Saliva as the Stress Biomarker after Fasting Exposure on Adult Girls and Boys

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ABSTRACT
This experiment was conducted to determine the fasting effects on anthropometric parameters and salivary antioxidant properties in girls and boys. Saliva samples were collected from 18 healthy girls (control – 9 and fasting – 9 girls) and 12 healthy boys (control – 6 and fasting – 6 boys). Saliva was collected 4 times from all the subjects at a time interval of 2 h. For anthropometric parameters, body weight, body temperature, pulse rate (PR), systolic blood pressure (SBP), and diastolic blood pressure (DBP) were measured during each saliva collection time. Antioxidant parameters of all the saliva samples were evaluated. Most of the anthropometric parameters such as temperature, PR, SBP, and DBP of the fasting group were significantly (P < 0.5) decreases than the control group of both boy’s and girl’s in the different time intervals. Antioxidant properties were significantly (P < 0.5) higher in the fasting group of girls, but the insignificantly different levels were found in the boy’s group. This study was indicating that fasting affected the anthropometric parameters (PR, SBP, and DBP), mainly in the girls. Salivary antioxidant properties were mostly lower in the girls’ fasting group. Therefore, saliva may be a useful stress biomarker during fasting conditions.

Keywords: Antioxidant, 2,2-Diphenyl-1-picrylhydrazyl, Ferric reducing ability of plasma, Fasting, Saliva

INTRODUCTION
Saliva is a secreted watery substance (98%) produced by the salivary glands of animals, including humans. About 0.5–1.5 l/day saliva secreted in humans. Saliva contains lots of essential substances, such as electrolytes, mucus, antibacterial agents, epithelial cells, and many enzymes.1,2 Saliva has been used for the diagnosis of the disease since ancient times.3 Furthermore, saliva serves as an outstanding biomarker for other oral associated disease such as periodontal inflammation, oral squamous cell carcinoma, salivary gland tumors, and pancreatic cancer.4-7 The antioxidant properties of saliva have been found to have decreased in patients with periodontal disease.8 However, studies indicated that in Ramadan’s fasting condition, the rate of saliva secretion decreased, changed in saliva composition, etc.9

In addition, intermittent fasting has been found to play a critical role in health.10 Fasting helps alleviate hypertension and cardiovascular disease.11,12 No studies have yet been carried out to determine the role of human salivary antioxidants and changes in anthropometric parameters in a short fasting period. Hence, this study was intended to evaluate the effect of fasting in boys and girls on physiology and salivary antioxidant properties.

MATERIALS AND METHODS

Ethical Clearance
During this time of research, religious fasting was continued. Therefore, there was no need for expert committee clearance to carry out this study. However, all individuals have voluntarily participated in this experiment with their full consent. They were a postgraduate student at this university. Previously, they were aware of the method of collecting saliva. They were also aware of the normal level of all anthropometric parameters.

Study Site and Experimental Design
This study was conducted at Arni University Campus, Himachal Pradesh, India, in 2019. This has chosen boys and girls who have been found to have decreased in patients with periodontal disease.8 However, studies indicated that in Ramadan’s fasting condition, the rate of saliva secretion decreased, changed in saliva composition, etc.9

In addition, intermittent fasting has been found to play a critical role in health.10 Fasting helps alleviate hypertension and cardiovascular disease.11,12 No studies have yet been carried out to determine the role of human salivary antioxidants and changes in anthropometric parameters in a short fasting period. Hence, this study was intended to evaluate the effect of fasting in boys and girls on physiology and salivary antioxidant properties.

Prototypical STARD Diagram to Report the Flow of Girls and Boys Participants through the Study

Measurement of anthropometric parameters
All the subjects were measured in height using a measuring tape. Bodyweight was measured using a weight balance machine. The temperature was measured using a digital electronic thermometer.
with a heat sensor. Blood pressure (BP) meter (Omron automatic BP monitor NANZ A-31) was used to measure all subjects’ BP. The pulse rate (PR) was calculated when the BP was measured by the BP meter (NANZ A-31).

