Iron absorption and iron status are reduced after Roux-en-Y gastric bypass\textsuperscript{1–3}

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ABSTRACT
Background: Iron deficiency and iron deficiency anemia are common in patients who undergo gastric bypass. The magnitude of change in iron absorption is not well known.

Objective: The objective was to evaluate the effects of Roux-en-Y gastric bypass (RYGBP) on iron status and iron absorption at different stages after surgery. We hypothesized that absorption would be markedly impaired immediately after surgery and would not improve after such a procedure.

Design: Anthropometric, body-composition, dietary, hematologic, and iron-absorption measures were determined in 67 severe and morbidly obese women [mean age: 36.9 ± 9.8 y; weight: 115.1 ± 15.6 kg, body mass index (BMI: in kg/m\textsuperscript{2}); 45.2 ± 4.7] who underwent RYGBP. The Roux-en-Y loop length was 125–150 cm. Determinations were carried out before and 6, 12, and 18 mo after surgery. Fifty-one individuals completed all 4 evaluations.

Results: The hemoglobin concentration decreased significantly throughout the study (repeated-measures analysis of variance). The percentage of anemic subjects changed from 1.5\% at the beginning of the study to 38.8\% at 18 mo. The proportion of patients with low serum ferritin increased from 7.5\% to 37.3\%. The prevalence of iron deficiency anemia was 23.9\% at the end of the experimental period. Iron absorption from both a standard diet and from a standard dose of ferrous ascorbate decreased significantly after 6 mo of RYGBP to 32.7\% and 40.3\% of their initial values, respectively. No further significant modifications were noted.

Conclusion: Iron absorption is markedly reduced after RYGBP with no further modifications, at least until 18 mo after surgery. Am J Clin Nutr 2009;90:527–32.

INTRODUCTION
Surgical approaches are recognized as the most effective treatment of sustained weight loss in adults with morbid obesity. Several alternatives are available, including gastric banding, sleeve gastrectomy, biliopancreatic diversion, and Roux-en-Y gastric bypass (RYGBP) (1–3). RYGBP has shown highly satisfactory results, eg, weight loss, improvement of medical conditions, and long-term quality of life (3, 4). The postoperative mortality rate associated with this technique is low. In terms of adverse nutritional consequences, anemia is relatively common. Megaloblastic anemia, potentially indicative of vitamin B-12 or folate deficiency, is relatively uncommon, with prevalence estimates near 0.8\% (5). In contrast, iron deficiency and iron deficiency anemia are among the most prominent findings. Iron deficiency has been reported in 15–60\% of patients who undergo gastric bypass (6–9). Although prophylactic iron supplements have been recommended for women who undergo RYGBP, there is no agreement on the amount and form of iron that could efficiently prevent the development of iron deficiency. Of the potential causes of iron deficiency, frequently mentioned is the reduced capacity to absorb iron, but the experimental evidence to support this is limited (10). In the present study we determined iron status and iron absorption before and 6, 12, and 18 mo after RYGBP. We hypothesized that, compared with presurgical measurements, iron absorption would be significantly impaired 6 mo after surgery and that this impairment would persist at the subsequent follow-up time points. A secondary hypothesis was that iron deficiency would also persist after surgery, despite iron supplementation.

SUBJECTS AND METHODS

Subjects
Sixty-seven menstruating women were enrolled in this study. The mean (± SD) age of the subjects was 36.9 ± 9.9 y (range: 18–55 y), weight was 115.1 ± 15.6 kg (range: 88.4–156.4 kg), and body mass index (BMI; kg/m\textsuperscript{2}) was 45.2 ± 4.7. The patients were enrolled between August 2004 and December 2006. The subjects were evaluated before and 6, 12, and 18 mo after RYGBP (see details below). The inclusion criteria considered women with a BMI ≥ 40 or ≥ 35 with comorbidities and who

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had decided to undergo RYGBP at the Department of Surgery of the University Clinical Hospital. Fifty-one subjects completed the 18-mo follow up. Main initial anthropometric and hematologic characteristics of the individuals who abandoned the study were not significantly different from those who remained (data not shown). All patients who accepted enrollment into the study signed an authorized consent form. The study was approved by the Ethics Committee for Human Investigation of the Faculty of Medicine of the University of Chile.

