Effects of soy milk consumption on inflammatory markers and lipid profiles among non-menopausal overweight and obese female adults

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BACKGROUND: Few studies have evaluated the effects of soy milk replacement in the diet on inflammatory markers among non-menopausal overweight and obese female adults. We evaluated the effects of soy milk compared to cow's milk on inflammation and lipid profiles among non-menopausal overweight and obese female adults. METHODS: This cross-over randomized clinical trial was conducted on 24 overweight or obese women. There were two 4-week trial periods (soy milk period and cow's milk period). In the soy milk period, only one glass of soy milk (240 cc) was replaced instead of one glass of cow’s milk (240 cc). RESULTS: Fat contents in soy milk and cow's milk were 1 g per 100 ml and 1.5 g per 100 ml, respectively. Serum low density lipoprotein (LDL) level reduced significantly following soy milk period (mean percent change in soy milk period vs. cow's milk period: -11.22 ± 3.85% vs. -1.18 ± 2.82%; p = 0.01). Inflammatory factors and other lipid profiles did not change significantly after the soy milk period compared to the cow's milk period. In addition, soy milk could not reduce the weight of non-menopausal overweight and obese females. CONCLUSIONS: Soy milk replacement had beneficial effects on LDL levels in a short term trial among overweight and obese women. However, it had no significant effects on inflammatory markers and other lipid profiles among these non-menopausal overweight and obese adults.

KEYWORDS: Soy Milk, Inflammation, Obese, Overweight, Females

BACKGROUND

Obesity is one of the major health concerns worldwide.[11] Prevalence of obesity has increased dramatically in both developed[2] and developing countries.[3-5] Obesity is also a risk factor for other chronic diseases such as type 2 diabetes, metabolic syndrome and cardiovascular diseases.[11] There is a close relationship between obesity, overweight, and cardiometabolic risks[6] in which inflammation has a major effect.[7] In addition, obesity causes chronic inflammatory disorders and results in the development of insulin resistance and cardiovascular diseases.[8,9]

Some environmental factors are associated with obesity.[10,11] Nutrition is an important factor in both prevention and control of obesity.[12] Different dietary therapies have focused on treatment of overweight and obesity.[12,13] Among different suggested foods, soy products had important effects on weight and some cardiovascular risk factors.[14-16] The majority of previous studies have focused on soy beans, soy proteins, or soy nuts and few studies have considered soy milk. Recent papers emphasized that consuming whole soy is more effective than soy components alone on cardiovascular risk factors.[15,17] Soy milk is approximately close to a whole soy product since it contains all the useful components of soy. Soy milk components are isoflavones, essential fatty acids, phytosterols, good fats, and inositol.[17]

Soy is a rich source of herbal estrogens such as daidzein and genistein which may increase vascular reactions and production of nitric oxide and decrease inflammatory markers and oxidative stress.[18] Few studies on the effects of soy milk on inflammation have failed to show significant results.[19,20]

Compared to casein, soy protein has a greater antioxidative ability in preventing lipid oxidation.[21] Antioxidant species (isoflavonoids) may act to decrease oxidative damage to DNA, proteins, and lipids and thus reduce the risk of coronary artery disease.[22] Isoflavones could exert their potential effects on serum lipid profiles through different mechanisms including enhancement of bile acid excretion and reduction of cholesterol metabolism and insulin to glucagon ratio.[23] However, there have been conflicting results regarding the effects of soy milk consumption on lipid profiles.[24,25]

Previous research regarding the effects of soy milk consumption has mostly focused on
postmenopausal women and sick people. Few studies have been performed on non-menopausal female adults and overweight and obese subjects.

We selected soy milk from various soy products because we believed that soy milk is close to a whole soy product. Therefore, this study tried to determine the effects of soy milk consumption on inflammation and lipid profiles among non-menopausal overweight and obese female adults.

**METHODS**

**Participants**

Non-menopausal women in the age range of 20 to 50 years who had body mass index (BMI) more than 25 kg/m² were included in this study. The exclusion criteria were having allergy to soy products or cow’s milk, beginning to consume medications, incidence of chronic or acute diseases which prevented the patients to follow the research protocol.

