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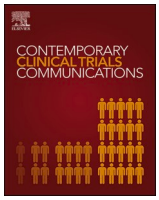

The effects of alpha-lipoic acid transdermal patch for local subcutaneous fat reduction: A randomized, placebo-controlled, clinical trial in overweight volunteers

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ABSTRACT

Background: Combating obesity is challenging, as anti-obesity compounds lose effectiveness or cause severe side effects when delivered via conventional routes. Thus, there is a need for new, effective treatment routes that are home-based and safe for long-term use. This double-blind, placebo-controlled clinical trial aimed to investigate the efficacy of a biocellulose transdermal patch containing α-lipoic acid (ALA), an anti-obesity compound, in reducing subcutaneous fat accumulation.

Methods: One hundred and sixteen overweight participants (average age 37.96 ± 7.80 years) were recruited for the study. They were randomly assigned to apply either the calcium citrate nanoparticle-encapsulated ALA transdermal patch or a placebo on their arm. The participants' body weight, height, blood lipid profile (cholesterol, triglyceride, low-density lipoprotein, and high-density lipoprotein), arm circumference, triceps skin fold, and subcutaneous fat thickness were recorded at baseline and at the 2-week follow-up.

Results: The mean arm circumference did not show any significant difference from baseline, whereas the triceps skinfold and subcutaneous fat thickness showed a significant reduction. The 2-week treatment did not significantly alter the plasma LDL, HDL, and triglyceride levels of the participants, but it significantly reduced the total cholesterol level.

Conclusion: This study reports the successful reduction of subcutaneous fat of the calcium citrate nanoparticle-encapsulated ALA transdermal patches. The transdermal patches could be used as a safe and effective home-based solution for combating obesity.

1. Introduction

In recent years, excessive fat accumulation in the human body has emerged as one of the most serious medical conditions worldwide, and more attention has been paid to the morbidity, mortality, and social embarrassment associated with being overweight [1,2]. This increase in public health awareness has created motivation among many to adopt healthier lifestyles and resolve their health conditions. Overweightness is associated with adipose tissue, which is mainly categorized according to anatomical location; the tissue located around the viscera is categorized as visceral fat, and the tissue presented in subcutaneous areas is categorized as subcutaneous fat [3]. Different types of adipose tissue may be associated with different health consequences of obesity [4]. Though multiple studies have reported that visceral adipose tissue poses a greater risk factor for metabolic diseases than subcutaneous adipose tissue, subcutaneous fat constitutes 80 % of the fat accumulated in the body [5–7]. Excessive subcutaneous fat storage under the skin results in the shift of fat accumulation to other areas such as the liver, the heart, or skeletal muscle and is related to obesity-associated insulin resistance and inflammation [8]. Moreover, the increase in

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subcutaneous adipose tissue deposition also contributes to the development of physiological conditions, such as knee osteoarthritis, as well as the deterioration of psychological comfort [9–11].

With the resulting complications from excess subcutaneous fat accumulation, it is crucial to find effective management of these conditions in the form of behavioral changes in parallel with adjunctive treatments. Complementary therapies and natural compounds have gained increasing recognition for their role in human health, particularly in the prevention and treatment of chronic diseases. Many of these natural compounds are known for their antioxidant and anti-inflammatory properties. ALA has demonstrated benefits in treating diabetes, obesity, and cardiovascular diseases by improving insulin sensitivity, lipid profiles, and reducing inflammation [12]. Conjugated linoleic acids have shown potential in reducing inflammation, oxidative stress, and body fat [13]. Propolis and pycnogenol have shown beneficial effects as complementary therapies for managing inflammation and oxidative stress [14,15]. These natural compounds have shown potential in improving lipid profiles, reducing inflammation, and combating oxidative stress, which are key factors in the pathogenesis of obesity and fat accumulation [16–18].

Among natural compounds, ALA, also known as 1,2-dithiolane-3-pentanoic acid or thioctic acid, has emerged as a promising candidate for its antioxidant and anti-inflammatory properties [19]. ALA is known to improve lipid metabolism, reduce oxidative stress, and may facilitate adipose tissue decrease [20,21]. However, the efficacy of oral administration can be limited by poor absorption and lack of target-specific delivery. To overcome these limitations, innovative approaches such as transdermal patches have been explored. These delivery systems have the potential to improve the bioavailability of ALA and other natural compounds [22]. For instance, a previous in vivo study demonstrated that the use of transdermal patches containing nanofibers loaded with curcumin resulted in a 4 to 7 percent decrease in the overall amount of adipose tissue as measured by whole-body magnetic resonance imaging [23]. In addition, a study in C57BL/6J mice showed that transdermal delivery of metformin using dissolving microcroneedles and iontophoresis patches is able to reduce fat pad size and improve energy metabolism [24].

Building on these findings, our previous study demonstrated a novel slow-release transdermal drug delivery system using calcium citrate nanoparticles (CaCitNPs), which have been shown to be effective drug carriers [25–27]. Therefore, the use of a transdermal patch loaded with CaCitNPs-encapsulated ALA has promising potential for improving the efficacy of subcutaneous fat reduction. In this study, we further develop and clinically evaluate a transdermal patch based on a nanoparticle delivery system. This patch is constructed from a biocellulose membrane loaded with CaCitNPs-encapsulated ALA. The objective is to assess the safety and efficacy of this innovative modality in reducing subcutaneous fat accumulation, offering a promising approach to localized fat reduction in the context of obesity management.

2. Material and methods

2.1. Preparation and characterization of ALA-loaded CaCitNPs

The protocol for the preparation of the ALA-CaCitNPs solution was modified from our previous study. Briefly, 100 mg of ALA (Sigma-Aldrich, MA, USA) were dissolved in a 0.375 M NaOH solution and then treated with a 1.00 M trisodium citrate solution at room temperature for 10 min. Next, 3.25 mL of a 2.00 M calcium chloride solution was added to the solution while stirring. After a milky suspension was created, it was stirred for an additional 30 min at room temperature. The solution was centrifuged, and the residual solid was washed with deionized water before being vacuum-dried overnight; the resulting product was a white solid. To characterize the particle size and shape, an ALA-CitNPs solution was analyzed under a Hitachi H-7650 transmission electron microscope (TEM) equipped with a charge-coupled device (CCD) camera (Hitachi High-Technologies Corporation, Tokyo, Japan). The surface charge of CaCitNPs was measured by the zeta-potential Malvern Zetasizer Nano Series (Malvern Panalytical, Malvern, UK).

