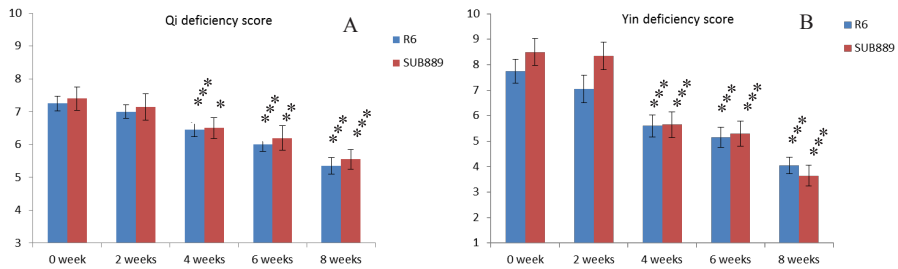


Figure 2. R6 and SUB889 decrease deficiency scores significantly. Effect of two formulae on Qi deficiency score (A) and Yin deficiency score (B) in MetS patients at time points of week 0, 2, 4 ,6 and 8 (blue bar: the R6 group; red bar: the SUB889 group).Data represent the mean± standard error of 20 patients and were analyzed by Wilcoxon signed rank test (* $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$ vs. 0 week). At each time point Qi or Yin deficiency scores were similar between R6 and SUB889 group

Associations between WM diagnosis and CM diagnosis

To investigate the associations between WM diagnostic clinical parameters and CM diagnostic scores for Qi/Yin deficiency, correlation analyses (Spearman's rho) were applied to the clinical parameters and deficiency scores containing all subjects with the R6 and SUB889 treatment (n=40) in week 0, 4 and 8. The results are shown in Supplementary Table S2. Qi- and Yin-deficiency scores were found to be highly correlated to systolic blood pressure (SBP) (Fig 3). Blood pressure is the diagnostic standard for hypertension according to WM, and the regulation of blood pressure involves a system intervention including the kidney, and the central or peripheral nervous systems in WM [26]. There is no concept of hypertension in CM, but according to symptoms it could be divided into three major CM

syndromes, including deficiency syndrome [27]. There are many studies pointing out the relationship between hypertension and Yin-/ Qi-deficiency. Although the mechanism behind this relationship is still unknown, deficiency in general may be a basic pathogenesis related to hypertension [28–30].

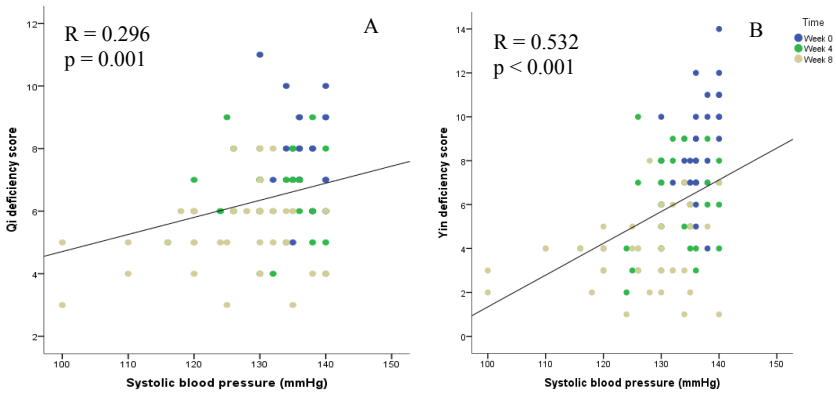


Figure 3. Relationship between systolic blood pressure and Qi deficiency score (A) and Yin deficiency score (B) during 8-week treatment of the two Rehmanniae Radix formulae. Graphs depict linear regression lines as well as Spearman correlation coefficients and their p values.

Changes of metabolites

Fasting blood samples collected from subjects treated with R6 (n=20) and SUB889 (n=20) at T0 and T8 were analyzed with a LC/MS method to obtain lipid profiles and with a GC/MS method to obtain global profiles. There were 95 and 127 metabolites measured with the LC/MS and GC/MS platforms respectively. After data pre-processing and quality checking, three statistical outliers were detected and therefore removed—one from the R6 group (patient ID: 37) and two from the SUB889 group (patient ID: 18, 40).

Paired t-tests and independent t-tests were applied to the normalized metabolomics data of the remaining 37 patients, and results are shown in Supplementary Table S3 (R6: week 8 vs. week 0; SUB889: week 8 vs. week 0). In the R6 group, 75 metabolites changed significantly after 8 weeks of treatment ($p < 0.05$), and most of the decreased metabolites were lipids, which consisted of six classes: cholesteryl esters (ChEs, n=8), fatty acids (FAs, n=7), phosphatidylcholines (PCs, n=12), triglycerides (TGs, n=16), diglycerides (DGs, n=2) and sphingomyelins (SPMs, n=18). The downregulated lipids measured by metabolomic profiling are consistent with the decrease of clinical parameter T-C and LDL-C during the R6 treatment. In the SUB889 group, 13 metabolites were found significantly upregulated, and

most of them were organic acids, including malic acid, fumaric acid, pyruvic acid and a few FAs, while 5 metabolites including glucose were found to be significantly downregulated.

