Original Article

Salivary and Serum Matrix Metalloproteinase (MMP)-2, MMP-9 and Their Tissue Inhibitors (TIMP)-1 and TIMP-2 in Young Obesity

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Objective: To assess the levels of MMP-9, MMP-2, and TIMP-1 and TIMP-2, and the MMP-9/TIMP-1, MMP-2/TIMP-2 ratios in serum and saliva in young individuals with obesity without comorbidities.
Methods: 24 people with obesity, 24 overweight and 24 normal weight individuals were studied and grouped by body fat mass percentage by bioelectrical impedance analysis (BIA). Salivary and serum MMPs and TIMPs levels were measured by slot-blot analysis.
Results: Individuals with obesity and overweight had higher MMP-9 levels in saliva comparing to normal weight (p <0.01 and p <0.05 respectively). People with obesity had higher MMP-2 levels than normal weight in saliva (p <0.05). Overweight had lower TIMP-1 levels in saliva than normal weight (p <0.05). We found higher net MMP-9 activity (higher MMP-9/TIMP-1 ratio) in saliva (p <0.01) and higher activity of MMP-2 (MMP-2/TIMP-2 ratio) in serum in overweight comparing to normal weight (p <0.05). Overall, no significant differences were found in serum results.
Conclusion: Obesity is a chronic inflammatory disorder early associated with dysregulated MMP-9 and MMP-2 activity affecting oral and systemic health.

Key words: Obesity, Matrix Metalloproteinases, Saliva, Inflammation

Introduction

Over the last decades, obesity and its consequences have become a worldwide health problem. The World Health Organization defines overweight and obesity as an abnormal or excessive fat accumulation that presents a risk to health. It also states that a crude population measure of obesity is the body mass index (BMI ≥ 25 kg/m^2 is considered overweight and BMI ≥ 30 kg/m^2 is the cut off value to consider obesity) estimating that 300 million of adults worldwide have obesity and more than 1 billion are overweight (1–3). Obesity is a multifactorial disease influencing not only body weight homeostasis but also insulin resistance, circulating lipid levels, arterial blood pressure and coagulation (4). Cardiovascular disease, type-2 diabetes and cancer are some of the subsequent comorbidities. Increased obesity-associated oxidative stress is probably due to the presence of the excessive adipose tissue itself, as adipocytes and preadipocytes have been identified as a source of proinflammatory cytokines including tumor necrosis factor-alpha (TNF-α), interleukin-1 (IL-1), and interleukin-6 (IL-6); subsequently, a major detrimental impact of obesity is related to an induced state of chronic low grade inflammation (5,6). In addition to metabolic and cardiovascular abnormalities, obesity has been linked with impairment in oral health status such as periodo-

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ntitis, gingivitis, caries and xerostomia (7–9). Oral pathologies may arise when bacterial pathogens are capable to cause disease in a debilitated oral environment, stimulating the release of more proinflammatory cytokines and inflammatory mediators from various host cells. There is a resultant increased proteolytic activity in the oral environment associated with periodontal disease status (10,11). Additionally, there is a clear evidence that inflammatory mechanisms play a role in atherogenesis, which is a process characterized by vascular remodeling and accumulation of lipids and fibrous elements in the large arteries (4). As a result, Matrix Metalloproteinases (MMPs) and their tissue inhibitors (TIMPs) are implicated in those chronic remodeling processes and therefore in obesity (11–13). The MMP family comprises a structurally and functionally related group of zinc-dependent endopeptidases which play a pivotal role in adipose tissue development and remodeling by the degradation of components of the Extracellular Matrix (ECM) namely collagen, elastin, fibronectin and proteoglycans (11,14,15). Despite the regulation of MMPs activity being very complex, changes in TIMPs levels are important under pathological conditions as TIMPs directly downregulate MMPs activity (16). It is well recognized that an imbalanced ratio between MMPs and TIMPs could lead to uncontrolled degradation of the ECM. The subsequent clinical and subclinical inflammation could predispose to pathological disorders (2,16–19). In fact, the interest in assessing MMPs' level in obesity has increased over the last years, being considered potential inflammatory biomarkers through their proteolytic action. Several lines of evidence suggest a possible functional role of MMP-2 in obesity, being one of its main roles the degradation of type IV collagen, a major component of basal membranes. The protease also exhibit activity toward growth factor-binding proteins and growth factor receptors, which are known to be involved in obesity (20). It has also been suggested that MMP-2 plays an important role in adipose tissue development, (21,22) also being pivotal to inflammation (23) and vasoconstriction (24). On the other hand, MMP-9 has a wide range of action being expressed by various cell lines such as keratinocytes, fibroblasts, osteoclasts, eosinophils, neutrophils and macrophages. It is known to degrade many components of the ECM during both physiological and pathological processes, namely in cardiovascular and atherosclerotic disease, and periodontal disease. Also, MMP-9 activity is regulated primarily by TIMP-1 forming a 1:1 stoichiometric and non-covalent inhibitory complex, (11,17,25) whereas TIMP-2 plays a dual role in regulating the processing of pro-MMP-2. Low TIMP-2 levels are required for the pro-MMP-2 cleaving to occur and high TIMP-2 levels inhibit MMP-2. (26,27) Therefore, this study aims to assess the impact of body weight on health status in young and apparently healthy adults with obesity, through the MMP-9, MMP-2, TIMP-1 and TIMP-2 levels, ratios and proteolytic activity evaluation in serum and saliva.

