Acute hormonal and immune responses after a bi-set strength training

Denis Foschini1,2
denisfoschini@gmail.com
Jonato Prestes1,3
jonatop@gmail.com

1 Faculdade de Fisioterapia e Educação Física
2 Universidade Metodista de São Paulo/SP
3 Faculdade de Ciências da Saúde

ABSTRACT: The purpose of this study was to investigate the effects of strength training on immune cells, testosterone and cortisol by the alteration in the order of the exercises and rest interval. 9 males were accomplished, with 22.2 ± 1.9 years old and a minimal experience of 12 months in strength training. The multiples sets and bi-set method were accomplished. The samples were collected before and immediately after exercise. There was a significant increase in cortisol levels, total leukocytes, neutrophils, lymphocytes and monocytes. There were no alterations in testosterone and testosterone/cortisol ratio. The alteration in the exercise order and duration of interval modified the immune cells response and increased cortisol levels, constituting a differentiated stimulus for the individuals.

Keywords: strength training; immune system; hormones.

Correspondence to:
Rua Luis da Silva Leite, 85, Vila Formosa, São Paulo, SP, CEP 0.3377-070.

Submitted: September / 2007 Accepted: November / 2007

INTRODUCTION

The adaptive mechanisms and effects, especially hormonal and immunological of the strength training still need to be systematically elucidated. This exercise type is dependent of the variation of some factors: the specific exercise movement, the used load, the duration of the repetitions, interval between the series and exercises, type and speed of the muscular action and order of the exercises. Differences in the duration of the recovery interval between the series influence the neuroendocrine and immunological answers to the strength training¹. Mayhew et al.² analyzed apprentices of strength training that accomplished a session of 10 series with 10 repetitions at 65% of 1RM in the leg press exercise, using a recovery interval of 1 minute and 3 minutes after the series. The authors verified that shorter recovery intervals promoted more pronounced leukocytosis and higher elevation in the circulating lymphocytes, monocytes and neutrophils 3, when compared with more lingering intervals. The strength training with short recovery intervals (60 seconds) induces more significant increases in the catecholamine, cortisol, testosterone and growth hormone (GH), that are agents that causes the redistribution of the leukocytes during and after the exercise⁴.

Training sessions with enough intensity and volume can elevate the acute answer of the testosterone and of the cortisol⁵. Schwab et al.⁶ registered significant elevations in the testosterone front at one of them, that incorporated forced repetitions; however, the cortisol presented higher elevation in the most intense protocol. It was demonstrated that 6 series of 1RM in the crouching with 2 minutes of interval significantly increased the serum cortisol concentrations⁷. Differently of the testosterone that causes Catholic functions as the lipolysis, protein degradation and reduction in the protein synthesis in the muscular cells⁸.

In this sense, few works investigated the hormonal and immunological answers for the alteration in the order of the exercises or for the accomplishment of bi-set exercises (two exercises in sequence without rest). Being like this, the objective of this study was to analyze the acute effect of a strength training session on the cells of the immune system, cortisol and testosterone, altering the exercises order, type and time of interval between the series in trained individuals.

MATERIALS AND METHODS

Selection of the Subjects

The criterion adopted for inclusion of the volunteers in the study had as prerequisites: to be male, minimum experience of 12 months in strenght training, accomplishing the protocol with objective of muscular hypertrophy (Intensity between 6 to 12 maximum repetitions) and to be using the same exercises selected for the study, however in different order (Table 1). The individuals were excluded with known history of cardiovascular and respiratory

<table>
<thead>
<tr>
<th>Variables</th>
<th>X</th>
<th>SD</th>
<th>min</th>
<th>max</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total Body Mass (Kg)</td>
<td>77.8</td>
<td>12.3</td>
<td>60.6</td>
<td>106.5</td>
</tr>
<tr>
<td>Height (cm)</td>
<td>175.9</td>
<td>5.5</td>
<td>165</td>
<td>185</td>
</tr>
<tr>
<td>Age (anos)</td>
<td>22.2</td>
<td>1.9</td>
<td>20</td>
<td>25</td>
</tr>
<tr>
<td>Fat %</td>
<td>10.6</td>
<td>4.7</td>
<td>4.7</td>
<td>19.1</td>
</tr>
<tr>
<td>Fat Mass (Kg)</td>
<td>8.9</td>
<td>5.2</td>
<td>2.8</td>
<td>20.4</td>
</tr>
<tr>
<td>Thin Mass (Kg)</td>
<td>69.8</td>
<td>8</td>
<td>57.8</td>
<td>86.1</td>
</tr>
</tbody>
</table>

The values are expressed on mean ± DP. Sample mean (X), standard deviation (sd), minimum value (min) and maximum value (max).

