Simulated Digestion of the Pigmented Legumes’ (Black Chickpea (*Cicer arietinum* L.) and Brown Lentil (*Lens culinaris* Medikus) Phenolics to Estimate Their Bioavailability

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Abstract

This study simulated the gastrointestinal digestion (GID) of black chickpeas (BC) and brown lentils (BL). BC phenolics increased from 105.01 to 141.86 mg GAE/100 g DW while the BL phenolics decreased from 143.26 to 132.70 mg GAE/100 g DW after cooking. In contrast, the remaining flavonoids after cooking were higher in BL (325.55 mg RE/100 g DW). After *in vitro* GID, moderate levels of flavonoids were detected in the colon (OUT) fractions (144.36 and 104.22 mg RE/100 g DW for cooked BC and BL, respectively). The highest TAA levels were detected as by CUPRAC assay, in cooked and *in vitro* GID BC (517.03 mg TEAC/100 g DW) and BL (604.98 mg TEAC/100 g DW) samples. Catechin was the most abundant compound detected in BC samples, while gallic acid was the most abundant in BL. BC and BL have unique and superior benefits for health when compared with conventional legumes. The possible interactions between their remaining phenolics and other bioactive components in the colon are promising for their widespread consumption.

Keywords Black chickpea · Brown lentil · Bioavailability · Legumes

Introduction

Legumes are among the most important food groups in the world, with their rich nutrient profile and low price. Although animal proteins are still the main protein source for most of the world’s population, changes in consumers’ nutrition trends have led to the pursuit of new sources/alternative proteins for human and animal consumption. Legumes have received considerable critical attention as they are significant dietary plant protein sources with high-quality proteins and peptides, in addition to well-balanced essential amino acids. Moreover, legume flours are becoming popular as gluten-free alternatives. Their total dietary fiber, resistant starch, vitamins, minerals and bioactive components are important contributors to their benefits in controlling and preventing various metabolic diseases, including diabetes [1]. Numerous studies have revealed that the consumption of legumes is effective to decrease levels of chronic diseases (obesity, coronary heart disease, type 2 diabetes mellitus, cardiovascular diseases, and cancers, as well as to address aging and gut health) in addition to their effects against the inflammation, LDL-cholesterol oxidation and DNA strand break [2–5]. Among their bioactive components, phenolic compounds are significant contributors to the positive health benefits of legumes, with their anti-allergenic, anti-atherogenic, antimicrobial, antioxidant, anti-thrombotic, cardioprotective, and vasodilatory effects. In pigmented legumes, phenolics also contribute to the seed color and sensory characteristics [6].

Chickpeas are the third most cultivated pulse after dry beans and peas. According to the FAO, total chickpea production was 15 million tons in 2020, while total lentil production was more than 6.5 million tons [7]. The global legume market was valued at about 45 billion US dollars in 2017, and it is projected to reach 75.8 billion US dollars by 2025 [8]. The coat color and size of the seed divide lentils into two main groups: 1) green lentils and 2) red lentils. Brown lentils (*Lens culinaris* Medikus) are smaller and more circular in comparison to green lentils. Lentil carbohydrates
have beneficial health effects, such as controlling and preventing diabetes mellitus, coronary heart disease, and cancer [9]. The primary polyphenolic compounds in lentil seed coats are tannins, phenolic acids (mainly ferulic acid), and flavonoids such as flavonol glycosides, anthocyanin, and tannins [1, 10]. Chickpeas are generally grouped into two types, desi and kabuli, and black chickpeas belong to the desi type [11]. Although their unique nutritional value has been attributed to their proteins and active peptides, chickpeas also have a variety of bioactive compounds, such as flavonoids of formononetin, biochanin A, and their corresponding glycosides and various biological activities (antioxidant, antihypertensive, hypocholesterolemic, and anticancer) [12–14]. Darker seed coat color in legumes generally correlates with different flavonoids of flavonol glycosides, anthocyanins, and tannins [1, 10]. Bioavailability generally refers to the extent of absorption and transportation of nutrients to body tissues. In that sense, it is considered one of the major factors in the health effect evaluation of foods. Cooking is required for legume consumption; most legumes are consumed only after cooking. Although some knowledge has been acquired about the phenolic profiles of different legumes and the effect of cooking/heat treatments on the bioaccessibility of polyphenols in some common varieties [6, 10, 13, 15], studies on the bioavailability of pigmented variants remain very limited [16–18]. Therefore, this study aimed to determine the effects of simulated in vitro digestion on the phenolic acids, flavonoids, and antioxidant activity of pigmented and relatively uncommon legume types, specifically the black chickpea (Cicer arietinum L.) and brown lentil (Lens culinaris Medikus), and to elucidate their health benefits.