Materials and Methods

Participating Subjects

150 individuals aged between 18 and 35 years old volunteered to participate in the study. They were grouped according to their body fat mass percentage in normal weight (female: <33%, male: 8-19%), overweight (female: ≥ 33-39%, male: ≥ 19-25%) and individuals with obesity (female: >39%, male: > 25%) (28). All participants were subjected to specific exclusion criteria. Smokers and individuals with symptoms of inflammation, oral cavity pathologies or any other chronic disease were excluded of this investigation. We selected 3 groups with the same number of participants and with different body fat mass. As a result, 72 individuals were selected for study. Saliva and serum samples were collected per individual resulting on a total of 144 samples, i.e. 24 serum samples and 24 saliva samples taken for each of the three different studied groups.

The body fat mass percentage and muscle mass were carried out using the specific equipment of bioelectrical impedance analysis Akem, model BIA-101 (Akem Srl, Florence, Italy, 2004) previously calibrated to reference values within the biokinetics laboratory of the *Faculdade de Ciências do Desporto e Educação* Física of *Universidade de Coimbra*.

The subjects were prepared and electrodes were applied according to the manufacturing operating procedures. A very low excitation current (800 μA) with a constant frequency (50 kHz) was allowed to flow in order to obtain two measured parameters: reactance (Rz) and total resistance (Xc). Those values were introduced into the equipment software Bodygram 1.3 that calculated both the body fat mass and muscle mass percentages.

Circumferences and Waist-Hip Ratio

A measuring tape of 1.5 m length and 0.5 cm width was used for body circumferences measurement, with a 0.1 cm scale and a spring return mechanism (Gulick tape) to ensure that the tape was always fully stretched. In the measurement process, the tape was always aligned tightly avoiding skin compression. Three measurements were obtained for each relevant body area and a mean was calculated according to American College of Sports Medicine recommendations (ACSM, 2008). Waist-hip ratios were calculated dividing the waist circumference by the hip circumference (ACSM, 2008).

Body Mass Index

The body mass index was used to assess the relation between subjects' weight and height. The body weight (in Kg measured with a Terraillion balance with 0.1 Kg sensitivity) was obtained and divided by the squared height (m) giving the body mass index in kg/m (ACSM, 2008). Height was measured using a Jofre stadiometer (in cm with 0.1 cm sensitivity).
The study complies with the principles of the Helsinki Declaration. All participants gave their written informed consent.

Sample Collection

Serum and unstimulated saliva samples were collected from subjects who had refrained from eating and drinking for at least 3 hours, by direct draining into an ice-cold saliva collection tube, between 08:00 am and 10:00 am (in order to respect the circadian rhythm) within the Biomedical Laboratory Sciences Laboratory of the Coimbra Health School, Polytechnic Institute of Coimbra. Saliva samples, about 2 ml in volume, were centrifuged at 12,000g for 30 minutes at 4ºC and the supernatant stored at -80ºC until analysis. Serum samples were centrifuged at 3500g for 10 minutes and stored at -80ºC until analysis.

Clinical Biochemistry

The serum levels of glucose, total cholesterol, high density lipoprotein cholesterol (HDL), low density lipoprotein cholesterol (LDL) and triglycerides were determined in the Prestige 24i automated analyzer using the Cormay: Prestige 24i GLU, Prestige kits 24i CHOL, Prestige 24i HDL Direct, Prestige 24i TG (PZ Cormay SA, Poland).

Total Protein Analysis

The total protein quantification on serum samples was performed on the Prestige 24i automated analyzer using the total Protein LQ kit (PZ Cormay S.A., Poland). On saliva samples, the DC Protein kit (Bio-Rad, Hercules, CA, USA) was used.

Slot Blot Analysis

The slot-blot analysis was performed according to Caseiro et al. (10) Serum samples were diluted in Tris buffered saline (TBS) (10 mM Tris, 500 mM NaCl) to give a final protein concentration of 0.02 μg/μL and a final volume of 100 μL was pipetted into the wells. Saliva samples were diluted in a TBS solution (10 mM Tris, 500 mM NaCl) to give a final protein concentration of 0.01 μg/μl and a final volume of 100 μL was pipetted into the wells. The nitrocellulose membranes - Hybond ECL Nitrocellulose Membrane (GE Healthcare, Pittsburgh, USA) were blocked in a 5% solution (m/v) of skimmed milk powder diluted in TBS-Tween (TBS-T). Membranes were incubated for 1 hour and 30 minutes at room temperature under constant agitation with anti-MMP-9 primary antibody (Clone 36020; R & D systems, Minneapolis, USA) or primary anti-MMP-2 antibody (Clone 101721; R & D systems, Minneapolis, USA), or anti-TIMP-1 primary antibody (Clone 63515; R & D systems, Minneapolis, USA), or anti-TIMP-2 primary antibody (Clone 127711; R & D systems, Minneapolis, USA), diluted 1: 500. Membranes were washed three times for 10, 15 and 10 minutes respectively with TBS-T and incubated for 1 hour and 30 minutes with a secondary antibody (alkaline phosphatase conjugated goat anti-mouse, Bio-Rad, Hercules, USA) in a dilution 1:3000. The detection was performed using the Immun-Star ™ AP Chemiluminescent Protein Detection System kit (Bio-Rad, Minneapolis, USA) and exposed to a Kodak BioMax Light Film photographic film (Carestream Health, Rocherter, USA) on a cassette (Kodak® X-OMAT Casset, Carestream Health, Rocherter, USA) for 3 minutes. The optical density analysis was performed using the GELDOCTM XR + image acquisition system (Bio-Rad Hercules, USA) and ImageLab® Version 3.0

