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Effects of high fat diet on salivary α -amylase, serum parameters and food consumption in rats

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ABSTRACT

Salivary α -amylase, a major protein in saliva, has been described as a marker for sympathetic nervous system activity, hence for metabolic energy balance. In this context, its expression in overweight and obesity is of interest. Rats fed with a diet enriched with sunflower oil differentially gained weight yielding two subgroups according to their susceptibility (OP) or resistance (OR) to obesity. Elevated plasmatic levels of leptin in the OP subgroup and altered plasmatic lipid profiles (lower triglycerides and higher total cholesterol/HDL ratio compared to controls) in OR subgroup were observed. Animals from OP subgroup presented higher α -amylase expression and activity even prior to the dietary treatment, suggesting that this salivary protein may constitute a putative indicator of susceptibility for fat tissue accumulation. After 18 weeks of high-fat diet consumption, salivary α -amylase levels did not significantly changed in OP subgroup, but increased 3-fold in OR subgroup. The raise of α -amylase for the latter might represent an adaptation to lower starch intake. These results suggest that salivary α -amylase secretion might be useful to predict susceptibility for weight gain induced by high-fat diet consumption.

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1. Introduction

During the past decades, obesity prevalence has enormously increased worldwide, reaching epidemic proportions in Europe.¹ An imbalance between energy intake and output, many times involving the consumption of high amounts of fat, is the main reason.

Sunflower oil is one of the most consumed liquid fats worldwide, mainly used for frying foods and in salads, being preferred by many people for its neutral taste.² Sunflower oil is rich in unsaturated fatty acids, such as linoleic acid (n-6 PUFA) and oleic acid (n-3-PUFA).³ Despite some controversy, oils with high contents of unsaturated fatty acids may induce weight gain without most of the deleterious effects associated with saturated fat. Some studies reported that diets rich in n-6 PUFA decrease plasmatic levels of triacylglycerol and total cholesterol, as well as liver fat, improving metabolic status,⁴ insulin sensitivity^{5,6} and cardiovascular function,⁷ comparatively to saturated fatty acids.

Saliva has a variety of proteins with important roles in oral health and food perception, some of which have been suggested to influence ingestive behaviour.^{8,9} The interest in the study of this fluid increased in the last decade due to methodological developments, which revealed its potential as a source of biomarkers of pathological and physiological conditions, helping in the understanding of biological mechanisms.¹⁰ Nonetheless, relatively few studies were found about salivary protein composition in obesity.¹¹

The levels of salivary α -amylase, a protein present in considerable amounts in mixed saliva and responsible for the hydrolysis of starch, are generally referred as a marker of stress and an indicator of sympathetic nervous system activity,¹² which in turn plays an essential role in metabolic homeostasis. Several studies have found elevated levels of this salivary protein concomitant to overall changes in norepinephrine in response to stress.¹³ Autonomic nervous system influences the regulation of salivary secretion, with sympathetic nervous system activation decreasing salivary flow and increasing salivary protein output. The stimulation of beta-adrenergic receptors from salivary parotid glands increases alpha-amylase secretion.¹⁴

The relation between obesity and sympathetic nervous system activity has been somehow controversial. Low activity of this branch of the autonomic nervous system has been suggested to be a risk factor for weight gain and obesity development.¹⁵ However, some evidences point that the sympathetic neural transmitter neuropeptide Y (NPY) is involved in stress-induced augmentation of diet-induced obesity.¹⁶ From our knowledge it has not been addressed whether the expression and enzymatic activity of salivary alpha-amylase differs in obesity.

Similarly to humans, in some rodent strains there are subjects prone and subjects resistant to obesity induced by high-fat diet consumption.¹⁷ Although the factors underlying this different propensity are yet poorly understood, these animals constitute a good model to study obesity. This study had three main objectives: to investigate how salivary alpha-amylase levels relate to rats susceptibility for weight gain; to evaluate the effects of the consumption of a high-fat diet in

salivary alpha-amylase secretion; and to assess the individual's responses to high-fat diet, by evaluating biochemical markers of metabolic status.

