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Salivary protein analysis of street runners after a real sporting event

Análise de proteínas salivares de corredores de rua após evento esportivo real

Análisis de las proteínas salivales de los corredores de la calle después de un evento deportivo real

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Abstract
The aim was to characterize acute exercise-induced changes in salivary proteins of street runners. Saliva samples from 12 adult male athletes were collected before and immediately after a street race. Two groups were formed based on the distance covered, 5 km (n=4) and 10 km (n=8). Samples were subjected to depletion of amylase, albumin and immunoglobulin G. Then they were concentrated, digested and analyzed by nano-UPLC-tandem nano-ESI-MSE. A total of 69 proteins were identified. Significant changes were observed in the expression of 15 proteins in the 5 km group and 13 proteins in the 10 km group. Among the proteins with altered expression, only 7 had already been described in the literature in similar models (Alpha-Amylase 1, Lactoperoxidase, Alpha Skeletal Muscle Actin, Cystatin-B, Cystatin- SN, Cystatin- SA and Androgen-regulated Protein 3B in the submaxillary gland). This study has shown that street running induces acute changes in the salivary proteome. The results obtained add to the limited data available in the literature in the search for a better understanding of the acute effects of exercise.
Keywords: Proteomics; Mass Spectrometry; Exercise; Saliva.

Resumo
Objetivou-se caracterizar as alterações induzidas pelo exercício físico agudo nas proteínas salivares de corredores de rua. Amostras de saliva de 12 atletas adultos do sexo masculino foram coletadas antes e imediatamente após uma corrida de rua. Dois grupos foram criados com base na distância percorrida, 5 km (n=4) e 10 km (n=8). As amostras foram submetidas às depleções de amilase, albumina e imunoglobulinas G. Em seguida, foram concentradas, digeridas e analisadas com nano-UPLC tandem nano-ESI-MSE. No total 69 proteínas foram identificadas. Observou-se alterações significativas na expressão de 15 proteínas no grupo de 5 km e 13 proteínas no grupo de 10 km. Dentre as proteínas com expressão alterada, apenas 7 já tinham sido descritas na literatura em modelos semelhantes (Alfa Amilase 1, Lactoperoxidase, Actina músculo esquelético alfa, Cistatina-B, Cistatina-SN, Cistatina-SA e Proteína 3B regulada por andrógeno na glândula submaxilar). Este estudo demostrou que a corrida de rua promove alterações agudas no proteoma salivar, os resultados obtidos juntam-se aos poucos dados disponíveis na literatura na busca de compreender melhor os efeitos agudos do exercício.
Palavras-chave: Proteômica; Espectrometria de Massas; Exercício; Saliva.

Resumen
El objetivo fue caracterizar las cambios inducidos por el ejercicio físico agudo en las proteínas salivales de los corredores callejeros. Se recogieron muestras de saliva de 12 atletas masculinos adultos antes e inmediatamente después de una carrera de calle. Se crearon dos grupos en función de la distancia recorrida, 5 km (n = 4) y 10 km (n = 8). Las muestras fueron sometidas a agotamiento de amilasa, albúmina e inmunoglobulina G. Luego fueron concentradas, digeridas y analizadas con nano-UPLC tándem nano-ESI-MSE. En total se identificaron 69 proteínas. Se observaron cambios significativos en la expresión de 15 proteínas en el grupo de 5 km y 13 proteínas en el grupo de 10 km. Entre las proteínas con expresión alterada, solo 7 ya habían sido descritas en la literatura en modelos similares (Alfa Amilasa 1, Lactoperoxidasa, Alfa Actina del Músculo Esquelético, Cistatina-B, Cistatina-SN, Cistatina-SA y Proteína 3B regulada por andrógenos en glándula submaxilar). Este estudio demostró que correr en la calle promueve cambios agudos en el proteoma salival, los resultados obtenidos se unen a los pocos datos disponibles en la literatura en la búsqueda de una mejor comprensión de los efectos agudos del ejercicio.
Palabras clave: Proteómica; Espectrometria de Masas; Ejercicio; Saliva.

1. Introduction

The promotion of physical activity is an important public health strategy, as prevention and treatment of diseases (Tuso, 2015). The health benefits associated with regular physical activity are responsible for risk reduction by 20 to 30% of more than 25 chronic diseases and premature death (Warburton & Bredin, 2016).

Street running has been characterized as an easy to perform and inexpensive sport (Hespanhol, 2015). Moreover, long-distance running competitions attract more participants each time, gaining popularity and supporting healthier lifestyles (Niemelã, 2016).

However, improper performance of exercise may be associated with several adverse health effects. The probable impairments are in the majority associated with overtraining caused by the imbalance of exercise intensity and body recovery (Cadegiani & Kater, 2017). The overtraining syndrome can lead to impairment of quality of life characterized by a series of psychological, biochemical, physiological and immunological changes, with extensive symptomatology: persistent fatigue, mood swings, depression, sleep disturbances and others (Alexandrova et al, 2017).

