

# Lipid Profile Parameters under influence of Periodontitis Associated with Chronic Stress: An Animal Model Study

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## ABSTRACT

**Background:** The present study compared the effect of two chronic stress models associated with periodontitis induced in rats on partial lipid profile parameters.

**Materials & Methods:** Forty-eight rats were divided into 4 groups: physical stress+periodontitis, variable stress+periodontitis, periodontitis and control. Physical stress and variable stress occurred over 60 days. After the first ten days of the stress test, periodontitis was induced by ligature. After 60 days of experimentation, the animals underwent incision and visualization of the posterior vena cava, and blood punctures were performed under a vacuum. Impartial and trained examiners performed the analysis of the parameters: low-density lipoprotein, high density lipoprotein, triglycerides and cholesterol.

**Results:** The lipid parameters and cholesterol were significantly lower in the variable stress group than in the ligature and control groups. The physical stress group was not statistically different from the other groups. The triglyceride level was highest for the control group and statistically different from the levels in groups variable stress and physical stress. The physical stress group had the lowest triglyceride level, which was statistically different from that of the control group. There was no statistical difference between physical stress and variable stress with respect to low-density lipoprotein; a similar finding was obtained for ligature and control. The variable stress group had the lowest low-density lipoprotein level, which was statistically different from those of the ligature and control groups. High density lipoprotein levels showed no statistical differences between groups.

**Conclusion:** Despite the limitations of the methodology, it seems that the stress model variable associated with periodontitis improved lipid parameters in the study.

**Key Words:** Metabolism lipid, stress, periodontitis, rat.

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## Introduction

Although periodontal disease has been viewed as a local oral infection it seems that due to its pathologic mechanisms, periodontitis could affect the systemic

health. Therefore, researchers are trying to identify what cells, tissues and functions could be altered by development and progression of periodontal disease aiming at elucidating the possible relationship between periodontitis and systemic diseases such as diabetes,

respiratory and cardiac disorders<sup>1-2</sup>

Human lipidic profile is related to the metabolism of plasma lipoproteins, which are composed of lipids (cholesterol, phospholipids and triglycerides). Patients who demonstrate lipidic changes compose high risk groups for heart disease, which leads to serious consequences and negatively impact their quality of life<sup>3</sup>. Interestingly, lipidic profiles can be influenced by stress as firstly demonstrated by Selye<sup>4-5</sup> and confirmed by Fazel & Danesh<sup>6-8</sup>.

Iacopino & Cutler<sup>9</sup> reviewed the literature and reported that it was not clear whether the changes occurring in immune cell function associated with periodontitis could dysregulate lipid metabolism through processes involving pro-inflammatory cytokines. On the other hand, evaluation of lipidic markers in the blood demonstrated an association with periodontal status<sup>10</sup>. There are controversial findings in the literature, Machado et al.<sup>11</sup> did not find a significant association between lipidemia and the presence or severity of periodontitis. Moreover, Katz et al.<sup>12</sup> conducted a large cohort study of 10,590 Israeli military service men and women. Although no significant association was observed among the women, in the men, the presence of periodontal pockets was positively associated with higher cholesterol and low-density lipoprotein (LDL) blood levels.

In addition to its impact on lipidemia, stress has been

suggested as a risk factor for periodontal status<sup>13</sup>. Stress seems to alter the host immune-inflammatory response is closely associated with periodontal disease pathogenesis, especially in relation to cytokine production<sup>14</sup>. However, the reported results are still controversial<sup>15</sup>. In animals, demonstrated that groups with periodontitis show a more extensive accumulation of lipids in the aorta than in the non-periodontitis animals<sup>16</sup>.

Considering that stress has become a common systemic condition in adults and that chronic periodontitis is more prevalent, it would be reasonable to consider that they could act together. To verify whether this association occurs, we selected an outcome that is an important systemic parameter: the lipidic profile. Therefore, it was hypothesized that under chronic stress, rats with ligature-induced periodontitis would show an increase in the levels of cholesterol, triglycerides, very low density lipoprotein (VLDL) and high density lipoprotein (HDL). In the present study, the lipidic parameters of periodontitis rats that were under physical or variable stress were compared.