2.2. In vitro cytotoxicity analysis of ALA-loaded CaCitNPs

In this study, the PrestoBlueTM cell viability assay was conducted to determine the effect of ALA-CaCitNPs on cellular viability (Invitrogen, CA, USA). Human cell types, including keratinocytes (HaCaT) and human dermal fibroblast cells (HDFa), were utilized. All cells were seeded at a density of 5x103 cells in 45 μL cell culture media in 96-well plates and incubated for 24 h to allow cell adhesion. Cells were stimulated with ALA, CaCitNPs, and CaCitNPs loaded with ALA for 24 h. As a negative control, untreated cells were employed. The reducing environment within metabolically active cells converts the non-toxic resazurin in the PrestoBlue reagent to an intensely red-fluorescent dye. Fluorescence intensity was measured with a microplate reader with excitation at 560 nm and emission at 560 nm (Thermo, Varioskan Flash Multimode, MA, USA).

2.3. Preparation of intervention and in vitro ALA released profile

The biocellulose transdermal patch used in this study was produced at the Nanobiomedicine Laboratory, Faculty of Medicine, Chulalongkorn University, Thailand. The transdermal patch contained ALA incorporated with CaCitNPs solution, while the placebo contained deionized water. The 8 × 8 cm membranes were soaked in an ALA-CaCitNPs solution and shaken at 100 rpm, room temperature, for 12 h to allow the complete absorption of the solution. After the total solution absorption, the SEM analysis of the CaCitNPs-encapsulated ALA transdermal patch was determined by the JSM 6480LV scanning electron microscope (Jeol, Tokyo, Japan). The percent release of ALA from the patch was measured with a microplate reader (Thermo, Vari-oskan Flash Multimode, MA, USA) at 330 nm absorbance. The ALA series concentrations were prepared for use as standard curves. ALA-CaCitNPs solution-loaded patches were tested for drug profile release in sodium acetate buffer (pH 4.55 and 5.45 to mimic natural skin pH); the unloaded patch was also prepared as a normalization control. The level of release of ALA was calculated as a percentage accumulation at each time point.

2.4. Study design and ethical approval of the study

This double-blind, placebo-controlled study aimed to investigate the efficacy and safety of CaCitNPs-encapsulated ALA transdermal patches in reducing subcutaneous fat accumulation. This study was conducted according to the guidelines established in the Declaration of Helsinki, the CONOSRT guidelines for clinical trial reporting. The trial design was registered with ClinicalTrials.gov (NCT03062163). Ethical approval for the study was obtained from the Research Ethics Review Committee for Research Involving Human Research Participants, Health Sciences Group, Chulalongkorn University (approval number 122.1/59) in accordance with the International Conference on Harmonization-Good Clinical Practice (ICH-GCP).

2.5. Study participants

Overweight volunteers were recruited through advertisements. The eligibility criteria included having a BMI (body mass index) of more than 25 kg/m^2 and being over 18 years old. Participants were excluded if they had an underlying disease, chronic health condition, participated in other weight loss programs, were prescribed any weight loss medication, were pregnant or lactating, or had a history of being clinically allergic to ALA and calcium citrate. After receiving a full explanation of the purpose and nature of all procedures involved, the volunteers signed a written informed consent form prior to their participation in this study.

The sample size for this study was calculated using G*Power 3.1.9.4 software, which is widely used for determining sample sizes in clinical trials. A two-tailed hypothesis test was employed with the following parameters: alpha (α): 0.05 (5 % significance level), power (1-β): 0.95 (95 % statistical power), effect size (Cohen's d): 0.7, which was calculated based on the difference change in fat thickness as observed in the previous study [28], and allocation ratio: 1:1 between the treatment and placebo groups. Using these parameters, the sample size was calculated to be 55 participants per group. To account for potential dropouts, we enrolled 58 participants per group, resulting in a total of 116 participants.

2.6. Randomization and blinding

The study applied a simple random sampling approach to randomly assigned participants into one of two groups: the treatment group, which received the CaCitNPs-encapsulated ALA transdermal patch, or the placebo group. Randomization was performed in a 1:1 ratio using a computer-generated randomization sequence. An independent researcher, who was not involved in any other aspects of the study, oversaw this process to ensure that treatment allocation remained concealed from both the participants and the research team. To maintain blinding, both the placebo and the CaCitNP-encapsulated ALA patch were identical in appearance, texture, and packaging, preventing participants and researchers from distinguishing between the two. All participants were blinded to their group assignments throughout the study. The researchers who conducted the assessments of body measurements and analyzed the data were also blinded to the treatment allocation to minimize any bias in the outcome evaluations. Blinding was maintained until the completion of the data analysis phase.

2.7. Procedure

The study period consisted of a 2-week treatment phase in which the participants were either given the placebo or the CaCitNPs-encapsulated ALA transdermal patch. Each participant was instructed to place the patches for 6 h per day on the inner side of their upper arms. Assessments of body weight, height, blood lipid profile (cholesterol, triglyceride, HDL, and LDL), arm circumference, triceps skin fold, and subcutaneous fat thickness of participants were recorded at baseline and at the 2-week follow-up. Arm circumference was measured from the midpoint between the tip of the acromial process and the tip of the olecranon; the triceps skin fold and thickness of the subcutaneous fat layer were measured at the posterior surface in the same area as the measurement of arm circumference. A marking pen was used for marking out areas of measurement. Triceps skin fold was assessed using Harpenden skinfold calipers, and subcutaneous fat thickness was measured by ultrasound (GE LogiQ P6 Ultrasound System, GE Healthcare). Measurements of arm circumference, triceps skin fold, and subcutaneous fat thickness were taken three times and averaged at both baseline and after two weeks. The blood lipid profile, including total cholesterol, triglyceride, high-density lipoprotein (HDL), and low-density lipoprotein cholesterol (LDL), was investigated by the Department of Laboratory Medicine, King Chulalongkorn Memorial Hospital, Bangkok, Thailand, following the standard protocol. During the study period, participants were instructed to maintain their usual lifestyle.