software (Bio-Rad Hercules, USA). The samples were analyzed at the same time, with the same conditions to minimize variability. To determine the fold difference or change between various samples the data was normalized by designating one sample as reference, and the values of optical density (O.D.) ratio expressed as arbitrary units.

Statistics

Statistical calculations were performed with the GraphPad Prism version 5.0 for Windows (GraphPad Software, San Diego, California, USA) and IBM SPSS Statistics version 23. The data normal distribution was assessed using the Shapiro-Wilk test. The values are presented as mean ± standard deviation. The optical density measurements of the different studied groups were evaluated using the ANOVA - 1 Factor, Kruskall Wallis tests and Dunn's multiple comparisons test. The correlation between variables was evaluated through the Spearman Rho correlation coefficient and Pearson R correlation according to the data normality. The differences / correlation between groups were considered statistically significant when a random error $p < 0.05$ was assumed with a confidence level of 95%.

Results

The 150 volunteers to participate in the study, aged between 18 and 35 years old were distributed according to their body fat mass by 3 groups of the same number of participants (n=24). The characterization of body fat mass by bioimpedance method enabled the distribution of the volunteers by groups. Saliva and serum samples were collected per individual resulting on a total of 144 samples. The individual data of the different cohorts (individual characteristics, body composition and biochemical markers) are described in table 1. The individual characteristics of the study population showed a similar age of all participants, and a well balance population according to gender in overweight and obese groups. In normal weight group the number of males was lower than females. In respect to blood pressure, the results showed higher results in individuals with obesity comparing to normal weight ($p < 0.01$), but those results do not classify our population with obesity as hypertensive. In regards to body composition, the group of obese showed a significant higher body fat mass percentage comparing to overweight ($p < 0.01$) and to normal weight individuals ($p < 0.001$) as expected. Concerning the waist-hip ratio, significant differences exist between normal weight and subjects with obesity ($p < 0.001$). The evaluation of the BMI only showed a higher trend between normal weight and obese groups. The biochemistry analysis, namely the fasting glycaemia, cholesterol and triglycerides results do not show significant differences between all groups.

Table 1 – Individual, anthropometric and biochemical profiles of studied cohorts.

	Normal weight	Overweight	Individuals with obesity
Individual characteristics			
Gender (n)			
Female	20 (83.33 %)	12 (50.00 %)	13 (54.17 %)
Male	4 (16.67 %)	12 (50.00 %)	11 (45.83 %)
Age (years)	19.75±2.63	20.13±1.94	20.38±2.45
Blood pressure (mmHg)			
Systolic	112.7±7.27	115.4±8.36	120.9±10.05$^{\#}$
Diastolic	68.99±6.25	71.15±7.59	76.00±7.16$^{\#}$
Body composition			
Body mass index (kg/m^2)	20.18±1.39	22.72±1.28	27.15±2.92
Body fat mass (%)	27.30±4.26	28.96±6.69	36.71±6.74$^{\S\ ¥}$
Waist-hip ratio (cm)	0.73±0.04	0.76±0.04	0.77±0.06$^{\#}$
Biochemical markers (mmol/L)			
Fasting glycaemia	5.09±0.48	5.30±0.34	5.36 ±0.49
Total cholesterol	4.66±1.21	4.60±0.85	4.67±0.97
HDL cholesterol	1.40±0.22	1.41±0.24	1.46±0.36
LDL cholesterol	2.91±1.09	2.85±0.67	2.82±0.82
Triglycerides	0.71±0.29	0.75±0.30	0.85±0.39

The data of the individual characteristics are presented as mean ± standard deviation; $^{**}p < 0.01$; $^{***}p < 0.001$; $^{\#}$ = ** normal weight vs individual with obesity; § = ** overweight vs individuals with obesity; $^{¥}$ = *** normal weight vs individuals with obesity.

MMP-9 Levels

We found no significant differences in MMP-9 serum levels between normal weight (11.68 ± 3.55), overweight (12.96 ± 3.55) and individuals with obesity (11.89 ± 3.95) groups (Fig. 1A). However, a significantly higher MMP-9 saliva level was found in overweight (12.49 ± 5.26, p <0.05) comparing to normal weight (9.69 ± 5.07). There were also significant differences between the normal weight and the group with obesity (13.64 ± 5.47, p <0.01) (Fig. 1B).

