Twenty-four-hour plasma tryptophan concentrations and ratios are below normal in obese subjects and are not normalized by substantial weight reduction1–3

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ABSTRACT
Background: Plasma tryptophan concentrations and the ratio of tryptophan to other large neutral amino acids (plasma tryptophan ratio) are reportedly low in obese subjects. The plasma tryptophan ratio predicts brain tryptophan uptake and serotonin production. If this ratio is low in obese subjects, serotonin function may also be low. Plasma tryptophan concentrations and ratios have been measured only at single time points in obese subjects; it is not known whether low values for these 2 variables persist throughout a 24-h period.

Objective: Our objective was to determine whether plasma tryptophan concentrations and ratios in obese subjects are lower than those in normal-weight subjects throughout a 24-h period and whether they increase when body weight is reduced.

Design: Plasma tryptophan concentrations and ratios were examined in obese subjects before and after weight loss and in nonobese control subjects. Blood samples were drawn frequently throughout the 24-h period. An insulin tolerance test was also used to determine whether weight loss altered the ability of insulin to modify plasma concentrations of tryptophan and of the other large neutral amino acids.

Results: Plasma tryptophan concentrations and ratios in obese subjects were low at all times; these effects persisted after weight reduction. Plasma concentrations of all the large neutral amino acids decreased during insulin infusion in all the groups.

Conclusions: The low 24-h plasma tryptophan ratios in obese and formerly obese subjects suggest that brain tryptophan uptake may be continuously diminished and may remain below normal despite weight reduction. Am J Clin Nutr 2003;77:1112–8.

KEY WORDS Obesity, weight loss, plasma tryptophan, large neutral amino acids, serotonin, very-low-energy diet, insulin

INTRODUCTION

Serotonin neurons in the brain participate in the control of appetite. In general, serotonin neurons function in neuronal circuits that diminish food intake (1–4). Hence, treatments that enhance serotonin function reduce food intake, whereas those that diminish serotonin function stimulate food intake (5, 6). The synthesis of serotonin in the brain is controlled in part by the availability of its amino acid precursor, L-tryptophan. Tryptophan pools in the brain, in turn, are influenced by the uptake of tryptophan from the circulation. This uptake process depends on a competitive, saturable transport carrier shared by tryptophan and several other large neutral amino acids (LNAAs), principally tyrosine, phenylalanine, and the branched-chain amino acids (BCAAs) leucine, isoleucine, and valine (7, 8). Because of competitive transport, brain tryptophan uptake and, ultimately, serotonin synthesis are influenced by plasma concentrations not only of tryptophan but also of the other LNAAs. The plasma ratio of the concentration of tryptophan to the sum of the concentrations of the other LNAAs (tyrosine + phenylalanine + leucine + isoleucine + valine) (the plasma tryptophan ratio), which summarizes this competitive relation, has been proven to be a useful and reliable predictor of brain tryptophan uptake and central serotonin synthesis (9, 10).

Plasma tryptophan ratios are below normal in obese subjects (11, 12) and may decrease with dieting (13, 14), an effect that may partly be responsible for the high relapse rate after diet-related weight loss (15) (ie, brain serotonin production remains low and stimulates appetite; 1). Obese subjects are often insulin resistant, and diminished insulin action may cause low plasma tryptophan ratios (11, 16) because of the peripheral effects of insulin on amino acid uptake and utilization (17, 18).

Currently, however, only a single, short report indicates that plasma tryptophan ratios remain below normal after weight reduction (13). Furthermore, the connection of diminished insulin action to a low plasma tryptophan ratio in obesity is a controversial subject (19–21). In addition, it is not known how weight reduction may influence the amino acid effects of insulin in relation to changes in the plasma tryptophan ratio. Therefore, we conducted a study in obese subjects who were undergoing marked weight reduction via consumption of a very-low-energy diet to determine 1) whether plasma tryptophan...
ratios are lower in obese persons than in normal-weight persons, 2) whether plasma tryptophan ratios remain low after weight reduction, and 3) whether insulin-induced changes in plasma amino acid concentrations account for changes in plasma tryptophan ratios before and during weight reduction. In this study, comparisons were made in obese subjects before and after weight reduction; comparisons were also made between obese subjects and a group of normal-weight subjects studied at the same time. In addition, to document the extent of any changes in plasma tryptophan ratios, plasma amino acid concentrations were measured at 2-h intervals over a 24-h period.