Materials and Methods

This section is described in the Supplementary Materials.

Results and Discussion

Total Phenolic Content (TPC)

The TPC values of black chickpea and brown lentil samples are given in Table S1. TPC in bound fractions was higher in BC and BL samples. BC samples had a TPC value of 105.01 mg GAE/100 mg DW. Cooking increased the TPC (35.1%) in all fractions ($p > 0.05$). For BL samples, the TPC was 143.26 mg GAE/100 mg DW. TPC decreased by about 7.4% after cooking and this was thought to be related to the slight decrease in phenolics, especially in the bound fraction (68.83 mg GAE/100 mg DW). Steam cooking caused no significant changes on the TPC in the free, bound, and total fractions of both samples ($p > 0.05$). Similar TPC values were found in studies by Heiras-Palazuelos et al. [13] and Fratianni et al. [19], although the TPC of different green lentil cultivars were found as higher than the current findings by another group [20]. Desi varieties have been reported to contain a relatively higher content of TPC than kabuli types due to the darker seed coat and smaller seed size [11, 13]. Similar to the reported findings from the literature [21], raw lentils have slightly higher TPC compared to raw chickpeas. Domestic cooking has been reported to decrease the TPC of chickpeas [22, 23] and lentils [24] significantly. In contrast, other studies have reported slight or no decrease in the TPC of chickpeas after steaming [11, 25], or significant increases in TPC of fava beans, soybeans, lentils, and peas after cooking [21] and in some colored bean varieties [16]. There may be two main factors affecting the extent of phenolic compound loss or increase in legumes: 1) the type of legume and 2) the two-sided effect of cooking, which either decreases the amount of heat-sensitive phenolic components or releases some complex/bound phenolics [17, 21]. Other reports have emphasized the simultaneous occurrence of partial free phenolics released by the effect of thermal degradation, and an increase in bound fractions due to the interactions of phenolic acids with macromolecules in the food matrix during cooking and cell disruptions [21].

Total Flavonoid Content (TFC)

TFC values are given in Table S1. TFC in bound fractions for BC and BL samples were slightly higher than in free fractions. Before cooking, BC samples had higher TFC than BL samples. Steam cooking decreased TFC in all BC and BL fractions. The most dramatic decreases were measured in the bound BC fraction (55%) and accordingly in the TFC of BC (72.9%), which was statistically significant ($p < 0.05$). In the BL samples, a similar trend was evident (57% decrease in TFC), since steam cooking decreased the TFC both in the free (39.7%) and bound (17.3%) fractions ($p > 0.05$). In the literature, significant differences in TFC have been attributed to the different extraction methodologies and genotypes [20]. According to the findings by Heiras-Palazuelos et al. [13], the TFC was similar in free fractions and slightly higher in bound fractions of different desi types of chickpeas. In contrast, TFC in brown chickpeas was reported as being significantly lower in a study by Parikh et al. [10]. It has been reported that desi chickpeas have more TFC than kabuli types, due to the presence of glycosides of luteolin, myricetin, and quercetin [6, 22]. In another study, cooking caused an average 60% decrease in TFC among six different lentil varieties, similar to our findings [24].
Total Antioxidant Activity (TAA)

TAA results measured by three different methods are given in Table S2. For BC samples before cooking, TAA in the bound fractions (65.2–89.0%) were higher than in the free fractions. This trend was also evident for bound fractions of BL samples (72.5–73.6%). CUPRAC assay gave the highest TAA among all three assays. Steam cooking increased TAA in the free fraction for both BC (p < 0.05) and BL samples (p > 0.05). In contrast, TAA in bound fractions and the sum of the two fractions (free and total) were the similar for CUPRAC and DPPH assays, but not for ABTS assay. According to these methods, the TAA in bound fractions decreased by about 12.5–29.7% after cooking. The TAA found by DPPH and CUPRAC assays correlated positively and significantly (p < 0.01) with TPC results (0.856 and 0.648, respectively) and also with each other (0.793) (p < 0.01). According to the literature, 20 lentil cultivars have a higher TAA when detected by DPPH assay, which might be related to the cultivars and methodology [20]. Colored chickpea lines have been reported to have higher antioxidant activity than the kabuli type [11].