2. Materials and methods

2.1. Treatment of animals

Twenty outbred female Wistar rats 4 weeks old (160–223 g) were maintained on a 12 h/12 h light/dark cycle at a constant temperature (22 °C) and humidity (65%) with ad libitum access to food and water. The animals were single caged in standard rat cages and maintained on a standard diet (SAFE A04) for 4 weeks acclimation. After that time animals were randomly divided in two dietary groups (control $N = 6$ and sunflower oil treated $N = 14$), with the control group receiving the same standard diet of the pre-trial period and the treatment group receiving a high fat diet rich in sunflower oil during 18 weeks, both ad libitum.

The experimental high-fat diet was prepared by soaking the standard feed in sunflower oil during 4 days, followed by drying in an oven (temperature below 40 °C) and then stored, protected from light and oxygen. Sunflower oil used to prepare the high-fat diet was obtained from a commercial trademark available for human consumption. Fatty acid composition was measured by gas chromatography (Supplementary Table S1). Analyses of the macronutrient content of the used rodent diets were performed in the Laboratory of Animal Nutrition of the ICAAM, University of Évora: sunflower oil enriched diet containing 14.5% total protein, 48.2% carbohydrates, 14.7% total fat, with an energetic content of 3.8 kcal/g and standard diet containing 17.4% total protein, 53.7% carbohydrates, 2.7% total fat, with an energetic content of 3.1 kcal/g.

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During both the acclimation and experimental periods the animals were weighed weekly and food consumption monitored, by weighing the amount distributed and the refusals. At the end of the experiment the animals were euthanized by exsanguination under anaesthesia and blood collected for further analysis. All animal procedures were approved by the scientific committee, and were supervised by a scientist trained by the Federation of European Laboratory Animal Science Associations (FELASA), and conformed to Portuguese law,¹⁸ which followed European Union Laboratory Animal Experimentation Regulations.

2.2. Oral glucose tolerance test

Oral glucose tolerance test (OGTT) was performed at the beginning, in the middle and at the end of dietary treatment. After overnight (16-h) fasting, blood samples were collected from the tail vein followed by oral administration of glucose (75 g/kg of body weight). Blood glycaemia was measured in fast and after 30, 60, 90 and 120 min after glucose bolus administration, using a hand-held glucometer (Accu-Check, Roche). Whole blood glucose levels were expressed in mg/dl.

2.3. Serum biochemical parameters

Blood obtained by heart puncture, after 18 weeks experiment, was collected in EDTA coated tubes and centrifuged at 3000 rpm and room temperature for 15 min.

Plasma concentrations of insulin and leptin were measured by enzyme-linked immunosorbent assay (ELISA). For insulin a competitive ELISA was used [Mouse anti-rat insulin serum (ref. BT53-3033-39 Linco Research, USA) and rat-insulin standards (ref. 813K, Linco, Research, USA)] whereas leptin determination was performed using a commercial kit according to the manufacturer's instructions (EZRL-83K Millipore). Plasma concentrations of total and HDL cholesterol, as well as triglycerides were measured using enzymatic tests (Sentinel Diagnostics, Milano, Italy). Precision and reproducibility of measurements were monitored by using a multi-parameter control for quantitative clinical chemistry determinations (Clin Chem Control 1, and Clin Chem Control 2, Sentinel Diagnostics, Milan, Italy).

2.4. Saliva sample collection and protein quantification

Whole mouth saliva was collected from each individual animal under parasympathetic stimulation, to increase flow, facilitating saliva collection. Animals were injected with pilocarpine (7 mg/kg body weight s.c.) and saliva aspirated from the mouth using a micropipette and collected to a tube maintained on ice. Although pilocarpine administration can lead to small increases in amylase activity, the dose used was the same for all the animals, to assure that any change in protein levels would only reflect experimental treatment. Collections were performed on each individual in two different days before the beginning of the dietary treatment and at the end of the 18 weeks experimental period. The two individual different collections from the beginning were pooled. After saliva collection, samples were centrifuged at $13,000 \times g$, for 5 min to remove food and cell debris, supernatant aliquoted and stored at -80°C until analysis. Bradford method (BioRad Protein Assay) was used for total protein quantification.