SUBJECTS AND METHODS

Subjects

The original study group consisted of 18 obese patients (2 men, 16 women) and 18 sex- and aged-matched nonobese subjects (22). The 9 obese patients that completed the weight-loss program and their age- and sex-matched nonobese counterparts formed the groups examined in this study. All the subjects were nondiabetic and healthy. The women were studied during midfollicular phase of the menstrual cycle. All the subjects gave informed consent. The revised Helsinki 2 Declaration was observed, and the study was approved by the Copenhagen Municipal Ethical Committee.

Weight-loss program

The obese subjects participated in a structured, outpatient weight-loss program. The goal of the program was to achieve ideal body weight; the time interval needed to achieve the goal was tailored to each subject and differed between subjects. During the initial phase of the program, the subjects consumed a commercial, very-low-energy liquid diet (Oluf Mørk Biochemie Ltd, Copenhagen) that provided 1.6 MJ energy/d and contained the following nutrients (in mg/d): protein, 56 400; lipid, 4800; carbohydrate, 29 700; calcium, 800; phosphorus, 400; iron, 18; zinc, 15; copper, 3; iodine, 0.15; manganese, 3.8; chromium, 0.12; selenium, 0.12; molybdenum, 0.12; vitamin A, 1.0; vitamin D₃, 0.01; vitamin E, 10; vitamin K, 0.14; thiamine, 1.5; riboflavin, 1.7; vitamin B-6, 2.2; vitamin B-12, 0.003; biotin, 0.2; niacin, 19; vitamin C, 60; folic acid (a monoglutamyl form of folate), 0.1; pantothenic acid, 7. The patients visited the outpatient clinic weekly for body weight measurements and nutrition counseling. When body weight had decreased to ≈130% of ideal body weight (6–17 mo), the patients were instructed to discontinue the liquid diet and begin consuming a 5.0 MJ/d diet of normal food items; in doing so, the patients used the diet and nutrition materials (including food exchange tables) that had been supplied to them and discussed during the nutrition counseling sessions (23). When ideal body weight was achieved or when no further weight could be lost, the patients were instructed to begin consuming a basic, 8-MJ/d diet (15% of energy from protein, 55% from carbohydrate, and 30% from fat), which was individually adjusted for each patient to obtain energy balance. Energy intake was further adjusted down or up for each subject on a weekly basis (during weekly dietary counseling sessions) to reach a final, stable body weight (eg, if body weight began to increase, daily energy intake was reduced). Sampling in the postobese patients was performed after body weight had remained stable (±1.5 kg) for ≥1 mo after switching to the last diet program.

Experimental design

For the obese subjects, the design included 2 blood-sampling periods. First, before the weight-loss phase of the study, we conducted a 24-h sampling period, which was followed the next day by a 2-h insulin sensitivity test. Second, after the weight-loss period, the 24-h sampling period was repeated and was again followed the next day by a 2-h insulin sensitivity test. The normal-weight control subjects underwent the same procedures but were studied on only one occasion.

Twenty-four-hour study

For each 24-h sampling period, the obese and the control subjects were admitted to the metabolic ward at the Hvidovre University Hospital at 0730. A heparinized cannula (Viggo AB, Vingmed, Denmark) was placed in a forearm vein. Blood withdrawal commenced 30 min after venipuncture through a nonthrombogenic catheter (Carmeda; Viggo AB) inserted through the cannula and connected to a constant withdrawal pump. The withdrawal rate was 3.5 mL/h, and the collection tubes were changed at 20-min intervals over the succeeding 24-h period (22). After collection, the samples were centrifuged at 1200 × g and 4°C for 20 min, and the resulting plasma samples were stored at −70°C until divided into aliquots and assayed. For amino acid measurements, equal aliquots of plasma samples from 6 sequential collections were combined to constitute a single, 2-h sample. In this manner, a series of samples consisting of 12 sequential, 2-h samples covering the 24-h period was generated for each subject.