2.8. Statistical analysis

The Kolmogorov-Smirnov test (with Dallal-Wikinson-Lilliefor P value) was used to evaluate abnormal data distribution, and the paired T-test was used to evaluate normal data distribution. Abnormally distributed data was analyzed using the Wilcoxon signed rank test. Analysis for the mean and standard deviations of each parameter was performed using GraphPad Prism statistical software version 9.5.1.

3. Results

3.1. Characterization of ALA-loaded CaCitNPs

The size and morphology of the ALA-loaded CaCitNPs were observed by TEM analyses. The TEM photograph of ALA-loaded CaCitNPs revealed that the NPs had a spherical morphology with a diameter of approximately 20 nm (Fig. 1A). Zeta potential measurements showed that CaCitNPs presented a negative surface charge (−24.7 mV) (Fig. 1B).

3.2. In vitro cytotoxic effect of ALA-loaded CaCitNPs

The viability of HaCaT and HDFa cells following exposure to ALA, CaCitNPs, and ALA-loaded CaCitNPs for 24 h is shown in Fig. 2A and B, respectively. Exposure to H2O2 significantly decreased the viability of HaCaT and HDFa cells, whereas ALA, CaCitNPs, and ALA-loaded CaCitNPs showed no obvious toxicity compared to the control group.

3.3. Characterization of a CaCitNPs-encapsulated ALA transdermal patch

The CaCitNPs-encapsulated ALA transdermal patch used in this study was prepared by loading ALA-CaCitNPs solution on an 8 × 8 cm biocellulose membrane, as shown in Fig. 3. The SEM morphologies of biocellulose membranes are shown in Fig. 4A. The SEM photograph of the prepared ALA-loaded CaCitNPs patch showed the distribution of ALA-CaCitNPs on the membrane (Fig. 4B).

3.4. Drug release profile of ALA-loaded CaCitNPs

The release profile of ALA from CaCitNPs-encapsulated ALA-loaded membranes in a sodium acetate buffer solution is shown in Fig. 5. In the first 30 min, 30 % of free ALA was released, and >60 % of free ALA was released after 180 min. This demonstrates the continuous release of ALA from the biocellulose transdermal patches.

3.5. General characteristics of the study participants

Of the 122 eligible participants screened for inclusion in the trial, 116 were enrolled and randomly assigned to one of two study groups. In group I, 58 participants received a CaCitNPs-encapsulated ALA transdermal patch, whereas in group II, 58 participants received a placebo patch. Out of the 116 eligible participants, 10 dropped out by the last follow-up, and the data from the two participants who did not complete the study's interventions were excluded. In total, the data from 52 participants in group I and 52 participants in group II were collected and analyzed (Fig. 6). 97 % of all participants were female. The demographic and baseline clinical characteristics of the participants are shown in Table 1. The mean age of all participants was 37.96 + 7.80 years, and the mean height was 1.59 + 0.07 m. The mean body weight and body mass index of the participants were 73.69 + 12.47 kg and 29.04 + 4.04 kg/m^2, respectively. No obvious trend was observed between the two groups. All the demographic and clinical characteristic parameters did not significantly change between baseline and the 2-week follow-up.

3.6. Effect of ALA-loaded CaCitNPs

To evaluate the effectiveness of the transdermal patch in reducing subcutaneous fat volume, three parameters of arm circumference, triceps skinfold, and thickness of the subcutaneous fat layer were measured as objective outcomes. The results were analyzed by comparing data between baseline and 2 weeks after using the patch. In addition, the mean differences in pre- and post-intervention were compared between the placebo and treatment groups, as shown in Table 2. At the 2-week follow-up, the mean arm circumference did not show significant change compared to baseline, and both the mean circumferences of the

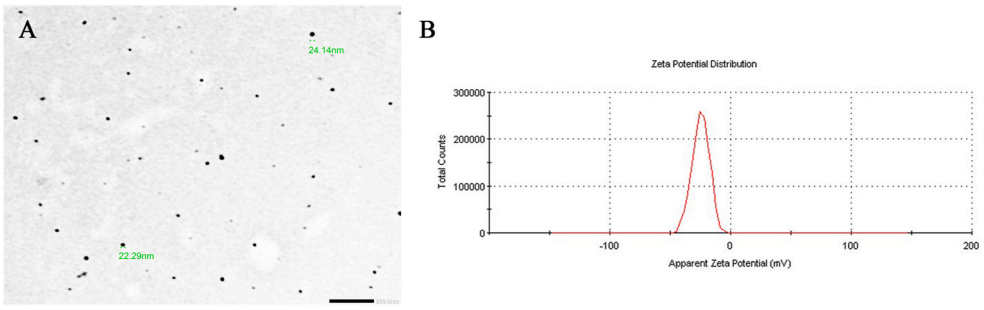

Fig. 1. Characterization of ALA-loaded CaCitNPs by TEM; the scale bar indicates 200 nm (A) and zetasizer (B).

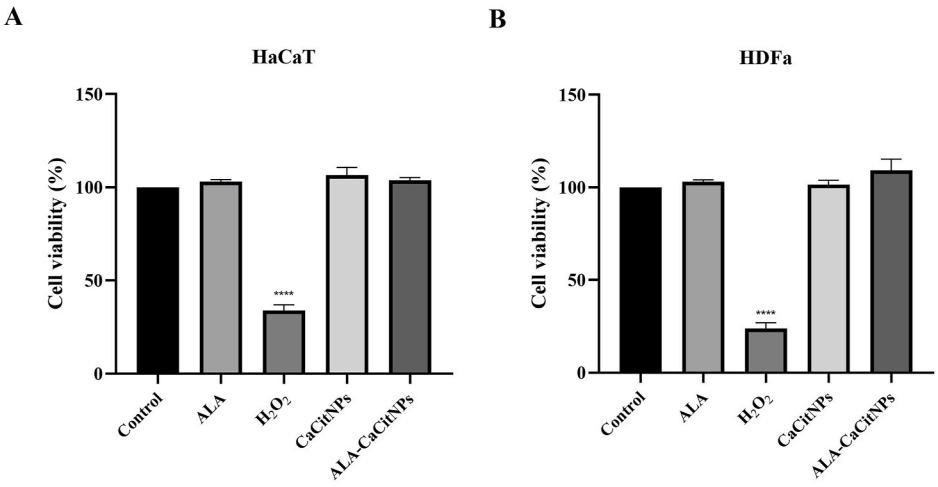

Fig. 2. Effect of ALA-loaded CaCitNPs on the viability of HaCaT cells (A) and HDFa cells (B) at 24 h of exposure.