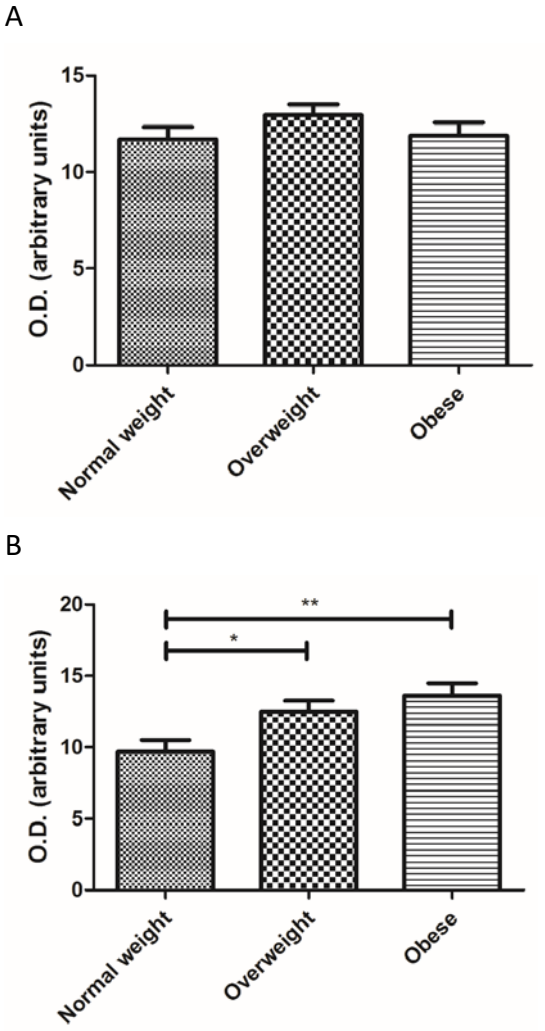


*Figure 1. A - MMP-9 serum levels in normal weight, overweight and obese by slot blot analysis; B - MMP-9 saliva levels in normal weight, overweight and obese by slot blot analysis.*p <0.05 **p<0.01*

TIMP-1 Levels

No significant differences were found between normal weight (4.99 ± 1.21), overweight (4.85 ± 0.88) and individuals with obesity (4.73 ± 1.26) (Fig. 2A) in TIMP-1 serum levels. However, in saliva the normal weight group showed increased TIMP-1 levels (38.09 ± 18.31) comparing to overweight (30.16 ± 14.74; p <0.05) and people with obesity (33.23 ± 11.41) (Fig.2B).

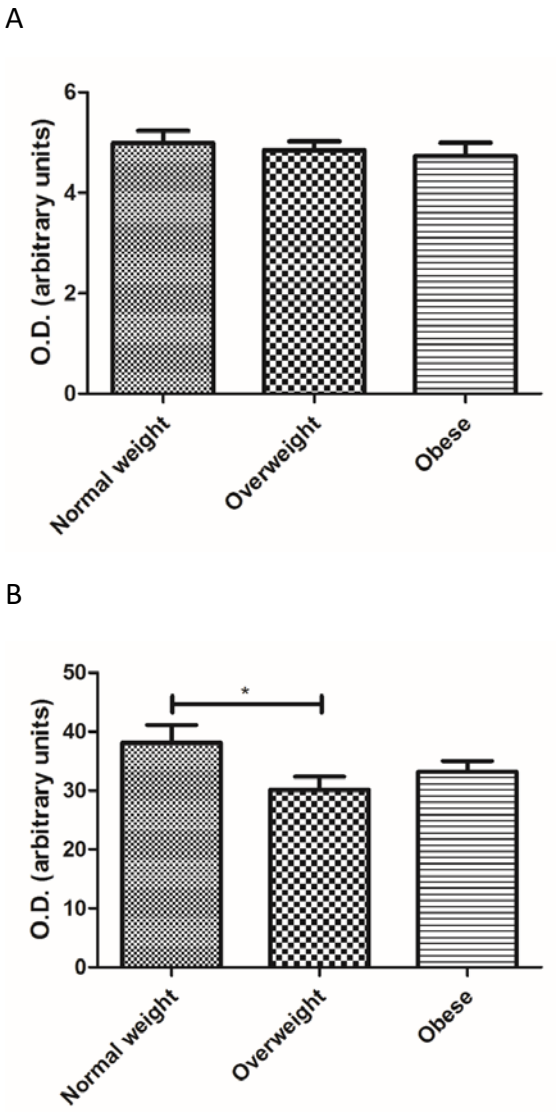


*Figure 2: A-TIMP-1 serum levels in normal weight, overweight and obese by slot blot analysis; B-TIMP-1 saliva levels in normal weight, overweight and obese by slot blot analysis. *p <0.05*

MMP-9 / TIMP-1 Ratio

The ratio of MMP-9 and TIMP-1 serum levels showed no significant difference. The ratio average value was 2.45 ± 0.98 in normal weight group, 2.69 ± 0.89 in overweight and 2.63 ± 1.31 in the participants with obesity (Fig. 3A). We found a significantly higher MMP-9/TIMP-1 ratio in saliva in overweight (0.49 ±

0.28; p <0.001) and individuals with obesity (0.42 ± 0.18; p <0.05) comparing to normal weight (0.33 ± 0.31) (Fig. 3B).