The sample size was calculated based on the formula suggested for cross-over trials:\[^{26}\]:

\[ n = \frac{\left(2\sigma^2 + Z_1 \alpha^2 + Z_1 \beta^2\right) S^2}{2 \Delta^2} \]

where \( \alpha \) (type 1 error) was 0.05, \( \beta \) (type 2 error) was 0.10, \( S \) (the variance of CRP) was 0.1, and \( \Delta \) (the difference in mean of CRP) was 0.2.\[^{27}\] We considered CRP as the principal variable.\[^{26}\] Therefore, \( n = \frac{\left(2\times1.96^2 + 1.28^2\right) 0.1^2}{2 \times 0.2^2} = 13 \). According to the formula mentioned above, 13 patients were needed for adequate power.

In this research, 30 females volunteered to participate. All of them had BMI more than 25 kg/m². Patients were not on specific medications and did not have any specific disease. All participants were requested to sign the written consent form. This study was approved by the research council and ethics committee of Food Security Research Center, Isfahan University of Medical Sciences and Department of Nutrition, School of Public Health, Tehran University of Medical Sciences in Iran (289249). It was also registered in the Iranian Registry of Clinical Trials (IRCT 201107052839N3). We followed the Consolidated Standards of Reporting Trials (CONSORT) statement in writing this manuscript.

**Study procedures**

This two-week treatment, two-period crossover randomized clinical trial was conducted on non-menopausal overweight or obese female adults in 2010 in Isfahan, Iran. After a two-week run-in period, subjects were randomly assigned to consume a diet containing cow’s milk or a diet with one glass (240 cc) of soy milk for four weeks. For allocating the subjects to different groups, random sequencing generated in SPSS (SPSS Inc., Chicago, IL, USA) was used. In this study patients were not blinded. They had to use soy milk in one period of trial and cow’s milk in the other. Each patient received two diets. The wash-out period was two weeks. At the end of the run-in period, randomization was conducted. All patients were on a weight reducing diet. Foods were not prepared for the participants, i.e., they were prescribed with a diet but had to prepare their own food. Only soy milk and cow’s milk were given to the patients. They were asked not to change their usual physical activity level during the study.

**Diets**

We prescribed two diets for each patient: 1) diet with cow’s milk and 2) diet with soy milk. Both diets had the following macronutrient composition: 50-60% carbohydrates, 15-20% protein, and < 30% total fat. Macronutrient intake of each patient was checked by their food records.

We used the equation suggested by the Food and Nutrition Board, Institute of Medicine to calculate the calorie requirement of each participant.\[^{28}\] In both periods, we reduced 200 to 500 kcal/day in the diet of the subjects based on their BMI range. In the soy milk period, one glass of soy milk (240 cc) was replaced instead of one glass of cow’s milk (240 cc). Fat percentage of cow’s milk and soy milk were 1.5% and 1%, respectively.

A nutritionist described the benefits of each diet, educated the subjects on how to record their food intake and the way of using the exchange list. We prescribed individual diets for each participant and provided an exchange list for each subject during the study period.

We assessed the adherence of subjects by analysis of the 3-day food records. No significant differences were found between the prescribed amounts and consumed amounts of the 5 food groups.

**Measurements**

Weight was measured on digital scales to the nearest 0.1 kg with minimal cloths and without shoes. Blood samples were collected after patients had fasted for 12 hours overnight. Separate tubes were used to store sodium citrate buffers for plasma and serum. Tubes were centrifuged at 4°C and 500 g for 10 minutes.\[^{27,29}\] We tried to perform the tests on the same day for all participants. Whenever testing was impossible, plasma samples were frozen promptly (-70°C). Serum total cholesterol, low-density lipoprotein cholesterol (LDL-
C) and triglyceride concentrations were measured by commercially available enzymatic reagents (Pars Azmoon, Iran) on a BT-3000 (Biotechnica) autoanalyzer. High density lipoprotein cholesterol (HDL-C) was also measured using a photometric enzyme assay (Pars Azmoon, Iran). High sensitivity C-reactive protein (hs-CRP) and interleukin-6 (IL-6) were evaluated by enzyme linked immunosorbent assay (ELISA) on serum (IBL International, Germany). Inter- and intra-assay coefficients of variation were both < 5% for all measurements. Standard and control solutions were used for all measurements and standard curves were plotted for all of these standardized measurements. The laboratory was blinded to groupings.