Changes During in vitro Gastrointestinal Digestion (GID)

Effect of In vitro Digestion on TPC

In vitro GID was applied directly to raw and cooked samples at each fraction: PG, IN, OUT. The methanolic extracts of raw samples for total free and bound forms were accepted as 100%. The changes in TPC of BC and BL samples after in vitro GID are summarized in Table 1. In vitro GID was applied directly to BC and BL samples (both raw and cooked) and TPC was measured. The TPC of in vitro GID changed between 51.43 and 295.60 mg GAE/100 mg DW. The highest TPC was measured for the colon fraction (OUT) of the BL raw sample (295.60 mg GAE/100 mg DW). For all samples, TPC increased in the PG, ranging from 140.1 to 195.5%. The highest increase was measured for the cooked BC-Raw and BL-Cooked (around twofold increases) samples. The increase in TPC after gastric digestion was in agreement with the findings in other studies, and might be explained by the breakdown of chemical bonds, the activity of digestive enzymes, and the consequent release of phenolics [26]. These findings were in parallel with previous studies on the pigmented bean varieties of Mexico [16] and six different unpigmented pulses [21]. After colon digestion, the TPC values of the samples declined to either half or one-third of the initial values (p < 0.05), except for the BL-Raw sample (maintained TPC as in the PG fraction). The decreased amounts of TPC in the IN fraction (absorption in the small intestine) were similar to that reported in the

Table 1 The effect of in vitro digestion on total phenolic (TPC) and flavonoid contents (TFC)

| TPC (mg GAE/100 mg DW) | BC-Raw | BC-Steamed Cooked | BL-Raw | BL-Steamed Cooked |
|------------------------|--------|-------------------|--------|-------------------|
| Initial                | 105.01 ± 17.43b (100%) | 141.86 ± 9.98b (100%) | 143.26 ± 19.82b (100%) | 132.70 ± 19.12b (100%) |
| PG                     | 214.76 ± 16.58a (204.4%) | 198.74 ± 7.14a (140.1%) | 223.52 ± 56.31b (156.0%) | 259.44 ± 11.40a (195.5%) |
| IN                     | 52.77 ± 2.53c (50.3%) | 51.43 ± 6.99c (36.3%) | 229.03 ± 14.70b (159.9%) | 58.09 ± 10.19c (32.3%) |
| OUT                    | 217.92 ± 24.87a (207.5%) | 196.96 ± 25.75a (138.8%) | 295.60 ± 18.60a (206.3%) | 221.95 ± 6.99a (167.3%) |
| TFC (mg RE/100 g DW)   | BC-Raw | BC-Steamed Cooked | BL-Raw | BL-Steamed Cooked |
| Initial                | 1342.76 ± 187.56c (100%) | 364.55 ± 36.69a (100%) | 1074.29 ± 238.29a (100%) | 325.55 ± 71.94a (100%) |
| PG                     | 71.38 ± 5.53c (5.3%) | 154.12 ± 45.94b (42.3%) | 36.62 ± 6.25c (3.4%) | 12.69 ± 18.89c (3.9%) |
| IN                     | 316.50 ± 12.76b (23.6%) | 2.28 ± 1.29b (0.6%) | 16.55 ± 0.52c (1.6%) | 6.63 ± 3.78c (2.0%) |
| OUT                    | 100.35 ± 8.11a (7.5%) | 144.36 ± 32.75b (39.6%) | 138.70 ± 37.87b (12.9%) | 104.22 ± 22.02b (32.0%) |

Values are the means of triplicate measurements ± standard deviations. Different letters in the same column represent significant differences at p < 0.05. BC Black chickpeas; BL Brown lentils.