Experimental design

The study was intended to produce minimal alterations to the regular clinical and medical management of the patients at the Department of Surgery undergoing RYGBP while simultaneously testing an improved vitamin and mineral supplement directed to reduce the potential nutritional consequences of this surgical procedure. All patients followed the routine standard procedures after surgery. During the first month, they consumed 500 mL/d of a liquid diet made of chicken breast, egg white, spinach, carrots, and potatoes that provided 800 kcal, 60 mg protein, and 3.9 mg Fe among other nutrients. A full medical evaluation was carried out at the end of this month. At this time, 1 unit of vitamins (TOL12; Saval Laboratories SA, Santiago, Chile) was prescribed to all patients to be administered intramuscularly. This preparation contained 200 mg thiamine chloride, 100 mg pyridoxine chloride, and 10 mg cyanocobalamin. A solid diet divided into 5 or 6 meals was also prescribed. It provided 1000 kcal and 60 g protein. According to a standard procedure in Chile, the medical evaluation in the third month included hematologic tests. If anemia or the risk of this condition was observed, iron supplements were prescribed. The supplements provided 40–315 mg elemental Fe (ferrous sulfate and ferrous fumarate salts) daily to be used during specified periods of treatment. Treatments with specific doses of iron were determined by the severity of anemia or by the extent of risk of this condition. These procedures were repeated at 6 mo and every 6 mo thereafter.

During the evaluation scheduled at the first month after surgery, the subjects were randomly allocated (using a random numbers table and carried out by a member of the Department of Nutrition not related to this study) into 2 groups: group A received the “standard vitamin and mineral supplementation” and group B received the “improved vitamin and mineral supplementation.”

The standard vitamin and mineral supplementation consisted of the routine vitamin and mineral supplementation regimen established at the University of Chile Clinical Hospital, which considered the use of a vitamin-mineral supplement (Laratobe; Bayer Laboratories, Santiago, Chile) and 2 tablets of a calcium and vitamin D supplement daily (Elcal D; Andromaco Laboratories, Santiago, Chile). The contents of vitamins and minerals supplied by these supplements are shown in Table 1. The “improved vitamin and mineral supplementation” consisted of a specially designed vitamin-mineral supplement that provided at least the Recommended Dietary Allowance of selected nutrients (11, 12) and 2 tablets of a calcium and vitamin D supplement daily (Elcal D-PLUS; Andromaco Laboratories). The total contents of vitamins and minerals supplied by these supplements are shown in Table 1. The amount of supplements consumed throughout the study was carefully recorded in a monthly register provided to the subjects and by counting the number of pills remaining in the container at the end of each month. In addition, the medical registers were periodically evaluated for any indication of additional iron supplementation recorded. When iron supplementation was noted, the subject was contacted by telephone to determine the extent of supplementation.

A series of anthropometric, body composition, dietary, and hematologic evaluations were conducted in all patients before the surgical procedure and 6, 12, and 18 mo after gastric bypass. In a subset of 36 individuals, randomly selected within each group, iron-absorption tests were carried out at the same evaluation periods.

Surgical procedure

Details of the surgical technique are available elsewhere (4). Briefly, it consisted of a 95% distal gastrectomy, resection of the excluded stomach, and retention of a small gastric pouch (15–20 mL). Resection of the excluded stomach was carried out to reduce the risk of gastric ulcer and bacterial proliferation in the remaining stomach and also to avoid potential complications if carcinoma developed. Then, an end-to-side gastro-jejunostomy with circular staple No. 25 was performed. The length of the Roux-en-Y loop was 125–150 cm, in accordance with the current practice of clinical centers in Chile expressed in the 2005 panel of experts’ consensus (13).

Anthropometric and body-composition evaluations

Weight (kg) was measured to the nearest 0.1 kg on a digital scale (Seca; Vogel & Halke GMBH & Co, Hamburg, Germany), and height (m) was measured to the nearest 0.1 cm with a scale-mounted stadiometer per standardized procedures (14). BMI was calculated as weight (kg)/height squared (m). Body fat mass and fat-free mass were measured by dual-energy X-ray absorptiometry (DEXA) with a Lunar DPX-L densitometer (Lunar, Madison, WI).