Saliva Collection
The saliva was collected in the sample collection tube by the spitting method. The saliva collection started at 10 am and ended at 4 pm. The time interval for the collection of saliva was 2 h. After the saliva was collected, the entire sample was stored in a deep freeze (−80°C) until the antioxidant parameters were analyzed.

Analysis of Antioxidant Parameters
2,2-Diphenyl-1-picrylhydrazyl (DPPH)\(^{[13]}\) and ferric reducing ability of plasma (FRAP)\(^{[14]}\) were performed for antioxidant parameter analysis. The whole test was performed in a triplicate test series. All participants had a discussion class about these methods and the use of all these methods.

Statistical Analysis
We selected 18 girls (nine in each group) and 12 boys (six in each group). We did so in accordance with the standard statistical method to select a minimum of six experimental subjects. Statistical analysis was performed using version 22.0 of the SPSS. All datasets have been executed for the t-test. ANOVA was performed to determine the level of significance between the different time intervals of two groups.

Results
Anthropometric Parameters
No adverse events occurred during the measurement of anthropometric parameters and saliva spitting at different

- Potentially eligible participants (Girls) n = 25
- Excluded n = 05
  - Reason 1 (n = 3) Digestion problem
  - Reason 2 (n = 4) Fever
- Eligible participants n = 20
- No index test n = 2
  - Reason 1 (n = 2) Absent
- Index text negative n = 0
- Index text positive n = 18
- Index text inconclusive n = 0
- Reference standard n = 9
  - Reason (n = 9) As per the experimental design nine volunteers were grouped as control group
- No reference standard n = 9
- Final diagnosis
  - Fasting group (n = 9)
  - Control group (n = 9)
times of both sexes. There is no significant variation in the bodyweight of girls and boys between control and fasting groups at different periods [Table 1]. The temperature in fasting boys and girls increased significantly ($P < 0.5$) [Table 2]. The PR in boys showed a significant decrease ($P < 0.5$) between control and fasting communities at all times. Whereas in the girls' fasting group, the PR decreased significantly at 12 pm and 4 pm. There was no significant difference between the different time ranges in the control and fasting categories for both boys and girls [Table 2]. In boys and girls, systolic BP (SBP) showed a significant ($P < 0.5$) difference (decreased pattern) at different time intervals within the fasting community. The girls' fasting group showed only a significant reduction in the pattern of diastolic BP (DBP) [Table 2].

**Antioxidant Parameters**

The result of the FRAP level after the $t$-test in girls showed a significant increase in the fasting group level ($P < 0.5$) at 10 am, 12 pm, and 2 pm but with the exception of 4 pm. In the case of boys, the FRAP level increased significantly, except at 12 pm. A significant decreasing pattern of FRAP levels has been identified in the fasting group of both sexes [Table 3]. Both the fasting groups of girls and boys showed a significant decline in DPPH levels in the fasting group. It has also been found that the DPPH level has increased at different times in both sexes [Table 3].

**Discussion**

**Anthropometric Parameters**

BWs findings showed no significant improvement in both sexes. The result is consistent with the analysis that no significant differences were recorded in BW. Our findings also suggest that the reduction of body weight in fasting conditions requires a minimum of 48 h. The trend in body temperature decreased significantly during a different time period, primarily in the fasting community. This may be due to the effect of fasting on the fasting community of counteracting the amount of body energy.

Furthermore, due to the fasting state, this can be attributed to the lower metabolic rate in the body. Lower metabolic processes are expected to reduce the lower secretion of thyroid hormone, which is the primary hormone for body temperature regulation after the body's basal metabolic rate increases or decreases. Another effect may be a decrease in glucose use and,
Table 1: Body weight of girls and boys in control and fasting conditions at a different time intervals

| Group | Time       | Control     | Fasting    |
|-------|------------|-------------|------------|
| Boys  | 10 am      | 52.39±1.73* | 52.30±2.02* |
|       | 12 pm      | 52.39±1.73* | 52.30±2.02* |
|       | 2 pm       | 52.39±1.73* | 52.30±2.02* |
|       | 4 pm       | 52.39±1.73* | 52.30±2.02* |
| Girls | 10 am      | 57.97±4.67* | 58.33±4.19* |
|       | 12 pm      | 54.97±4.67* | 58.33±4.19* |
|       | 2 pm       | 54.97±4.67* | 58.33±4.19* |
|       | 4 pm       | 54.97±4.67* | 58.33±4.19* |