To avoid these types of complications, it is important to monitor sports practice with the aim of ensuring a load compatible with the individual and his rest. Therefore, it is crucial to assess the health status of the individual and the physiological effects of the activity in order to optimize the physical activity, with the aim of promoting the associated benefits at the expense of possible disorders (Bourdon et al, 2017).

In this context, there is a growing interest in non-invasive methods in routine monitoring of athletes. The ease and speed of sample collection and the 'low stress' method with minimal interference with the athlete's training routine and competitions removes the need for a controlled laboratory environment and allows samples to be collected in the usual sporting environment (Lindsay & Costello, 2016).

Saliva moves into the limelight among body fluids because it has a heterogeneous origin and its composition varies depending on different stimuli, these stimuli include physical activity. Its composition is complex (enzymes, hormones, antibodies, antimicrobial substances, growth factors, salts, ions and others) and has different origins: serum, bronchial and nasal secretions, food and microorganisms. In addition, saliva is an ideal tool to analyze the stress associated with physical activity, since it has biomarkers that allow a chronic and acute analysis of the effects of exercise; there are studies in different sports that analyze different parameters (Palacios et al, 2015; Lindsay & Costello, 2016).

The application of "omics" science in the sports context aims to provide new insights into the physiological changes induced by physical activity, using new computational resources and big data. Proteomics consists of the application of technologies, mainly mass spectrometry, to study the expression of proteins in a given biological matrix in a specific physiological context. Currently, a hypothesis-free and non-targeted proteomics approach is used to understand metabolic responses to physical stress through the analysis of tissue samples, cell and biological extracts, biofluids such as blood, urine

and sweat (Bongiovanni et al, 2015; Aslam et al, 2017; Prado et al, 2017). However, there are few published studies that have analyzed salivary proteomics to assess physical activity, although the analytical potential of this fluid has been demonstrated in previous studies (Zauber et al, 2016; Franco-Martínez et al, 2020).

Therefore, this study aims to characterize changes in salivary proteins of street runners induced by acute physical training.

2. Methodology

2.1 Studied Population

Before data collection, all subjects signed an informed consent form of the project, approved by the Research Ethics Committee of the Universidade de Fortaleza (# 56841216.4.0000.5052). This study is a descriptive cross-sectional study carried out with a group street runners. A group of 12 amateur street runners were recruited to voluntarily participate in this study. All subjects were male, adult (34.7 ± 9 years), members of a sports advisory team, and had completed a competitive street running event in Fortaleza- CE (Brazil). The N sample was determined by non-probabilistic sampling, in other words, expediency to be accessible for this research. Runners formed two groups, the 5 km test group (5 km; n = 4) and the 10 km test group (10 km; n = 8). All subjects underwent screening, through the use of an adapted version of AHA /ACSM "Preparticipation Questionnaire" - AAPQ, to determine health eligibility to participate in this study (Whitfield et al, 2014). Physical activity level was assessed using a validated version of the "International Physical Activity Questionnaire" - IPAQ in Brazilian adults (Garcia et al, 2013). The study included males over 18 years of age who were regularly physically active (≥150 minutes of moderate-intensity physical activity per week), who had participated in sports counseling for at least 6 months, and who participated in the sports event. Volunteers with a record of serious diseases and the use of medications or supplements that alter body responses to exercise were also excluded.

2.2 Anthropometric evaluation and urinalysis

Height and weight were measured with a portable stadiometer (Alturexata, Belo Horizonte, BR) and an automatic balance (Gtech, Guandong, CN) at intervals of 0.1 cm and 0.5 kg, respectively. Waist and hip circumferences were also measured. Body measurements were performed according to the standardized method approach STEPS (Who, 2017).

Urine samples were collected before and immediately after the race. For urine collection, volunteers were given an appropriately labeled collection tube and instructed to clean the genital area with an alcohol swab and dry well. After cleaning, volunteers were instructed to put on a pair of gloves, dispose of the first stream of urine in the toilet, and collect the second stream of urine in the collection tube. After collection, the tubes were immediately frozen and stored in a freezer at -80ºC until the day of analysis.Urine density was evaluated using the Uriclin-10TM kit (Laborclin, Pinhais, BR) according to the manufacturer's instructions.

2.3 Saliva Samples

Saliva was collected in a nonstimulated manner, by a passive flow method using centrifuge tubes that were sterilized before and immediately after the race. After collection of 2,5 mL of saliva, samples were immediately stored in dry ice and kept at -80°C until use. They were then thawed and centrifuged at 14000 x g for 20 min at 4°C to remove insoluble materials, food, and cellular debris. The total protein concentration of each supernatant was determined by the Bradford method (Bradford, 1976).