2.5. Western blot

For Western blot, SDS PAGE separation of 3 μg protein from each sample was performed using 12% gels run at a constant voltage of 100 V. The separated proteins were transferred to a PDVF membrane by electroblotting using a Tris-glycine buffer system, and the protein patterns were visualized by staining with Ponceau S dye (0.3% w/v in 10% acetic acid) for 10 min. The protein profiles were developed by washing away excess dye with distilled water. For Western blot the membrane was incubated with anti- α -amylase primary antibody (Sc-46657, Santa Cruz Biotechnology; 1:200 dilution). A solution consisting in 5% non-fat dry milk in TBS-Tween 20 was used for blocking, for 2 h, with agitation at room temperature followed by incubation with primary antibody overnight at 4°C . Salivary amylase bands were detected with an alkaline phosphatase-linked secondary antibody against mouse IgG (anti-mouse, GE Healthcare, 1:10,000 dilution) using a chemi-fluorescent substrate (ECL Plus Western Blotting Detection

Reagents, GE, Healthcare). Membranes were revealed using a transilluminator (Bio-Rad Gel-doc system) and a semi-quantitative analysis of salivary amylase expression was carried out by using Quantity One software (BioRad).

2.6. Salivary α -amylase enzymatic activity

Dinitrosalicylic acid assay¹⁹ was used for measuring the starch-hydrolyzing activity of salivary α -amylase. The reaction mixture consisted 100 μL of 1% starch solution, 100 μL of 20 mM phosphate buffer (pH 7.0) and 50 μL saliva (diluted to a protein concentration in enzymatic assay of 1.5–2.5 $\mu\text{g}/\text{ml}$ in the same phosphate buffer). After incubation at 37°C for 20 min, in a thermostable water bath constant stirring, the reaction was stopped by the addition of 100 μL DNS reagent (1% dinitrosalicylic acid, 0.2% phenol, 0.05% sodium sulfite, 1% sodium hydroxide). Samples were then placed in a boiling water bath for 5 min, and subsequently cooled down to room temperature. Further, 200 μL of sodium and potassium tartrate (40%) and 900 μL of bidistilled water were added to samples. Absorbance was measured using a spectrophotometer (Hitachi U200) at 540 nm against a blank prepared using the identical method, except the enzyme solution, which was added to the mixture after the addition of the DNS solution. The absorbance values were then converted to glucose equivalent using a standard curve (0.2–2.0 mg/mL). The results were expressed as mmol glucose/min/mg protein and as a percentage of control (group receiving standard diet) considered as 100%.

2.7. Statistical analysis

Considering the objectives of the study, different statistical approaches were carried out. After the end of experiment, the measured and calculated variables were analyzed with descriptive statistics. Subsequent analysis was carried out using a median analysis and establishing a ranking according to the percentile distribution of the variables: weight at the end of the trial, weight gain throughout the test and weight gain relative to the initial weight. The animals from sunflower oil treatment were divided into two subgroups based on the results of the previous analysis. The normality and homoscedasticity were tested respectively by Kolmogorov-Smirnov and Levene methodologies. Variables such as body weight, total food consumption and total energy consumption were tested according ANCOVA, with the initial body weight as a covariant. Serum biochemical parameters were tested according ANOVA-GLM. When significant differences were found, post hoc pooling was performed by Tukey's method. Pearson correlations between different variables were carried out, taking into account the level of significance and the value of R. All statistical analyses were performed by SPSS 20 software.