Means bearing the same superscript (*) in a column differ insignificantly (P>0.05)

Table 2: Difference of anthropometric parameters in control and fasting group of both sexes during the different time intervals

| Parameter     | Group | Time       | Control     | Fasting    |
|---------------|-------|------------|-------------|------------|
| Temperature   | Boys  | 10 am      | 97.17±0.21* | 97.15±0.24* |
|               |       | 12 pm      | 96.92±0.47* | 96.98±0.34* |
|               |       | 2 pm       | 96.35±1.14* | 96.95±0.33* |
|               |       | 4 pm       | 97.38±0.76* | 97.52±0.34* |
|               | Girls | 10 am      | 96.67±0.42* | 97.02±0.34* |
|               |       | 12 pm      | 98.32±0.63* | 96.28±0.46* |
|               |       | 2 pm       | 96.33±0.47* | 96.72±0.56* |
|               |       | 4 pm       | 96.44±0.52* | 96.01±0.54* |
| Pulse Rate    | Boys  | 10 am      | 79.50±4.51* | 68.83±4.09* |
|               |       | 12 pm      | 75.17±3.91* | 69.00±3.33* |
|               |       | 2 pm       | 77.17±3.83* | 68.00±3.77* |
|               |       | 4 pm       | 87.00±6.89* | 74.89±4.89* |
|               | Girls | 10 am      | 83.89±11.47* | 84.22±5.46* |
|               |       | 12 pm      | 94.44±3.69* | 81.78±5.52* |
|               |       | 2 pm       | 87.44±6.37* | 84.11±5.52* |
|               |       | 4 pm       | 96.89±3.81* | 82.44±3.81* |
| Systolic blood pressure | Boys | 10 am      | 119.33±3.43* | 129.17±6.76* |
|               |       | 12 pm      | 120.33±4.33* | 110.67±10.27* |
|               |       | 2 pm       | 120.33±4.33* | 116.33±3.35* |
|               |       | 4 pm       | 119.17±3.17* | 121.83±8.98* |
|               | Girls | 10 am      | 109.33±3.84* | 101.33±0.04* |
|               |       | 12 pm      | 100.56±3.4*  | 101.67±3.67* |
|               |       | 2 pm       | 104.00±4.34* | 93.78±3.01*  |
|               |       | 4 pm       | 109.33±4.26* | 95.67±4.01*  |
| Diastolic blood pressure | Boys | 10 am      | 80.67±3.01* | 76.83±3.28* |
|               |       | 12 pm      | 72.00±3.86* | 71.50±4.16* |
|               |       | 2 pm       | 72.00±3.86* | 67.33±2.64* |
|               |       | 4 pm       | 77.67±3.54* | 71.17±4.71* |
|               | Girls | 10 am      | 77.11±3.12* | 72.00±2.48* |
|               |       | 12 pm      | 73.00±2.36* | 68.00±1.95* |
|               |       | 2 pm       | 73.11±3.09* | 63.22±2.27* |
|               |       | 4 pm       | 76.44±3.21* | 63.56±3.89* |

Means bearing the different superscripts (a,b) in a column differ significantly (P<0.05). *Signifies the difference in a row (P<0.05)

