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Re: ''Annurca Apple Nutraceutical Formulation Enhances Keratin Expression in a Human Model of Skin and Promotes Hair Growth and Tropism in a Randomized Clinical Trial'' by Tenore *et al.*

(*J Med Food* 2018;21:90–103)

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Dear Editor,

We read with interest the article by Tenore *et al.*,[1] which includes a report on the results of a preclinical study of the bioactivity of Annurca apple polyphenolic extract (AMS) and a report on the results of a clinical trial of AMS supplementation in patients. The article is misleading about its clinical trial design, a design that, as described by the investigators, raises concerns over the validity of the results as the study and results do not appear to support the conclusion that AMS can effectively increase hair density, weight, and keratin content.

The greatest concerns stem from the following: (1) the lack of randomization between active treatment and placebo control conditions, and (2) the lack of a true double blind on placebo control and active AMS observations.

Recruited patients appear to have been randomized, but not between placebo control and active AMS supplement treatment groups. Rather, patients were randomized to one of the two active AMS treatment groups (*i.e.*, receiving either an AMS or an AMSbzs formulation of the study treatment). After randomization, each patient received placebo treatment for 4 weeks and then received the assigned active supplement formulation for 8 weeks. No rationale was provided for this study design, which contradicts the investigators' assertion in the methods that this was a ''placebo-controlled trial.'' The investigators go on to present as ''Effects of Annurca Apple Supplements on Clinical Parameters,'' in Table 2 of their article, questionable results that appear to have confounded treatment effect estimates by including the placebo period data in the effect estimates. That is, the percent changes from ''t 0'' at ''t 30'' and ''t 60'' appear to correspond to changes from baseline (according to values presented in Table 1, the Table 2 ''t 0'' values are from the baseline visit on all 250 patients randomized before the placebo treatment, not at the beginning of supplement treatment after placebo treatment) after both 1 month of placebo treatment *and* 1 month of active supplement treatment and after 2 months of active supplement treatment, respectively.

Regardless, even if the placebo period data were not in the effect estimates, by the design described by the investigators, there were no observations made under the placebo condition that could have adequately represented the potential outcomes of the patients receiving the active treatment. As such, it would have been impossible to distinguish what proportion of any observed changes in the study's primary and secondary clinical endpoints were caused by either active treatment's effects and which were caused by the placebo's effect. Since the investigators did not analyze results between the randomized groups in the trial, their study design amounts to what Campbell and Stanley referred to as ''The One-Group Pretest-Posttest Design'' and introduced it as a ''bad example'' having its internal validity jeopardized by confounding and only ''worth doing where nothing better can be done.''[2]

There is good reason to be concerned about confounding in the article's results. Human hair growth undergoes seasonal changes and the investigators were not clear on what time of the year study patients were on their placebo and then on their active supplement treatment. They noted that, ''It has also been reported that the anagen ratio undergoes seasonal changes: it rises to a maximum in March and falls to a minimum in September.'' The article they

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cited on this matter specifically found that, ''In the scalp the proportion of follicles in anagen reached a single peak of over 90% in March, and fell steadily to a trough in September. The number of shed hairs reached a peak around August/September, when least follicles were in anagen. At this time the average loss of hairs was about 60 per day, more than double that during the preceding winter.''[3]

This observation suggests that, since patient recruitment occurred in November and the ''clinical test was performed from January to July,'' some of the changes in the clinical endpoints summarized in Table 2, specifically those observed during the placebo course finished by study patients in January or earlier and active supplement treatment course finished in March or earlier, would likely have been confounded by and actually attributable to seasonal changes in human hair growth. It is not clear from the article what proportion of the study's results were subject to this source of bias.

No efforts appear to have been made to adjust the estimates or inferences in the study for this seasonal bias, nor does it appear advisable, given the narrow calendar window in which the study was conducted. Importantly, the concern about seasonal effects is only one example of a potential confound from the universe of specifiable and unspecifiable confounds and, even if it could be dismissed, the design would still be inadequate to support conclusions.

It follows from the nonconcurrent placebo control design that the study investigators would be aware that the patients were being supplied with placebo first and then active treatment. Therefore, it does not seem plausible that there could be a true double blind in the management and treatment of this study's patients.

We cannot know exactly what impacts these two important sources of bias may have had on the study's results. Although there are also numerous other concerns regarding the clinical trial, it is clear that, by its design alone, the trial is inappropriate for establishing causal effects of AMS supplementation and the published article is inaccurate and misleading in its conclusions.

DISCLAIMER

The opinions expressed are those of the authors and do not necessarily represent those of the NIH or any other organization.

AUTHOR DISCLOSURE STATEMENT

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