Body fat redistribution after weight gain in women with anorexia nervosa¹–³


ABSTRACT
Background: Body image distortions are a core feature of anorexia nervosa (AN). Increasing evidence suggests that the fat distribution immediately after weight restoration in patients with AN differs from the distribution typical of healthy adult women.
Objective: The purpose of this study was to assess body fat distribution before and shortly after normalization of weight in women with AN.
Design: Body composition and fat distribution were assessed by anthropometry, dual-energy X-ray absorptiometry, and whole-body magnetic resonance imaging in 29 women with AN before and after weight normalization and at a single time point in 15 female control subjects. Hormone concentrations were also evaluated in patients and control subjects.
Results: During 10.1 ± 2.9 wk (range: 4–17.3 wk) of treatment, patients with AN gained 12.2 ± 3.6 kg, and refed weight (54.1 ± 4.2 kg) did not differ significantly from that of control subjects (54.7 ± 4.4 kg). Waist-to-hip circumference ratio (P < 0.006), total trunk fat (P < 0.003), visceral adipose tissue (P < 0.006), and intramuscular adipose tissue (P < 0.003) were significantly greater in the weight-recovered patients than in the control subjects. In contrast, after refueling, total subcutaneous adipose tissue and skeletal muscle mass did not differ significantly between the patients and control subjects. In patients with AN, serum cortisol decreased and serum estradiol increased significantly with refeeding but not to control concentrations.
Conclusions: In women with AN, normalization of weight in the short term is associated with an abnormal distribution of body fat. The implications of these findings for the long-term psychological and physical health of women with AN are unknown.

KEY WORDS
Body fat distribution, anorexia nervosa, visceral adipose tissue, whole-body MRI

INTRODUCTION
Psychological distortions about body image and body size are hallmark features of anorexia nervosa (AN) (1). However, in the underweight state, although patients may feel “huge,” studies have documented that all components of body composition are severely diminished (2–4). Total body fat, as well as total body muscle, is markedly reduced, consistent with the starved, underweight state.

Clinical experience suggests that many patients specifically fear that weight gain will result in a disproportionate and unacceptable increase in abdominal girth. The few studies that have examined changes in body composition with weight normalization suggest that patients may not gain weight in an evenly distributed pattern (5–8). Specifically, there are indications that body mass increases in the trunk region disproportionately to the extremities (6–8). These studies, although suggestive, are limited by incomplete weight restoration and suboptimal measurement techniques.

The goal of the current study was to determine, with the use of anthropometric measures, dual-energy X-ray absorptiometry (DXA), and whole-body magnetic resonance imaging (MRI), whether the pattern of body fat distribution of patients with AN is normal immediately after full-weight restoration.

SUBJECTS AND METHODS

Subjects
Subjects were 29 women with AN and 15 healthy, control women aged between 18 and 45 y. Subjects with AN were patients receiving treatment at the Eating Disorder Research Unit at the New York State Psychiatric Institute (NYSPI), Columbia University Medical Center (CUMC), who met Diagnostic and Statistical Manual of Mental Disorders, 4th edition (1) criteria for AN, including amenorrhea. The exceptions were one subject who had regular menses and one subject who had oligomenorrhea. Subjects were recruited from referrals by physicians and mental health workers or by contacting the clinic directly.

Control subjects were thin, healthy, weight stable, regularly menstruating, young women without histories of eating disorders or other significant psychiatric or medical history, with a body mass index (BMI; in kg/m²) similar to weight-restored patients.
with AN (19.5–21.0) recruited from the Columbia University undergraduate and CUMC campuses. Weight stability was defined as within 2 kg of current weight for the previous 6 mo by self report.

Most of the subjects had not taken any medications (except for acetaminophen and ibuprofen), including oral contraceptives, for a minimum of 4 wk before testing; one patient had been medication-free for only 2 wk before testing.

All subjects gave written consent before participation in the study. This study was approved by the Institutional Review Boards of the NYSPI, Columbia University, and St Luke’s–Roosevelt Hospital Center.