Table 3: Difference of FRAP, DPPH, and ABTS level in control and fasting group

| Parameter     | Group | Time       | Control     | Fasting    |
|---------------|-------|------------|-------------|------------|
| FRAP          | Boys  | 10 am      | 1.11±0.37*  | 1.39±0.31** |
|               |       | 12 pm      | 1.09±0.37*  | 1.00±0.31*  |
|               |       | 2 pm       | 1.03±0.37*  | 1.30±0.31** |
|               |       | 4 pm       | 1.01±0.34*  | 1.17±0.34** |
| DPPH          | Boys  | 10 am      | 0.89±0.27*  | 1.14±0.28** |
|               |       | 12 pm      | 0.91±0.27*  | 1.07±0.23** |
|               |       | 2 pm       | 0.91±0.24*  | 1.15±0.26** |
|               |       | 4 pm       | 1.03±0.25*  | 0.28±0.03** |
| Uric acid     | Boys  | 10 am      | 49.92±21.98* | 49.97±21.93* |
|               |       | 12 pm      | 49.92±21.87* | 49.90±21.86* |
|               |       | 2 pm       | 46.62±1.97* | 23.27±1.83* |
|               |       | 4 pm       | 44.67±1.95* | 21.45±4.50* |
|               | Girls | 10 am      | 24.21±1.55* | 35.85±5.89* |
|               |       | 12 pm      | 22.17±1.01* | 24.99±1.54* |
|               |       | 2 pm       | 24.18±1.56* | 25.11±1.55* |
|               |       | 4 pm       | 23.19±1.54* | 25.19±1.54* |

Means bearing the different superscripts (a,b) in a column differ significantly (P<0.05) and *signifies the difference in a row (P<0.05). FRAP: Ferric reducing ability of plasma, DPPH: 2,2-Diphenyl-1-picrylhydrazyl, ABTS: 2,2’-Azino- bis(3-ethylbenzothiazoline-6-sulfonic acid

Antioxidant Parameters

Fasting induces activation of lipid burning in the body and decreases the metabolic rate of rest at the optimum concentration of oxygen.$^{15,20}$ Animals must reduce the metabolic rate in response to a long period of fasting. This change in metabolic status may be associated with higher levels of oxidative stress.$^{26}$ Earlier studies showed an increase in salivary alpha-amylase levels during exercise.$^{13}$ Dental caries caused oxidative stress to the oral cavity. Salivary antioxidants were higher during dental caries.$^{26,29}$ Another study reported cigarette smoking and exercise in the oral cavity-induced oxidative stress and increased the antioxidant capacity of the saliva.$^{16}$ Total antioxidant capacity and uric acid saliva have also been reported to have changed during occupational exposure to mining dust.$^{15}$ This may be because the oxidative stress in the oral cavity is attenuated. Thus, the salivary antioxidant properties have been modified under various stressful conditions. In our study, it was found that the levels of antioxidant parameters in the fasting group were significantly lower than those in the control group of both sexes. As a result, this finding suggested a decrease in salivary antioxidant capacity for extended fasting in both sexes.$^{22,23}$ It may be due to the fasting stress that affects the salivary properties of antioxidants. However, at the initial stage of the experiment, antioxidant power was higher for both sexes in the fasting group. This happened because all the people were well used to having breakfast in the morning. Due to the fasting stress, the antioxidant properties have been triggered to fight stress in the body. The antioxidant strength in the fasting girl community was slightly lower at the final stage of the experiment. Fasting was also mainly influenced by the salivary properties of girls. Earlier research has shown that the salivary properties differed between the sexes.$^{28}$ In the category of girls, anthropometric parameters have often been changed. As a result, major salivary changes in girls could most likely be due to this cause.

Overall findings have been reported that fasting has mostly changed in anthropometric parameters in girls and, as a result, antioxidant parameters have also been significantly modified in girls. Fasting, therefore, triggered major changes in the
properties of antioxidants in a short-term fasting state. In the end, saliva can act as a good potent stress biomarker under stressful conditions.

**Conclusion**

During a different time interval, especially in children, the fasting condition affected anthropometric parameters (PR, SBP, and DBP). Salivary antioxidant activity was also reduced for fasting groups of children. Likewise, in fasting conditions, saliva may be a useful biomarker for oxidative stress. Research will be carried out with regard to the large population.

**Significant Statements**

- For the 1st time, this study reports on salivary antioxidant properties after short-term exposure in boys and girls.
- The fasting condition affected anthropometric parameters (PR, SBP, and DBP) at different time intervals, especially in girls.
- The antioxidant properties in the fasting group were higher than in the control group of both sexes.
- Salivary antioxidant properties were mainly lower in fasting groups, both boys and girls. Fasting caused a reduction in the antioxidant properties of saliva.
- In fasting conditions, saliva can be a valuable biomarker for oxidative stress.

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**Conflicts of Interest**

All the authors are declaring that there are no any conflicts of interest related with this publication.

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