3. Results

3.1. Body weight and food intake

At the end of acclimation time (week 0), animals were randomly assigned to two experimental groups [control

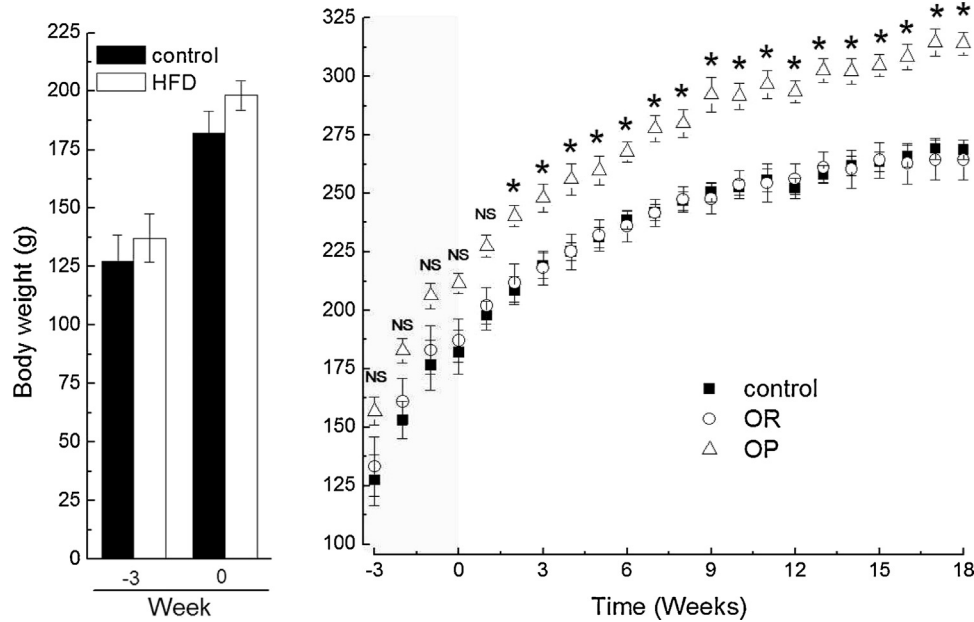


Fig. 1 – Initial body weights (A) and growth rate (B) of control (Cont; N = 6) and treatment (HFD; N = 13) groups. OR = obese resistant (N = 7); OP = obese prone (N = 6). Week –3 represents the time at which the animals were allocated to individual cages for acclimation, whereas week 0 represents the start of high-fat diet administration to randomly selected animals. Data are presented as means \pm SEM. (*significant for $P < 0.05$) (ANCOVA analysis using the initial body weight as a covariant).

(N = 6) and high-fat treated (N = 14)], which received dietary treatment for 18 weeks. At this time mean body weights from control and from the group treated with the high fat diet did not present significant differences (Fig. 1A).

After the 18 weeks all animals, including controls, had gained weight. However, at this time, for the animals from the high-fat dietary group, there was a large dispersion in the values, suggesting the existence of animals with different propensity to weight gain. The ratio between the weight gain and initial body weight was used to allocate animals from high-fat dietary group in two subgroups. Since quite different ratios were observed, medians were used to create more homogeneous groups according to the respective percentiles. Analysis of variance was one ANCOVA to correct the possible effect of the different initial body weights. The two distinct subgroups, from the high-fat treatment group were constituted by: (i) individuals whose body weight gains and final body

weights remain similar to control group (obese resistant, OR; N = 7); (ii) individuals for which body weight gains and final body weights were at least 10% higher than in control group (obese prone, OP; N = 6) (Fig. 1 and Table 1), according to the criteria reported by others.²⁰ One rat from the high-fat diet treatment group was excluded from the analysis because it was identified as outlier. This was due to its abnormal weight gain relatively to its final body weight, as evidenced by the statistical analysis (abnormal value in the box-plot after using the Z-score procedure afterwards confirmed by the Dixon test).

Food intake differed among the groups. The animals maintained on the high-fat diet ingested a lower amount of food compared to controls (2.60 ± 0.06 kg control vs 2.38 ± 0.08 kg for OP and 2.12 ± 0.039 kg for OR groups treatment; values for the total amount consumed in the 18 weeks of experiment). Considering total energy consumption,

Table 1 – Body weight and food consumption.