Dietary evaluation

During each evaluation period, the patients were interviewed by a dietitian, and a 3-d record that corresponded to 2 weekdays

### TABLE 1

<table>
<thead>
<tr>
<th>Nutrient</th>
<th>Standard supplement</th>
<th>Improved supplement</th>
</tr>
</thead>
<tbody>
<tr>
<td>Calcium (mg)</td>
<td>640</td>
<td>1000</td>
</tr>
<tr>
<td>Zinc (mg)</td>
<td>7.5</td>
<td>15</td>
</tr>
<tr>
<td>Iron (mg)</td>
<td>—</td>
<td>18</td>
</tr>
<tr>
<td>Copper (μg)</td>
<td>1000</td>
<td>900</td>
</tr>
<tr>
<td>Selenium (μg)</td>
<td>15</td>
<td>55</td>
</tr>
<tr>
<td>Manganese (mg)</td>
<td>1.5</td>
<td>—</td>
</tr>
<tr>
<td>β-Carotene (mg)</td>
<td>3</td>
<td>3</td>
</tr>
<tr>
<td>Vitamin C (mg)</td>
<td>250</td>
<td>100</td>
</tr>
<tr>
<td>Vitamin E (mg)</td>
<td>200</td>
<td>200</td>
</tr>
<tr>
<td>Folic acid (μg)</td>
<td>—</td>
<td>400</td>
</tr>
<tr>
<td>Vitamin A (μg)</td>
<td>—</td>
<td>200</td>
</tr>
<tr>
<td>Vitamin D (IU)</td>
<td>250</td>
<td>800</td>
</tr>
</tbody>
</table>
and 1 weekend day was completed. The data registered was analyzed by using a computer program (Food Processor II; ESHA Research, Salem, OR) to calculate energy and nutrient intakes by using a database that contained locally generated nutrient composition data as well as information from the literature (15).

Iron-absorption tests

On day 1 of the study, the subjects were fed 100 g of the standard liquid diet used during the first month after surgery, which was labeled with 111 kBq $^{55}$Fe. The iron content was adjusted to provide $\approx 3$ mg Fe. A dose of ferrous ascorbate labeled with 37 kBq $^{59}$Fe was administered on day 2, which contained 3 mg elemental Fe (molar ratio of iron to ascorbic acid: 1:2). On day 14, a fasting blood sample (30 mL) was obtained by antecubital puncture. Ten milliliters were used to assess iron-status indexes as indicated below. Circulating iron radioactivity (cpm/mL) was measured in the remaining 20 mL by using a liquid scintillation counter (Beckman LS-5000 TD; Beckman Instruments, Fullerton, CA) according to the double-isotope method of Eakins and Brown (16). The percentage of iron absorbed was calculated based on blood volume, which was estimated by using the Tulane tables (17) and assuming an incorporation of 80% of the radioactive iron into red blood cells (18). Because the blood volume in obese subjects is low in relation to actual body weight, mainly because the amount of blood in adipose tissue is lower than in lean tissue, the fraction of the excess weight was used to calculate weight-normalized blood volume (19). For this purpose we estimated blood volume by using the following formula:

$$\text{Blood volume} = \text{volume } A + \left[ \left( \text{volume } B - \text{volume } A \right) \times 0.20 \right] \quad (1)$$

where volume $A$ was estimated by using ideal weight, and volume $B$ was estimated by using actual weight. Ideal weight was calculated by using the method of Hamwi (20).

Hematologic evaluation

Hemoglobin and mean cell volume (MCV) were assessed by using a Coulter counter (CELL-DYN 1700; Abbott Diagnostics, Abbott Park, IL). Serum iron, total-iron-binding capacity, and transferrin saturation (TS) were determined by the method of Fischer and Price (21). Zinc protoporphyrin (ZPP) was measured by using a ZP Hematofluorometer (model 206D; AVIV Biomedical Inc, Lakewood, NJ). Serum ferritin (SF) was assessed by using the method suggested by the International Anemia Consultative Group (22). All women with hemoglobin concentrations $<12.0$ g/dL were classified as having anemia. Those who had normal hemoglobin concentrations with $\geq 2$ abnormal biochemical measurements of iron status (MCV $<80$ FL, ZPP $>70$ $\mu g/\text{dL}$ red blood cells, TS $<15\%$, or SF $<12$ $\mu g/\text{L}$) were classified as having iron deficiency. Iron deficiency anemia was defined as a hemoglobin concentration $<12.0$ g/dL plus $\geq 2$ abnormal biochemical measurements of iron status.