A hierarchical cluster analysis was performed on the top 30 metabolite ratios of metabolites$_{T8/T0}$ (Supplementary Table S3, Ratio) ranked by the independent t-tests, since these metabolites show the largest difference in response to R6 versus SUB889. Euclidean distance was used as the similarity measure and average linkage (clustering uses the centroids of the observations) was used as the clustering algorithm. The clustering results were visualized as a heatmap with the samples as the rows and metabolites as the columns, and each colored cell corresponding to a normalized metabolite ratio value (Fig 4). Most patients were found to be clustered correctly in their respective treatment group, and the misclassification rate was 16.22% (6 subjects misclassified). Comparing the two groups, metabolites in the R6 group, especially SPMs, PCs and FAs, were downregulated (color blue), while in the SUB889 treatment group the metabolites from these classes were relatively stable or slightly increased (color red).

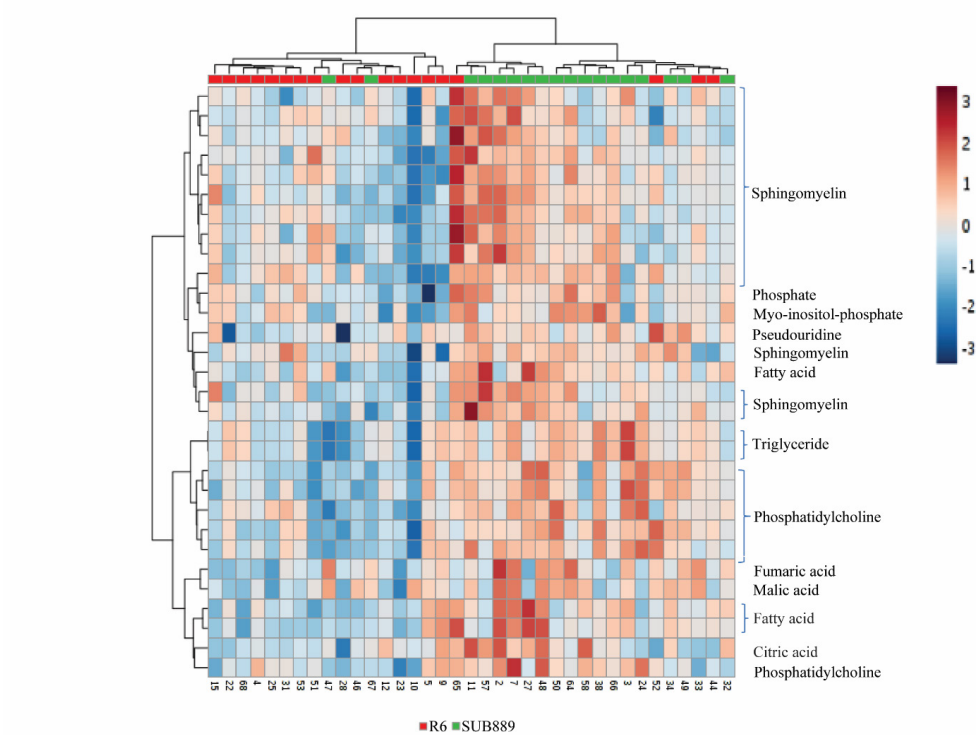


Figure 4. Heatmap of the R6 group and the SUB889 group based on the top 30 metabolite ratios (T8/T0) of the selected metabolites. The horizontal axis represents the patients: patient IDs were listed at the bottom and

grouped by treatment; the vertical axis represents 30 selected metabolic ratios, in which similar metabolites cluster in rows.

Hypothetical mechanisms of the two Rehmanniae Radix formulae

In the present study, responses of subjects to R6 or SUB889 were measured at multiple levels, and it is interesting to investigate the mechanisms of these two formulae by combing metabolomics data and clinical parameters. Metabolites which were changed after 8 weeks (Supplementary Table S3, T0 vs. T8) of treatment were selected. These selected metabolites and clinical lipid parameters (TG, HDL-C, LDL-C) were mapped on metabolite pathways taken from KEGG [31].

R6 treatment significantly decreased lipid related metabolites (FAs, SPMs, TGs, DGs, PCs and ChEs) and clinical parameters (T-C and LDL-C) (Fig 5A). The decrease of lipids may be explained by the increased insulin level. Insulin can activate the synthesis of lipids and inhibit their degradation [32]. R6 treatment might stimulate insulin secretion, leading to reductions of the plasma lipids and LDL-C; downregulated FAs indicated that R6 may also promote the β-oxidation of FAs for energy generation. Since the lipid levels substantially declined during the R6 treatment, the BMI and waist circumference were reduced after 8 weeks. In addition, decreased SPMs could lead to changes in cell membrane composition and related signaling pathways. Many studies have already revealed the role of SPMs and related signaling pathways in obesity, insulin resistance, and other metabolic disorders [33–35]. It is reported that R6 may affect endocrine and excretory systems related pathways, such as peroxisome proliferator-activated receptor (PPAR) signaling pathway and aldosterone-regulated sodium reabsorption, which play important roles in lipid metabolism, glucose uptake and blood pressure regulation [36,37].

However, lipid levels were not substantially changed during the SUB889 treatment, only a few lipid metabolites were slightly altered (Fig 5B). Malic acid/malate and fumaric acid/fumarate, which are important intermediates of the tricarboxylic acid (TCA) cycle, increased dramatically after 8 weeks, indicating that the TCA cycle may be stimulated with the SUB889 treatment. Besides, as an important intermediate of gluconeogenesis and glycolysis, pyruvic acid/pyruvate was also upregulated significantly, which may also induce the TCA cycle. Therefore, we hypothesize that SUB889 did not change the lipid homeostasis in plasma, but it might treat MetS by regulating glucose metabolism (gluconeogenesis, glycolysis) and the TCA cycle to achieve an improvement of clinical outcomes in MetS patients.