Protocol

All patients were admitted to the Eating Disorders Service of the General Clinical Research Unit at NYSPI/CUMC. For the first 1–2 wk of hospitalization, patients were weighed daily on a beam balance scale (Detecto, Webb City, MO) and were encouraged to consume calories in the form of food. Liquid nutritional supplements (Ensure or Ensure Plus; Ross Nutritional, Columbus, OH) were added if necessary to prevent further weight loss. Patients underwent testing after a 1–2-wk period of medical and weight stabilization before the onset of formal weight gain and again after normalizing and maintaining ≥90% ideal body weight (9) (approximately equivalent to a BMI of 19.5) for 2–4 wk. Menstruating subjects were tested during the follicular phase of the cycle.

The testing battery included morning blood samples (fasting samples obtained for 22 of 29 patients, 5 of 15 control subjects), self-report questionnaires and interviews for assessing nutritional intake and activity, and body composition. Anthropometry, DXA, and total-body MRI for body composition assessment were performed at the Body Composition Unit of the New York Obesity Research Center at St Luke’s–Roosevelt Hospital Center.

Body composition

Anthropometry was performed with the use of a standard, cloth tape measure. Waist circumference was measured in millimeters at the level immediately below the lowest ribs, and hip circumference was measured below the iliac crest (10). Two trained assessors performed all assessments. For each patient, the same assessor performed both the low-weight and weight-restored measurements.

DXA (DPX-L; GE Systems, Madison, WI) was performed to obtain total-body and regional fat and lean soft tissue. Body fat distribution was determined by DXA with the use of the method of Grinspoon et al (7) described by Hadigan et al (11) that calculated extremity fat as a percentage of total fat (extremity fat mass/total fat mass) and trunk fat as a percentage of total fat (trunk fat mass/total fat mass).

Whole-body MRI (1.5T; GE Systems, General Electric, Milwaukee, WI) was done to evaluate total-body and regional adipose tissue and skeletal muscle mass (12). Cross-sectional images were analyzed for subcutaneous adipose tissue (SAT), visceral adipose tissue (VAT), intramuscular adipose tissue (IMAT), total adipose tissue (TAT), and skeletal muscle (SM) by 3 trained observers who used VECT image analysis software (Slice-O-Matic, Montreal, Canada) and total volumes were calculated as reported by Shen et al (12). Initial volume results were reported in liters and converted to kilograms by multiplying volume of tissue by reference densities (SM: 1.04 g/L; adipose tissue: 0.92 g/L) (13). Intracranal correlation coefficients for agreement among multiple readers were 0.99 (CI: 0.89, 1.0) for SM, 0.99 (CI: 0.81, 1.0) for SAT, and 0.95 (CI: 0.58, 0.99) for VAT (14).

Assays for cortisol, estradiol, and testosterone were conducted on serum. Cortisol, estradiol, and testosterone were each measured by a commercial solid-phase, chemiluminescent immunoassay (Immulite; Diagnostic Products Co, DPC, Los Angeles, CA). The polyclonal antibodies used are highly specific with low cross-reactivity to other steroids or hormones. Assay sensitivity for cortisol is 0.2 µg/dL with intraassay and interassay CVs of 7.6% and 10.2%, respectively. Assay sensitivity for estradiol is 20 pg/mL with intraassay and interassay CVs of 9.3% and 10.5%, respectively. Assay sensitivity for testosterone is 10 ng/dL with intraassay and interassay CVs of 7.4% and 9.8%, respectively.

After the testing battery was complete, patients with AN began the weight gain phase of treatment. Modest variations of the following treatment protocol have been in use for the past 20 y at NYSPI and are of established utility (15). Treatment consisted of a structured behavioral program aimed at normalizing weight and eating patterns. Patients were prescribed 3 meals daily plus a snack with sufficient caloric content to gain ≥1 kg/wk. If the patients were unable to gain weight with food alone, additional calories in the form of liquid nutritional supplement were added. Exercise was not permitted on the unit at any time during weight gain. In addition to the behavioral protocol, patients were seen in individual therapy, with supportive and cognitive-behavioral elements, 3–5 times weekly and in group and family therapy. The weight-gain phase continued until the patient reached 90% of ideal body weight.

Statistical analyses

Clinical variables were compared between patients and control subjects before weight gain and after normalization of weight with the use of Student’s t test. Paired t tests were used to compare clinical variables in the subjects with AN before and after weight gain. Linear regression models were constructed to evaluate the relation between concentrations of cortisol, estradiol, and testosterone and body fat distribution. Analyses were performed by using SPSS for WINDOWS (version 10.1, Chicago, IL). Means ± SDs are reported. Two-tailed tests were used. The Bonferroni correction was used to adjust for multiple comparisons by multiplying the unadjusted P value by 3. Corrected P values are reported. Significance level was set at 0.05.