The samples in the initial conditions were accepted as (100%) and changes are given accordingly.
The alkaline conditions of the small intestine and bile salt secretions may have caused changes in chemical structures, and their degradation or formation of new compounds [26]. Compared to initial values, increases in OUT fractions were observed for all samples ($p < 0.05$). This increase may be explained by the limited bioavailability in the serum (IN) to make these phenolics available for microbial metabolism in the large intestine (OUT), exerting beneficial effects on gut and systemic health through modulation of gut microbiota metabolism [27]. The fibers from beans and lentils were reported to contain associated hydroxybenzoic and hydroxycinnamonic compounds, flavan-3-ols, procyanidins, flavonols, and flavones [28]. Therefore, interactions of phenolic compounds with indigestible polysaccharides may lead to the formation of hydrogen and hydrophobic linkages, decreasing bioaccessibility after intestinal digestion by restricting the diffusion of the enzymes to their substrates as an entrapping matrix [29]. Therefore these commodities may only be absorbed after the activity of microorganisms in the intestinal lumen [21, 30]. In contrast, Zhang et al. [3] depicted that, although TPC after in vitro gastric digestion decreased (22%), the intestineally digested TPC increased to 51% in cooked Canadian green lentils (cultivar Greenland). However, they did not mention the fraction remaining in the GI tract [3]. According to Lafarga et al. [21], the TPC was significantly higher after the gastric and intestinal phases of digestion of white chickpea and green lentil samples when compared to the initial stage. Therefore, the bioavailability of colored lentil and chickpea varieties may be considered quite different than that of common pulses.

**Effect of in vitro Digestion on TFC**

The effect of in vitro GID on the TFC is given in Table 1. For all samples, TFC decreased significantly after gastric digestion ($p < 0.05$). The drop during gastric digestion (PG) was relatively limited in the BC cooked sample (57.7%) when compared with the remaining raw and cooked samples (around 3.4–5.3% of the initial TFC). Therefore, cooking significantly preserved the TFC during gastric digestion in BC (around 40%), but not in BL. However, during intestinal digestion, no such effect was detected, since the TFC remaining in all samples changed around 0.6–2.0% of the initial levels. In the colon fraction (OUT), the TFC was better retained in both the cooked BC and BL samples (32–39.6%) when compared to their uncooked counterparts (7.5–9.6%). In the literature, green lentils have a contradictory trend. According to the findings of Zhang et al. [20] the bioavailable TFC after in vitro gastric digestion was 31% of the initial value, although intestinal digestion increased TFC bioavailability to 67% [20]. However, another study revealed that the TFC of Pinto beans after GID was around 1.26 to 4.39%, similar to the current results except for the BC raw samples (23.6%) [15]. The low amount of in vitro bioavailability during digestion (oral, stomach, and small intestine digestion), in contrast to the higher levels detected in the large intestine (OUT) by microbial metabolism, was in agreement with the findings of a previous study on three different legumes [31].

**Effect of in Vitro Digestion on TAA**

TAA was measured for each fraction of GID (PG, IN, OUT). The initial extracts were accepted as 100% and the results of the three different assays are given in Table 2. TAA during GID was between 2.33 and 927.38 mg TEAC/100 g DW, with CUPRAC results being the highest among the three methods. Gastric digestion (PG) significantly decreased the TAA content in all samples (cooked and raw) ($p < 0.05$). The most dramatic decreases were found by the ABTS assay. A general evaluation of the changes during in vitro GID revealed that the TAA decrease in PG was opposite to that of TPC but similar to TFC. Among the PG results, cooking was protective for the TAA of both BC and BL samples according to the DPPH assay (around 20% higher TAA in cooked BC and BL than in the raw counterparts). The other assays depicted no significant effects of cooking during gastric digestion. This might be related to the sensitivity of the DPPH assay to the acidic conditions of gastric digestion [32]. According to ABTS and DPPH assays, further decreases ($p < 0.05$) were detected in the serum (IN) of BC and BL samples, with no significant differences due to cooking. However, according to the CUPRAC assay, significant increases ($p < 0.05$) in the TAA of both the cooked BC (to 36.6%) and BL (to 74.2%) were observed, although significant decreases were evident in the uncooked samples (to 4.7 and 8.8%, respectively, for BC and BL). The CUPRAC assay, working at a pH of 7, might be better for a more sensitive evaluation of intestinal digestion [29]. Therefore, TAA increases due to digestion in the cooked BC and BL samples were better detected. The OUT results by CUPRAC assay also supported this finding, since the TAA was higher in the uncooked samples. The other two assays were not effective or sensitive enough to identify significant differences among the PG and OUT fractions. Akillioglu and Karakaya [15] revealed a significant drop in the TAA after in vitro digestion of Pinto beans by DPPH assay, and related the findings to the insoluble indigestible fractions such as proanthocyanidins that are fermented in the colon [15]. In contrast, Zhu et al. [31] reported that different bound phenolics were extremely low after in vitro gastric digestion, and were released during colonic fermentation, according to the FRAP assay. Moreover, they highlighted significant correlations with TFC and TAA, rather than TPC. Therefore, our findings showed that some flavonoids that are found in pigmented BC and BL might become more bioavailable through cooking.
increasing TAA in the OUT fraction. However, this activity was evident only with the FRAP assay, so it is important to emphasize the use of different TAA assays during studies.