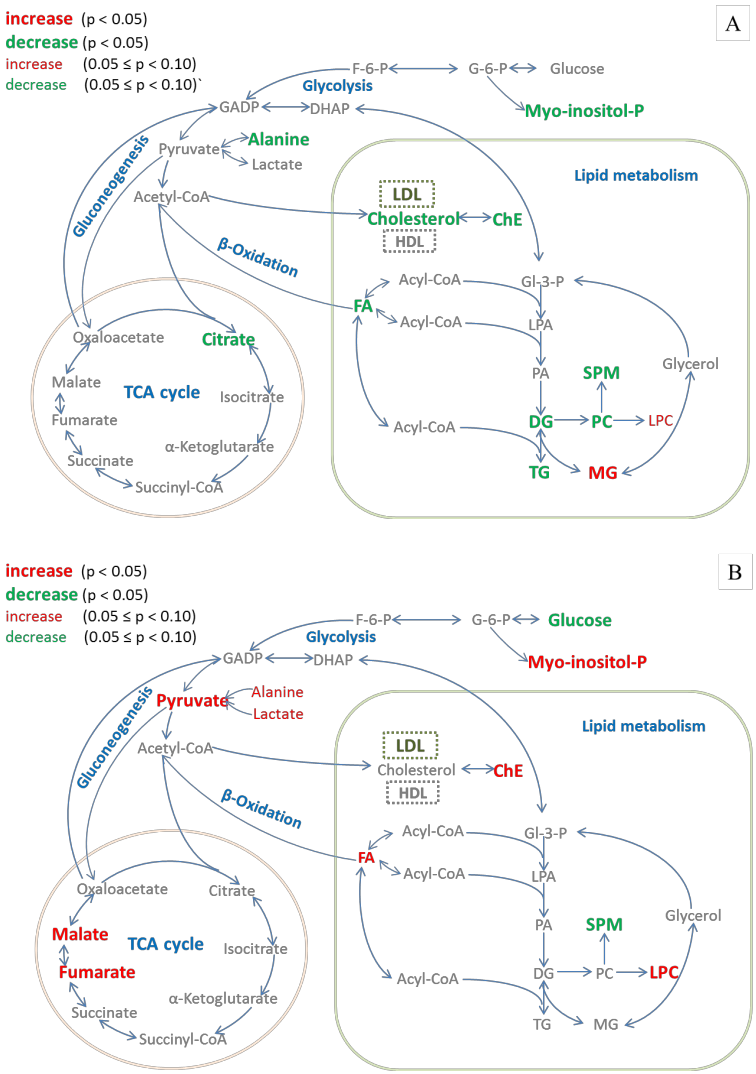


Figure 5. Pathway analysis of metabolites which were affected by 8-week treatment of R6 (A) or SUB889 (B).

It is likely that the formulae both lead to improvement of MetS even though the herbal components have effects on a variety of different metabolic targets. This indicates that these pathways are interconnected in the regulation of human physiological states. Multi-herbal formulae consisting of numerous active components inducing complex interactions within the human body, might therefore be especially suitable for treating complex diseased (e.g. MetS) and offer valuable alternatives to Western single target medicines. Since the R6 and SUB889 formulae have very similar effects with respect to CM, we assume that the regulation of lipid metabolism, glucose metabolism or the TCA cycle by different formulae may result in the

same therapeutic outcomes. From a Chinese medical point of view, one Western medical defined disease can be treated with different approaches, much according to the CM statement 'Tong Bing Yi Zhi' (same disease, different treatment). However, in this study there was no placebo group included. The similar effectiveness may be due to other factors (e.g. placebo effect) than the herbal formula in the treatment of MetS. Therefore, a placebo-controlled randomized study is needed to validate our findings in the future.

Conclusions

In conclusion, this study shows that R6 and SUB889 have similar effects on the treatment of MetS by improving CM deficiency syndromes and WM clinical outcomes (central obesity and systolic blood pressure). R6 is more effective in decreasing lipid profiles compared to SUB889, indicating that the two formulae target different metabolic pathways in the patients. Even though the two multi-herbal Rehmanniae Radix formulae induce different metabolic changes, the therapeutic effect can be similar according to both CM and WM diagnosis. In the future, SUB889 may be developed as a candidate treatment for MetS patients or the prevention of cardiovascular disease. Further studies are needed with larger cohorts as well as healthy control groups to elucidate the mechanism of actions for the two Rehmanniae Radix formulae in MetS.

References

1. Alberti, K. G. M. M., Zimmet, P. & Shaw, J. The metabolic syndrome - A new worldwide definition. *Lancet* **366,** 1059–1062 (2005).
2. Eckel, R. H., Alberti, K. G. M. M., Grundy, S. M. & Zimmet, P. Z. The metabolic syndrome. *Lancet* **375,** 181–183 (2010).
3. Lv, C., Yang, S. & Du, J. Research mentality of pathology and semiotics of Traditional Chinese Medicine and in metabolic syndrome. *Chinese J. Integr. Tradit. West. Med.* **27,** 799 (2007).
4. Diabetology branch of China Association of Traditional Chinese Medicine. Diagnostic criteria for patients with diabetes and metabolic syndrome by Chinese medicine. *World J. Integr. Tradit. West. Med.* **6,** 177–149 (2011).
5. Jiang, M. *et al.* Syndrome differentiation in modern research of traditional Chinese medicine. *J. Ethnopharmacol.* **140,** 634–642 (2012).
6. Li, S. Network Systems Underlying Traditional Chinese Medicine Syndrome and Herb Formula. *Curr. Bioinform.* **4,** 188–196 (2009).
7. Jia, W. *et al.* The rediscovery of ancient Chinese herbal formulas. *Phyther. Res.* **18,** 681–686 (2004).
8. van der Greef, J. Perspective: All systems go. *Nature* **480,** S87–S87 (2011).
9. Zhang, A., Sun, H., Yan, G., Wang, P. & Wang, X. Metabolomics for Biomarker Discovery: Moving to the Clinic. *Biomed Res. Int.* **2015,** 1–6 (2015).
10. Liu, W., Xiong, X. & Wang, J. Discussion on the treatment of hypertension by Liuwei Dihuang

Wan. *China J. Tradit. Chinese Med. Pharm.* **28,** 3329–3333 (2013).