2.4 Prepare of saliva sample

First, samples were subjected to a homemade potato starch affinity column (Merck, St Louis, MO, EUA) to reduce the level of alfa-amylase according to a previously mentioned protocol (Deutsch et al, 2008). This method was used to ligate the alfa-amylase present in human saliva and increase the identification of low abundance proteins. The device consisted of a 5 mL plastic syringe with a 0,22 mm filter at the tip (Whatman FP 30/0.45). The syringe was filled with 1000 mg of potato starch and 600 mL of water, then pressure was applied by hand for 20s to hydrate the substrate. Then, 1 mL of saliva was divided into two aliquots of 500 mL, pressed by hand, and filtered with a syringe for 120 seconds.

Subsequently, 500µL of saliva was filtered across a 0,22 µM membrane and 150 µL of the filtered saliva was reserved into a 1 mL column application "Hitrap Albumin & IgG Depletion" (GE Healthcare, USA) coupled with a "FPLC ÄKTApurifier 10" system (GE Healthcare, USA). First, the column was balanced with five volumes of Tris-HCl balance buffer 20mM, ph 7.4, containing NaCl 0,15 M, then 150 µL of filtered saliva was applied. Eight volumes of balance buffer were added to wash out proteins that did not interact with the column matrix, and five volumes of glycine-HCl 0.1 M pH 2.7 buffer were added to elute the interacted protein fraction. Chromatographies were performed at a constant flow of 1mL/min using 200 µL samples and protein elution was monitored by absorbance at 218, 260 and 290 nm. Three consecutive depletion chromatographies were performed on each 500µL filtered plasma sample. Finally, the filtered protein solutions were dialyzed and concentrated to 1 µg/µL on ultrafiltration devices with a molecular weight cutoff of 3 kDa (Vivaspin® 6, GE Healthcare, Chicago, IL, EUA) and spun at 10000 x g for 30 min at 8°C.

2.5 Tripsin digestion

Each sample containing 50 µL of protein was denatured with 0.2% RapiGest TM SF (Waters, Milford, MA, USA), reduced with 10 mM dithiothreitol, alkylated with 10 mM iodoacetamide, and enzymatically digested with trypsin (Promega, Madison, WI, USA). At the end of this process, the samples were centrifuged and the supernatant was transferred to new flasks containing ultrapure water and 0.1% formic acid. After this step, the samples were centrifuged and the supernatant was transferred to new flasks where 5 µL of internal standard, alcohol dehydrogenase (ADH, 50 fmol, access code P00330 in SwissProt), 85 µL of acetonitrile solution containing 3% and 0.1% formic acid were added. The final concentration of protein and ADH was estimated to be 250 ng / µL and 25 fmol / µL, respectively, in a final volume of 200 µL.

2.6 Proteomic analysis LC-MS

Quantitative and qualitative experiments were performed with nano-UPLC "tandem" nano-ESI-MSE on digested samples using reversed-phase chromatography on peptides with 3 to 40% acetonitrile (v/v) containing -.1% formic acid for 90 min, held at a flow rate of 600 nL/min for 100 min in a central "nanoACQUITY UPLC" system. A 1.7 µm, 100 µm x 10 cm "nanoACQUITY C18 UPLC BEH" reversed phase column was used in conjunction with a 5 µm, 180 µm x 23 mm SCX precolumn. All analyzes were performed using electrospray ionization in positive ESI(+) ion mode and a "NanoLockSpray" source. Independent data acquisition (MSE) was performed using a SYNAPT HDMS G1 (Waters, Manchester, UK) programmed to automatically switch between standard MSE MS (3 eV) and high energy collision MSE (12-50 eV) applied to trap "t-wave" cells CID (collision induced dissociation) in the presence of argon gas. The collision cell transfer was set to 1eV, with 1s, at low and high energy. After time-of-flight ("TOF") analysis, m/z spectra were collected from 50 to 3000. However, the RF offset of the quadrupole was set for efficient collection of LC /MS data from m/z 300-3000 to ensure that any massless values below m/z 300 observed in the LC /MSE data were from the collision cell only. Thus, the low-mass values were known CID fragmentation products and not the result of source fragmentation. The Protein Lynx Global Server (PLGs) v. 2.4 package, which includes the ExpressionE v. 2.4 program, was used to process the spectra and perform database searches. PLGs