**Major Phenolic Compounds**

Five main phenolic compounds were identified for BC (gallic acid, catechin, rutin, kaempherol, and quercetin dihydrate) and six for BL (gallic acid, catechin, epicatechin, coumaric acid, rutin, and quercetin dihydrate). They are shown in Table S3. Catechin was the most abundant compound detected in the BC samples. The highest amount was measured in the BC Cooked-OUT fraction at 579.80 mg/100 g. Cooking decreased the catechin content of the free fraction, while liberating the bound form. Although gastric digestion caused further increases to a total of 348.27 mg/100 g, no catechin was detected at the end of GID. Catechin (a member of the flavan-3-ols) has been reported to have a low GID bioavailability. However, in the colon (OUT) it was measured as 66% more than in the cooked sample [33]. Gallic acid was also high; cooking almost doubled the gallic acid in both the free and bound fractions, although with restricted in vitro bioavailability after GID. In the colon (OUT) it was measured at around 30% of the total cooked fragment. Rutin was relatively bioavailable, although its total amount was lower. Gallic acid was the most abundant phenolic compound (431.37 mg/100 g) in the BL-Cooked Bound samples. However, only a limited amount (around 4%) was bioavailable after GID, while it was around 30.8% in the colon (OUT). Catechin became measurable in BL after cooking and had one-fourth the bioavailability after in vitro GID, but it was also detected in BL-Cooked OUT (210 mg/100 g). Although their contents were lower, epicatechin and coumaric acid were found to be bioavailable after in vitro GID, with as much as 64 and 39% of the total cooked BL, respectively. A poor selectivity of the HPLC method for gallic acid and catechin has been noted in a previous study, correlated with the polar substances coming from the enzymes and/or the simulated digestion fluids used in the simulated digestion model affecting the bioavailability of other phenolics [17]. Moreover, different pH levels during GID have been reported to cause changes in the structures of the polyphenols such as catechin, affecting their bioavailability [34]. Hydroxybenzoics, hydroxycinnamicas, flavonols, flavones, and flavanones, alongside isoflavones, have been detected as the main phenolic compounds in two chickpea varieties [22]. The major common phenolic acids in chickpea samples of five kabuli and one desi chickpea types were p-hydroxybenzoic and gentisic acids, and the desi also contained glycosides of luteolin, myricetin, and quercetin [6]. Rutin, quercetin, and

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**Table 2** Effect of in vitro digestion on total antioxidant activity (TAA)