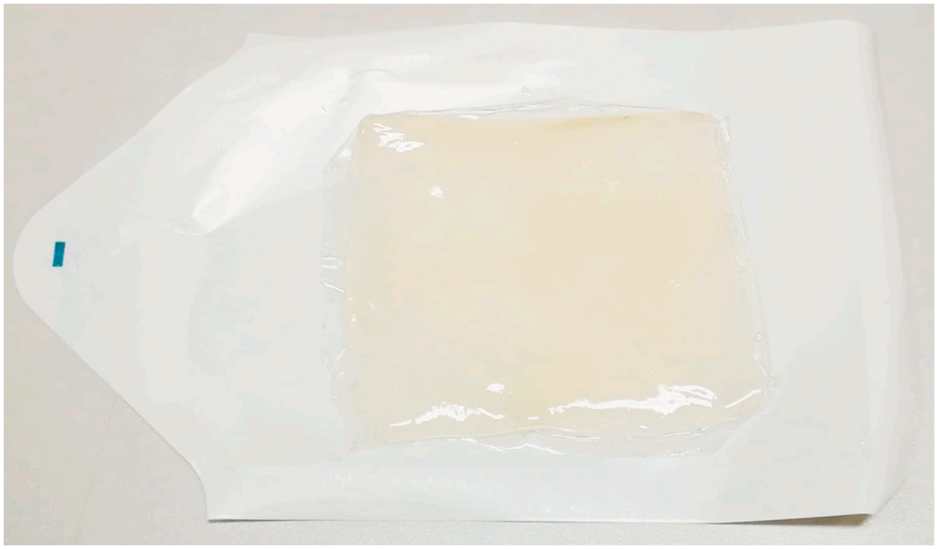

Fig. 3. The ALA-loaded CaCitNPs transdermal patch used in the study.

two groups from before and after treatment showed no significant difference from placebo (−0.04 ± 1.31; −0.17 ± 0.96, p = 0.172). However, triceps skinfold showed a significant reduction after two weeks of intervention compared to placebo (−0.58 ± 1.97; 0.29 ± 3.01, p = 0.010), and a significant reduction in subcutaneous fat thickness was also observed from the ultrasonography (−0.19 ± 0.23; −0.10 ± 0.25, p = 0.007). The example photos demonstrate the reduction in the subcutaneous fat layer after the 2-week intervention (Fig. 7).

3.7. Blood lipid profile

To evaluate the effect of the transdermal patch on the systemic reduction of fat, the blood lipid profiles, including low-density lipoprotein (LDL), high-density lipoprotein (HDL), triglyceride, and total cholesterol, of the participants were evaluated. The mean values for four basic blood lipid parameters are shown in Table 3. When comparing data between baseline and follow-up outcomes, there were no

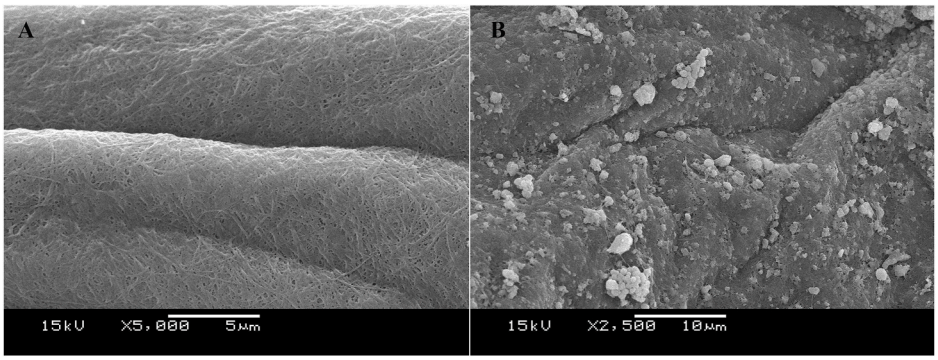

Fig. 4. Representative SEM micrographs of the CaCitNPs-encapsulated ALA transdermal patch. (A) SEM image of the biocellulose membrane loaded with DI water. (B) SEM image of a biocellulose membrane loaded with ALA-CaCitNPs.

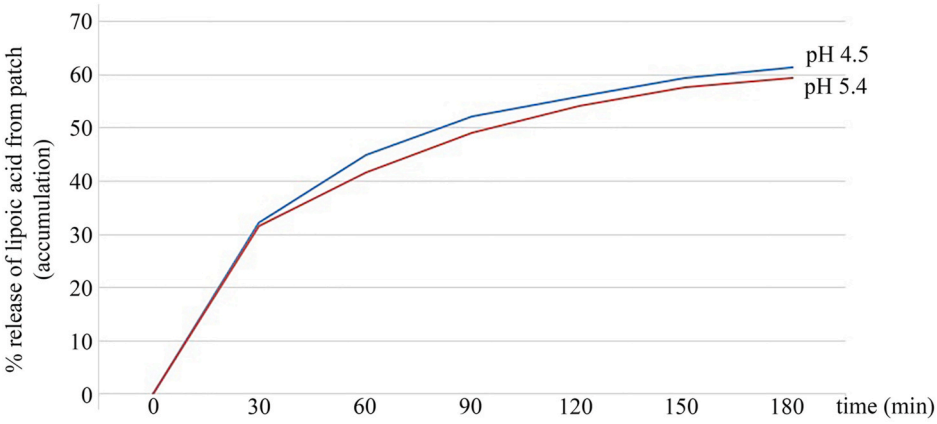

Fig. 5. The in vitro release profile of ALA from transdermal patches.