RESULTS

Demographic information for the subjects is presented in Table 1. Low-weight patients with AN, as expected, had reduced weight and BMI compared with the control subjects. The mean BMI of weight-restored patients (20.65 ± 0.90) was not significantly different from that of control subjects (20.61 ± 1.1). The mean age was not significantly different (P ≥ 0.15) between the patients with AN (24 ± 5 y) and the control subjects (27 ± 6 y). Mean time between low-weight and weight-restored assessments was 10.1 ± 2.9 wk (range: 4–17.3 wk).
Body composition

Anthropometric measurements

The waist-to-hip ratio (WHR) of patients with AN at low weight (0.80 ± 0.05) did not differ significantly from that of control subjects (0.79 ± 0.03). After normalization of weight, the WHR of patients (0.84 ± 0.05) was significantly greater (P ≤ 0.006) than that of the control subjects.

Dual energy X-ray absorptionometry

The mean percentage of body fat of patients with AN at low weight (9.3 ± 6.4%) was significantly lower (P ≤ 0.003) than that of control subjects (25.9 ± 4.4%; Table 2). At low weight, trunk fat as a percentage of total fat in patients (46.7 ± 5.8%) was not different (NS) from control subjects (44.9 ± 3.5%), but extremity fat as a percentage of total fat in patients (45.0 ± 6.7%) was reduced (P ≤ 0.012) compared with control subjects (50.7 ± 3.9%). With weight gain, trunk fat as a percentage of total fat increased significantly (46.7 ± 5.8% compared with 50.7 ± 5.4%, P ≤ 0.003) without a concomitant rise in extremity fat. The percentage of trunk fat (50.7 ± 5.4% and 44.9 ± 3.5%, P ≤ 0.003) was significantly higher and the percentage of extremity fat (43.9 ± 5.8% and 50.7 ± 3.9%, P ≤ 0.003) was reduced in weight-restored patients compared with control subjects, respectively.

Magnetic resonance imaging

The average masses of SM and TAT and of other adipose tissue compartments (SAT, VAT) except IMAT of the patients with AN at low weight were significantly lower than those of control subjects (Table 2). After weight normalization, mean TAT and SM masses were not significantly different between

### Table 1

| Clinical characteristics of patients with anorexia nervosa (AN) before and immediately after weight gain and of healthy control subjects |
|-----------------------------------------------|-----------------|-----------------|-----------------|-----------------|
| AN patients (n = 29)                          | Control subjects (n = 15) | Low-weight AN patients compared with control subjects | Weight-restored AN patients compared with control subjects | Low-weight AN patients compared with weight-restored AN patients |
| Low-weight                        | Weight-restored       | ≤ 0.150         | ≤ 0.003         | NS              | ≤ 0.003         |
| Age (y) 23.5 ± 4.9                                      | 27.0 ± 6.3                                     | 0.150           | 0.003           | NS              | 0.003           |
| Weight (kg) 42.0 ± 4.5                                   | 54.1 ± 4.2                                     | 0.003           | NS              | ≤ 0.003         |
| BMI (kg/m²) 15.95 ± 1.6                                  | 20.65 ± 0.90                                   | 0.003           | NS              | 0.003           |
| Duration of illness (y) 7.38 ± 4.35                     | 20.61 ± 1.1                                    | 0.003           | NS              | ≤ 0.003         |
| Duration of amenorrhea (mo) 28.5 ± 32.2                 | 20.65 ± 1.1                                    | 0.003           | NS              | ≤ 0.003         |

1 Adjusted for multiple comparisons.
2 P value determined by independent-sample t test comparisons.
3 P value determined by paired t test comparisons.
4 * P value determined by paired t test comparisons.
5 SD (all such values).