Palavras-chave: Treinamento de força, sistema imune, hormônios..
diseases, diabetes, hypertension, hormonal disorder, muscular lesion (last 12 months), besides those that were administering or had administered medication or supplements in the 6 months that preceded the beginning of the study. However, were selected 9 individuals seemingly healthy. The participants’ anthropometric characteristics are presented in the table 1. The experiment was approved by the Committee of Ethics in Research of the Universidade Metodista de São Paulo. ZIP CODE - UMESP (074709/05), being in agreement with the Helsinki declaration. All the participants were informed in full detail about the used procedures and they agreed in participating in voluntary way to the study, signing term of informed consent.

**Nutritional recommendations**

The participants of the study received a individualized breakfast in agreement with their body composition, guided by a qualified professional, being offered the macronutrients by the amount in g / Kgr in the percentages of macronutrients established by American College of Sports Medicine and Dietitians Canada Joint Position Statement ².

**Test of maximum force (1RM)**

One day after the anthropometric evaluations, the tests of 1 maximum repetition were accomplished (1RM). After the general warming (10 minutes of walk in rolling mat), the individuals executed a series of specific warming of eight repetitions approximately the 50% of the estimated 1-RM (in agreement with the load that the participants accomplished before the beginning of the study). Afterwards for other series of three repetitions at 70% of the estimated 1-RM for the first exercises of the sequence (fly with dumbbells and thread hammer - (fly with halters e hammer washer), the test was accomplished for all of the exercises, following the same order of the training session (sloping fly with dumbbells, hammer washer, supine sloping, scott washer, supine straight and direct washer - (inclined fly with halters, hammer washer, inclined supine, scott washer, straight supine e direct washer). The subsequent liftings were simple repetitions with progressively heavier loads. Was repeated the test until that the 1-RM was certain. The rest interval between the series was of three minutes and the number of attempts to the determination of the maximum load was of three, following the descriptions of Matuszak et al.¹° . All of the procedures for determination of the dynamic maximum strenth, besides the standardization of the angulation of movements followed the descriptions of Brown and Weir¹¹ . 2 exercises a day were selected, being these test sessions separated by 48 hr, to avoid influences in the determination of the maximum loads (in the week of the tests the participants didn’t accomplish any type of physical exercise).

**Training protocol**

The protocol followed the recommendations of American College of Sports Medicine¹². The strength training with multiple series (method that uses more than a series for muscular group) and in bi-set (consists of accomplishing two exercises without passive “stopped” interval). The session was accomplished 72 h after the strength tests. Three exercises were accomplished for horizontal adduction of shoulders, and three exercises for elbows flexing. All the participants used in their training program the exercises selected for the protocol, however the method and the order of the exercises were different. For horizontal adduction of shoulders the exercises were: straight supine, sloping supine and sloping fly with dumbbells-halters and for elbows flexing the exercises were: direct washer, scott washer and hammer washer. In all of the exercises, 3 series were accomplished looking for the largest possible number of repetitions at the 70% of the calculated 1RM, individually, starting from the test of maximum strength. The table 2 presents how the exercises were combined, being the volunteers guided to accomplish each combination without “passive” interval between the series (total rest - “stopped”). For instance, in the combination 1 the sloping fly was accomplished with dumbbells-halters, when finishing, the individual accomplished the hammer thread, afterwards went back to the sloping fly. The procedure was accomplished 3 times in each combination and then the individual went for the execution of the next combination. That procedure was adopted for the three combinations.

**Sanguine collections**

Before beginning the training session, the individuals were submitted to absolute rest for 30 minutes, afterwards was accomplished the first collection of blood, where 20 ml of blood were collected. The same amount of blood was collected immediately after the end of the training session. At the times of 24 and 48hr after the end of the training session 10 ml of blood were collected. After the collections, the blood was centrifuged by 10 minutes at 5000g and stored at - 20°C. The materials used in the collection were all discarded, appropriately labeled and of recognized quality. A qualified technician accomplished the blood collection. The analyses were accomplished in the Laboratory of Clinical Analyses of the Universidade Metodista de São Paulo (LABMESP).