## Materials and Methods

### Experimental groups

For the present study, 48 adult Wistar rats (*Rattus Norvegicus*) of an average initial weight of 230 g were

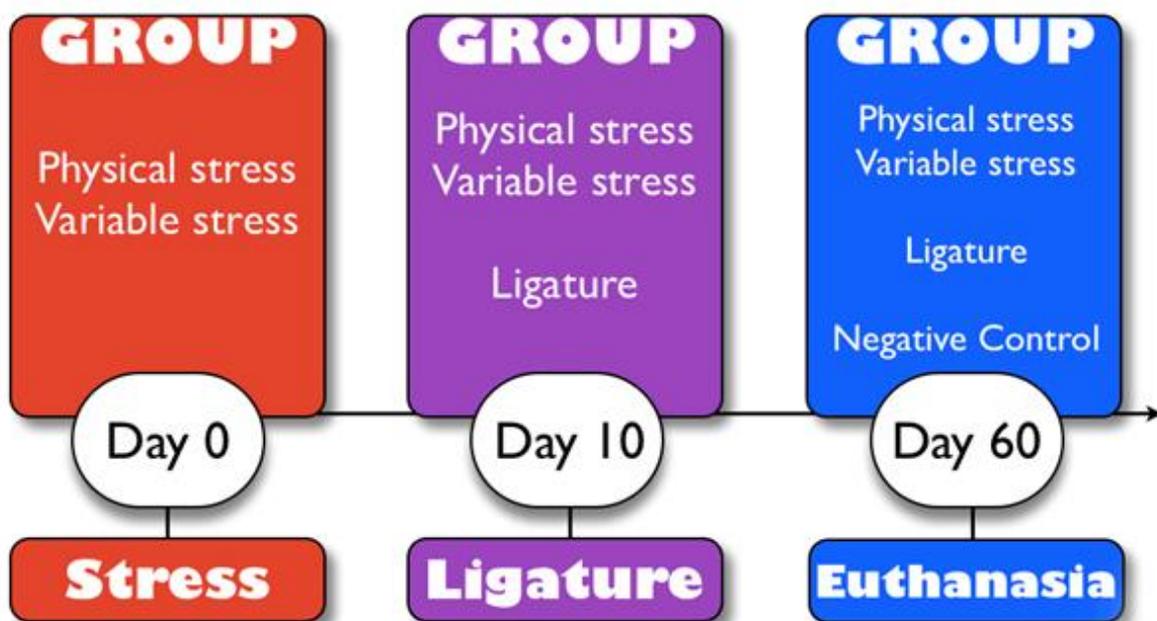


Fig. 1: Experimental procedures overtime in test and control group.

selected and allowed to adapt to the new environment for four weeks. Six animals were kept in each housing box (polyethylene 16x40x30) with standard feed and water *ad libitum* under a light/dark cycle of 12 hours that was temperature and humidity controlled at 23° C and + 40%, respectively. The experiment was approved and registered by the ethics committee in animal experimentation (2009-051).

Initially, animals were randomly divided (by a research assistant) into four experimental groups, described by the following:

1. PSP Group: physical stress + periodontitis (n=12).
2. VSP Group: variable stress + periodontitis (n=12).
3. PG Group: periodontitis (n=12).
4. CG Group: negative control (n=12).

After being exposed to, physical stress (PSP) or variable stress (VSP) for 10 days, animals in these groups underwent experimental induction of periodontal disease (described below) at this time. The periodontitis group also underwent experimental induction of periodontal disease; i.e in the first 10 days of the experiment, this group was not subjected to induction of periodontitis. Ligatures were kept in position until the end of the study (day 60).

The animals in the PSP and VSP groups were respectively exposed to physical stress and variable stress. After being exposed to stress for ten days, these groups went through the process of ligature induced periodontitis described below.

The animals in the PG group also went through the same process on the 10 day of the study, but were not exposed to stress within the initial 10 days. The ligatures remained in position until the end of the study (day 60). Finally, the negative control group did not receive any type of intervention; however, this group was maintained in the same environment as the other groups (Figure 1).

### Experimental periodontal disease

To induce periodontal disease in groups, the physical stress, variable stress and periodontitisligature animals received general anesthesia through intramuscular administration of 0.1 ml ketamine hydrochloride (Dopalen, AgribRANDS. Saúde Animal, Paulínia, SP,

Brazil) per each 10 g of body weight.

After anesthesia, a #4 sterile silk suture thread was wrapped around the second upper right molar<sup>17</sup>.

### Stress Induction

The models of physical stress were immobilization and immobilization associated with exposure to cold applied for two months, six times a week, at various times on alternate days. The models of variable stress<sup>18</sup> were exposure to intermittent light, 24 hours isolation, oral cavity examination, crowded environment, smell of blood and noise. One type of variable stress was conducted each day between 6:00 and 18:00. Stress was induced as previously described<sup>19</sup> as following.