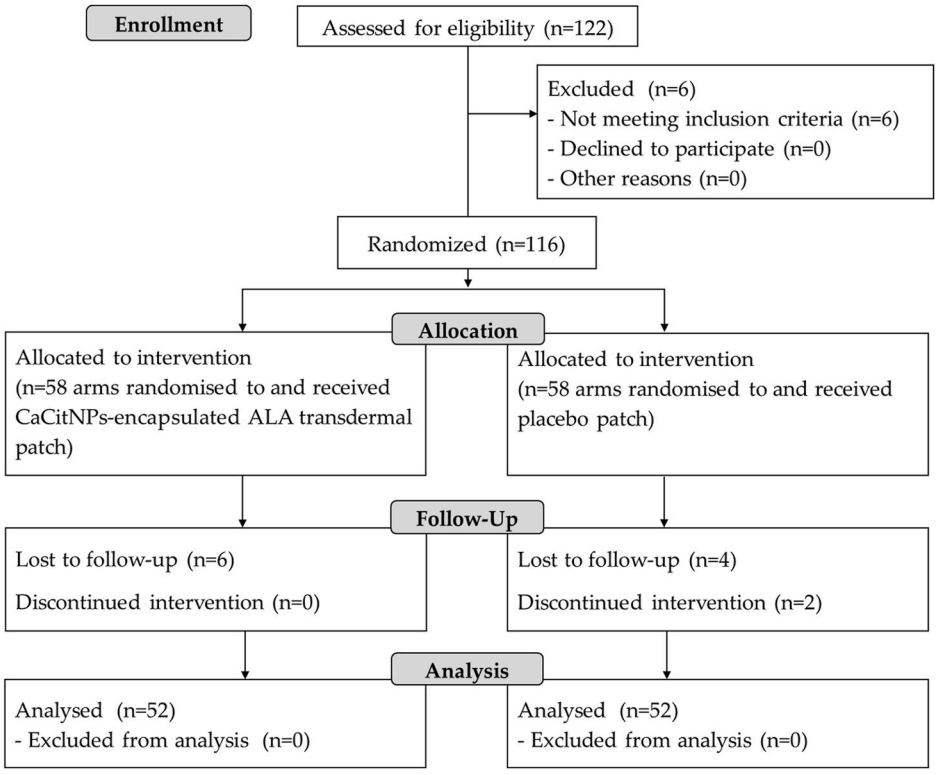

Fig. 6. Flow diagram of the study participants.

Table 1
Baseline characteristics of the participants.

Variables	Placebo	Transdermal patch		Total
	Mean + SD (n = 52)	Mean + SD (n = 52)	p-Value	Mean + SD (n = 104)
Age (year)	38.88 ± 8.34	37.04 ± 7.19	0.229	37.96 ± 7.80
Weight (kg)	72.96 ± 12.65	74.41 ± 12.37	0.556	73.69 ± 12.47
Height (m)	1.59 ± 0.07	1.58 ± 0.07	0.657	1.59 ± 0.07
Body mass index (kg/m^2)	28.67 ± 4.29	29.41 ± 3.77	0.352	29.04 ± 4.04

significant changes in blood lipid profile in both the placebo and CaCitNPs-encapsulated ALA transdermal patch-treated groups. While the mean differences between pre- and post-intervention were no significant changes in the participants' plasma LDL, HDL, and triglyceride levels, total cholesterol levels were significantly reduced compared to placebo (3.94 ± 5.37; −0.44 ± 1.90, p = 0.0001).

3.8. Adverse events

The post-treatment evaluation of the treatment area revealed that no participants experienced any adverse effects from using the transdermal patch. Within the treatment group, two participants complained of localized itching at the patch application site, which resolved spontaneously after removing the intervention. Overall, participants reported satisfaction with the transdermal patch.

4. Discussion

In this study, we developed an CaCitNPs-encapsulated ALA transdermal patch as a painless yet effective intervention for reducing human subcutaneous adipose tissue. The efficacy and safety of the CaCitNPs-encapsulated ALA transdermal patch were clinically evaluated. Our results revealed that the thickness of the subcutaneous fat layer and triceps skinfold had decreased in the area exposed to the transdermal patch. There were no reports of any adverse side effects resulting from the use of this transdermal patch. One of the assessment outcomes, which

Table 2
Outcome variables at time of recruitment and 2 weeks follow up of placebo or CaCit-NPs-encapsulated ALA transdermal patch treatment.

	Placebo			Transdermal patch			Mean differences between baseline and 2 week follow-up		
	Baseline	2-week follow-up	p-Value	Baseline	2-week follow-up	p-Value	Placebo	Transdermal patch	p-Value
Arm circumference (mean ± SD, cm)	30.48 ± 3.46	30.31 ± 3.76	0.242	30.31 ± 3.53	30.26 ± 3.79	0.828	−0.17 ± 0.96	−0.04 + 1.31	0.172
Triceps skinfold (mean ± SD, mm)	28.83 ± 6.05	29.12 ± 7.03	0.809	28.42 ± 6.25	27.84 ± 5.93[a]	0.036	0.29 ± 3.01	−0.58 ± 1.97[a]	0.010
Subcutaneous fat thickness (mean ± SD, cm)	1.29 ± 0.49	1.18 ± 0.48[b]	0.005	1.29 ± 0.49	1.09 ± 0.37[c]	0.0001	−0.10 ± 0.25	−0.19 ± 0.23[b]	0.007

[a] $p < 0.05$.
[b] $p < 0.01$.
[c] $p < 0.001$.