**Table 2. Exercises Combinations.**

<table>
<thead>
<tr>
<th>Combination 1</th>
<th>Combination 2</th>
<th>Combination 3</th>
</tr>
</thead>
<tbody>
<tr>
<td>Horizontal adduction of shoulder</td>
<td>Elbow flexing</td>
<td></td>
</tr>
<tr>
<td>Inclined Fly with halters</td>
<td>Hammer washer</td>
<td>Direct Washer</td>
</tr>
<tr>
<td>Sloping Fly with dumbbells</td>
<td>Direct Washer</td>
<td></td>
</tr>
<tr>
<td>Inclined Supine</td>
<td>Scott washer</td>
<td></td>
</tr>
<tr>
<td>Supine sloping Supine</td>
<td>Scott washer</td>
<td></td>
</tr>
<tr>
<td>Supine Straight</td>
<td>Direct Washer</td>
<td></td>
</tr>
<tr>
<td>Supine Straight</td>
<td>Direct Washer</td>
<td></td>
</tr>
</tbody>
</table>

40 Fit Perf J, Rio de Janeiro, 6, 1, 40, Jan/Feb 2007
**Determination of the subjective perception of pain**

The subjects were guided to classify the muscular pain “Before”, immediately “After”, “24hr” and “48hr” after the training session. It was given to the individuals a sheet with a 10 cm line that represents a subjective scale of pain perception. In that scale of analog visual (VAS, Visual Analog Scale) (Illustration 1), one of the extremities (0 cm) contains a verbal expression, “little pain” and the other extremity13 “a lot of pain” (10 cm). The subjects marked a place in that line that evidenced the pain that they were feeling after “palpation” in the area of the larger breastplate, brachial and brachial biceps, requested in the training. The distance in centimeters of the extremity 0 cm to the suitable point for the subject was measured with a scale and considered as the pain measure.

**Statistical analysis**

Parametric and non-parametric tests were set following the recommendations of Diggle et al.14. To analyze dependent data in two and four times, the Generalized estimate equations (GEE) was adopted, with gamma and multinomial distribution (non parametric). That equation analyzes the longitudinal data in different distribution types and compares between the times14. The SPSS for Windows version 11.0 statistical package was used.

**RESULTS**

The mean for the serum concentration of the testosterone variable in the analysis accomplished “Before” the intervention was 14.7 + 4.7 nmol / L and immediately “After” it was 16.5 + 22.8 nmol / L (table 3). Those data demonstrate that the testosterone concentration did not alter with the intervention when analyzed by Odds Ratio (OR) or chances Reason (p = 0.336). The mean serum concentration of cortisol “Before” the intervention was 343.2 + 115.5 nmol / L and “After” 516.8 + 181.6 nmol / L (table 3). Those values represent significant increase of 51% (p = 0.001). The T/C reason did not alter with the intervention where the mean of the T/C reason “Before” the intervention was 0.048 + 0.026 and immediately “After” the intervention was verified 0.035 + 0.017 of reason between the serum testosterone and cortisol concentrations (table 3).

There was increase of 39% in the number of circulating leukocytes (p < 0.05) “After” the intervention (9585.6 + 2017.6 mm$^3$) in relation to the time “Before” of the intervention (6888.9 + 1199.3 mm$^3$) (table 4). The number of circulating neutrophils was 28% higher in the time “After” the intervention (4683.9 + 2038.7 mm$^3$) in relation to the value found “Before” the accomplishment of the protocol (3663.8 + 1371.3 mm$^3$) (table 4).

The lymphocytes went to the cells that suffered larger influence of the protocol adopted in the present study. There was increase of 77% of the number of circulating lymphocytes in the time “After” the intervention (p < 0.05). “Before” the beginning of the (table 4) training session the mean was of 2080.3 + 627.2 mm$^3$, increasing to 3678.4 + 809.4 mm$^3$ immediately “After”.

Regarding the monocytes number, increase of 57% was observed in the time “After” the intervention (p < 0.05) when compared to the time “Before” the training (769.7 + 187.1 mm3 “Before” and 1209.8 + 351.9 mm3 “After”) (table 4).