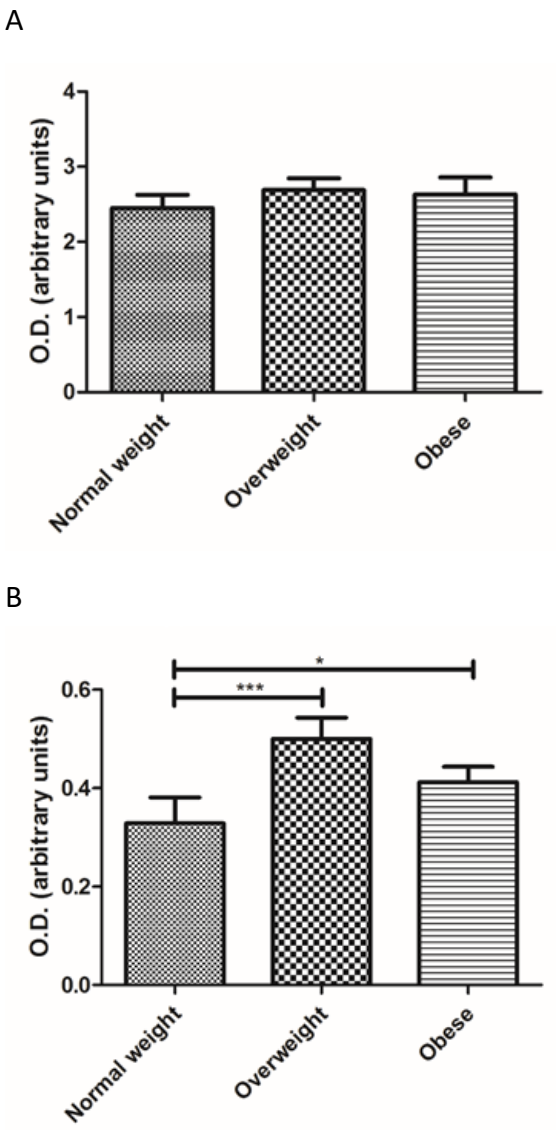


*Figure 3: A-MMP-9/TIMP-1 ratio in serum; B-MMP-9/TIMP-1 ratio in saliva. *p <0.05 ***p<0.001*

MMP-9 and TIMP-1 Correlation Between Serum and Saliva

Correlation analysis of MMP-9 serum and saliva levels showed no significant results (r = 0.1535; p <0.05) (Fig. 4A). Also, no significant correlation was found between TIMP-1 serum and saliva levels (r = 0.01379; p <0.05) (Fig. 4B).

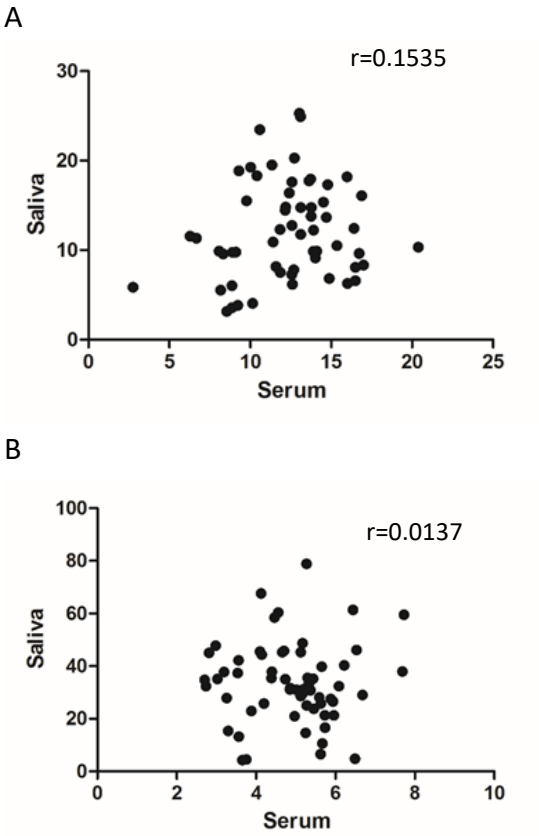
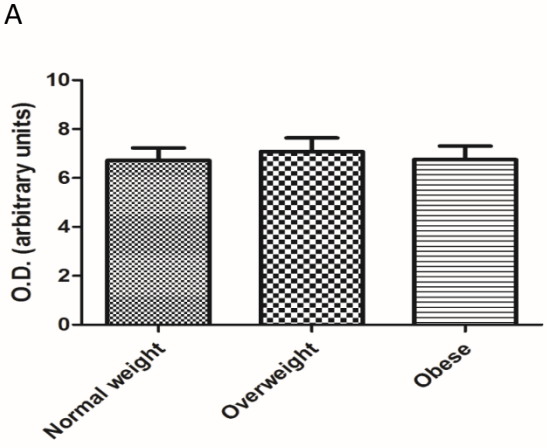


Figure 4: A- Serum/saliva MMP-9 levels correlation; B- Serum/saliva TIMP-1 levels correlation.