Statistical analysis
Dietary intake was analyzed by N4 software. Paired t-test was used for comparing the mean values of all variables at the end of the two different diet periods. The percent change for each variable was calculated by the formula (E-B/B) × 100, where E was the value at the end of treatment and B was the baseline value. Distribution of serum triglyceride level, CRP, and IL-6 was not a normal distribution. Therefore, we reported the geometric means for these variables. Groups were compared using the percent change in paired t-test analysis.

All results were considered significant if the two-tailed P-value was < 0.05. Statistical analyses were performed using SPSS13 for Windows (SPSS Inc., Chicago, IL, USA).

RESULTS

Of the 30 participants, 24 non-menopausal overweight and obese female adults completed the study. During the study, two patients were diagnosed with digestive problems and did not continue the study. One subject encountered a problem in blood testing. Moreover, 3 participants did not follow the study protocol and their data was hence not available (Figure 1).

The mean age of the subjects was 37.7 ± 1.3 years. Mean BMI was 30.85 ± 0.83 kg/m². All participants were married. None of them were smokers and no one was on specific medications.

Macronutrients in each dietary period were evaluated according to the 3-day dietary records (Table 1). Except two women who complained about bloating, there were no complications for consuming soy milk.

Weight did not change significantly after soy milk period compared to the cow’s milk period (mean percent change of weight: -1.75 ± 0.28 in the soy milk period vs. -1.62 ± 0.36 in the cow’s milk period; p = 0.79).

Physical activity levels did not change during the entire study period. All the patients were housewives and did not have any exercise. Their physical activity was similar and it was checked by a questionnaire. Baseline and final values of inflammatory markers and lipid profiles are shown in Table 2. The final values of LDL-C were significantly different between the two groups. However, there were no significant differences regarding the inflammatory markers and other lipids at baseline and at the end of the study. The results of IL-6 and total cholesterol were marginally significant. Following the diet with soy milk, triglyceride, total cholesterol, HDL-C, LDL-C, and CRP reduced but IL-6 increased. Following the diet with cow’s milk, triglyceride, HDL-C, and LDL-C decreased but total cholesterol, IL-6, and CRP increased.

Mean percent changes in inflammatory markers and lipid profiles are presented in Table 3. No significant changes appeared regarding inflammation and serum lipid concentration. Only serum LDL-C level reduced significantly after soy milk replacement in the diet. Total cholesterol showed marginally significant change after soy milk replacement in the diet.

Compositions of soy milk and cow’s milk are presented in Table 4.

Table 1. Macronutrients in each dietary period

<table>
<thead>
<tr>
<th>Nutrients</th>
<th>Dietary intakes (per day)</th>
<th>Cow’s milk (n = 24)</th>
<th>Soy milk (n = 24)</th>
<th>p²</th>
<th>Wash-out (n = 24)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Energy (kcal)</td>
<td>2104.6 ± 87.0³</td>
<td>2112.9 ± 91.0</td>
<td>0.82</td>
<td>2200.2 ± 97.1</td>
<td></td>
</tr>
<tr>
<td>Protein (% of energy)</td>
<td>16.8 ± 0.5</td>
<td>16.9 ± 0.5</td>
<td>0.76</td>
<td>15.9 ± 0.4</td>
<td></td>
</tr>
<tr>
<td>Total fat (% of energy)</td>
<td>30.0 ± 0.2</td>
<td>31.0 ± 1.1</td>
<td>0.20</td>
<td>31.5 ± 1.2</td>
<td></td>
</tr>
<tr>
<td>Carbohydrate (% of energy)</td>
<td>52.6±1.3</td>
<td>51.9 ± 1.2</td>
<td>0.24</td>
<td>50.6 ± 2.0</td>
<td></td>
</tr>
</tbody>
</table>

³Cow’s milk period: In this period patients had a weight reducing diet. General recommendations for macronutrient composition of the diet were: carbohydrates 50-60%; protein 15-20%, and total fat < 30%. All patients received 1 to 2 glasses of cow’s milk during this period.