All the subjects were studied under weight-stable conditions. On the day of each study, the subjects were provided with an 8-MJ/d basic diet (15% of energy from protein, 55% from carbohydrate, 30% from fat). They were instructed to modify this diet to match their normal daily diet as closely as possible. The subjects were allowed normal, modest physical activity during the sample collection period.

Insulin tolerance study

On the day after each 24-h sampling period (after an overnight fast), an intravenous injection of 0.1 IU insulin (Actrapid Human; Novo-Nordisk A/S, Gentofte, Denmark)/kg body wt was administered at 0800 after catheter insertion. Venous blood samples were obtained 15 min before and 30, 60, and 120 min after insulin infusion. Because of technical difficulties, this test was not performed on one of the obese subjects and one of the control subjects. The omission of these subjects from this portion of the study did not significantly modify the anthropometric characteristics of the remaining group from those of the original group (data not shown).

Analytic methods

Anthropometric measurements

Waist and hip circumferences were measured while the subjects were standing. The waist-to-hip ratio was calculated and used as an index of abdominal fat distribution. [The waist was defined as the minimal abdominal circumference located midway between the lower rib margin and the iliac crests; the hip was defined as the greatest circumference over the great trochanters (24).] Body mass index was calculated as weight (kg)/height² (m). Body weight was measured to the nearest 0.1 kg with the use of an electronic scale (Seca 707; Seca, Copenhagen). Body composition was estimated with the use of dual-energy X-ray absorptiometry on a
TABLE 1

Anthropometric characteristics of the subjects

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Obese subjects (n = 8 F, 1 M)</th>
<th>After weight loss</th>
<th>Control subjects (n = 9 F, 1 M)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (y)</td>
<td>30.3 ± 2.2</td>
<td>30.3 ± 2.2</td>
<td>28.6 ± 1.1</td>
</tr>
<tr>
<td>Height (m)</td>
<td>1.70 ± 0.03</td>
<td>1.70 ± 0.03</td>
<td>1.70 ± 0.02</td>
</tr>
<tr>
<td>Weight (kg)</td>
<td>110.7 ± 6.7c</td>
<td>80.4 ± 6.2a</td>
<td>66.5 ± 1.8a</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>38.5 ± 1.5b</td>
<td>27.8 ± 1.4a</td>
<td>23.1 ± 0.4a</td>
</tr>
<tr>
<td>Waist (cm)</td>
<td>114.3 ± 6c</td>
<td>94.7 ± 7b</td>
<td>75.4 ± 1.1</td>
</tr>
<tr>
<td>Waist-to-hip ratio</td>
<td>0.93 ± 0.04b</td>
<td>0.88 ± 0.03a</td>
<td>0.78 ± 0.02b</td>
</tr>
<tr>
<td>Lean body mass (kg)</td>
<td>44.2 ± 2.1b</td>
<td>48.8 ± 3.9b</td>
<td>38.2 ± 2.5b</td>
</tr>
<tr>
<td>Percentage body fat (%)</td>
<td>53.6 ± 3.0b</td>
<td>37.0 ± 2.1b</td>
<td>35.5 ± 2.7b</td>
</tr>
</tbody>
</table>

*SEM. Values in the same row with different superscript letters are significantly different, P < 0.05.

Norland XR-36 densitometer (software version 2.4; Norland Medical Systems, Basingstoke, United Kingdom) (22).

Biochemical measurements

Plasma LNAA concentrations (except tryptophan concentrations) were quantitated with the use of a Beckman 6300 Amino Acid Analyzer equipped with a fluorometric detector (Beckman Instruments, Palo Alto, CA) (10). Plasma tryptophan concentrations were measured by using the method of Denckla and Dewey (25) as modified by Lehmann (26) and Bloxam and Warren (27). Plasma insulin concentrations were measured with a commercial kit (28). The plasma ratio of the concentration of tryptophan to the sum of the concentrations of the other LNAAAs was calculated by using molar concentrations as follows: tryptophan/(tyrosine + phenylalanine + leucine + isoleucine + valine).

Statistical analysis

The data are expressed as means ± SEMs and were analyzed by using one-way analysis of variance or two-factor (treatment group, time) repeated-measures analysis of variance with interaction; the level of significance was set at P < 0.05 for main effects and interactions. Post hoc testing was performed by using a Bonferroni multiple range test or Tukey’s honestly significant difference multiple range test; the level of significance was set at P < 0.05. STATGRAPHICS SG PLUS 7.0 software (Graphic Software Systems Inc, Rockville, MD) was used for the calculations.