MMP-2 Levels

No significant differences were noted in MMP-2 serum levels between normal weight (6.72 ± 2.78), overweight (7.081 ± 3.10) and people with obesity (6.76 ± 2.99) (Fig. 5A).

In saliva, MMP-2 levels were found to be higher in individuals with obesity (13.05 ± 5.26) comparing to overweight (12.67 ± 5.24) and normal weight groups (9.97 ± 6.25). Significant differences were found between normal weight and subjects with obesity (p <0.05, Fig. 5B).



B

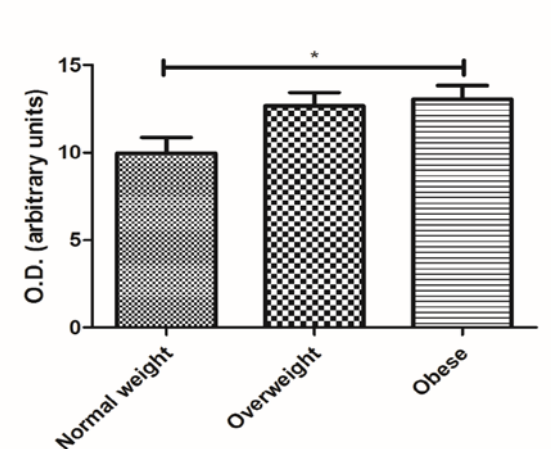


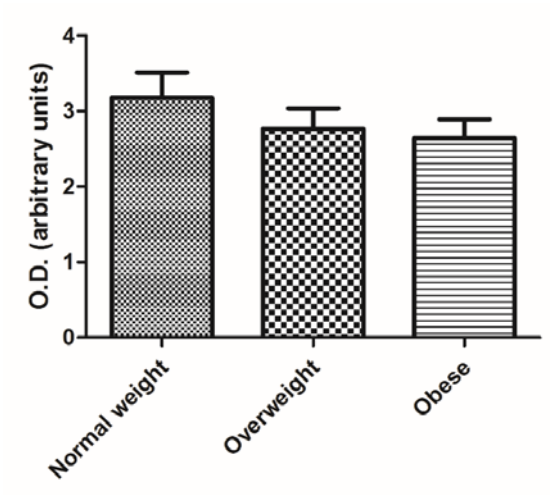
*Figure 5: A- MMP-2 serum levels in normal weight, overweight and obese by slot blot analysis; B- MMP-2 saliva levels in normal weight, overweight and obese by slot blot analysis. *p <0.05*

TIMP-2 Levels

TIMP-2 levels in serum were higher in normal weight individuals (3.18 ± 1.59) comparing to overweight (2.77 ± 1.38) and subjects with obesity (2.64 ± 1.37), however, the differences were not statistically significant (Fig. 6A).

The TIMP-2 levels in saliva were 19.93 ± 14.37 in normal weight group, 25.39 ± 17.17 in overweight and 21.76 ± 13.34 in individuals with obesity. However, the differences were not statistically significant (Fig. 6B).

A



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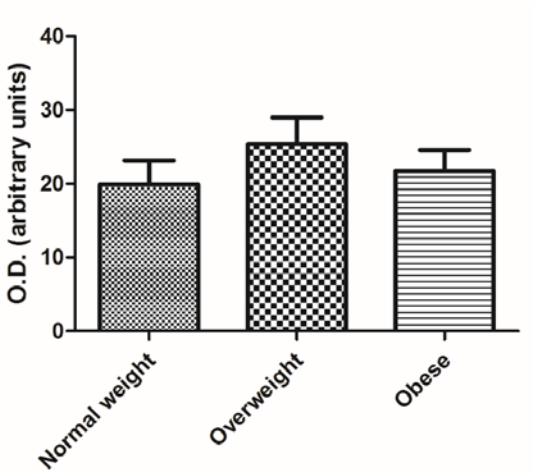
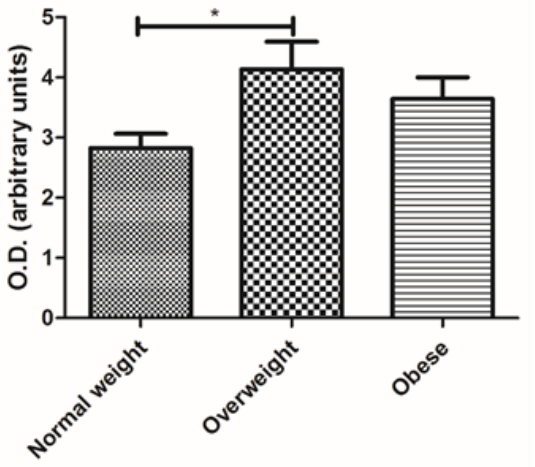


Figure 6: A-TIMP-2 serum levels in normal weight, overweight and obese by slot blot analysis; B-TIMP-2 saliva levels in normal weight, overweight and obese by slot blot analysis.