	Control	OP	OR
Body weight (week –3) (g)	127.5 \pm 11.0	156.8 \pm 6.0	133.4 \pm 8.6
Body weight (week 0) (g)	182.1 \pm 9.5 ^a	211.5 \pm 4.4 ^a	187.1 \pm 9.2 ^a
Final body weight (g)	268.7 \pm 4.0 ^a	314.2 \pm 5.0 ^b	267.3 \pm 8.4 ^a
Total food consumption (kg)	2.60 \pm 0.06 ^a	2.38 \pm 0.08 ^b	2.12 \pm 0.04 ^b
Total energy consumption (Kcal) [#]	8.07 \pm 0.19 ^a	9.06 \pm 0.30 ^b	8.06 \pm 0.15 ^a

OR = obese resistant; OP = obese prone. Values are means \pm SEM.

^{a,b}For each parameter, different letters represent significant differences among means. Mean comparisons were performed using the ANCOVA analysis with the initial body weight as covariant and $P < 0.05$.

[#] Calculated values according to the energy content after nutritional analysis (3.8 kcal/g for the high fat diet and 3.1 kcal/g for the standard chow diet).

Table 2 – Serum biochemical parameters measured after 18 weeks of a high fat or control diet.

	Control	OP	OR	Ref values [#]
Insulin (ng/mL)	0.75 ± 0.02 ^a	0.75 ± 0.07 ^a	0.85 ± 0.05 ^a	0.60–0.90 ^{21,22}
Leptin (ng/mL)	1.69 ± 0.17 ^{a,b}	2.39 ± 0.41 ^b	1.34 ± 0.17 ^a	1.37–2.29 ²³
TG (mg/dL)	79.1 ± 6.8 ^a	61.0 ± 7.3 ^a	45.3 ± 2.8 ^b	40.0–230.0 ^{24,25}
Total cholesterol (mg/dL)	127.0 ± 9.4 ^a	129.6 ± 7.4 ^a	119.3 ± 8.8 ^a	70.0–125.0 ²⁵
HDL (mg/dL)	60.3 ± 3.6 ^a	52.7 ± 2.5 ^a	43.5 ± 5.2 ^b	38.0–63.0 ⁴
Total cholesterol/HDL	2.12 ± 0.14 ^a	2.48 ± 0.14 ^a	2.86 ± 0.27 ^b	

OR = obese resistant; OP = obese prone. Data are presented as mean ± SEM.
[#] Normal mean values for female Wistar adult rats.
^{a,b}For each parameter, different letters represent significant differences among means. Mean comparisons were performed using ANOVA analysis for $P < 0.05$.

however, the caloric intake was 12.3% higher in the OP subgroup compared to both the control and OR subgroups (Table 1).

3.2. Blood biochemical parameters

All animals presented normal oral glucose tolerance tests both at the beginning and at the end of the experiment. In both times the mean glycaemic levels, from all the groups, ranged from 68 to 90 mg/dL glucose following overnight fast, increased to 100–115 mg/dL 30 min after the administration of the oral bolus of glucose and returned to fasting values at 60 min (results not shown), indicating that no significant differences in glucose tolerance were induced by the sunflower oil enriched diet. Accordingly, fasting plasmatic insulin levels, at the end of the dietary treatment, were similar among the groups (Table 2). Concerning fasting plasmatic leptin levels, differences were observed, with OP subgroup presenting values almost twice higher than the OR subgroup (Table 2).

Being leptin a hormone that promotes satiety, the relation between leptin plasmatic levels and energy consumption was investigated. A strong positive correlation was observed for the animals fed the high fat diet ($r = 0.71$; $P = 0.003$), i.e., higher plasmatic leptin levels were associated with higher energy consumption (Fig. 2B), indicating higher ingestion.

The consumption of the sunflower oil enriched diet induced differential changes in lipid profiles among OR and OP subgroups. A 40% decrease in the plasmatic levels of

triglycerides was observed in OR subgroup while OP presented values similar to the ones from controls. Concerning total cholesterol no differences were observed between the groups. However, high-density lipoprotein (HDL) cholesterol was approximately 30% lower in the OR subgroup and, accordingly, total cholesterol to HDL ratio was significantly increased in this subgroup (Table 2).