11. Xue, Y., Zhu, B. & Luo, R. The application and study of Liu Wei Di Huang Wan in Diabetes. *Morden J. Integr. Tradit. Chinese West. Med.* **14,** 1925–1927 (2005).
12. Zheng, X. *et al.* Efficacy of Liuwei Dihuang Pill on the Chronic Kidney Disease Induced by Diabetes Mellitus and Hypertensive Diseases. *West China Med. J.* **26,** 490–492 (2011).
13. van Wietmarschen, H., van Der Greef, J., Schroën, Y. & Wang, M. Evaluation of symptom, clinical chemistry and metabolomics profiles during Rehmannia six formula (R6) treatment: An integrated and personalized data analysis approach. *J. Ethnopharmacol.* **150,** 851–859 (2013).
14. Zheng, X. *et al.* Efficacy of kidney-nourishing therapy on the chronic kidney disease induced by metabolic syndrome: a randomized controlled trial. *Morden Prev. Med.* **38,** 4513–4514 (2011).
15. Huang, C.-Y., Tsai, Y.-T., Lai, J.-N. & Hsu, F.-L. Prescription pattern of chinese herbal products for diabetes mellitus in taiwan: a population-based study. *Evid. Based. Complement. Alternat. Med.* **2013,** 201329 (2013).
16. Xie, P., Guo, L., Zhao, Y., Bensky, D. & Stoeger, E. Searching Clue of the Relationship between the Alteration of Bioactive Ingredients and the Herbal 'Property' Transformation from Raw Rehmanniae Radix to Steam-Heating-Processed Rehmanniae Radix. *Chin. Med.* **5,** 47–60 (2014).
17. Chang, W.-T. *et al.* Traditional processing strongly affects metabolite composition by hydrolysis in Rehmannia glutinosa roots. *Chem. Pharm. Bull. (Tokyo).* **59,** 546–552 (2011).
18. Zhang, R. X., Li, M. X. & Jia, Z. P. Rehmannia glutinosa: Review of botany, chemistry and pharmacology. *J. Ethnopharmacol.* **117,** 199–214 (2008).
19. Maciocia, G. in *Diagnosis in Chinese Medicine* 929–941 (2012).
20. Zhu, B. & Wang, H. in *Diagnostics of Traditional Chinese Medicine* 142–149 (2010).
21. Koek, M. M., Muilwijk, B., Van Der Werf, M. J. & Hankemeier, T. Microbial metabolomics with gas chromatography/mass spectrometry. *Anal. Chem.* **78,** 1272–1281 (2006).
22. Draisma, H. H. M. *et al.* Similarities and differences in lipidomics profiles among healthy monozygotic twin pairs. *OMICS* **12,** 17–31 (2008).
23. Xia, J., Sinelnikov, I. V., Han, B. & Wishart, D. S. MetaboAnalyst 3.0--making metabolomics more meaningful. *Nucleic Acids Res.* 1–7 (2015). doi:10.1093/nar/gkv380
24. McCrum-Gardner, E. Which is the correct statistical test to use? *Br. J. Oral Maxillofac. Surg.* **46,** 38–41 (2008).
25. Tomizawa, S. Measures of departure from marginal homogeneity for contingency tables with nominal categories. *Stat.* **44,** 425 (1995).
26. Wang, J. & Xiong, X. Control strategy on hypertension in chinese medicine. *Evid. Based. Complement. Alternat. Med.* **2012,** 284847 (2012).
27. Wang, J. & Xiong, X. Evidence-based chinese medicine for hypertension. *Evid. Based. Complement. Alternat. Med.* **2013,** 978398 (2013).
28. Wang, J., Xiong, X. & Liu, W. Traditional chinese medicine syndromes for essential hypertension: a literature analysis of 13,272 patients. *Evidence-Based Complement. Altern. Med.* **2014,** 1–19 (2014).
29. Xu, H. & Cai, Y. Research development of TCM constitution in patients with hypertension. *Chinese J. Inf. Tradit. Chinese Med.* **18,** 104–105 (2011).
30. Zhang, Y. & Wang, J. Morden research of TCM syndromes in hypertension. *Chinese J. Inf. Tradit. Chinese Med.* **9,** 78–80 (2002).

31. Kanehisa, M. *et al.* Data, information, knowledge and principle: back to metabolism in KEGG. *Nucleic Acids Res.* **42,** D199–D205 (2014).

32. Saltiel, A. R. & Kahn, C. R. Insulin signalling and the regulation of glucose and lipid metabolism. *Nature* **414,** 799–806 (2001).

33. Mitsutake, S. *et al.* Dynamic modification of sphingomyelin in lipid microdomains controls development of obesity, fatty liver, and type 2 diabetes. *J. Biol. Chem.* **286,** 28544–28555 (2011).

34. Holland, W. L. & Summers, S. A. Sphingolipids, insulin resistance, and metabolic disease: New insights from in vivo manipulation of sphingolipid metabolism. *Endocr. Rev.* **29,** 381–402 (2008).