MMP-2 / TIMP-2 Ratio

In serum, the MMP-2/TIMP-2 ratio is significantly higher in overweight 4.14 ± 3.77 comparing to normal individuals 2.83 ± 1.84 (p <0.05) (Fig. 7A). The population with obesity also showed a higher ratio (3.65 ± 3.04) in comparison with normal weight participants. In saliva, no significant differences were found assessing MMP-2/TIMP-2 ratio, indeed, there is a trend showing higher results in subjects with obesity (0.95 ± 0.78) comparing to overweight (0.79 ± 0.59) and normal weight (0.77 ± 0.65) (Fig. 7B).

A



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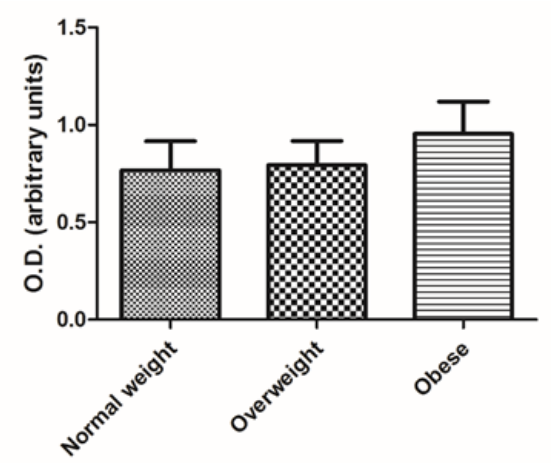

*Figure 7: A-MMP-2/TIMP-2 ratio in serum; B-MMP-2/TIMP-2 ratio in saliva. *p <0.05*

MMP-2 and TIMP-2 Correlation Between Serum and Saliva

A weak positive linear correlation was noted between MMP-2 serum and saliva levels (r = 0.244; p <0.05) (Fig. 8A) and a low negative linear correlation was found between TIMP-2 serum and saliva levels (r = -0.454; p <0.001, Fig. 8B).

A

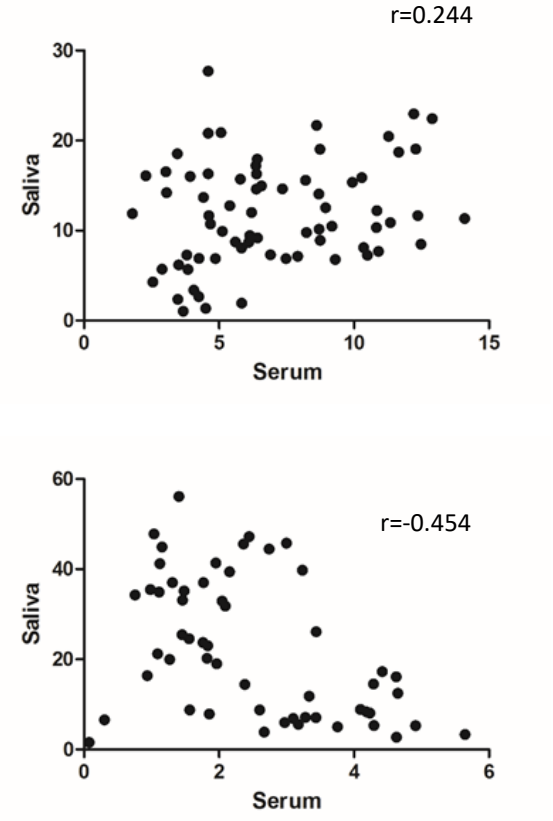

*Figure 8: A- Serum/saliva MMP-2 correlation; B-Serum/saliva TIMP-2 correlation. *p <0.05 ***p<0.001*

Discussion

MMP-2 and MMP-9 and their inhibitors have been associated with a broad range of diseases with a chronic inflammatory background, having been studied in childhood obesity (20,29), hypertension (24), obesity and cardiac function (30), and inflammation and obesity (7,31). However, controversy exists due to a multitude of studies showing elevated serum levels of MMP-9, MMP-2 and TIMP-1 in individuals with obesity (7,17,18,29,32), whereas other studies reporting no differences in young subjects with obesity (4,33). Interestingly, our study reveal more significant results

in saliva comparing with serum. To the best of our knowledge, no published data exists about salivary levels of MMPs and inhibitors in obesity to compare with.

This study demonstrates an increase in salivary MMP-9 levels in overweight (p <0.05), and individuals with obesity (p <0.01; Fig. 1B) and a decrease in salivary TIMP-1 levels (p <0.05 normal weight vs overweight group; Fig 2B) in individuals with greater body fat mass, whereas no differences were found in serum. Therefore, no correlation between saliva and serum was noted in MMP-9 or TIMP-1 (Fig. 4). Our serum results are in concordance with studies that found no differences for those parameters in obesity (4,33), but disagree with Derosa et al that found elevated circulating MMP-9 levels in adults with obesity (7). However, our results showed a positive trend in which serum MMP-9 discreetly increases in overweight and in subjects with obesity while serum TIMP-1 levels are slightly decreasing (Fig. 1A and Fig. 2A, respectively). The evaluation of a larger population could have enabled to obtain significant differences also in serum results. The serum MMP-9/TIMP-1 ratio suggests it through a slight positive trend with greater body mass

percentage (Fig. 3A). In saliva, we found a significant increase in MMP-9/TIMP-1 ratio indicating that individuals with obesity have a higher net MMP-9 activity, favorable to increased ECM proteolysis (Fig. 3B. The results showed significant raised salivary MMP-2 levels (p <0.05 between normal weight and people with obesity; Fig. 5B) while no differences were noted in serum (Fig. 5A). In respect to TIMP-2 we found no differences in serum or saliva levels (Fig.