3.3. Salivary α -amylase expression levels and enzymatic activity

The main aim of the present experiment was to study salivary α -amylase secretion in individuals with different susceptibilities to obesity. Differences in this salivary protein expression levels and enzymatic activity were observed among the experimental groups. Before the beginning of dietary treatment, where all animals were fed standard rodent diet, OP subgroup presented higher expression levels of α -amylase when compared to both the control and OR groups, being these last two groups similar (Fig. 3A).

After the chronic consumption of sunflower oil enriched diet, OR subgroup presented an increased expression of salivary α -amylase comparatively to the other experimental groups (Fig. 3B). It is noteworthy that when compared to the expression levels prior to the treatment, α -amylase expression was significantly increased in the OR subgroup, whereas it was maintained in the control and OP subgroup (Fig. 3B).

In concordance with protein expression, the salivary α -amylase enzymatic activity from whole saliva collected at the beginning of the trial (when animals were all maintained on a standard rodent chow diet) was 1.6 fold higher in OP individuals (Fig. 4).

At this time point, α -amylase enzymatic activity was strongly correlated with future individual body weight gains ($r = 0.70$; $P = 0.032$), i.e., individuals which gained more weight during the 18 weeks high fat diet treatment presented higher salivary α -amylase activities (Fig. 5A). Interestingly, this relation (Pearson correlation coefficient) is even higher considering only the animals that fed the high fat diet ($r = 0.76$; $P = 0.042$) (Fig. 5B).

In the contrary, at the end of the dietary treatment the α -amylase activity in OP subgroup was similar to the controls while in the OR subgroup α -amylase activity was 3-fold higher. Notice that, compared to the beginning of the trial, salivary α -amylase enzymatic activity in OR subgroup was increased 3 times (Fig. 4).

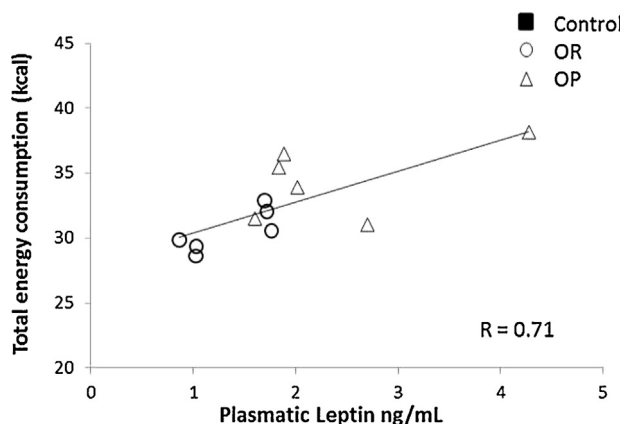


Fig. 2 – Correlation between plasmatic leptin levels and total energy consumption.

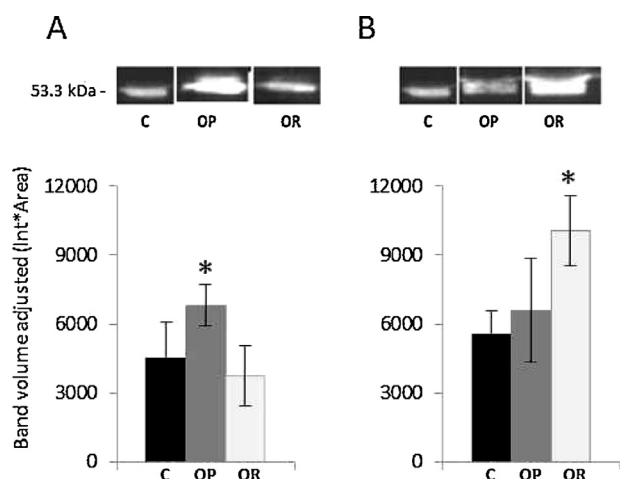


Fig. 3 – Representative Western blot analysis of α -amylase in mixed saliva samples from control (C), obese-prone (OP) and obese resistant (OR) animals. A: before dietary treatment; B: after 18 weeks dietary treatment; Lower panels show α -amylase expression quantified by densitometric analysis of this protein immunoreactive band. Data are presented as mean \pm SEM. (*differences between high fat diet subgroups and control are significant for $P < 0.05$).