²Soy milk period: All the recommendations were the same as the cow’s milk period. Only one glass of soy milk was replaced instead of cow’s milk.

²P-values for differences between the two periods (paired t-test)

⁴Wash-out: In this period, patients used the same diet they were using before the study.

⁵All values are mean ± standard errors.
A total number of 35 overweight and obese female adults were assessed for eligibility

Excluded (n = 5)
- Did not meeting inclusion criteria (n = 2)
- Refused to participate (n = 3)

At the end of the run-in period, all 30 overweight and obese female adults were randomly allocated to receive a diet with either cow’s milk or soy milk

Randomized and allocated to intervention (n = 30)

Among all subjects, 15 were randomly assigned to the diet with cow’s milk and 15 to the diet with soy milk in the first 4 weeks of trial

In total, 2 overweight and obese female adults did not continue the study because of digestive problems

The wash-out period was started with 15 subjects in the diet with cow’s milk and 13 subjects in the diet with soy milk

At the end of the wash-out period, 13 subjects started the diet with cow’s milk and 15 subjects began the diet with soy milk for another four weeks.

One participant in the diet with soy milk group and two subjects in the diet with cow’s milk group did not follow the study protocol. One person in the diet with cow’s milk group was excluded after being diagnosed with problem in blood testing.

A total of 24 overweight and obese female adults completed the study

The data of 24 overweight and obese female adults were included in all analyses

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### Table 2. Baseline and final values of inflammatory markers and lipid profile

<table>
<thead>
<tr>
<th>Metabolic variables</th>
<th>Cow’s milk* (n = 24)</th>
<th>Soy milk* (n = 24)</th>
<th>p*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Triglyceride (mg/dl)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Baseline</td>
<td>120.58 ± 9.39</td>
<td>127.21 ± 9.04</td>
<td>0.28</td>
</tr>
<tr>
<td>Final</td>
<td>114.71 ± 6.78</td>
<td>116.08 ± 9.03</td>
<td>0.86</td>
</tr>
<tr>
<td>Total cholesterol (mg/dl)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Baseline</td>
<td>195.75 ± 6.36</td>
<td>200.0 ± 7.38</td>
<td>0.28</td>
</tr>
<tr>
<td>Final</td>
<td>205.04 ± 8.80</td>
<td>198.25 ± 8.11</td>
<td>0.051</td>
</tr>
<tr>
<td>HDL-C (mg/dl)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Baseline</td>
<td>58.21 ± 2.38</td>
<td>55.42 ± 2.36</td>
<td>0.06</td>
</tr>
<tr>
<td>Final</td>
<td>56.25 ± 2.26</td>
<td>54.21 ± 2.54</td>
<td>0.13</td>
</tr>
<tr>
<td>LDL-C (mg/dl)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Baseline</td>
<td>114.92 ± 5.05</td>
<td>117.96 ± 5.07</td>
<td>0.27</td>
</tr>
<tr>
<td>Final</td>
<td>113.54 ± 6.40</td>
<td>104.89 ± 6.71</td>
<td>0.03</td>
</tr>
<tr>
<td>Hs-CRP (mg/l)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Baseline</td>
<td>3.73 ± 0.60</td>
<td>4.43 ± 1.04</td>
<td>0.41</td>
</tr>
<tr>
<td>Final</td>
<td>3.86 ± 0.76</td>
<td>4.19 ± 0.84</td>
<td>0.53</td>
</tr>
<tr>
<td>IL-6 (pg/ml)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Baseline</td>
<td>16.64 ± 1.50</td>
<td>17.3 ± 1.62</td>
<td>0.72</td>
</tr>
<tr>
<td>Final</td>
<td>19.33 ± 1.86</td>
<td>23.29 ± 1.98</td>
<td>0.08</td>
</tr>
</tbody>
</table>

HDL-C: High density lipoprotein cholesterol; LDL-C: Low density lipoprotein cholesterol; Hs-CRP: High-sensitivity C-reactive protein; IL-6: Interleukin-6

*Cow’s milk period: In this period patients had a weight reducing diet. General recommendations for macronutrient composition of the diet were: carbohydrates 50-60%, protein 15-20%, and total fat < 30%. All the patients received 1 to 2 glasses of cow’s milk during this period.