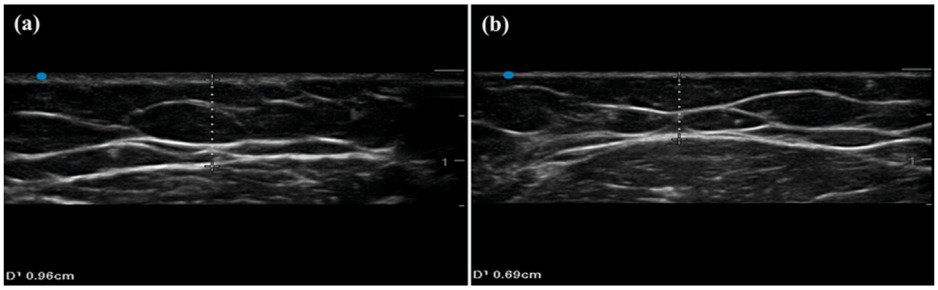

Fig. 7. Ultrasonography of the representative participant at pretreatment (A) and after 2 weeks of treatment with a CaCitNPs-encapsulated ALA transdermal patch (B).

Table 3
Blood lipid profile of the participants before and after the intervention.

	Placebo			Transdermal patch			Mean differences between baseline and 2 week follow-up		
	Baseline	2-week follow-up	p-Value	Baseline	2-week follow-up	p-Value	Placebo	Transdermal patch	p-Value
Cholesterol	206.50 ± 29.11	206.9 ± 29.20	0.938	206.30 ± 28.48	202.30 ± 27.05	0.470	−0.44 ± 1.90	3.94 ± 5.37[a]	0.0001
High-density lipoprotein (HDL)	48.37 ± 7.69	48.10 ± 7.10	0.853	48.17 ± 7.57	48.04 ± 6.90	0.924	0.26 ± 2.28	0.13 ± 2.20	0.760
Triglyceride	163.00 ± 62.90	162.90 ± 62.30	0.991	162.20 ± 62.79	160.80 ± 60.64	0.912	0.13 ± 2.74	1.32 ± 4.27	0.094
Low-density lipoprotein (LDL)	130.00 ± 19.16	129.3 ± 17.92	0.845	129.6 ± 20.89	129.0 ± 20.67	0.876	0.71 ± 2.96	0.63 ± 1.60	0.869

[a] $p < 0.0001$.

showed no significant decrease after using the patch, is arm circumference. However, it is difficult to interpret the changes in upper arm muscles, laxity of skin, and subcutaneous fat volume by measuring the circumference of the arm.

The participants' blood lipid profile at the 2-week follow-up had significantly reduced total blood cholesterol. However, this statistical significance may not be clinically significant, as drug interventions to lower blood cholesterol levels should have a potency of 30 % or more. This is in accordance with previous studies that showed oral administration of ALA could improve lipid profiles [29,30]. Although not all blood lipid parameters showed statistically significant changes, plasma LDL, HDL, and triglyceride levels demonstrated trends toward improvement, consistent with findings from other studies. ALA supplementation has been linked to positive effects on lipid metabolism and weight loss. However, similar to our results, a previous trial of ALA supplementation reported no significant reduction in plasma triglyceride levels, despite observing other beneficial outcomes, such as weight loss [31,32]. These findings suggest that while ALA may favorably influence lipid profiles and body composition, its effects on specific lipid parameters, such as triglycerides, may be limited or require a longer treatment duration to achieve significant results.

The original prototype of the transdermal patch of a nanoparticle-based drug delivery system demonstrated its effectiveness in subcutaneous adipose tissue reduction. Though there were no scientific reports on this innovative strategy in animal models, our results suggest that the transdermal patch may be an efficacious and safe subcutaneous fat reduction intervention for humans. The efficacy of the CaCitNP-encapsulated ALA transdermal patch might result from ALA's ability to reduce fat accumulation via both direct and indirect mechanisms. ALA has been well-established as a potent antioxidant that plays a crucial role in mitochondrial bioenergetic reactions, enabling it to scavenge reactive oxygen species (ROS) and regenerate endogenous antioxidants such as glutathione [33,34]. In addition, ALA has been shown to modulate several biochemical pathways associated with fat metabolism, promoting fat oxidation, reducing lipid synthesis, and improving insulin sensitivity, all of which contribute to its anti-obesity effects [33,35]. These effects are likely mediated by the interaction of ALA with signaling pathways such as AMPK and PPAR, which play essential roles in energy homeostasis and fat metabolism [35,36]. The ability of ALA to regulate these pathways may help explain the improvements in body composition and fat reduction observed in this study.

In this study, we delivered ALA topically using a nanoparticle-based drug delivery system, which offers several advantages over oral supplementation, including improved absorption and bioavailability. Oral administration of ALA is often limited by poor absorption and first-pass metabolism in the liver, resulting in lower therapeutic efficacy [36]. By contrast, the localized delivery of ALA through a transdermal patch bypasses the digestive system and the first-pass effect, allowing for more effective targeting of subcutaneous adipose tissue. The use of nanoparticle formulations can significantly improve the stability and controlled release of ALA, ensuring prolonged exposure to the target tissues [37]. The nanoparticle-based delivery system used in this study has been reported to be outstanding materials for drug delivery due to their high availability, low cost, safety, biocompatibility, and slow biodegradability [25–27]. Nanoparticles with smaller particle sizes, such as the CaCitNPs used in this study, are effective in crossing the stratum corneum and reaching subcutaneous fat tissue, which could enhance bioavailability and therapeutic efficacy [26]. Although the penetration mechanisms of the compounds in this transdermal patch are still unknown, our in vitro drug release investigation revealed sustained release of ALA from the patch at pH 4.5 and 5.4, which is the natural skin pH [38]. This may explain the significant reduction in triceps skinfold thickness and subcutaneous fat layer observed in our study, despite the short duration of the intervention.

Although the results of this study are promising, several limitations should be acknowledged. The short duration of the study may not have been sufficient to capture long-term changes in fat metabolism or blood lipid profiles. Additionally, the sample size was relatively small, and future research with a larger population may yield more robust findings. Further studies should also explore the effectiveness of the CaCitNP-encapsulated ALA transdermal patch in other areas of the body and over longer periods to assess its long-term efficacy and safety.