**DISCUSSION**

This was the first study to evaluate the modifications on the hormonal and immune answers front to the bi-set training method, and also the manipulation of the acute variables of the training (alteration in the order of the exercises and manipulation in the type and time of interval between the series).

The strenght training can modulate the serum concentrations of the testosterone hormone in men6. The elevations found in the total testosterone can be attributed to the reduction in the plasmatic volume (influx of fluids for the sarcoplasm of the active muscular cell), adrenergic stimulation15, secretion stimulated by the lactate16 and potential adaptations in the testosterone synthesis and/or secretory capacity of the Leydig cells17. However, other studies did not evidence changes in the testosterone concentrations7,18.

In the present work, it was not found significant change in the total serum concentration of testosterone, in the studied individuals (table 3). In similar way, Ratamess et al.7 did not find alterations in the circulating testosterone after a protocol with 1 series of 10 repetitions, at 80-85% of 1RM in the crouching exercise. Häkkinen and Pakarinen4 did not also observe alterations in the total testosterone with 5 series of 10 repetitions in the crouching in women. Ahtiainen et al.6 did not register any change in the acute answer of the testosterone between 2 different protocols of similar number of repetitions, but with intensity lightly higher in one of the protocols.

In contradiction, Kraemer et al.19, registered significant increases in the testosterone after protocol with smaller intensity (50% of 1 RM) and with 5 series from 15 to 20 repetitions in the c. It was demonstrated that characteristic programs of the bodybuilding (moderate loads and high volumes) produce higher answers in the testosterone concentration than more intense trainings20.

Possibly the reduced duration of the training session of the present study, total of 25 minutes, have not been enough to alter the acute answer of this hormone, while, the works that found alterations in the testosterone used a superior duration period compared to the current (between 45 minutes and 1 hour, on average)21,22.
The cortisol stimulates the lipolysis, increases the protein degradation, and it still reduces the proteins synthesis. Due to its important role in the tissue remodeling, acute and chronic alterations of the cortisol during the strength training are frequently examined. A acute session of strength training can induce significant elevations of the cortisol and of the adrenocorticotropic hormone (ACTH), with similar answers between men and women. The reason between testosterone and cortisol T/C has been responsible factor for a 30% decrease in the T/C reason.

In agreement, Banfi et al. investigated the behavior of the T/C reason in the case the decrease of the reason T/C is higher than 30%, the individual can be in a situation of fatigue and/or an incomplete recovery, characterizing a catabolism condition, and harming the adaptive answer to the trainings.

In the present study it was not found significant difference in the T/C reason. The fact of the testosterone was not altered, it might have put upon the significant increase of the cortisol. In function of the increase of the intensity and maintenance of the training volume in the current study, it is reasonable to imagine that the volume of the training is more important than the intensity to unchain alteration in the T/C reason, in apprentices of force training.

In agreement, Banfi et al. investigated the behavior of the T/C reason in sprint skaters that intensely training, and they verified that the abrupt increase in the training volume was the main responsible factor for a 30% decrease in the T/C reason.

Uchida et al. did not find alteration in the T/C reason after a strength training session in trained women, in that case there was 8-12 RMs in each one of the series. Studies that used number of series and repetitions similar to the present protocol verified increase in the plasma’s cortisol concentration.

The reason between testosterone and cortisol T/C has been used either as indicative of adaptation or to establish the training overload. The reason between these two hormones can be a parameter of the relationship of muscular anabolism / catabolism. According to Vervoorn et al. and Banfi et al. in the case the decrease of the reason T/C is higher than 30%, the individual can be in a situation of fatigue and/or an incomplete recovery, characterizing a catabolism condition, and harming the adaptive answer to the trainings.

In the present work, significant increase of 51% (table 3) was demonstrated in the concentration of the serum cortisol front to the applied strength training session. Tends in view that, in this protocol the recovery interval was active (with the accomplishment of another strength exercise, combined series); and stayed at the most for 1 minute until that was again accomplished another series in the same exercise, it can be suggested that this interval was sufficiently intense to provoke the alterations observed in the serum cortisol concentration, as well as it was detected in the works above that used between 1 minute and 1 and a half minute of recovery. As well as, Kraemer et al., that registered that the accomplishment of 8 series of 10RM in the leg press exercise with 1 minute of interval, increased more significantly the acute answer of the cortisol, than the same protocol with rest period of 3 minutes.