Soy milk period: All the recommendations were the same as the cow’s milk period. Only one glass of soy milk was replaced instead of cow’s milk.

P-values are for comparisons between the two periods (paired t-test)

All values are mean ± standard errors.

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**DISCUSSION**

The results of the present study which was conducted on non-menopausal overweight and obese female adults revealed that four weeks of soy milk consumption could reduce LDL-C levels. However, soy milk replacement in the diet had no significant effects on weight, inflammatory markers, and other blood lipid levels. Previous studies regarding the effects of soy had focused on older adults, mostly post-menopausal women and sick subjects. However, few studies have evaluated female adults and overweight or obese subjects. Furthermore, we assessed the effects of soy milk instead of other forms of soy products since we believed that soy milk, with most useful components of soy, is close to a whole soy product.

Soy milk replacement did not enhance weight loss in the present study. Previous clinical trial studies in Iran indicated no significant changes in weight following the consumption of various soy products.\(^{30-33}\) According to the mentioned studies, beneficial components in soy products seem to be effective on cardiometabolic and cardioenral abnormalities independent of weight change.\(^{30-33}\) Our short-term trial was similar to most studies which showed no beneficial effects of soy products on weight.\(^{30-33}\)

There have been conflicting results regarding the effects of soy components on inflammatory factors and endothelial function in humans.\(^{34-40}\) Phytoestrogen tablet consumption had neutral effect on serum concentration of CRP.\(^{38}\) Via the activation of nuclear factor-kappa B (NF-kB), CRP could be induced from IL-6.\(^{41,42}\) Soy isoflavones could block the nuclear transloca-tion of NF-kB and inhibit its activation.\(^{43,44}\) Probably the effects of isoflavones can have, at least a partial, meditative role on CRP expression through the modula-tion of the NF-kB-dependent pathway. However, the exact mechanisms of the effects of soy on inflammatory factors and endothelial function are not yet known. In this clinical trial study, soy milk did not have significant effects on CRP and IL-6 among healthy subjects. These results were similar to the results of two recent clinical trial studies on healthy women.\(^{39,40}\) This might be related to the low amount of isoflavone intake in the current study.

In a recent study, soy milk could reduce LDL-C (25%) and total cholesterol (11%) and increase HDLC (20%). However, no change in triglyceride was observed among healthy subjects.\(^{41}\) In this study, soy milk could reduce LDL-C without changing other serum lipid indices among adults with normal lipid profile. In contrast to other fats, soy milk’s fat did not have any adverse effects.\(^{42}\) Many components of soy products, e.g. isoflavones,\(^{46}\) saponins,\(^{47}\) and β-conglycinin (7S globulin),\(^{48}\) could have beneficial effects on lipid profile. Some clinical trial studies have defined that soy 7S globulin protein can upregulate LDL receptors in

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**Table 3. Mean percent changes in inflammatory markers and lipid profile separated by the two periods**

<table>
<thead>
<tr>
<th>Variables</th>
<th>Cow’s milk (^1) (n = 24)</th>
<th>Soy milk (^2) (n = 24)</th>
<th>(p) (^3)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Triglyceride (mg/dl)</td>
<td>0.12 ± 5.40(^4)</td>
<td>-7.81 ± 3.81</td>
<td>0.18</td>
</tr>
<tr>
<td>Total Cholesterol (mg/dl)</td>
<td>4.68 ± 2.45</td>
<td>-0.78 ± 1.73</td>
<td>0.06</td>
</tr>
<tr>
<td>HDL-C (mg/dl)</td>
<td>-2.65 ± 2.18</td>
<td>-2.17 ± 1.67</td>
<td>0.86</td>
</tr>
<tr>
<td>LDL-C (mg/dl)</td>
<td>-1.18 ± 2.82</td>
<td>-11.22 ± 3.85</td>
<td>0.01</td>
</tr>
<tr>
<td>Hs-CRP (mg/l)</td>
<td>70.91 ± 65.03</td>
<td>56.13 ± 29.43</td>
<td>0.82</td>
</tr>
<tr>
<td>IL-6 (pg/ml)</td>
<td>28.52 ± 14.29</td>
<td>61.58 ± 18.44</td>
<td>0.11</td>
</tr>
</tbody>
</table>