35. Straczkowski, M. *et al.* Relationship between insulin sensitivity and sphingomyelin signaling pathway in human skeletal muscle. *Diabetes* **53,** 1215–21 (2004).

36. Liang, X., Li, H. & Li, S. A novel network pharmacology approach to analyse traditional herbal formulae: the Liu-Wei-Di-Huang pill as a case study. *Mol. Biosyst.* **10,** 1014–22 (2014).

37. Li, S., Zhang, B., Jiang, D., Wei, Y. & Zhang, N. Herb network construction and co-module analysis for uncovering the combination rule of traditional Chinese herbal formulae. *BMC Bioinformatics* **11 Suppl 1,** S6 (2010).

Supplementary information

Method

Procedures for producing SUB889

Rehmanniae Radix, Polygoni Multiflori Radix, Cistanches Herba, Acanthopanacis Senticosi Radix, Atractylodis Rhizoma and Ephedrae Herba were refluxed with 80% ethanol for 2h, filtered through a gauze. The residue and three other herbs were extracted with boiling water twice, 2h each time. Then all filtrates were pooled and evaporated with rotary evaporation under vacuum at 70°C. In the end, soluble starch and dextrin (1:1) were added as the excipient into the herbal extract to produce the SUB889 pills.

Roles of herbs in SUB889 except for Rehmanniae Radix

In SUB889, Polygoni Multiflori Radix and Cistanches Herba were chosen as 'minister herbs'; Lycii Fructus, Polygonati Rhizoma, Ganoderma and Acanthopanacis Senticosi Radix were chosen as 'assistant herbs'. The 'emperor herb', 'minister herbs' and 'assistant herbs' all work together to increase the nurturing. Polygoni Multiflori Radix replenishes the vital Essence and Blood, expel Wind and counteract toxicity, as well as moistens the intestine and stimulates purgation. Cistanches Herba is used to warm Kidney and help Yang, improve vitality and reinforce Essence, moisten the intestine and stimulate purgation, to enhance the vital Yang and vital Yin power of Kidney. Lycii Fructus, Polygonati Rhizoma, Ganoderma and Acanthopanacis Senticosi Radix could reinforce Kidney, replenish vital energy and benefit Essence. Furthermore, the ingenuity in this formula is that Atractylodis Rhizoma and Ephedrae Herba were chosen as the 'envoy herbs' to improve the effectiveness of all the ingredients together and complement each other without side effects.

Tables

Table S1 Calculation rules of Qi and Yin deficiency scores

Symptom score criteria	Symptom name	Qi- or Yin-deficiency related
Main symptoms (0= mild or no symptom, 2= moderate symptom, 4= sever symptom)	Fatigue	Qi
	Heat sensation	Yin
	Hectic Fever	Yin
Secondary symptoms (0= mild or no symptom, 1= moderate symptom, 2=	Spontaneous sweating	Qi
	Taciturn	Qi

sever symptom)	Joints & Waist weakness	Qi
	Head Giddiness	Qi & Yin
	Tinnitus	Yin
	Insomnia & Dreamful	Yin
	Dry mouth & Pharynx	Yin
	Constipation	Yin
Tongue and pulse diagnostic symptoms (0= no symptom, 1= with symptom)	Tongue substance pale red	Qi
	Tongue substance red	Yin
	Tongue shape normal or enlarged	Qi
	Tongue shape thin	Yin
	Tongue coating yellow	Yin
	Pulse thready and weak	Qi
	Pulse weak	Yin

Symptoms were grouped as main, secondary and tongue and pulse diagnostic symptoms. All symptoms were scored by CM doctors according to the symptom score criteria. For example, each main symptom can be scored 0, 2 or 4, in which 0 indicates 'mild or no symptom', 2 indicates 'moderate symptom' and 4 indicates 'sever symptom'. For each patient, Qi- and Yin-deficiency scores were calculated by summing the related symptom scores separately.

Table S2. Spearman's correlation between Chinese medicine and Western medicine diagnosis

Clinical parameter	Qi deficiency score		Yin deficiency score	
	Correlation coefficient	p value	Correlation coefficient	p value
Body weight	0.07	0.35	0.14	0.05
Waist circumference	0.09	0.23	0.24	<0.001
BMI	0.08	0.25	0.06	0.41
Systolic blood pressure	0.23	<0.001	0.54	<0.001
Diastolic blood pressure	0.10	0.14	0.09	0.22
Plasma glucose	-0.04	0.66	0.09	0.35
Total cholesterol	0.02	0.85	0.02	0.80
Triglycerides	0.03	0.78	0.05	0.62
HDL-cholesterol	-0.06	0.49	-0.02	0.81
LDL-cholesterol	0.04	0.69	0.00	0.96
HbA1c	0.02	0.87	0.06	0.62
Plasma insulin	-0.08	0.41	0.04	0.68

p values calculated with paired t-tests (0 week v.s. 8 weeks)