6A, Fig. 6B). However, we found higher activity of MMP-2 (MMP-2/TIMP-2 ratio) in overweight comparing to normal weight in serum while only a positive trend was found in saliva (Fig. 7A and Fig. 7B respectively). Those results are in concordance with Belo et al that found increased activity of MMP-2 (MMP-2/TIMP-2 ratio) and reduced TIMP-2 levels in childhood with obesity (19). However, that study was performed in hypertensive children with obesity. Hypertension itself could have impacted the results being the cause of altered MMP and TIMP levels. While studies in saliva generally focus in the oral environment, here we corroborate that the analysis of saliva is also relevant at a systemic level and in obesity itself. No studies are available in obesity, but evidence exists about increased salivary MMP levels in diabetes (34), periodontitis (11,35) and oral cancer (36). Moreover, studies have shown that salivary inflammatory markers, including TNF-α, MMPs, IL-6 and IL-8 are associated with dental health and periodontitis (35). Therefore, this corroborates that the assessment of the MMP family and inhibitors in saliva has great potential as biomarkers, in order to monitor oral health and to screen the inflammatory and proteolytic status in obesity.

The BMI as a measure of body fat is inaccurate and can lead to bias in measuring the effects of obesity on health outcomes. In fact, it does not take age, sex, bone structure, fat distribution or muscle mass into consideration. For these reasons and others, BMI can misrepresent the quantity it is used to measure (37). Body fat percentage has been recommended as a more accurate measurement of body fatness (38). Consequently, in this study it has been used a bioimpedance method to carefully select and group patients according to the body fat mass percentage. In regard to our studied population with obesity, it showed a significant higher body fat mass percentage comparing to overweight ($p < 0.01$) and to normal weight individuals ($p < 0.001$). Concerning the waist-hip ratio, significant differences exist between normal weight and subjects with obesity ($p < 0.001$), when the BMI only showed a higher trend between normal and people with obesity. In spite of the blood pressure revealing higher results in individuals with obesity comparing to normal weight ($p < 0.01$), those results do not classify our population with obesity as hypertensive. Additionally, the fasting glycaemia, cholesterol and triglycerides results do not show significant differences between all groups. All those results emphasize that we endeavored to correctly select and characterize our cohorts, excluding any pathological status linked with an inflammatory profile as hypertension, diabetes or hypercholesterol -emia. Besides, we believe that it is relevant to think that the existing discrepant MMPs and TIMPs results in obesity could, at least in part, be due to difficulties in controlling all pathophysiological factors involved with the disease. In this way, the fact that BMI is widely used instead of bioimpedance could lead to study populations being inconsistently selected and mischaracterized, increasing the bias.

Serum or plasma is generally considered the first choice of specimen for testing given its fullness of biological information and relatively easy collection. It is also widely chosen for the MMP assessment in several diseases (30,33). Nowadays, saliva is not only used in the diagnosis of oral diseases, being relevant in infections, cancer, hereditary, autoimmune and endocrine diseases (39). In fact, salivary composition generally reflects the health status of an individual or disease susceptibility for oral and systemic pathologies. Moreover, the advantages of saliva in comparison with other bodily fluids for diagnostic purposes are given by its accessibility and noninvasive and easy collection (39,40).

In conclusion, this study show evidence indicating higher MMP-9 and MMP-2 and lower TIMP-1 levels in saliva in young obesity. Overall, no significant differences were found in serum results. We found higher net MMP-9 activity (higher MMP-9/TIMP-1 ratio) in saliva ($p < 0.01$) and higher activity of MMP-2 (MMP-2/TIMP-2 ratio) in serum in overweight comparing to normal weight ($p < 0.05$).

In this study we highlight that, in an early stage and even in young individuals, obesity is linked to significant salivary alterations. It can be suggested that obesity is early linked with direct negative consequences in oral health. On the other hand, saliva could have greater sensibility than serum for the evaluation of MMPs levels, reflecting both oral and systemic pathological changes in obesity. Therefore, this work represents an advance in biomedical science because it highlights the potential of saliva as a diagnostic fluid and MMPs as targets for the prevention, risk assessment and monitoring of obesity comorbidities.

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Geolocation Information

This investigation was carried out at Coimbra Health School, Rua 5 de Outubro, 3046-854 Coimbra, Portugal. Latitude: 40.19803360143075 or 40º 11' 53" N. Longitude: -8.461118191480636 or 8º 27' 40" W.

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