- a) **Immobilization:** animals in the physical stress group were exposed to an average temperature of 26°C by placing them in polyvinyl chloride tubes compatible with their body size. Then, the tubes were stopped up on both sides with metallic wire, enabling the animals to breathe while they were immobilized for 4 hours.
- b) **Immobilization and exposure to cold:** After immobilization as previously described, animals in the physical stress group were exposed to an average temperature of 7°C for a period of 4 hours.
- c) **Exposure to intermittent light:** The animals housing boxes were placed in larger boxes that blocked the entrance of natural light, and a 60 watt lamp was intermittently used for 4 hours.
- d) **Isolation:** The animals were individually separated in one new housing-box for a period of 24 hours while receiving food and water *ad libitum*.
- e) **Ligature examination:** Each animal was gently immobilized by the researcher, and then the ligature was examined by looking at the molar teeth with the aid of an n° 7 spatula.
- f) **Crowded environment:** All animals were grouped together in one new housing box for a period of 24 hrs while receiving food and water *ad libitum*.
- g) **Odor of blood:** Two plastic five-milliliter test tubes with small perforations in the upper region were filled with the same species blood together with an anticoagulant (EDTA, ethylene diamine tetra acetic acid) to release the odor into the interior of the housing box. This container was stabilized to

prevent the rats from contacting the product during the 4 hours of the procedure.

- h) **Noise:** The animals were placed in their house-boxes within a framework that prevented the entrance of light, and they were exposed to noise produced by musical sound at an intensity of 90 decibels for 4 hours.

### Collection and blood analysis

After the 60<sup>th</sup> day experimental time, animals were anesthetized through intramuscular administration of 0.1 ml ketamine hydrochloride, (Dopalen, Agribrands. Saúde Animal, Paulínia, SP, Brazil) associated with 0.05 ml xylazine hydrochloride (Rompun, Bayer. Saúde Animal, São Paulo, SP, Brazil) for each 100g of body weight. After anesthesia, the skin and abdominal wall at the base of the abdomen were diagonally incised, forming a V. After the displacement of the upper portion flap, access to the abdominal cavity was obtained. The internal organs were dislodged, enabling visualization of the posterior vena cava. Blood was collected by venous puncture using a 25x7 needle

(mg/dL) and very low density lipoprotein (VLDL). To calculate the levels of these lipidic markers, a photocolometric method using a spectrophotometer was performed (Femto 700S, São Paulo, SP); calibration of the equipment with standard solution was previously conducted (Bioclin – Quibasa, Belo Horizonte, MG, Brazil).

### Statistical analysis

Averages of lipidic markers of the experimental groups were compared using analysis of variance (ANOVA); Tukey and Bonferroni tests were used for comparisons between groups. All tests had a significance level of 5%. The statistical software used was SPSS (Statistical Package for the Social Sciencis SPSS Inc. Chicago, IL, USA).

### Results

#### Body Weight

There were no statistically significant differences ( $p>0.05$ ) among the body weights of the groups at any

**Table 1: Mean and standard deviation (M±sd) of animal whole body weight (g) during the study.**

Groups Periodontitis*	Physical stress + Periodontitis*	Variable stress + Periodontitis*	Periodontitis*	Negative Control*
1	232.75±22.07	252.41±29.08	252.41±16.40	253.08±32.71
2	242.16±19.94	252.50±36.02	254.83±15.23	255.33±37.70
3	246.16±20.10	256.83±29.91	255.91±11.58	259.91±50.65
4	258.16±22.55	266.23±30.63	260.75±12.45	265.33±25.68
5	255.66±22.85	267.16±26.00	268.91±11.57	266.66±31.98
6	260.91±20.38	252.58±27.90	264.25±16.77	266.41±29.30
7	257.83±21.41	274.16±26.38	267.41±12.99	271.08±32.30

No differences were observed ( $p>0.05$ ) considering the groups and periods of the tests.

(Vacutainer system, Becton Dickinson, Plymouth, UK) in a 5 ml tube with the anticoagulant ethylenediamine tetraacetic acid (EDTA), and the samples were carefully homogenized. After the blood puncture, the animals underwent euthanasia.

After centrifugation of the blood plasma at 3000 rpm for 10 minutes, the lipidic profile was determined through following markers: cholesterol (mg/dL), triglycerides (mg/dL), high density lipoprotein (HDL)

of the evaluation periods (Table 1).

### Lipidic Parameters

The lipidic parameters were significantly lower in the variable stress group than in the periodontitis and negative control groups. The physical stress group was not statistically different from the other groups. The triglyceride level was highest for the negative control group and statistically different from the levels in

Groups Parameter	Physical stress + Periodontitis*	Variable stress + Periodontitis*	Periodontitis*	Negative Control*
Cholesterol	54.07±7.45ab*	48.98±7.28a	62.94±11.43b	62.51±10.62b
Triglycerides	73.77±19.78ab	60.65±26.45a	99.85±38.63bc	118.53±45.60c
VLDL	14.75±3.95ab	12.13±5.29a	19.97±7.72bc	21.33±4.83c
HDL	61.05±9.47a	58.26±8.35a	68.99±12.35a	69.05±11.19a

\*different lower case letters within lines indicate significant differences among groups (p<0.05).

groups variable stress and physical stress. The physical stress group had the lowest triglyceride level, which was statistically different from that of the negative control group.