RESULTS

The obese and control groups were closely matched in age, sex, and height (Table 1). At the conclusion of the weight-loss program, the obese subjects had lost almost 30% of their initial body weight (30.3 ± 4.6 kg; range: 20–63 kg), due exclusively to a loss of body fat. The percentage body fat values in the obese group decreased almost to control values, although the normal-weight control subjects in this study, almost all of whom were women, had a somewhat higher mean body fat percentage than that estimated for the overall female Danish population (29). Although lean body mass appeared to increase when the obese subjects lost weight (Table 1), this effect was attributable to one subject, who showed an unusual increase in lean body mass during weight loss. If data for this subject are excluded from the calculation, the lean body masses of the obese subjects before (45.8 ± 1.5 kg) and after (45.3 ± 2.02 kg) weight loss did not differ significantly. Body mass index in the obese subjects decreased from 38.4 to 27.9, which approached but did not reach the values observed in the control subjects (22).

Twenty-four–hour study

Plasma tryptophan concentrations showed significant main effects of group and time (24 h); the group × time interaction was not significant. The least-squares mean values (in nmol/mL) for plasma tryptophan concentration differed significantly between all the treatment groups (control subjects, 73.3; obese subjects before weight loss, 64.1; obese subjects after weight loss, 57.7; P < 0.05 for any comparison, Bonferroni multiple range test) (Figure 1, left panel). Plasma tryptophan ratios did not show a significant time effect, but a significant group effect was present (Figure 1, right panel). The group × time interaction was not significant. Post hoc testing showed that the least-squares mean tryptophan ratio of the control subjects (0.093) differed significantly (P < 0.05, Bonferroni multiple range test) from that of the obese subjects either before (0.072) or after (0.071) weight loss.

FIGURE 1. Mean (± SEM) plasma tryptophan concentrations and ratios of tryptophan to other large neutral amino acids (plasma tryptophan ratios) in control subjects (●; n = 10) and in obese subjects (○; n = 9) before (○) and after (●) weight loss during a 24-h period of continuous sampling. For plasma tryptophan concentration, significant effects of treatment group (P < 0.05, ANOVA) and time (P < 0.05, ANOVA) were found; the interaction between treatment group and time was not significant. For plasma tryptophan ratio, a significant effect of treatment group (P < 0.05) was found; the effect of time and the interaction between treatment group and time were not significant.
loss but that the tryptophan ratios of the obese subjects before and after weight loss were not significantly different.

Plasma tyrosine and phenylalanine concentrations and the combined plasma concentrations of the BCAAs (ΣBCAAs) each showed significant main effects of time (24 h) and group (Figure 2). For all the amino acids (tyrosine, phenylalanine, ΣBCAAs), the group \times \text{time interaction term} was not significant. For all the amino acids, post hoc testing showed that least-squares mean plasma concentrations (in nmol/mL) in the obese subjects before weight loss (tyrosine, 88.5; phenylalanine, 132.2; ΣBCAAs, 908.3) differed significantly (\(P < 0.05\), Bonferroni multiple range test) from those of the control subjects (tyrosine, 83.7; phenylalanine, 109.6; ΣBCAAs, 793.5) and of the obese subjects after weight loss (tyrosine, 81.3; phenylalanine, 120.3; ΣBCAAs, 834.3) but that plasma concentrations did not differ significantly between the control subjects and the obese subjects after weight loss (Figure 2).

Fasting plasma insulin concentrations (single time point) showed a significant group effect (\(P < 0.05\)). Post hoc testing showed that the least-squares mean plasma insulin concentration in the obese subjects before weight loss (134.7 pmol/L) differed significantly (\(P < 0.05\), Tukey’s honestly significant difference multiple range test) from that of the control subjects (64.2 pmol/L) and of the obese subjects after weight loss (55.6 pmol/L) but that plasma concentrations did not differ significantly between the control subjects and the obese subjects after weight loss.

**Insulin infusion test**

In this study, plasma concentrations of tryptophan, tyrosine, phenylalanine, and ΣBCAAs all showed significant main effects of time and treatment group (\(P < 0.05\); for all of the amino acids, the interaction term (time \times \text{treatment group}) was not significant (Figures 3 and 4).