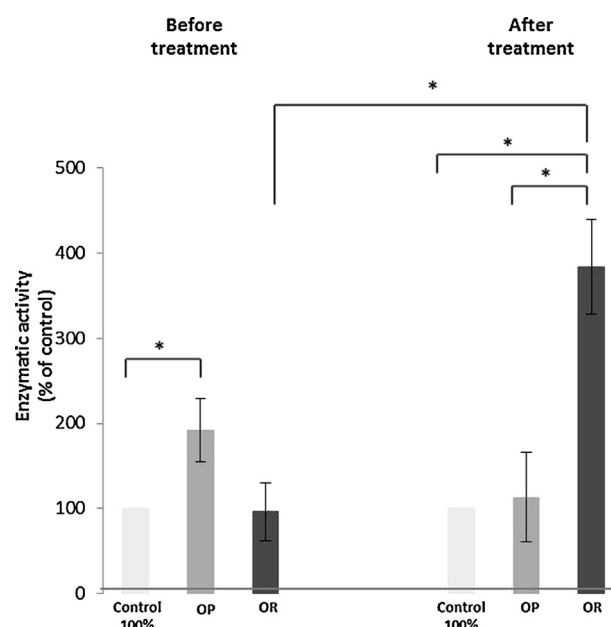


Fig. 4 – Salivary amylase activity of mixed saliva samples before and after dietary treatment. OP = obese prone; OR = obese resistant. Data are presented as mean \pm SEM. $P < 0.05$.

4. Discussion

The existence of individuals with propensity and individuals with resistance to weight gain has been referred for both animals and humans. In Wistar rats this differential response in body weight changes has been already referred for individuals submitted to high saturated fat diets^{26,27} and has been attributed to different causes such as overconsumption, due to these diets high palatability, or to the modulation of gene expression linked to lipogenesis.²⁸ Studies are not consensual in terms of the potential of diets rich in polyunsaturated fat for obesity development. Some authors refer similar²⁹ and others lower³⁰ body weight gains, induced by PUFA comparatively to the ones induced by saturated fat.

In the present study, a differential individual response in the rate of weight gain was observed for rats consuming a high-fat-enriched with sunflower oil diet. Despite being an unsaturated fat, the sunflower oil used in this study contains a considerable amount of n-6 PUFA over n-3 PUFA, the opposite of other oils such as olive or rapeseed oils,³ and a key factor in high-fat induced obesity.³¹ Our results support the fact that a high-fat diet rich in n-6 PUFA induce weight gain and eventually obesity in animals with genetic susceptibility, such as the OP subgroup.

Despite obesity development in susceptible animals, the effects of the tested diet appeared not to be as deleterious as the ones produced by saturated fat. High amounts of saturated dietary fats lead to insulin resistance in rats³² what can