Compliance

A member of our team (JC) provided a new container with a known number of vitamin and mineral pills at the beginning of every month throughout the study. At the time of distribution, the number of remaining pills from the previous month was counted. Compliance was assessed by comparing the total number of pills provided and those consumed during any given period.

Statistical analyses

Two-factor repeated-measures analysis of variance with treatment groups as a between-subjects factor and time as a within-subjects factor, followed by a Bonferroni test for multiple comparisons, regression and correlation analyses, and the McNemar test (23), were performed by using the SPSS 15.0 statistical software (SPSS Inc, Chicago, IL). Because SF concentrations and iron-absorption data have skewed distributions, the values were converted to logarithms before the statistical analysis was performed. The results were then retransformed into antilogarithms to recover the original units and are expressed as geometric means and ranges of $\pm 1$ SD or $\pm 1$ SE.

RESULTS

Compliance, defined as the number of vitamin and mineral pills consumed related to the total provided, was 81.1 $\pm 16.2\%$ (median: 84.2%) between 0 and 6 mo, 86.6 $\pm 11.5\%$ (median: 88.6%) between 6 and 12 mo, and 83.6 $\pm 13.0\%$ (median: 87.4%) between 12 and 18 mo of the study.

Main anthropometric changes and dietary information of selected nutrients are shown in Table 2. Six months after gastric bypass, body weight and fat mass had decreased by 27.9% and 37.8%, respectively. This trend continued until 12 mo after surgery, after which it remained stable. The dietary intake of energy, protein, and iron showed a U-shape trend. Mean supplemental iron intake during the 0–6, 6–12, and 12–18-mo periods was 10.2 $\pm 17.4$ mg/d (range: 0–105 mg/d), 17.2 $\pm 29.9$ mg/d (range: 0–122.7 mg/d), and 21.7 $\pm 37.6$ mg/d (range: 0–192.5 mg/d), respectively.

A 2-factor repeated-measures ANOVA was conducted to test the effects of time and type of supplementation (standard compared with improved) on a series of variables, such as hemoglobin, MCV, ZPP, TS, SF, and iron absorption. The type of supplementation had no effect on any of the variables. Thus, further analyses are presented and discussed considering both groups combined.

The subject’s iron-status indexes during the study are shown in Table 3 and Table 4. A significant reduction in hemoglobin concentration was observed throughout the study. After 18 mo of gastric bypass, the mean hemoglobin concentration was 87.6% of the initial value. The percentage of anemic subjects changed from 1.5% at the beginning of the study to 38.8% at 18 mo. The prevalence of iron deficiency anemia was 23.9% at the end of the experimental period. SF concentrations also decreased during the study; the percentage of subjects with low iron stores increased from 7.5% at baseline to 37.3% 18 mo after surgery. Changes in MCV and TS were not significant. Changes in mean ZPP were less marked, although significant, than those of hemoglobin and SF. Nevertheless, the number of individuals with abnormal values doubled when comparing final and initial results.

Iron absorption from both a standard diet and from a standard dose of ferrous ascorbate decreased significantly (Figure 1) after...
6 mo of the gastric bypass, representing 32.7% and 40.3% of their initial values, respectively. No further significant modifications were noted beyond this time point.