Table S3. The differences of metabolites with and between R6 and SUB889

Class	R6: week8 vs. week0			SUB889: week 8 vs. week 0			Ratio: R6 vs. SUB889	
	Metabolite	p value [c]	Regulation	Metabolite	p value [c]	Regulation	Metabolite	p value [d]
FA [a]	C16:0	0.015	Down	C10:0	0.018	Up	C10:0	0.001
	C16:1	0.007	Down	C17:0	0.020	Up	C16:0	0.034
	C17:0	0.010	Down	C20:1	0.023	Down	C16:1	0.025
	C18:0	0.002	Down				C17:0	0.047
	C18:1	0.003	Down				C18:1	0.020
	C20:1	0.016	Down				C14:0	0.100
	C22:6	0.049	Down				C18:2	0.052
	C10:0	0.054	Down				C20:1	0.087
	C14:0	0.074	Down					
SPM [a]	Heptadecasphing-4-enine/FAC24:0	0.009	Down	Sphingosine/FAC18:0	0.026	Down	Heptadecasphing-4-enine/FAC24:0	0.019
	Hexadecasphing-4-enine/FAC18:0	0.047	Down	Sphingosine/FAC17:0	0.069	Up	Hexadecasphing-4-enine/FAC20:0	0.020
	Hexadecasphing-4-enine/FAC20:0	0.020	Down				Hexadecasphing-4-enine/FAC22:0	0.023
	Hexadecasphing-4-enine/FAC24:1	0.003	Down				Hexadecasphing-4-enine/FAC24:1	0.016
	Sphingadiene/FAC24:0andC24:1	0.015	Down				Sphingadi-ene/FAC24:0andC24:1	0.024
	Sphingosine/FAC24:0andC24:1	0.001	Down				Sphingosine/FAC17:0	0.031
	Spingadiene/FAC20:0	0.016	Down				Sphingosine/FAC22:0	0.040
	Hexadecasphing-4-enine/FAC16:0	0.067	Down				Sphingo-sine/FAC24:0andC24:1	0.026

	Hexadecasphing-4-enine/FAC22:0	0.086	Down				Spingadiene/FAC20:0	0.008
	Sphingosine/FAC17:0	0.058	Down				Heptadecasphing-4-enine/FAC16:0	0.073
	Sphingosine/FAC18:0	0.058	Down				Hexadecasphing-4-enine/FAC16:0	0.077
	Spingadiene/FAC18:0	0.074	Down				Sphingosine/FAC16:0	0.055
							Spingadiene/FAC16:0	0.062
MG [a]	1-Monolinoleoylglycerol	0.041	Up					
	2-Hydroxybutanoicacid	0.008	Down	D-Glucose	0.031	Down	Citricacid	0.001
	Alanine	0.012	Down	Fumaricacid	0.030	Up	D-Ribulose-orD-Xylulose	0.036
	Cholesterol	0.030	Down	L(-)Malicacid	0.015	Up	Fumaricacid	0.005
	Citricacid	0.002	Down	L-Cystine	0.005	Up	Glycerol	0.032
	D-Glutamicacid	0.044	Down	Myo-inositol	0.031	Up	L(-)Malicacid	0.011
	HypoXanthine	0.046	Down	Myo-inositolphosphate	0.005	Up	L-Threonine	0.042
	Myo-inositol-1,2-cyclicphosphate	0.048	Down	Pseudouridine	0.018	Up	Monomethylphosphate	0.042
	Myo-inositolphosphate	0.026	Down	Pyruvicacid	0.049	Up	Myo-inositolphosphate	0.027
Oth-ers [a]	Phosphate	0.043	Down	1-Palmitoyl-L-alpha-lysophosphatidicacid	0.027	Up	N-Acetylaminomalonicacid	0.041
	VitaminE	0.008	Down	3-Methyl-2-oxo-valericacid	0.013	Down	Phosphate	0.019
	3-Methyl-2-oxo-valericacid	0.073	Down	1,5-Anhydro-D-Glucitol	0.085	Down	Pseudouridine	0.020
	4-Deoxyglucose	0.072	Down	2-Amino-1-butyricacid	0.072	Up	2-Hydroxybutanoicacid	0.047
	Creatinine	0.092	Down	Beta-Alanine	0.054	Up	Aminomalonicacid	0.042
	D-Ribose	0.062	Down	D-Maltose	0.056	Up	4-Methyl-2-oxovalericacid	0.052
	Monomethylphosphate	0.058	Down	Lacticacid	0.051	Up	Alanine	0.078
	Ureum	0.069	Down	L-Threonine	0.089	Up	Cholesterol	0.084

							Creatinine	0.081
							D(+)Glycericacid	0.081
							HypoXanthine	0.053
							L-Cysteine	0.091
							L-Lysine	0.055
							L-Valine	0.086
							Myo-inositol	0.058
							Ureum	0.088
							Uridine	0.051
							VitaminE	0.057
	C14:0	0.021	Down	C18:1	0.028	Up	C16:1	0.030
	C16:1	0.011	Down	C20:3	<0.0001	Down	C18:1	0.049
	C16:2	0.005	Down				C20:5	0.045
	C18:1	0.027	Down				C14:0	0.060
ChE [b]	C20:3	0.037	Down				C18:3	0.092
	C20:4	0.014	Down				C20:3	0.078
	C20:5	0.009	Down					
	C22:6	0.006	Down					
	C18:3	0.094	Down					
MG [b]	C18:2	0.023	Up					
	C34:1	0.038	Down					
	C36:2	0.027	Down					
DG [b]	C32:0	0.095	Down					
	C32:1	0.086	Down					
	C36:1	0.057	Down					
TG [b]	C44:1	0.024	Down				C56:4	0.025