**DISCUSSION**

These results show the presence of below-normal plasma tryptophan concentrations in obese subjects throughout the 24-h period. Moreover, after weight reduction, plasma tryptophan concentrations remained low. Because the plasma concentrations of the other LNAAs in the obese subjects before weight loss were moderately elevated above normal-weight control values at most
Figure 4. Mean (± SEM) plasma concentrations of tyrosine and phenylalanine and mean combined concentrations of branched-chain amino acids (ΣBCAAs; leucine + isoleucine + valine) in response to intravenous insulin infusion after an overnight fast in control subjects (○, n = 9) and in obese subjects (□, n = 8) before (●) and after (○) weight loss. Significant effects of treatment group (P < 0.05, ANOVA) and time (P < 0.05, ANOVA) were found for plasma tyrosine, phenylalanine, and ΣBCAAs. The interaction between treatment group and time was not significant for any of the variables.

times of day and night, the plasma tryptophan ratio was markedly below normal in the obese subjects (Figure 1). Despite some reductions in plasma LNAA concentrations with weight reduction, the plasma tryptophan ratio remained low because of persistently low tryptophan concentrations. No significant differences in the response of plasma LNAA concentrations to insulin infusion were noted between the treatment groups. This result suggests that the group differences in 24-h plasma LNAA variations cannot be attributed simply to differences in insulin sensitivity. Together, the findings suggest that obese subjects have a persistently low plasma tryptophan ratio, tryptophan uptake into the brain and, consequently, serotonin synthesis may be continuously low. They further suggest that because the ratio does not return to normal values in the postobese state, the brains of such subjects may continue to receive a below-normal flow of tryptophan and thus synthesize serotonin at an abnormally low rate.

The plasma concentrations of each LNAA in the normal-weight and the obese subjects varied significantly over the 24-h period, with values generally higher during the daytime than at night. Such variations appear to be fairly typical (30–33). These variations persisted in the obese subjects after weight reduction (Figures 1 and 2), presumably indicating the maintenance of a normal diurnal pattern of eating. Because the 24-h variations in tryptophan and the other LNAAAs tended to be parallel in all the treatment groups, no marked variation in plasma tryptophan ratios appeared over the 24-h period (Figure 1, right panel). This finding is similar to that noted by others (30, 31) and presumably indicates that all the subjects ingested ≥75 g protein/d, at least during testing days (30). The absence of marked daily variations in plasma tryptophan ratios may indicate that subjects insensibly control this ratio to prevent diurnal vagaries in tryptophan uptake into the brain. But it may also mean that the ratio is not controlled under normal metabolic conditions but simply reflects the sum of numerous metabolic activities that together and without being controlled modify plasma LNAA concentrations so that the plasma tryptophan ratio does not change. At present, these possibilities are untested.

Several other studies examined plasma tryptophan concentrations and plasma tryptophan ratios in obese and lean subjects. Almost all such studies measured a single time point. Although plasma tryptophan ratios have consistently been found to be lower in obese than in lean subjects (12, 13, 20, 34) [although not always (35)] and to remain low after weight reduction (36), the underlying basis for this effect has been variable. In some studies, tryptophan concentrations were low and the sum of the concentrations of the other LNAAAs was normal (12, 13); in other studies, tryptophan concentrations were low, and the sum of the concentrations of the other LNAAAs was high (34); and in still other studies, tryptophan concentrations were normal, and the sum of the concentrations of the other LNAAAs was high (12, 20). Because plasma amino acid concentrations vary diurnally and are subject to changes in diet and other metabolic phenomena (30, 32, 37), we felt that plasma LNAA concentrations and plasma tryptophan ratios should be examined throughout a 24-h period so that greater confidence could be gained regarding the influence of obesity and weight loss on plasma tryptophan concentrations and ratios. Under such conditions, both plasma tryptophan concentrations and plasma tryptophan ratios in obese subjects clearly fall below those in normal-weight control subjects at all times of day and night and remain low after weight reduction. Although the basis for the difference in tryptophan ratios can be seen as being due primarily to

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the difference in plasma tryptophan concentrations, the 24-h data for the other LNAAs indicate that the higher plasma concentrations of these amino acids in the obese subjects than in the normal-weight control subjects contribute to the below-normal tryptophan ratios in obese subjects at many times of day and night (Figures 1 and 2).