used a new algorithm to process raw data obtained from MS using ion properties, i.e., retention time, precursor/product intensity, and exact mass. A label-free proteomic approach was used for quantitative analysis. Subsequently, the PLGs generated a list of all precursors and products. This list contained the mass of precursor and product ions for each peptide to be searched against the non-redundant protein database, UniProtKB/ Swiss-Prot 57.1, under search conditions based on taxonomy [Human (Homo sapiens)]. Both quantitative and qualitative information were automatically generated by the software using standard parameters that required (i) at least three fragmented ions per peptide, (ii) at least seven fragmented ions per protein, and (iii) at least one peptide ion per protein. The search parameters were (i) enzyme - trypsin, (ii) fixed modification - carbamidomethylation of cysteines, (iii) variable modification - oxidation of methionine and phosphorylation of serine, threonine or tyrosine residues, (iv) initial tolerances for mass accuracy - 10 ppm for the precursor. To monitor False Discovery Rate (FDR), data were matched to a randomized Decoy database created using the original database so that amino acid abundances were preserved. The FDR was set at 4% according to the criteria described previously. It was determined by the number of random or reverse identifications (false positive rate - FPR) divided by the number of correct identifications (true positive rate - TPR), expressed as a percentage: FDR = FPR/TPR × 100. For each protein, the Expression^{E} program selected all corresponding peptides from the samples and compared their intensities for relative protein quantification. Using the intensity of a peptide of a known size, ADH, the program performed a self-standardization of the data sets. Fold changes were calculated using the mean peptide intensities of the two groups before and after training (T0/T1).The lists of proteins were then filtered to show only those present in all three repeated injections of each sample, from which an output table was generated. This table showed the names, accession codes, and expression levels of the proteins, indicating whether they were up-regulated (≥2 fold), down-regulated (≤0.5 fold), or had no significant differences between groups (0,5< expression level <2).

2.7 Data analysis

Post-exercise differential protein expression coding genes were used to determine terms associated with Gene Ontology using the tool PANTHER version 15.0 (http://www.pantherdb.org/).

2.8 Statistical analysis

GraphPad Prism v. 6.01 (GraphPad Software, California, USA) was used for descriptive analysis. An unpaired t-test was used to calculate statistical significance between the analyzed points of urine density. Descriptive analyzes were reported on the study variables as mean ± standard deviation. Differences were considered statistically significant at P values less than 0.05 (P< 0.05).

3. Results and Discussion

Anthropometric and performance parameters of runners are shown in Table 1. Runners had an average pace of 5,65 km/min during the race. The average urine density of runners before the race was 1025,42 ± 3,96 and 1022,92 ± 8,65 immediately after the race, with no significant difference (P = 0,373), indicating the maintenance of the average hydration status after the race.

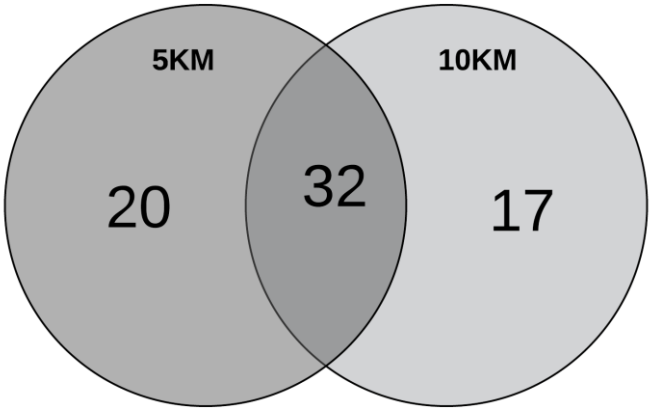
Table 1 - Averages from anthropometric caractheristics and sports performance from avalued street runners.

Parameters	Average values
Age (Years)	34,75 ± 11,09
BMI (kg/m²)	25,99 ± 3,07
Waist Circumference (cm)	82,71 ± 5,70
Hip Circumference (cm)	88,52 ± 5,11
Pace (min/km)	5,65± 0,8

Subtitle: The data was represented in average ± standard deviation (SD). Body Mass Index (BMI). Source: Authors.

After mass spectrometric analysis, a total of 69 proteins were identified. Of the total number identified, 32 proteins were identified in both groups analyzed, 20 proteins were found exclusively in the 5 km group, and 17 were found exclusively in the 10 km group (Figure 1).

Figure 1 – Venn's diagram displaying an overlap between proteins identified on groups of 5 and 10 k.



Source: Authors.

The effects of physical activity on health are diverse and complex. Analysis of changes in protein expression after running revealed that 4 proteins were down-regulated and 11 were up-regulated in the 5 km group. In the 10 km group, only 1 up-regulated protein and 12 down-regulated proteins were observed.

Table 2 - List from proteins differentially expressed in the saliva of street runners after a sporting event.