Another factor that might have led to the increase of the cortisol in the present study, was the number of accomplished series, 18 in the total and also the number of repetitions that were between 8-12 RMs in each one of the series. Studies that used number of series and repetitions similar to the present protocol verified increase in the plasma’s cortisol concentration.

### Table 3. Comparison of the testosterone and cortisol concentrations, and the T/C reason between the times: “Before” and “After” the intervention (n=9).

<table>
<thead>
<tr>
<th>Variables</th>
<th>X</th>
<th>dp</th>
<th>min</th>
<th>max</th>
<th>perc 25</th>
<th>perc 50</th>
<th>perc 75</th>
</tr>
</thead>
<tbody>
<tr>
<td>Testosterone Before (nmol/L)</td>
<td>14.7</td>
<td>4.7</td>
<td>9.4</td>
<td>24</td>
<td>11</td>
<td>12.5</td>
<td>18.2</td>
</tr>
<tr>
<td>Testosterone After (nmol/L)</td>
<td>16.5</td>
<td>5.2</td>
<td>6.5</td>
<td>22.8</td>
<td>13.6</td>
<td>16.1</td>
<td>21.9</td>
</tr>
<tr>
<td>Cortisol Before (nmol/L)</td>
<td>343.2</td>
<td>115.5</td>
<td>148.9</td>
<td>508.9</td>
<td>259.5</td>
<td>340.8</td>
<td>442.1</td>
</tr>
<tr>
<td>Cortisol After (nmol/L)</td>
<td>516.8*</td>
<td>181.6</td>
<td>216.7</td>
<td>750.6</td>
<td>411</td>
<td>456.4</td>
<td>719.9</td>
</tr>
<tr>
<td>T/C Reason Before</td>
<td>0.048</td>
<td>0.026</td>
<td>0.025</td>
<td>0.104</td>
<td>0.03</td>
<td>0.039</td>
<td>0.064</td>
</tr>
<tr>
<td>T/C Reason After</td>
<td>0.035</td>
<td>0.017</td>
<td>0.016</td>
<td>0.071</td>
<td>0.021</td>
<td>0.031</td>
<td>0.044</td>
</tr>
</tbody>
</table>

The data presented in the table display the sample mean (X), standard deviation (sd), minimum value (min), maximum value (max), 25 percentile, 50 percentile and 75 percentile. *Statistically significant difference of the concentration of the cortisol variable in the time “After” in relation to the time “Before.” Analyze for Odds Ratio (OR) or Chances Reason (p < 0.05).

### Table 4. Leukocytes (mm3), neutrophils (mm3), lymphocytes (mm3) and monocytes (mm3) number, in the times “Before” and “After” the intervention (n=9).

<table>
<thead>
<tr>
<th>Variables</th>
<th>X</th>
<th>dp</th>
<th>min</th>
<th>max</th>
<th>perc 25</th>
<th>perc 50</th>
<th>perc 75</th>
</tr>
</thead>
<tbody>
<tr>
<td>Leukocytes Before</td>
<td>6888.9</td>
<td>1199.3</td>
<td>5300</td>
<td>8630</td>
<td>5720</td>
<td>6850</td>
<td>8040</td>
</tr>
<tr>
<td>Leukocytes After</td>
<td>9585.6*</td>
<td>2017.6</td>
<td>7700</td>
<td>14090</td>
<td>7945</td>
<td>8950</td>
<td>10455</td>
</tr>
<tr>
<td>Neutrophils Before</td>
<td>3663.8</td>
<td>1371.3</td>
<td>1695</td>
<td>6472</td>
<td>2813.5</td>
<td>3300</td>
<td>4409</td>
</tr>
<tr>
<td>Neutrophils After</td>
<td>4683.9*</td>
<td>2038.7</td>
<td>2860</td>
<td>9300</td>
<td>3164.5</td>
<td>4157</td>
<td>5537</td>
</tr>
<tr>
<td>Lymphocytes Before</td>
<td>2080.3</td>
<td>627.2</td>
<td>1122</td>
<td>3080</td>
<td>1663.5</td>
<td>2034</td>
<td>2564</td>
</tr>
<tr>
<td>Lymphocytes After</td>
<td>3678.4*</td>
<td>809.4</td>
<td>2239</td>
<td>4664</td>
<td>3067.5</td>
<td>3922</td>
<td>4330.5</td>
</tr>
<tr>
<td>Monocytes Before</td>
<td>769.7</td>
<td>187.1</td>
<td>530</td>
<td>1013</td>
<td>597.5</td>
<td>770</td>
<td>952</td>
</tr>
<tr>
<td>Monocytes After</td>
<td>1209.8*</td>
<td>351.9</td>
<td>618</td>
<td>1656</td>
<td>861.5</td>
<td>1318</td>
<td>1465.5</td>
</tr>
</tbody>
</table>