The bases for the differences between studies in the changes in plasma LNAAs concentrations that produce below-normal plasma tryptophan ratios in obese subjects are presently unknown and mostly unexamined. Presumably, they include differences in insulin resistance, age, sex, and body mass index. Given the level of experimental detail provided in these studies, it is impossible to evaluate these possibilities. However, if an increase in plasma LNAAs concentrations, a decrease in plasma tryptophan concentrations, or both are almost always present in obese subjects and account for the below-normal plasma tryptophan ratios, it may be possible to inquire about the basis of these effects. Unfortunately, relatively few studies have examined this issue.

Regarding tryptophan, only a single relevant study appears to be available: Caballero et al (11) observed that the acute increase in plasma tryptophan concentration that followed oral tryptophan administration to obese subjects was less than that observed when the amino acid was given to normal-weight subjects. This result suggests that tryptophan metabolism may be greater in obese subjects than in normal-weight subjects, a possibility that is supported by studies in diabetic rats (38) and that could account for chronically low plasma tryptophan concentrations in obese persons. However, in the study by Caballero et al (11), baseline plasma tryptophan concentrations were not below normal before tryptophan administration.

Regarding the other LNAAs, particularly the BCAAs, elevations in their plasma concentrations, where present, could be due to insulin insensitivity. Insulin secretion lowers plasma concentrations of BCAAs (39), probably by promoting their net uptake into peripheral tissues (20, 40). The development of insulin insensitivity for amino acids in obesity could raise plasma BCAA concentrations. However, this potential mechanism has been a subject of debate. Thus, although some researchers have suggested that the insulin-induced decrease in plasma BCAA concentrations in obese subjects is lower than that in lean subjects (11, 20), other researchers have found it to be normal (21). However, Caballero and Wurtman (20) also suggested that if plasma BCAA concentrations (and insulin-related leucine disposal) are related to fat-free mass, no difference in insulin sensitivity is noted between obese and normal-weight subjects. Our data tend to support the view that BCAA disposal in response to insulin is not blunted in obese subjects, because we observed no marked differences between the study groups in the reduction in the plasma concentration of any LNAAs after insulin infusion (Figures 3 and 4). In addition, although lean body mass and plasma BCAA concentrations throughout the 24-h period were higher in our obese subjects before weight loss than in the normal-weight subjects, lean body mass did not decrease with weight loss, but plasma BCAA concentrations did (Table 1 and Figure 2; in contradistinction to the link between fat-free mass and plasma BCAAs suggested by others (20)). Therefore, these results suggest that although lean body mass may predict plasma BCAA concentrations under some circumstances (obese subjects compared with nonobese subjects), it is less predictive under other conditions (obese subjects before weight loss compared with obese subjects after weight loss). Conceivably, in the latter situation, the dietary regimen may have been a contributing factor, thus obscuring a simple link between fat-free mass and plasma BCAA (20).

Numerous studies implicate brain serotonin neurons in the control of appetite. The observation has repeatedly been made that drugs that enhance transmission across serotonin synapses generally suppress appetite, whereas those that diminish serotonin transmission stimulate food intake (1, 6, 41). Evidence suggesting that defects in the serotonin system may be etiologic of obesity has also accumulated (3, 4). In relation to the present findings, such results predict that when serotonin transmission is low, appetite will be stimulated. The persistently lower plasma tryptophan ratios observed throughout the day and night in the obese subjects than in the normal-weight control subjects support the notion that brain tryptophan uptake and serotonin synthesis in obese subjects may be abnormally low (11). The fact that the plasma tryptophan ratio remains persistently low after weight reduction further suggests that the formerly obese may struggle against a biochemical signal oriented toward increased appetite and food intake.

All the authors participated in the experimental design of the study. In addition, MHR designed the weight-loss program and the insulin infusion tests, JH supervised the execution of the study, LB conceived of and organized the study and performed all statistical analyses, and JDF conducted the amino acid measurements. All the authors participated in writing and editing the manuscript. None of the authors had any financial or personal interest in any of the organizations that sponsored this project.

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