Access Number	Protein Description	Gene	Fold Changes	
			5km Group	10km Group
P31025	Lipocalin-1	LCN1	↓2.89	-
P0CF74	Immunoglobulin lambda constant 6	IGLC6	↓2.39	-
P68871	Hemoglobin subunit beta	HBB	↓6.96	-
P04080	Cystatin-B	CSTB	↓2.2	↓3.35
P04745	Alpha Amylase 1	AMY1A	↑4.0	-
P02042	Hemoglobin subunit delta	HBD	↑2.17	-
P09228	Cystatin-AS	CST2	↑3.33	-
P01036	Cystatin-S	CST4	↑3.45	-
P01037	Cystatin-SN	CST1	↑4.0	-
Q9BYX7	Putative beta actin like protein 3	POTEKP	↑6.67	-
Q562R1	Beta actin like protein 2	ACTBL2	↑9.09	-
Q6S8J3	POTE ankyrin domain family member E	POTEE	↑20.0	↓2.29
P12273	Prolactin inducible protein	PIP	↑2.38	-
P22079	Lactoperoxidase	LPO	↑2.38	-
P02814	Submaxillary gland androgen regulated protein 3B	SMR3B	↑4.17	↑11.1
P01834	Immunoglobulin kappa constant	IGKC	-	↓2.77
P01833	Polymeric immunoglobulin recepto	PIGR	-	↓3.0
P01876	Immunoglobulin heavy constant alpha 1	IGHA1	-	↓3.6
P01877	Immunoglobulin heavy constant alpha 2	IGHA2	-	↓3.19
P01591	Immunoglobulin J chain	JCHAIN	-	↓3.82
P80188	Neutrophil gelatinase-associated lipocalin	LCN2	-	↓2.18
P02787	Serotransferrin	TF	-	↓2.39
P60174	Triosephosphate isomerase	TPI1	-	↓2.64
Q6P5S2	Protein LEG1 homolog	LEG1	-	↓4.44
P68133	Actin alpha skeletal muscle	ACTA1	-	↓6.05

Subtitle: Protein expression presented as up-regulated (↑), down-regulated (↓) and with no alteration in its expression (-) after the sport event. Source: Authors.

Gene Ontology analysis of the proteins whose expression was altered after the 5 and 10 km athletic events revealed that the major biological processes were affected: Retinal homeostasis (21.43%), sensory perception of taste (20.41%), transport of substances (16.33%) and glomerular filtration (13.27%) (Figure 2-A). Among the cellular components, the extracellular exosome (47.42%) and the dimeric secretion complex of immunoglobulin IgA (43.20%) stood out (Figure 2-C). As for molecular functions, those that received the highest percentage were cysteine-type endopeptidase inhibitory activity (55%), peroxidase activity (14%), and binding to immunoglobulin receptors (6%) (Figure 2-B).

Findings related to the immune system were expected because exercise induces changes in the immune system and it mediates many of its effects. A classic paradigm in immunology states that intense physical training creates a "window" after immunosuppression, which states that some immunological variables transiently decrease after intense physical activity (Peak et al, 2017; Nieman & Lila & Gillitt, 2019). The observed changes in proteins involved in peroxidase activity confirm the available evidence that physical activity promotes changes in the redox balance of the body and induces oxidative stress (Powers, Radak & Ji, 2016).

There are few previous studies that have looked at the analysis of salivary proteome profiles in a sporting context, one of which is the work of Zauber et al. (2012) who investigated ultra-endurance athletes during a 1060 km cycling race. This study found changes in the expression of similar salivary proteins despite shorter distance and duration. Zauber et al. (2012) identified a total of 34 proteins with significant changes in males comparing the exercise phase to the rest phase. Among those that showed significant changes, 5 proteins were also identified in the present study: Alpha-Amylase 1 (AMY1), Androgen-Regulated Protein 3B in the submaxillary gland (SMR3B), Cystatin-SN (CST1), Cystatin-SA (CST2), and Lactoperoxidase (LPO).