**DISCUSSION**

Micronutrient deficiencies after restrictive-malabsorptive procedures, such as biliopancreatic diversion and Roux en-Y gastric bypass (RYGBP), have commonly been reported (6–9, 13, 24). Biliopancreatic diversion is rarely used now. Whereas RYGBP is associated with micronutrient deficiencies, it continues to be widely used because of its greater effectiveness in terms of maintained weight loss after long periods of time compared with restrictive procedures, such as adjustable gastric banding (3). Iron deficiency has been found in 15–60% of the patients (9). This condition is present even after routine iron supplementation (25). In our study, after 18 mo of gastric bypass, more than one-third of the subjects became anemic. Of these, almost 60% were classified as having iron deficiency anemia. That means that although iron deficiency is the major determinant of anemia in these individuals, it is not exclusive, and deficiency of other nutrients involved in red blood cell synthesis may be present. Individuals with low or absent iron stores were also close to 40% at the end of the experimental period. Thus, despite our subjects having been prescribed a wide range of supplemental iron during each study period, it was insufficient to prevent the development of iron deficiency in a significant number of them.

At least 3 major factors can determine iron status in patient undergoing gastric bypass: reduced iron intake, reduced gastric capacity, and reduced absorptive surface area. As a result of the reduction in gastric capacity, diet volume is also reduced. In our study total dietary iron was significantly reduced after 6 and 12 mo but not after 18 mo of the surgery. The reduction in meat consumption after gastric bypass may be especially important. Low tolerance to red meat seems to be frequent in these patients (6). The consequence is less highly available iron in the diet. This situation was also observed in the present study. Our subjects consumed an average of 82.8, 44.5, 47.2, and 55.0 g meat/d before and 6, 12, and 18 mo after gastric bypass, respectively. On the other hand, chicken, turkey, and fish consumption did not change during the study (data not shown).

Second, surgery resulted in reduced gastric capacity and gastric juice volume. This condition is marked in RYGBP (4). The size of the gastric pouch left after gastric bypass is 15–20 mL. This has an important effect on iron digestion because gastric juice allows not only for iron release from its protein matrix but also solubilization and ionization of dietary iron (26). It also contributes to maintain iron on its reduced form, which is the form taken up by its transporter DMT1.

Third, surgery results in a reduction in the intestinal absorption surface and consequently in less absorption capacity. It should be noted that the length of the loop in the standard RYGBP in the United States is less than the 125–150 cm used in this study. Nevertheless, such a difference would not be a critical issue in patients with a BMI <50 according to Brolin’s observations, who reported that the Roux limb—in the range of 150 to 200 cm—does not result in more frequent nutritional sequelae compared with shorter Roux limb procedures (27). Iron incorporation to

**TABLE 2**

Anthropometric characteristics and dietary intakes of obese women before and after Roux-en-Y gastric bypass

<table>
<thead>
<tr>
<th></th>
<th>Month 0 (n = 67)</th>
<th>Month 6 (n = 58)</th>
<th>Month 12 (n = 56)</th>
<th>Month 18 (n = 51)</th>
<th>P2</th>
</tr>
</thead>
<tbody>
<tr>
<td>Weight (kg)</td>
<td>115.1 ± 15.6a</td>
<td>82.9 ± 11.7b</td>
<td>75.5 ± 10.8b</td>
<td>74.5 ± 11.5b</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>45.2 ± 4.7a</td>
<td>32.7 ± 4.0b</td>
<td>29.8 ± 4.1b</td>
<td>29.1 ± 4.2b</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Fat mass (%)</td>
<td>47.7 ± 4.6a</td>
<td>40.4 ± 6.8b</td>
<td>35.3 ± 7.4b</td>
<td>34.1 ± 8.4b</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Fat mass (kg)</td>
<td>54.5 ± 10.5a</td>
<td>33.9 ± 9.6b</td>
<td>27.3 ± 8.8b</td>
<td>26.0 ± 9.5b</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Energy intake (kcal/d)</td>
<td>1675</td>
<td>888</td>
<td>1031</td>
<td>1178</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Protein intake (g/d)</td>
<td>69.1 ± 21.4a</td>
<td>43.9 ± 16.2b</td>
<td>51.6 ± 15.7bc</td>
<td>54.5 ± 18.9b</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Iron intake (mg/d)</td>
<td>9.4 ± 4.8a</td>
<td>5.8 ± 2.6b</td>
<td>7.0 ± 2.5bc</td>
<td>8.3 ± 3.7b</td>
<td>&lt;0.0001</td>
</tr>
</tbody>
</table>

1 All values are means ± SDs. Values in a row with different superscript letters are significantly different, P < 0.05 (Bonferroni’s correction for multiple comparisons).
2 Repeated-measures ANOVA.
3 Iron intake exclusively from the diet.