**Table 4. Composition of soy milk and cow’s milk**

<table>
<thead>
<tr>
<th>Nutrients per 100 ml</th>
<th>Soy milk</th>
<th>Cow’s milk</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fat (g)</td>
<td>1</td>
<td>1.5</td>
</tr>
<tr>
<td>Carbohydrate (g)</td>
<td>3.5</td>
<td>4.9</td>
</tr>
<tr>
<td>Protein (g)</td>
<td>2.5</td>
<td>3.3</td>
</tr>
<tr>
<td>Sodium (mg)</td>
<td>40</td>
<td>50</td>
</tr>
<tr>
<td>Calcium (mg)</td>
<td>40</td>
<td>100</td>
</tr>
</tbody>
</table>

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\(1\) Cow’s milk period: In this period patients had a weight reducing diet. General recommendations for macronutrient composition of the diet were: carbohydrates 50-60%, protein 15-20%, and total fat < 30%. All patients received 1 to 2 glasses of cow’s milk during this period.

\(2\) Soy milk period: All the recommendations were the same as the cow’s milk period. Only one glass of soy milk was replaced instead of cow’s milk.

\(3\) P-values are for comparisons between the two diet periods (paired t-test).

\(4\) All values are mean percent change ± standard errors.
Previous clinical trials have shown an average 5-8% reduction in plasma total cholesterol in normcholesterolemic subjects who drank 350 ml soy milk daily (14.5 g soy protein), and in hypercholesterolemic subjects who drank 1 l/day soy milk (41.1 g soy protein) compared to those who consumed cow’s milk. One important difference between our study and the mentioned research was the amount of soy milk intake. We recommended only one glass of soy milk every day because of some reports including the adverse effects of high doses of isoflavones intake. As the weight loss was not significantly different between these two groups, we could not assume that more LDL-C reduction in soy milk period might be due to weight loss.

Most clinical trials have been conducted with soy protein (only some components of soy) and there have been few clinical trials with all parts of the soy, i.e. real whole soy. Recent publications have revealed higher positive effects from complete forms of soy products such as soy nut or soy milk on cardiovascular risk factors. Whole soy with all components of phytoestrogens, essential fats, plant amino acids, and isoflavones might have more benefits. It seems that a combination of soy protein, fatty acids, and phytoestrogens is more effective on cardiovascular risk factors than the purified phytoestrogens or isolated soy protein alone. Recent studies have shown different effects of various soy products. We used soy milk in the present study as we thought it was more palatable among the whole soy products.

A positive point of this study was the crossover design which was a strong method for conducting this trial. In this study, we just recommended food items, but soy milk and cow’s milk were provided for the subjects. Thus, pre-prepared food was not available for patients. In interpretation of the results, this limitation should be considered. We controlled exactly what the patients ate by analyzing the dietary intake which the participants recorded. Our results showed that soy milk could have beneficial effects on LDL-C, even though the diet in our study may not have been followed as carefully as in trials where prepared food was available. This study was conducted among non-menopausal women. Therefore, changes of blood estrogen level might have affected lipid profile variations. In order to have all the subjects in the same hormone phase, we assessed the biochemical measurements depending on their menstrual date.

Our trial should have good external validity since we conducted it on a sample of non-menopausal overweight or obese female adults without any specific disorders.

In conclusion, soy milk replacement in the diet could reduce serum LDL-C level among overweight and obese female adults. However, this replacement had no significant impact on inflammatory factors and other lipids.

ACKNOWLEDGMENTS

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