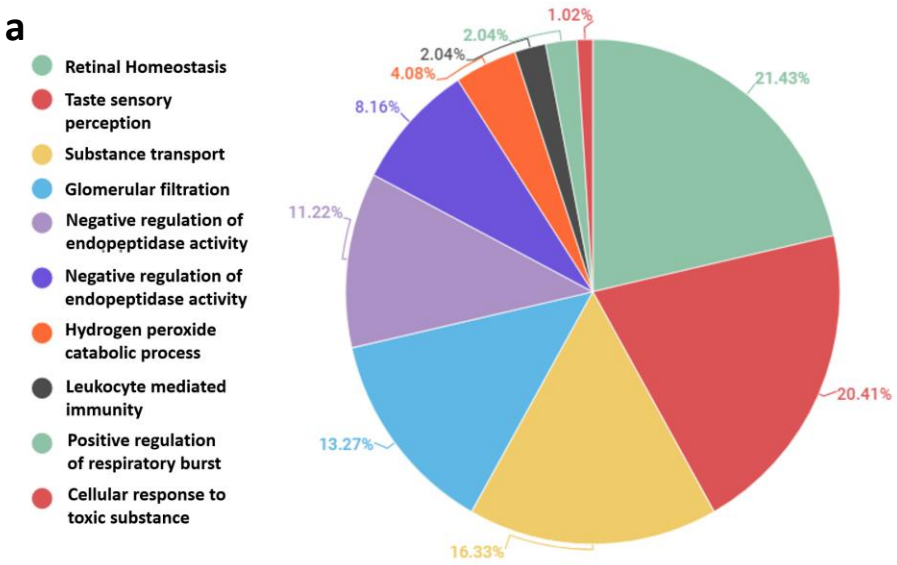
AMY1 is secreted by the parotid gland in response to adrenergic activity and is increased in situations of physical or psychological stress (Nater & Rohleder, 2009). It plays an important role in digestion and mucosal immunity and is considered a consistent indicator of blood markers of epinephrine and norepinephrine (Nater & Rohleder, 2009; Ihalainen et al, 2016). The results of this study are consistent with other authors who have found an increase in AMY1 activity and concentration with exercise (Zauber et al, 2016; Koibuchu & Suzuki, 2014; Ligtenberg et al, 2015). However, in this study, expression was increased only in the 5 km group, whereas no changes in expression were observed in the 10 km group.

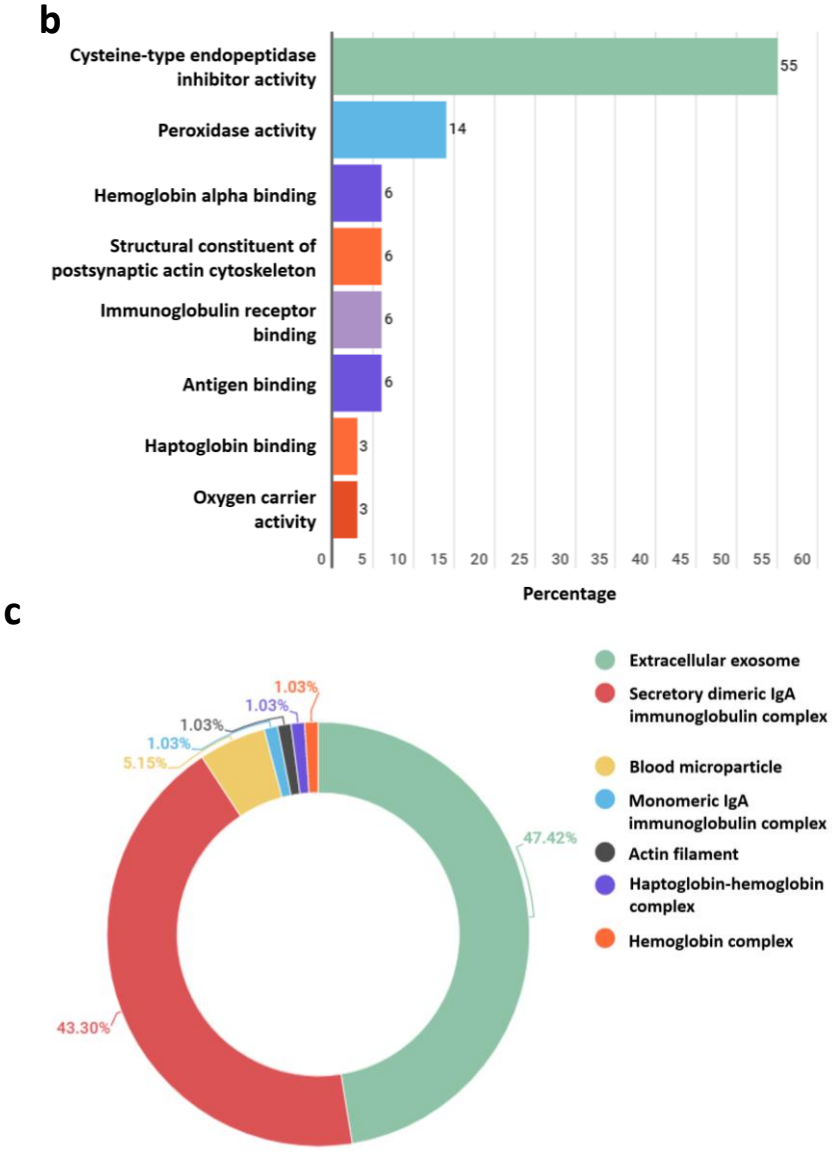
AMY1 is considered a noninvasive and effective marker of physical stress, and wearable monitoring systems have been introduced to assess stress by its activity (Yamaguchi et al, 2006; Peng et al, 2016). On the other hand, there are important differences in salivary proteome and AMY1 activity between men and women, indicating the need for bridges of specific sections by sex to analyze possible changes (z; Li, 2015).