**TABLE 3**

Iron-status indexes in obese women before and after Roux-en-Y gastric bypass

<table>
<thead>
<tr>
<th></th>
<th>Month 0 (n = 67)</th>
<th>Month 6 (n = 58)</th>
<th>Month 12 (n = 56)</th>
<th>Month 18 (n = 51)</th>
<th>P2</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hemoglobin (g/dL)</td>
<td>13.7 ± 0.9a</td>
<td>13.0 ± 1.1b</td>
<td>12.4 ± 1.0f</td>
<td>12.0 ± 0.9d</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Mean cell volume (fL)</td>
<td>86.2 ± 4.9</td>
<td>86.0 ± 4.8</td>
<td>86.1 ± 4.1</td>
<td>85.4 ± 5.4</td>
<td>NS</td>
</tr>
<tr>
<td>Zinc protoporphyrin (µg/D RBCs)</td>
<td>61.1 ± 19.6c</td>
<td>65.1 ± 24.5b</td>
<td>68.3 ± 20.4b</td>
<td>70.6 ± 23.4b</td>
<td>&lt;0.010</td>
</tr>
<tr>
<td>Transferrin saturation (%)</td>
<td>23.1 ± 7.8</td>
<td>25.4 ± 10.0</td>
<td>23.9 ± 10.6</td>
<td>24.0 ± 13.0</td>
<td>NS</td>
</tr>
<tr>
<td>Serum ferritin (µg/L)</td>
<td>37.5</td>
<td>28.7</td>
<td>19.4</td>
<td>13.4</td>
<td>&lt;0.0001</td>
</tr>
</tbody>
</table>

1 All values are means ± SDs, except where otherwise indicated. RBCs, red blood cells. Values in a row with different superscript letters are significantly different, P < 0.05 (Bonferroni’s correction for multiple comparisons).
2 Repeated-measures ANOVA.
3 Values are geometric mean ± 1 SD; range in parentheses.
the organism takes place at its greatest extent at the duodenum and initial portions of the jejunum, precisely the bypassed portions (28, 29). Iron-absorption studies in individuals undergoing bariatric surgery are limited. For instance, Stucki (30) reported reduced iron absorption in 7 subjects after bilipancreatic bypass. Rhode et al (10) evaluated patients after gastric bypass by measuring the change in plasma iron concentrations after a 50-mg dose of ferrous gluconate as a measure of iron absorption. This dose is well above physiologic conditions, however. In a recent report, we described a subject with persistent anemia 2 y after a RYGBP who did not respond to oral iron therapy. Despite a hemoglobin concentration of 9.6 g/dL, her iron absorption from a standard ferrous ascorbate dose was only 48%, which suggested a limited capacity of the intestine to respond. This subject improved her condition only after intravenous iron administration (31).

We assessed iron absorption using a widely accepted isotopic methodology (32, 33). Iron absorption from a standard test meal and from a standard dose of ferrous ascorbate was markedly limited, because iron absorption from both a standard meal and from standard ferrous ascorbate did not increase as would have been expected in individuals with reduced iron status who have an intact small intestine (43). In conclusion, we found that iron deficiency and iron deficiency anemia affect many patients after RYGBP. According to our data, an average supplemental iron intake of ≈20 mg/d is largely insufficient to prevent iron deficiency and iron deficiency as preservation of the signal system related to iron stores and iron absorption in the remaining sections of the intestine after RYGBP. Nevertheless, in quantitative terms its response is limited, because iron absorption from both a standard meal and from standard ferrous ascorbate did not increase as would have been expected in individuals with reduced iron status who have an intact small intestine (43).

In our study we found a negative correlation between SF and absorption capacity from a standard diet ($r = -0.58$, $P < 0.01$) and from a standard ferrous ascorbate solution ($r = -0.69$, $P < 0.001$) after 18 mo of gastric bypass, which might be interpreted as preservation of the signal system related to iron stores and iron absorption in the remaining sections of the intestine after RYGBP.
REFERENCES