The data presented in the table display the sample mean (X), standard deviation (sd), minimum value (min), maximum value (max), 25 percentile, 50 percentile and 75 percentile. *Statistically significant difference of the concentration of the variable in the time “After” in relation to the time “Before.” Analyze for Odds Ratio (OR) or Chances Reason (p < 0.05).
not alteration in the total testosterone concentration, as well as of circulating cortisol. The interval between the series was of 90 seconds (passive interval). In the present study, in function of the adopted method (bi-set), probably the stress has been larger, once the individuals finished an exercise and if they went to another combination, executing it without passive interval.

The leukocytosis (increase in the number of circulating leukocytes) occurs in response to intense and of short duration physical exercises. The effects of this type of physical exercise on the increase in the number of circulating leukocytes are mediated, at least partly, for the activation of the sympathetic nervous system29 and acute increase of the serum catecholamine levels during the exercise30. The leukocytosis can linearly increase in agreement with the elevation of the exercise intensity31.

A higher catecholamine’s secretion can occur in strength training sessions that use smaller recovery intervals (< 1 minute) when compared to protocols that use larger recovery periods (> 2 minutes)24,32. Other responsible mechanisms for the increase in the leukocytes migration induced by the exercise include: cytokines concentration elevation, changes in the body temperature, hydration state and increase in the sanguine flow33.

During an acute session of strength training, leukocytosis can be observed, together with lymphocytosis (increase in the number of circulating lymphocytes), monocytes (increase in the number of circulating monocytes) and neutrocytosis (increase in the number of circulating neutrophils)34,35,36. This leukocytosis was also observed in a strength protocol accomplished in the leg press exercise, with 8 series of 10RMs at 70-90% of 1RM, in subjects trained in strength34.

The strength training protocol of this study induced significant acute increase of 39% in the total counting of leukocytes, 28% in the neutrophils and 77% in the lymphocytes after the strength training session (table 4). Mayhew et al.2 found similar results with individuals in strength that accomplished a protocol with 10 series of 10 repetitions at 65% of 1RM in the leg press exercise, using a recovery interval of 1 minute and 3 minutes. These researchers observed that shorter recovery intervals (1 minute) promoted more pronounced leukocytosis and higher elevations in the circulating lymphocytes, monocytes and neutrophils, when compared with longer intervals (3 minutes). The similarities between the two studies can be related at the used intensity, 70% in this study, and 65% in the study of Mayhew et al.2, but mainly in the recovery period that was practically the same, of 1 minute to 1 and a half minute.

**FINAL CONSIDERATIONS**

The alterations of the acute variables of the strength training as recovery interval between the series and the training method, can produce significant modifications in the cells of the immune system, promoting the increase in the total concentrations of circulating leukocytes, neutrophils, lymphocytes (in larger scale) and monocytes. They also increase the concentrations of circulating cortisol, indicating this way that the training promoted an differentiated incentive in trained individuals. These physiologic answers can indicate the intensity of the intern training load, because, the external load is commonly accompanied by the strength tests. The hormonal and immunological dosages can be constituted as important tools in the attendance of the training loads along the year, aiding in the prevention of the overtraining syndrome and stress symptoms due to the excessive loads.

The knowledge of the testosterone and cortisol concentrations and the behavior of the T/C reason after a training session, can aid the trainers, physiologists and physical educators in the choice of the ideal protocol for the population that looks to potentiate the performance, the muscular hypertrophy and, above all, to improve the life quality. However, the use of the T/C reason, as well as the immune parameters, as stress indicative imposed to the organism by the strength training demands caution, once its behavior still not totally clear, needing more studies for elucidation of the subject.

**REFERENCES**