SMR3B is secreted by the submaxillary gland and is expressed by a family of genes that can produce opiorfin homologs (Koffler et al, 2013; Tong et al, 2008). Opiorfin has natural analgesic and antidepressant effects (Wisner et al, 2006). Under-expression of SMR3B has been found in infectious diseases (hepatitis) (Gonçalves et al, 2014) and neurological diseases (Ngounou Wetie et al, 2015). SM3B had its expression increased in both groups analyzed (5 and 10 km).

LPO is a glycoprotein that has a single polypeptide chain and is found in secretions from exocrine glands (Sarr et al, 2018). Salivary LPO plays a critical role in the nonspecific immune response associated with oral health. In the presence of hydrogen peroxide, it thus catalyzes the oxidation of salivary thiocyanate ions (SCN -) to products that exhibit antimicrobial activity (Magacz et al, 2019). LPO had increased expression only in the 5 km group.

Figure 2 - Distribution of proteins differentially expressed in *gene ontology* analysis of biological processes (a) molecuar function (b) and cellular components (c).





Source: Authors.

Cystatins are a superfamily of proteins whose main function in the saliva is to protect the oral cavity by inhibiting cysteine proteases (Manconi et al, 2017). Salivary CSTB showed reduced expression in both groups studied (5 km and 10 km) in this study. Plasma CSTB was inversely associated with exercise in a recent study, as well as other factors that may be related to exercise (Stattin et al, 2019). Unregulated expression of CSTB has been associated with several types of cancer (Ma, Chen & Petersen, 2017). In addition, there are still studies linking CSTB to inflammation, endotoxemia, and cardiovascular disease (Maher et al, 2014; Dencker et al, 2017).

CST1 is also discussed in the context of malignant neoplasms and identified as a potential tumor marker, as it is involved in inflammation, cell cycle, cell senescence, tumorigenesis, and metastasis (Liu & Yao, 2019). Cystatin-S (CST4) is identified as a potential biomarker in Sjögren's syndrome (autoimmune disease affecting the lacrimal and salivary producing glands) (Martini et al, 2017). Salivary CST2 has been associated with the severity of periodontal disease and its serum levels may reflect renal function (Techatanawat et al, 2019).

The study by Franco-martínez et al. (2020) aimed to investigate the influence of sex on the salivary proteome at rest and after an acute exercise session leading to muscle failure. The exercise model used was the resistance model (6 sets of 10 repetitions of total squat exercise). In the group of proteins identified as modulated after exercise in both sexes, two with a

similar variation in expression were also found in the present study: Actin Muscle Skeletal Alpha (ACTA1) and Cystatin-B (CSTB).

ACTA1 is a protein that plays very important roles in biological systems, including muscle contraction, maintenance of physical integrity of cells, and others (Pollard, 2016). Our results showed that it was under-expressed in the 10 km group after exercise. A previous study using plasma ACTA1 showed a significant decrease in its levels 1h after eccentric exercise (Tékus et al, 2017). However, plasma ACTA1 was identified as a possible marker of muscle injury in a study that found high serum levels of this protein in injured athletes (Martinez Amat et al, 2007).

One of the limitations of the study is the sample size, which may limit the potential to identify proteins that are modulated after training with reproducibility. However, this sample size is consistent with what has been reported in the literature on proteomic studies in a training context. This study was conducted in two training models (5 and 10 km street running), which requires work evaluating other modalities, with variations in duration and intensity. However, we highlight the innovative nature of the work, which used sophisticated analytical techniques that have great analytical power and significant cost in the sports setting.

4. Conclusion

It was found that street running promoted significant changes in the expression of 15 salivary proteins in the 5 km group and 13 salivary proteins in the 10 km group. Of the identified proteins, only 7 had been previously reported in the literature in similar experimental models (AMY1, SMR3B, LPO, ACTA1, CSTB, CST1, and CST2). This study adds to the few existing studies in the literature to better understand the acute effects of exercise on the salivary proteome. Further population studies are needed to understand interindividual variations in the salivary proteome, in addition to assessing time-of-day oscillations between genders and accounting for different exercise conditions.

Supplementary Table 1 - List of total proteins identified in street runners at a real sporting event.