### Table 2

| Measures of body composition assessed by dual-energy X-ray absorptionmetry (DXA) and magnetic resonance imaging (MRI) in patients with anorexia nervosa (AN) before and immediately after weight gain and in healthy control subjects |
|-----------------------------------------------|-----------------|-----------------|-----------------|-----------------|
| AN patients (n = 29)                          | Control subjects (n = 15) | Low-weight AN patients compared with control subjects | Weight-restored AN patients compared with control subjects | Low-weight AN patients compared with weight-restored AN patients |
| Body fat distribution, DXA                    |                  |                  |                  |                  |
| Body fat (%) 9.3 ± 6.45                        | 25.0 ± 5.3       | 25.9 ± 4.4       | NS              | 0.003           |
| Trunk fat (%) of total fat 46.7 ± 5.8           | 50.7 ± 5.4       | 44.9 ± 3.5       | 0.012           | 0.003           |
| Extremity fat (%) of total fat 45.0 ± 6.7       | 43.9 ± 5.8       | 50.7 ± 3.9       | 0.003           | NS              |
| Body composition, MRI                          |                  |                  |                  |                  |
| SM (kg) 14.0 ± 2.3                             | 17.0 ± 1.9       | 18.1 ± 2.2       | 0.003           | 0.207           |
| TAT (kg) 6.6 ± 2.7                             | 14.8 ± 3.2       | 14.3 ± 2.1       | 0.003           | NS              |
| SAT (kg) 5.9 ± 2.5                             | 13.0 ± 2.9       | 13.6 ± 1.9       | 0.003           | NS              |
| VAT (kg) 0.38 ± 0.19                           | 1.04 ± 0.5       | 0.59 ± 0.23      | 0.009           | 0.006           |
| IMAT (kg) 0.37 ± 0.22                          | 0.81 ± 0.25      | 0.51 ± 0.13      | 0.105           | 0.003           |

1 SM, skeletal muscle; TAT, total adipose tissue; SAT, subcutaneous adipose tissue; VAT, visceral adipose tissue; IMAT, intramuscular adipose tissue.
2 Adjusted for multiple comparisons.
3 * P value determined by independent-sample t test comparisons.
4 * P value determined by paired t test comparisons.
5 ± ± SD (all such values).
patients and control subjects. However, adipose tissue distribution differed significantly: VAT and IMAT were significantly elevated in patients with AN compared with control subjects, although SAT mass was not different between the groups.

Consistent with the absolute values, the relative proportions of SAT and VAT were also different between the groups. As a percentage of TAT, weight-restored patients had less SAT (88 ± 4% and 95 ± 10%, P ≤ 0.003) and more VAT (7 ± 3% and 4 ± 2%, P ≤ 0.003) than that of the control subjects, respectively.

Hormonal analyses

Results from hormonal analyses conducted on all morning blood samples (29 patients, 15 control subjects) and the subset of fasting blood samples (22 patients, 5 control subjects) were not significantly different. Thus, results from the larger sample are presented.

Mean serum cortisol of the low-weight patients was elevated compared with control subjects (P ≤ 0.003; Table 3), and mean serum estradiol was reduced (P ≤ 0.009). Serum testosterone concentrations were not significantly different between patients and control subjects (P = 0.345).

Despite normalization of weight, the average serum cortisol concentration of the patients did not change and remained higher than that of control subjects. Serum estradiol concentrations, however, increased with weight gain but remained below control concentrations. In addition to one subject who had regular menstrual cycles despite low weight, 5 additional subjects resumed menstruating with weight normalization. Mean serum testosterone concentration was unchanged with weight gain.

For the patient group only, we fit a series of regression models to assess the relation between VAT after weight normalization, hormone concentrations before and after weight normalization, and initial VAT. In a multiple linear regression model, with VAT after weight normalization as the dependent variable and with cortisol concentration at low weight, cortisol after weight normalization, estradiol concentration at low weight, estradiol concentration after weight normalization, testosterone concentration at low weight, and testosterone concentration after weight normalization as the independent variables, the set of cortisol at low weight (β = −0.541, P = 0.036) and testosterone concentration after weight normalization (β = −0.724, P = 0.019) was significant.

### DISCUSSION

The present study found that, immediately after weight restoration to a normal weight, women with AN have an adipose tissue distribution that differs significantly from healthy control subjects. These findings were consistently observed with several different methods that all suggested disproportionate central adipose tissue deposition with weight recovery: elevated WHR, total trunk fat, and VAT. In addition, the adipose tissue interspersed between muscle fibers, IMAT, was also significantly greater in weight-recovered patients with AN than in control subjects.