	C46:0	0.029	Down				C56:6	0.025
	C46:1	0.049	Down				C50:3	0.076
	C48:1	0.008	Down				C52:5	0.099
	C48:2	0.013	Down				C56:5	0.068
	C48:3	0.042	Down					
	C50:1	0.013	Down					
	C50:2	0.006	Down					
	C50:3	0.007	Down					
	C50:4	0.037	Down					
	C52:2	0.007	Down					
	C52:5	0.048	Down					
	C54:2	0.030	Down					
	C56:4	0.002	Down					
	C56:5	0.028	Down					
	C56:6	0.002	Down					
	C44:0	0.093	Down					
	C50:5	0.052	Down					
	C52:3	0.061	Down					
	C54:3	0.099	Down					
SPM [b]	C14:0	0.006	Down	C15:0	0.040	Up	C14:0	0.024
	C14:1	0.006	Down	C24:0	0.055	Up	C14:1	0.029
	C15:0	0.030	Down				C15:0	0.049
	C16:0	0.005	Down				C16:0	0.022
	C16:1	0.014	Down				C16:1	0.032
	C18:0	0.014	Down				C22:0	0.005
	C18:1	0.030	Down				C23:1	0.026
	C22:0	0.030	Down				C24:1	0.039

	C23:1	0.032	Down				C24:2	0.025
	C24:1	<0.0001	Down					
	C24:2	0.005	Down					
PC [b]	C32:0	0.001	Down				C32:0	0.033
	C32:1	0.001	Down				C32:1	0.006
	C34:0	0.019	Down				C34:1	0.007
	C34:1	0.001	Down				C36:3	0.014
	C34:3	0.038	Down				C36:4	0.013
	C36:1	0.009	Down				C36:5	0.012
	C36:3	0.011	Down				C38:5	0.015
	C36:4	<0.0001	Down				C34:3	0.073
	C36:5	0.001	Down				C36:1	0.086
	C38:4	0.001	Down				C38:4	0.067
	C38:5	0.001	Down					
	C38:6	0.002	Down					
LPC [b]	C18:2	0.093	Up	C18:1	0.018	Up	C16:1	0.043

a: analyzed in GC/MS platform

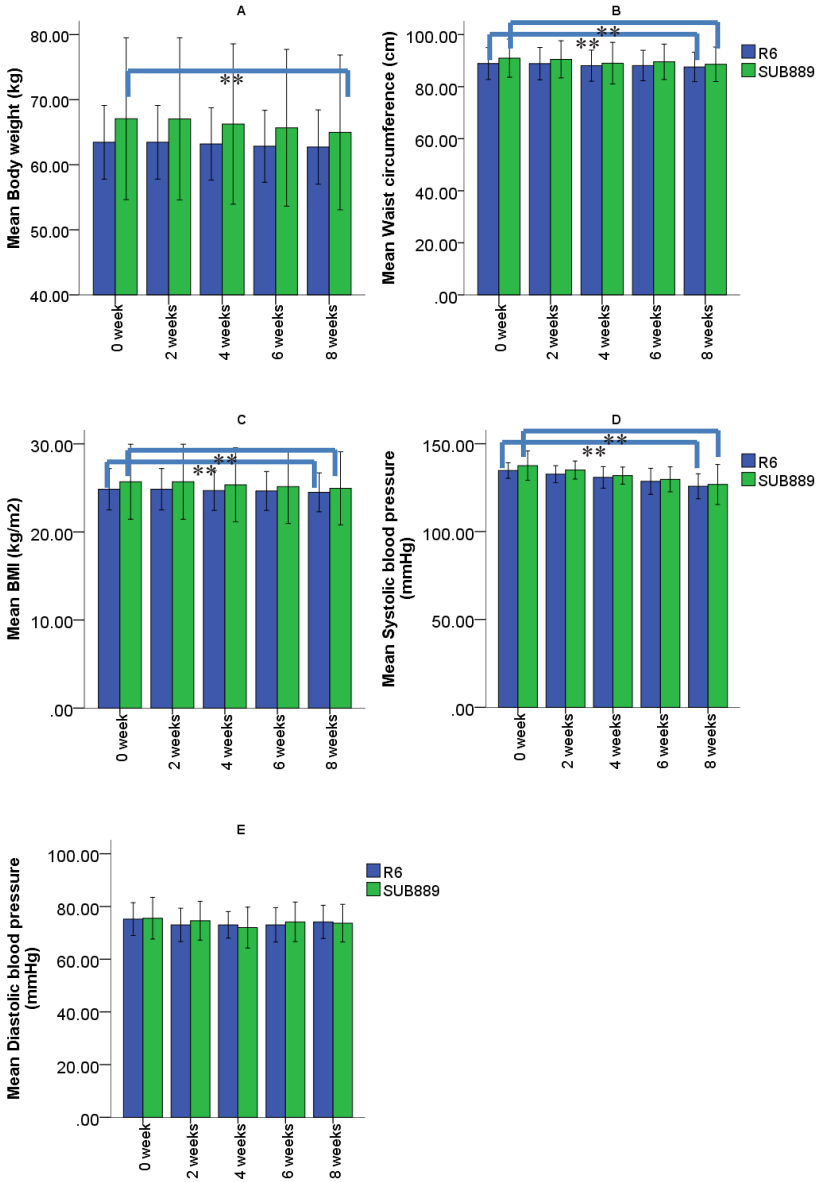
b: analyzed in LC/MS platform

c: p values calculated with paired t-tests

d: p values calculated with independent t-tests

ChE, cholesteryl ester; DG, diglyceride; FA, fatty acid; LPC, lysophosphatidylcholine; MG, monolinoleoglycerol; PC, phosphatidylcholine; SPM, sphingomyelin; TG, triglyceride.