5. Conclusion

In summary, according to the nanoparticle characterization, CaCitNPs-encapsulated ALA was successfully prepared. Our results showed that neither CaCitNPs nor ALA-loaded CaCitNPs are obviously cytotoxic toward human cells, including HaCaT and HDFa. The in vitro release profile revealed a sustained release of ALA from CaCitNPs. Our data demonstrated that the CaCitNPs-encapsulated ALA transdermal patches are effective in reducing subcutaneous fat in the arms. Based on these findings, this novel strategy could be used as a convenient and effective home-based solution for reducing subcutaneous fat accumulation.

CRediT authorship contribution statement

Kanidta Sooklert: Writing – review & editing, Writing – original draft, Visualization, Validation, Project administration, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **Sasin Thamakaison:** Writing – review & editing. **Siwaporn Nilyai:** Validation, Investigation. **Sarocha Cherdchom:** Investigation. **Rojrit Rojanathanes:** Resources. **Amornpun Sereemaspun:** Writing – review & editing, Methodology, Conceptualization.

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Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Data availability

Data will be made available on request.

References

[1] M. Tremmel, U.-G. Gerdtham, P.M. Nilsson, S. Saha, Economic burden of obesity: a systematic literature review, Int. J. Environ. Res. Publ. Health 14 (4) (2017) 435.
[2] C.M. Apovian, Obesity: definition, comorbidities, causes, and burden, Am. J. Manag. Care 22 (7 Suppl) (2016) s176–s185.
[3] A. Marcadenti, E.O. de Abreu-Silva, Different adipose tissue depots: metabolic implications and effects of surgical removal, Endocrinol. Nutr. 62 (9) (2015) 458–464.
[4] M.M. Ibrahim, Subcutaneous and visceral adipose tissue: structural and functional differences, Obes. Rev. 11 (1) (2010) 11–18.
[5] S. Laforest, J. Labrecque, A. Michaud, K. Cianflone, A. Tchernof, Adipocyte size as a determinant of metabolic disease and adipose tissue dysfunction, Crit. Rev. Clin. Lab Sci. 52 (6) (2015) 301–313.