### Concordance with previous studies

Forbes (16) was the first to examine body fat distribution in a cross-section of underweight women with AN. Using the WHR as a measure of body fat distribution, he reported no difference in the WHR between women with AN and healthy control subjects. Our results replicate this first examination of body fat distribution in patients with AN.

Our results are generally consistent with and extend previous reports that document a central adiposity phenotype in weight-recovered patients with AN (5–8, 17, 18). Orphanidou et al (5) assessed body composition by DXA in patients with AN, and studies by Iketani et al (6), Grinspoon et al (7), and Scaletti et al (8), also using DXA, included healthy control subjects. All reported, as expected, that at low weight patients had reduced body fat mass that increased with weight gain. Grinspoon et al (7) extended the findings of Iketani et al (6) by measuring not only absolute fat mass but also by analyzing the relative proportions of fat in the trunk compared with the extremities before and after weight gain. At baseline, trunk fat as a percentage of total fat was not statistically different between the patient and control groups. With weight gain, however, trunk fat as a percentage of total fat increased but the percentage of extremity fat did not, thus supporting a relative truncal obesity in refed women with AN. A limitation of the study of Grinspoon et al (7), overcome in the present study, is that patients were still underweight at the end of the trial, leaving uncertain the effect of more complete weight restoration on body fat distribution. Our study confirms the findings of Grinspoon et al (7) and extends them to a population with more complete weight restoration.

### Table 3

Morning serum hormone concentrations in patients with anorexia nervosa (AN) before and immediately after weight gain

<table>
<thead>
<tr>
<th>Hormone</th>
<th>AN patients (n = 29)</th>
<th>Control subjects (n = 15)</th>
<th>Low-weight AN patients compared with control subjects&lt;sup&gt;2&lt;/sup&gt;</th>
<th>Weight-restored AN patients compared with control subjects&lt;sup&gt;2&lt;/sup&gt;</th>
<th>Low-weight AN patients compared with weight-restored AN patients&lt;sup&gt;2&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cortisol (μg/dL)</td>
<td>20.0 ± 4.9&lt;sup&gt;a&lt;/sup&gt;</td>
<td>17.23 ± 6.5</td>
<td>≤0.003</td>
<td>≤0.003</td>
<td>≤0.114</td>
</tr>
<tr>
<td>Estradiol (pg/dL)</td>
<td>23.4 ± 7.4</td>
<td>30.7 ± 13.4</td>
<td>≤0.009</td>
<td>≤0.045</td>
<td>≤0.009</td>
</tr>
<tr>
<td>Testosterone (ng/dL)</td>
<td>82.4 ± 37.6</td>
<td>80.3 ± 35.1</td>
<td>≤0.345</td>
<td>≤0.408</td>
<td>NS</td>
</tr>
</tbody>
</table>

<sup>1</sup> Adjusted for multiple comparisons.
<sup>2</sup> P value determined by independent-sample t test comparisons.
<sup>3</sup> P value determined by paired t test comparisons.
<sup>4</sup> x ± SD (all such values).
To more directly quantify abdominal fat, Mayo-Smith et al (17) used single-slice computed tomography (CT) to measure SAT and VAT in women with AN. Although limited by the cross-sectional nature of the study, Mayo-Smith et al (17) reported that the proportion of VAT to TAT in the L4–L5 CT image was significantly higher in patients with AN (0.4) compared with control subjects (0.14). In the current study, total body VAT as a percentage of total body adipose tissue, assessed by multiple-slice MRI, was also higher in low-weight patients (5.7%) compared with control subjects (4.1%) and further increased with weight gain (to 7.0%). Zamboni et al (18), using single-slice CT at the L4–L5 level, replicated and extended the findings of Mayo-Smith et al (17) by reporting that with a mean 7.3 kg weight gain, SAT increased by 212%, and VAT increased by 117%. They, too, concluded that patients with AN gain abdominal fat but were unable to address whether there is a distinct preference to deposit weight centrally because they did not measure adipose tissue in other regions (eg, the extremities). The patients in the study of Zamboni et al (18) also had incomplete weight restoration at the time of retesting (BMI: 17.5 ± 2.0), and a control group was not evaluated. With the use of total-body MRI and with more complete weight normalization (a mean weight gain of 12.2 kg and BMI of 20.6), our study extends the findings of Zamboni et al (18) and shows increases in total body SAT and VAT of 141% and 209%, respectively.