There was no statistical difference between physical stress and variable stress with respect to VLDL; a similar finding was obtained for periodontitis and negative control. The variable stress group had the lowest VLDL level, which was statistically different from those of the periodontitis and negative control groups. HDL showed no statistical differences between groups (Table 2).

## Discussion

Cardiovascular disease is a major public health problem in the world. Young people are exposed to the most severe risk factors for this illness, including obesity, smoking, bad eating habits, lack of exercise and stress<sup>20</sup>. The risk factors described indicate that poor metabolic control due to dyslipidemia, emotional factors, and the absence of insulin interferes with the activation of the hypothalamic-pituitary-adrenal axis. Among the neurohormones involved, cortisol and adrenaline have a direct effect on immunoinflammatory responses<sup>21</sup>.

Simple indicators such as a lipid profile associated with the dental clinical history and examination could therefore be used by periodontists to give some idea of how the treatment of periodontitis could aid in the treatment of heart disease or emotional problems and vice-versa<sup>10</sup>. However, the development of such indicators remains difficult<sup>22</sup>.

The study results were surprising. Although there was an infection in the animal's mouth and a stressor with a

systemic manifestation, there was still a "protective effect" in the vascular system, especially for the stress variable, associated with the induction of chronic periodontal disease by ligature<sup>18</sup>. The indicator values in the stressed groups were lower than those in the control group. Studies show that medical triglycerides, cholesterol and VLDL can predict cardiac pathology<sup>6</sup>.

It is possible that the stress models chosen, particularly the stress variable, might have caused some kind of protection for the cardiovascular system because humans are subjected to a heavy load of stress and their responses are often unpredictable, especially in terms of mental health<sup>6</sup>. However, the protective effect of stress was not confirmed with respect to the lipid profile<sup>23</sup>.

In most studies of stress associated with ligature-induced periodontitis in rats, the degree of stress is statistically different from models of stress induced by 12 hours of restraint. The stress load in such models is close to the limit of survival of the organism, so the findings of such models may be linked to a stage that Selye described as the burnout stage. In the proposed model of this study, we sought the stage described by Selye as the resistance phase, where the animal responds to stimuli, including serious systemic changes, without coming close to collapse. Such a response may have stimulated the cardiovascular system to decrease the parameters chosen for evaluation<sup>4,5</sup>.

It is known that lipids are associated with carrier proteins for transport to the liver and other organs; such activity can directly affect platelet-mediated inflammatory events<sup>24</sup>. This response usually starts in the vessel wall and also involves structures such as smooth muscle, endothelium and inflammatory cells<sup>21</sup>. Such a process seems not to have occurred in animals only receiving periodontitis when compared to the

negative control group. One hypothesis is that the adopted model of periodontitis may have brought about a significant systemic response, although other studies with a similar induction of disease show the systemic response to the induction of periodontitis<sup>17</sup>.

Periodontal health is related to a balance between microorganisms and host response. Some of these microorganisms are considered pathogens and might lead to disease<sup>25</sup>. Because of the similarities between the periodontal anatomy of rats and humans, animal models represent an important tool to study periodontal diseases<sup>26</sup>. In rats, this disease may be induced through changes in diet<sup>27</sup>, inoculation of periodontal pathogens or bacterial toxins as well as through ligature insertion in gingival crevice<sup>28</sup>.

One item to note is the feeding of the animals. The type of food is linked to problems in the cardiovascular system. This study was designed such that the food intake and the feed itself would be the same for all groups. The body masses of the groups were not statistically different between the beginning and end of the experiment. This shows an important measure of overall health<sup>29</sup>.

Modulation of stress has been studied by many researchers in the field of psychoneuroimmunology (PNI). However, it remains generally controversial whether periodontitis can be epidemiologically linked to stress. Contributing to the difficulty in establishing such a linkage is the absence of studies with longitudinal design, as well as the large variability in the methods of the available studies<sup>15,23</sup>. A link has also been shown between heart disease and periodontitis, as well as between stress factors and the outcomes of major illnesses; however, there is still a lack of good quality evidence to support such a link<sup>29</sup>.

It is known so far that there is a link between the stress triad, ligation and periodontitis, and greater efforts are needed to try to connect stress with periodontitis and serum lipids in rats. In conclusion, despite the limitations of the methodology, it seems that the stress model variable associated with periodontitis improved lipid parameters in the study).

## Conclusion

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associated with periodontitis improved lipid parameters in the study).

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