[6] L.M. Sipe, C. Yang, J. Ephrem, E. Garren, J. Hirsh, C.D. Deppmann, Differential sympathetic outflow to adipose depots is required for visceral fat loss in response to calorie restriction, Nutr. Diabetes 7 (4) (2017) e260, e260.
[7] A. Vecchié, F. Dallegri, F. Carbone, A. Bonaventura, L. Liberale, P. Portincasa, G. Frühbeck, F. Montecucco, Obesity phenotypes and their paradoxical association with cardiovascular diseases, Eur. J. Intern. Med. 48 (2018) 6–17.
[8] U. Smith, B.B. Kahn, Adipose tissue regulates insulin sensitivity: role of adipogenesis, de novo lipogenesis and novel lipids, J. Intern. Med. 280 (5) (2016) 465–475.
[9] A. Ruhdorfer, W. Wirth, T. Dannhauer, F. Eckstein, Longitudinal (4 year) change of thigh muscle and adipose tissue distribution in chronically painful vs painless knees–data from the Osteoarthritis Initiative, Osteoarthritis Cartilage 23 (8) (2015) 1348–1356.
[10] A. Rosengren, K. Teo, S. Rangarajan, C. Kabali, I. Khumalo, V.R. Kutty, R. Gupta, R. Yusuf, R. Iqbal, N. Ismail, Psychosocial factors and obesity in 17 high-, middle- and low-income countries: the Prospective Urban Rural Epidemiologic study, Int. J. Obes. 39 (8) (2015) 1217–1223.
[11] K. Preiss, D. Clarke, P. O'Brien, X. de la Piedad Garcia, A. Hindle, L. Brennan, Psychosocial predictors of change in depressive symptoms following gastric banding surgery, Obes. Surg. 28 (2018) 1578–1586.
[12] M. Shanaida, R. Lysiuk, O. Mykhailenko, N. Hudz, A. Abdulsalam, T. Gontova, O. Oleshchuk, Y. Ivankiv, V. Shanaida, D. Lytkin, Alpha-lipoic acid: an antioxidant with anti-aging properties for disease therapy, Curr. Med. Chem. 13 (10) (2024) 1228.
[13] H.D. Putera, R.I. Doewes, M.N. Shalaby, A.A. Ramírez-Coronel, Z.S. Clayton, W. K. Abdelbasset, S.S. Murtazaev, A.T. Jalil, P. Rahimi, E. Nattagh-Eshtivani, The effect of conjugated linoleic acids on inflammation, oxidative stress, body composition and physical performance: a comprehensive review of putative molecular mechanisms, Nutr. Metabol. 20 (1) (2023) 35.
[14] E. Nattagh-Eshtivani, A. Gheflati, H. Barghchi, P. Rahbarinejad, K. Hachem, M. N. Shalaby, W.K. Abdelbasset, G. Ranjbar, D. Olegovich Bokov, P. Rahimi, The role of Pycnogenol in the control of inflammation and oxidative stress in chronic diseases: molecular aspects, Phytother Res. 36 (6) (2022) 2352–2374.
[15] E. Nattagh-Eshtivani, N. Pahlavani, G. Ranjbar, J. Gholizadeh Navashenaq, A. Salehi-Sahlabadi, T. Mahmudiono, M. Nader Shalaby, M. Jokar, M. Nematy, H. Barghchi, Does propolis have any effect on rheumatoid arthritis? A review study, Food Sci. Nutr. 10 (4) (2022) 1003–1020.
[16] I. Pérez-Torres, V. Castrejón-Téllez, M.E. Soto, M.E. Rubio-Ruiz, L. Manzano-Pech, V. Guarner-Lans, Oxidative stress, plant natural antioxidants, and obesity, Int. J. Mol. Sci. 22 (4) (2021) 1786.
[17] A. Esteghamati, T. Mazaheri, M.V. Rad, S. Noshad, Complementary and alternative medicine for the treatment of obesity: a critical review, Int. J. Endocrinol. Metabol. 13 (2) (2015).
[18] H. Barghchi, Z. Dehnavi, E. Nattagh-Eshtivani, E.R. Alwaily, A.F. Almulla, A. K. Kareem, M. Barati, G. Ranjbar, A. Mohammadzadeh, P. Rahimi, The effects of Chlorella vulgaris on cardiovascular risk factors: a comprehensive review on putative molecular mechanisms, Biomed. Pharmacother. 162 (2023) 114624.
[19] D. Tibullo, G. Li Volti, C. Giallongo, S. Grasso, D. Tomassoni, C.D. Anfuso, G. Lupo, F. Amenta, R. Avola, V. Bramanti, Biochemical and clinical relevance of alpha lipoic acid: antioxidant and anti-inflammatory activity, molecular pathways and therapeutic potential, Inflamm. Res. 66 (2017) 947–959.
[20] A.R. El Barky, S.A. Hussein, T.M. Mohamed, The potent antioxidant alpha lipoic acid, J. Plant Chem. Ecophysiol 2 (1) (2017) 1016.
[21] A.E. Huerta, P.L. Prieto-Hontoria, M. Fernández-Galilea, X. Escoté, J.A. Martínez, M.J. Moreno-Aliaga, Effects of dietary supplementation with EPA and/or α-lipoic acid on adipose tissue transcriptomic profile of healthy overweight/obese women following a hypocaloric diet, Biofactors 43 (1) (2017) 117–131.
[22] T. Snyman, Formulation, In-Vitro Release and Transdermal Diffusion of Alpha-Lipoic Acid, 2009.
[23] A.R. Ariamoghaddam, B. Ebrahimi-Hosseinzadeh, A. Hatamian-Zarmi, R. Sahraeian, In vivo anti-obesity efficacy of curcumin loaded nanofibers transdermal patches in high-fat diet induced obese rats, Mater. Sci. Eng., C 92 (2018) 161–171.
[24] M. Abbasi, Z. Fan, J.A. Dawson, S. Wang, Transdermal delivery of metformin using dissolving microneedles and iontophoresis patches for browning subcutaneous adipose tissue, Pharmaceutics 14 (4) (2022) 879.
[25] M. Chalermnon, S. Cherdchom, A. Sereemaspun, R. Rojanathanes, T. Khotavivattana, Biguanide-based synthesis of 1, 3, 5-triazine derivatives with anticancer activity and 1, 3, 5-triazine incorporated calcium citrate nanoparticles, Molecules 26 (4) (2021) 1028.
[26] S. Cherdchom, W. Keawsongsaeng, W. Buasorn, N. Rimsueb, P. Pienpinijtham, A. Sereemaspun, R. Rojanathanes, P. Aramwit, Development of eugenol-embedded calcium citrate nanoparticles as a local anesthetic agent, ACS Omega 6 (43) (2021) 28880–28889.
[27] N. Rimsueb, S. Cherdchom, V. Aksornkitti, T. Khotavivattana, A. Sereemaspun, R. Rojanathanes, Feeding cells with a novel "trojan" carrier: citrate nanoparticles, ACS Omega 5 (13) (2020) 7418–7423.
[28] N. Pahlavani, E. Nattagh-Eshtivani, A. Amanollahi, G. Ranjbar, H.A. Aghdaei, J. G. Navashenaq, Z. Shabaninezhad, N.R. Sharahi, M. Maleki, M. Malekahmadi, Effects of microwave technology on the subcutaneous abdominal fat and anthropometric indices of overweight adults: a clinical trial, J. Cosmet. Dermatol. 21 (4) (2022) 1482–1488.
[29] G. Derosa, A. D'Angelo, D. Romano, P. Maffioli, A clinical trial about a food supplement containing α-lipoic acid on oxidative stress markers in type 2 diabetic patients, Int. J. Mol. Sci. 17 (11) (2016) 1802.
[30] N. Li, W. Yan, X. Hu, Y. Huang, F. Wang, W. Zhang, Q. Wang, X. Wang, K. Sun, Effects of oral α-lipoic acid administration on body weight in overweight or obese subjects: a crossover randomized, double-blind, placebo-controlled trial, Clin. Endocrinol. 86 (5) (2017) 680–687.
[31] G. Bobe, A.J. Michels, W.-J. Zhang, J.Q. Purnell, C. Woffendin, C. Pereira, J.A. Vita, N.O. Thomas, M.G. Traber, B. Frei, A randomized controlled trial of long-term (R)-α-lipoic acid supplementation promotes weight loss in overweight or obese adults without altering baseline elevated plasma triglyceride concentrations, J. Nutr. 150 (9) (2020) 2336–2345.
[32] E.H. Koh, W.J. Lee, S.A. Lee, E.H. Kim, E.H. Cho, E. Jeong, D.W. Kim, M.-S. Kim, J.-Y. Park, K.-G. Park, Effects of alpha-lipoic acid on body weight in obese subjects, Am. J. Med. 124 (1) (2011) 85, e1.
[33] S. Park, U. Karunakaran, N. Ho Jeoung, J.-H. Jeon, I.-K. Lee, Physiological effect and therapeutic application of alpha lipoic acid, Curr. Med. Chem. 21 (32) (2014) 3636–3645.
[34] L. Rochette, S. Ghibu, A. Muresan, C. Vergely, Alpha-lipoic acid: molecular mechanisms and therapeutic potential in diabetes, Can. J. Physiol. Pharmacol. 93 (12) (2015) 1021–1027.
[35] N. Namazi, B. Larijani, L. Azadbakht, Alpha-lipoic acid supplement in obesity treatment: a systematic review and meta-analysis of clinical trials, Clin. Nutr. 37 (2) (2018) 419–428.
[36] A.K. Tripathi, A.K. Ray, S.K. Mishra, S.M. Bishen, H. Mishra, A. Khurana, Molecular and therapeutic insights of alpha-lipoic acid as a potential molecule for disease prevention, Revista Brasileira de Farmacognosia 33 (2) (2023) 272–287.
[37] C. Bellini, F. Mancin, E. Papini, R. Tavano, Nanotechnological approaches to enhance the potential of α-lipoic acid for application in the clinic, Antioxidants 13 (6) (2024) 706.
[38] H. Lambers, S. Piessens, A. Bloem, H. Pronk, P. Finkel, Natural skin surface pH is on average below 5, which is beneficial for its resident flora, Int. J. Cosmet. Sci. 28 (5) (2006) 359–370.