Hormonal effects

At baseline we observed the classic hormone pattern observed in patients with AN (19–21). With recovery, serum cortisol concentrations remained elevated and estrogen concentrations remained low. Serum testosterone concentrations in patients did not differ from those in control subjects either at baseline or with recovery. Grinspoon et al (7) reported a strong association between change in percentage of trunk fat and baseline 24-h urinary-free cortisol, as well as end-of-study cortisol and increase in trunk fat. We found that serum cortisol at low weight and testosterone after weight gain were significant predictors of postweight gain VAT. The full implication of an association between testosterone concentrations and VAT is unclear. Because of likely changing sex hormone-binding globulin concentrations, total serum testosterone may not accurately reflect free testosterone concentrations.

Similarly, we confirm the finding of Grinspoon et al (7) of an absence of significant relation between estradiol and body fat distribution in the AN population. However, because only a small number of patients resumed regular cycling with weight gain, additional work is necessary to exclude an effect of estradiol on fat accumulation.

Potential clinical implications

It is unknown whether the tendency to accumulate abdominal fat, particularly VAT, during acute weight recovery has a significant negative psychological effect. It is conceivable that those who gain the most trunk fat and VAT are also the most distressed about their body shape and, thus, more prone to relapse. If this disproportionate abdominal fat is an acute effect of weight gain and redistributes with long-term maintenance of normal weight, supportive therapy might suffice to help the patient tolerate the body distortions until redistribution. Alternately, if the changes are more permanently induced, a more targeted, cognitive approach might be necessary to promote self-acceptance.

The implications of increased VAT in this population of young women deserve further exploration. Although increased VAT has been associated with the metabolic syndrome in other disorders (22), there are no definitive studies in AN of other features of metabolic syndrome (eg, insulin resistance, activated inflammatory pathways). Similarly, although VAT has been implicated as a significant risk factor for cardiovascular disease (23), few studies have examined long-term increased rates of morbidity and mortality from cardiovascular disease in this population (24).

Study limitations

There are several limitations to this study. Because of the structured, inpatient setting, patients were able to gain weight relatively rapidly (mean time between assessments was ∼10 wk). Whether a slower rate of weight gain would be associated with a more normal distribution of body fat is uncertain. However, in the study of Grinspoon et al (7) a smaller weight gain over 9 mo was also associated with a greater increase in truncal fat than in extremity fat. Thus, the effect of rate of weight gain on body fat distribution is an area for further study.

Additionally, the subjects in the current study had maintained normal weight for a relatively short duration. Whether body fat distribution would further equilibrate and become more normal with persistent weight maintenance is an important unanswered question. A study by Wentz et al (25), that compared the bone density and body composition of women and men with AN 11 y after onset of illness (some of whom still carried the diagnosis) with that of normal, healthy control subjects found significant differences between the groups. Percentages of body fat and body fat distribution were both significantly different between the groups: patients with AN had reduced percentage of body fat (despite similar BMI) compared with control subjects, but control subjects had higher trunk fat-to-extremity fat ratio. It is suggested that changes in body composition and body fat distribution continue to evolve with long-term weight maintenance, but a prospective, longitudinal study is essential to address this question definitively.

Another limitation relates to the age of the participants. Although this study recruited only adult women with AN, AN is an illness that often begins during adolescence. Although a recent study by Misra et al (26) suggested that teenagers with AN do not develop central accumulation of fat with weight gain, the distribution of body fat as weight gain that occurs in adolescents with AN requires further study.

Conclusions

In summary, the current study confirms and extends the accumulating evidence to support patients’ contentions that body fat is not normally distributed during recovery, being disproportionately deposited around the waist and in the abdominal cavity. The implications of these findings for the long-term psychological and physical health of individuals with AN are unknown.

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LM, BTW, RNP, STH, DG, RLL, and MPW were responsible for the study concept and design. LM, JW, EK, and DG were responsible for the acquisition of data. LM, BTW, RNP, SBH, DG, MKP, and RLL were responsible for the analysis and interpretation of the data. LM, BTW, RNP,
SBH, DG, MKP, RLL, and DG were responsible for drafting the manuscript. MKP provided the statistical expertise. LM, BTW, RNP, SBH, DG, and RLL supervised the study. None of the authors had a financial conflict of interest in relation to this study.

REFERENCES