|                      | BC-Raw                  | BC-Steam Cooked          | BL-Raw                  | BL-Steam Cooked          |
|----------------------|-------------------------|--------------------------|-------------------------|--------------------------|
| ABTS (mg TEAC/100 g DW) | 172.80 ± 6.84a (100%)   | 204.99 ± 19.29a (100%)  | 209.71 ± 6.81a (100%)  | 230.37 ± 11.14a (100%)  |
| PG                   | 23.58 ± 0.16b (13.6%)   | 16.32 ± 3.05b (8.0%)    | 29.10 ± 1.42b (13.9%)  | 26.48 ± 1.18b (11.5%)   |
| IN                   | 11.09 ± 0.39c (6.4%)    | 10.53 ± 0.03c (5.1%)    | 10.32 ± 0.03c (4.9%)   | 10.14 ± 0.45c (4.4%)    |
| OUT                  | ND                      | ND                       | ND                      | ND                       |
| CUPRAC (mg TEAC/100 g DW) | ND                      | ND                       | ND                      | ND                       |
| Initial              | 1559.27 ± 39.20a (100%) | 1413.02 ± 78.28a (100%) | 939.81 ± 60.57a (100%) | 815.57 ± 15.01a (100%) |
| PG                   | 372.37 ± 63.49c (23.9%) | 328.34 ± 31.87c (23.2%) | 271.06 ± 11.85c (28.8%)| 247.29 ± 7.02c (30.3%) |
| IN                   | 73.54 ± 1.98d (4.7%)    | 517.03 ± 68.00b (36.6%) | 82.63 ± 3.47d (8.8%)   | 604.98 ± 13.91b (74.2%) |
| OUT                  | 927.38 ± 51.01b (59.5%) | 138.93 ± 5.34d (9.7%)   | 711.54 ± 41.60b (75.7%)| 108.26 ± 15.10d (13.3%)|
| DPPH (mg TEAC/100 g DW) | BC-Raw                  | BC-Steam Cooked          | BL-Raw                  | BL-Steam Cooked          |
| Initial              | 65.29 ± 1.89a (100%)    | 49.38 ± 4.84a (100%)    | 73.68 ± 4.49a (100%)   | 61.67 ± 17.77a (100%)   |
| PG                   | 12.92 ± 1.42b (19.8%)   | 19.59 ± 1.51b (39.7%)   | 13.32 ± 0.16b (18.1%)  | 18.21 ± 1.63b (29.5%)   |
| IN                   | 3.33 ± 0.03c (5.1%)     | 5.22 ± 0.27c (10.6%)    | 2.33 ± 0.39c (3.2%)    | 4.52 ± 0.26c (7.3%)     |
| OUT                  | 4.40 ± 0.39c (6.7%)     | 3.98 ± 0.91c (8.0%)     | 7.75 ± 0.57c (10.5%)   | 5.89 ± 0.75c (9.6%)     |

*Values are the means of triplicate measurements ± standard deviations. Different letters in the same column represent significant differences at p < 0.05. PG, post gastric fraction leaving the stomach; IN, fraction entering the serum (dialyzable fraction); OUT, fraction remaining in the GI tract (undialyzable fraction). BC Black chickpeas, BL Brown lentils. The samples in the initial conditions were accepted as (100%) and changes are given accordingly.
delphinidin 3-glucoside have been detected only in the soaking water of black chickpeas, and similar to our findings, gallic acid was predominant, in addition to 4-hydroxybenzoic acid, syringic acid, delphinidin 3-glucoside, rutin, quercetin, and kaempferol 3-glucoside [17]. The destruction of the structural integrity of the vegetal tissue during cooking might have caused the measured increase following cooking [21]. Among 20 different lentil cultivars, kaempferol glycosides dominated the phenolic profile, followed by some catechin derivatives (catechin glucoside, catechin gallate, epicatechin glucoside) [20]. In a typical green lentil variety, p-hydroxy-benzoic acid, syringic acid, trans-p-coumaric acid, epicatechin gallate, quercetin-3-xylloside, quercetin-3-glucoside, and kaempferol-3-glucoside were the main phenolic [2].

Conclusion
This study demonstrated the simulated digestion of the pigmented and relatively uncommon legumes of black chickpeas (Cicer ariei tinum L.) and brown lentils (Lens culinaris Medikus). These legumes may play a key role in improving colon health, particularly via their flavonoids. Black chickpeas and brown lentils should be more commonly consumed for their health, particularly via their flavonoids. Black chickpeas and brown lentils were the main phenolic [2].

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Author Contributions D.N.E. created the study concept. B.E.K conducted the analyses. Z.T.C. helped with the analyses and methodology. Z.T.C. and B.E.K wrote the main manuscript text, and Z.T.C. prepared the tables. All authors reviewed the manuscript.

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Data Availability The datasets generated during and/or analyzed during the current study are available from the corresponding author on reasonable request.

Declarations

Ethics Approval Not applicable.

Consent to Participate Not applicable.

Consent for Publication Not applicable.

Conflict of Interest The authors state that there is no conflict of interest with respect to the objective, interpretation, and presentation of the results in this study.

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