Access Number	Protein Description	Gene	Average LFQ intensity (fmol)	
			5km Group	10km Group
P60709	Actin cytoplasmic 1	ACTB	-	172,96
Q8N4F0	BPI fold-containing family B member 2	BPIFB2	39,66	159,94
P23280	Carbonic anhydrase 6	CA6	-	128,61
P54108	Cysteine-rich secretory protein 3	CRIPS3	-	14,54
P06744	Glucose-6-phosphate isomerase	GPI	-	21,50
P09211	Glutathione S-transferase P	GSTP1	-	25,73
P01966	Hemoglobin subunit alpha	HBA	190,14	300,00
P02790	Hemopexin	HPX	-	11,77
P00738	Haptoglobin	HP	22,65	-
P01764	Immunoglobulin heavy variable 3-23	IGHV3-23	12,83	14,57
A0A0B4J1V0	Immunoglobulin heavy variable 3-15	IGHV3-15	-	41,95

A0A0C4DH30	Probable non-functional immunoglobulin heavy variable 3-16	IGHV3-16	18,72	43,01
B9A064	Immunoglobulin lambda-like polypeptide 5	IGLL5	-	246,99
P18510	Interleukin-1 receptor antagonist protein	IL1RN	9,76	33,71
P01615	Immunoglobulin kappa variable 2D-28	IGKV2D-28	-	44,34
Q5VSP4	Putative lipocalin 1-like protein 1	LCN1P1	-	95,63
P80188	Neutrophil gelatinase-associated lipocalin	LCN2	27,14	98,25
A5A3E0	POTE ankyrin domain family member F	POTEF	40,88	49,05
P0CG38	POTE ankyrin domain family member I	POTEI	-	32,13
P0CG39	POTE ankyrin domain family member J	POTEJ	-	3,76
P02812	Basic salivary proline-rich protein 2	PRB2	-	1799,71
P04280	Basic salivary proline-rich protein 1	PRB1	417,47	772,50
P05109	Protein S100-A8	S100A8	34,35	224,49
P06702	Protein S100-A9	S100A9	-	87,67
P20061	Transcobalamin-1	TCN1	-	86,18
Q14508	WAP four-disulfide core domain protein 2	WFDC2	-	32,81
P25311	Zinc-alpha-2-glycoprotein	AZGP1	121,41	586,05
P31947	14-3-3 protein sigma	SFN	3,11	-
P63104	14-3-3 protein zeta/delta	YWHAZ	2,74	-
P02768	Albumin	ALB	64,44	-
Q8N4F0	BPI fold-containing family B member 2	BPIFB2	39,66	159,94
P36222	Chitinase-3-like protein 1	CHI3L1	149,74	-
P23528	Cofilin-1	CFL1	5,17	-
P06733	Alpha-enolase	ENO1	10,58	-
Q01469	Fatty acid-binding protein 5	FABP5	16,51	-
P04264	Keratin, type II cytoskeletal 1	KRT1	11,44	-
P06870	Kallikrein-1	KLK1	38,89	-
P0DOY3	Immunoglobulin lambda constant 3	IGLC3	114,76	-
P62937	Peptidyl-prolyl cis-trans isomerase A	PPIA	9,67	-
P07737	Profilin-1	PFN1	20,04	-

P10599	Thioredoxin	TXN	15,51	-
P29401	Transketolase	TKT	6,34	-
P31025	Lipocalin-1	LCN1	41,17	-
P0CF74	Immunoglobulin lambda constant 6	IGLC6	94,14	98,87
P68871	Hemoglobin subunit beta	HBB	108,58	225,50
P04080	Cystatin-B	CSTB	74,10	494,12
P04745	Alpha Amylase 1	AMY1A	159,16	1001,21
P02042	Hemoglobin subunit delta	HBD	9,59	-
P09228	Cystatin-SA	CST2	85,71	518,89
P01034	Cystatin-C	CST3	25,49	-
P01036	Cystatin-S	CST4	924,95	3332,18
P01037	Cystatin-SN	CST1	749,41	3183,58
Q9BYX7	Putative beta actin like protein 3	POTEKP	12,61	-
Q562R1	Beta actin like protein 2	ACTBL2	9,54	51,29
Q6S8J3	POTE ankyrin domain family member E	POTEE	24,76	56,67
P12273	Prolactin inducible protein	PIP	58,15	235,54
P22079	Lactoperoxidase	LPO	17,33	75,79
P02814	Submaxillary gland androgen regulated protein 3B	SMR3B	1307,91	3491,69
P01834	Immunoglobulin kappa constant	IGKC	126,59	887,81
P01833	Polymeric immunoglobulin receptor	PIGR	181,04	1063,75
P01876	Immunoglobulin heavy constant alpha 1	IGHA1	193,24	1246,85
P01877	Immunoglobulin heavy constant alpha 2	IGHA2	159,73	1463,85
P01871	Immunoglobulin heavy constant mu	IGHMU	10,27	-
P01591	Immunoglobulin J chain	JCHAIN	34,24	253,88
P80188	Neutrophil gelatinase-associated lipocalin	LCN2	27,14	98,25
P02787	Serotransferrin	TF	29,91	203,47
P60174	Triosephosphate isomerase	TPI1	8,32	59,27
Q6P5S2	Protein LEG1 homolog	LEG1	27,31	179,20
P68133	Actin alpha skeletal muscle	ACTA1	-	17,14

Source: Authors.

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