Figures


A
Mean Body weight (kg)
80.00
70.00
60.00
50.00
40.00
0 week
2 weeks
4 weeks
6 weeks
8 weeks
**
B
Mean Waist circumference (cm)
100.00
80.00
60.00
40.00
20.00
.00
R6
SUB889
C
Mean BMI (kg/m2)
30.00
20.00
10.00
.00
D
Mean Systolic blood pressure (mmHg)
150.00
100.00
50.00
E
Mean Diastolic blood pressure (mmHg)

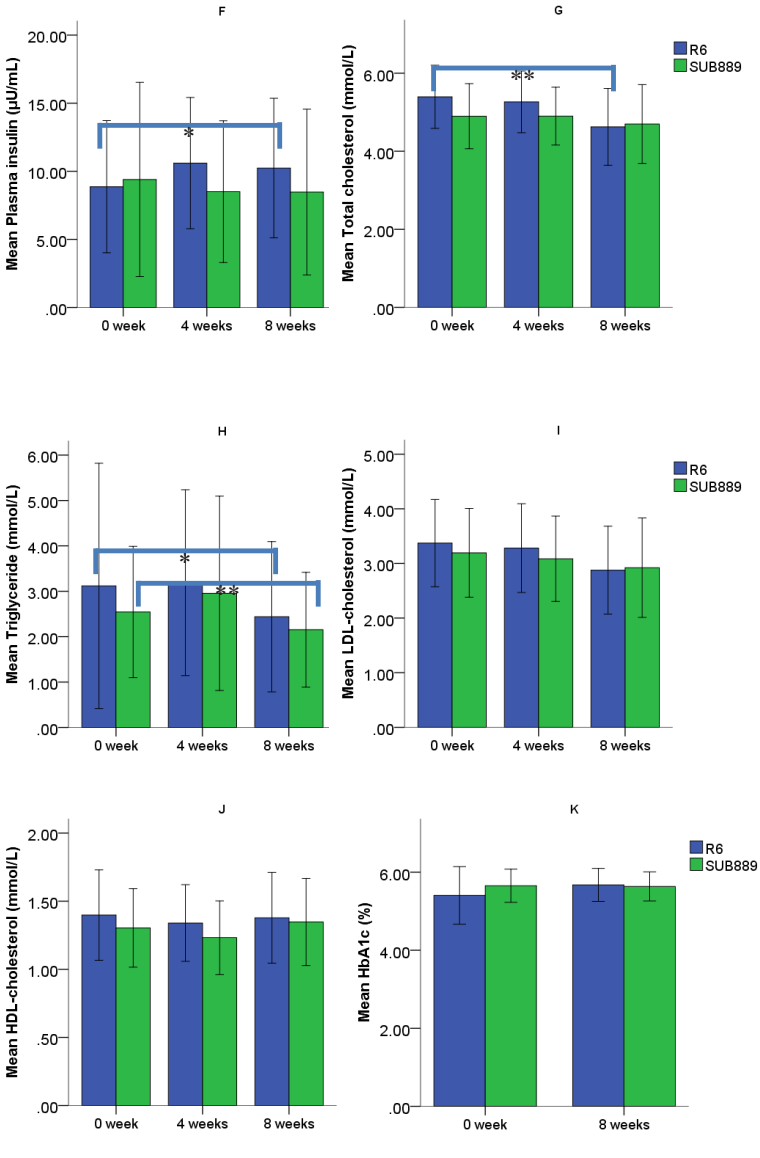


Figure S1. Bar plots of clinical parameters changing with treatment time in R6 and SUB889 group. A, body weight; B, waist circumstance; C, BMI; D, systolic blood pressure; E, diastolic blood pressure; F, plasma insulin; G, total cholesterol; H, triglycerides; I, LDL-cholesterol; J, HDL-cholesterol; K, glycated hemoglobin

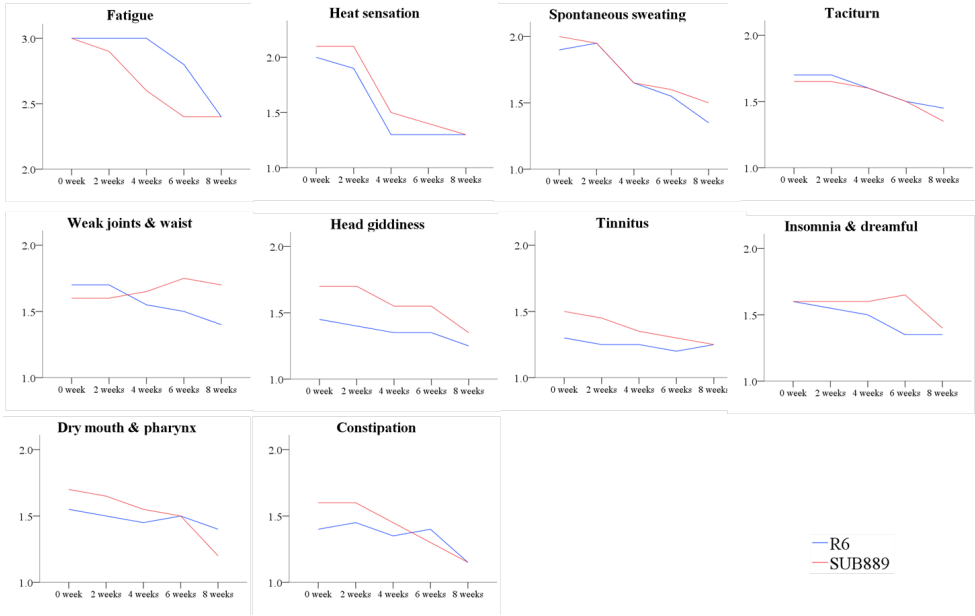


Figure S2. Symptom changes with treatment time. X-axis displays the treatment time, Y-axis display the symptoms scores.

6