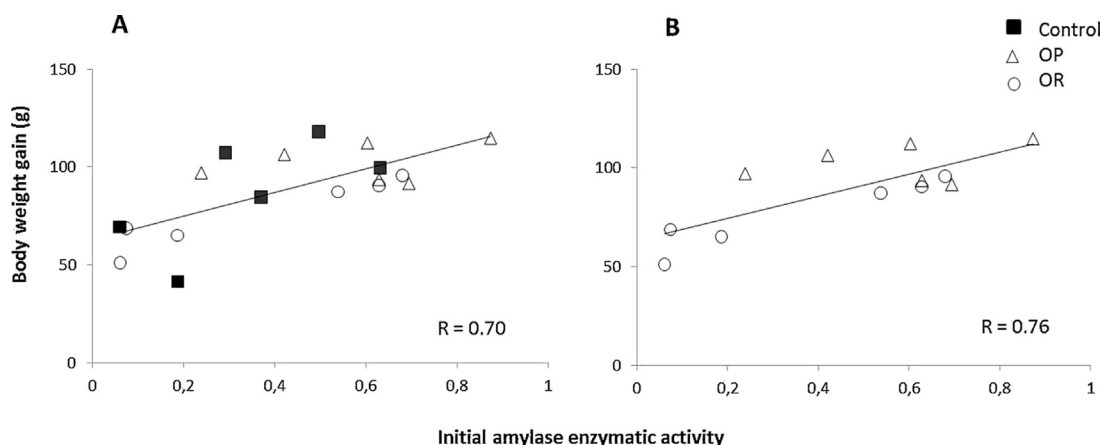


Fig. 5 – Correlation between body weight gains and α -amylase enzymatic activity from saliva collected before high-fat dietary treatment. (A) All groups; (B) OR and OP groups.

ultimately result in altered glucose tolerance and diabetes. In the present study, no changes in oral glucose tolerance tests or fasting insulinemia were observed as a consequence of the consumption of this high fat diet consumption for 18 weeks. The fatty acid composition may be a possible explanation for these results, since sunflower oil is rich in polyunsaturated fat, therefore not inducing insulin resistance, as suggested by others.³³

Accordingly with fasting leptin levels, it is possible to hypothesize that the weight gain observed in some of the animals (OP animals) can be the result of a degree of leptin resistance by these. Leptin is mainly produced by adipose tissue and is highly involved in the control of ingestion by inducing satiety. Despite the increased levels of plasmatic leptin in OP animals, higher energy intake was observed in this subgroup suggesting a reduced response to this hormone and a consequent lack of the fall in satiety, a factor already pointed out as responsible for diet induced obesity.³⁴

Lipid profiles were accessed with the aim of adding information on the metabolic effects of the fat used. These effects were observed to be dependent on the propensity for obesity. In OR subgroup the dietary treatment induced a decrease in triacylglycerol and HDL levels. The maintenance of total cholesterol resulted in an increase of total cholesterol/HDL ratio in this subgroup. Similar results were reported by other authors for sunflower oil enriched diets, for both triacylglycerol levels³⁵ and total cholesterol/HDL ratio.³⁶ Cholesterol is precursor of corticosteroids and androgens, which increased levels are ultimately related to insulin resistance.^{37,38} Although insulinemias and glucose tolerance curves were similar to the ones from control individuals, as discussed above, the hypothesis of OR individuals became less sensitive to insulin and/or present metabolic changes, if maintained for a longer period in a high fat diet, is not to discard. By the contrary, lipid profiles from OP individuals did not differ from control animals. Besides concluding that the polyunsaturated high fat diet did not induce severe dyslipidaemias in either OP or OR subgroups, for the period considered in this study, it is worthwhile to note that the effects of polyunsaturated fat are complex and should not be generalized, but instead should have into consideration the metabolic characteristics of each individual, where the genetic background is certainly playing a central role.

In the present study differences in the expression and enzymatic activity of salivary α -amylase, both before and after the high fat diet trial were observed among the groups. The α -amylase levels measured before animals entered the trial was higher in individuals which were further allocated to OP subgroup and were strongly correlated with the rate of body weight gain achieved during the experiment. Consequently, the levels of this protein seem to behave as a predictor of the susceptibility individuals have to become obese. The major role of this salivary protein in the oral cavity is to hydrolyse starch to maltose and maltotriose. It has been stated that considerable starch hydrolysis occurs in the oral cavity³⁹ and can continue after swallowing, due to the presence of partially digested starch, which appears to protect salivary α -amylase from acid inactivation.⁴⁰ Therefore, higher levels of this salivary enzyme could lead to a higher efficiency in starch digestion, what can be hypothesized to increase carbohydrate

absorption, contributing to the greater increase in body weight observed in OP animals.

Salivary α -amylase has been proposed as a marker of sympathetic nervous system activity.¹² Stimulation of beta-adrenergic receptors results in increases in salivary total protein and α -amylase output.¹⁴ Based on that, we can assume that OP animals presented a higher activity of this branch of autonomic nervous system, at an early age. Sympathetic nervous system activity is related to energy homeostasis, increasing energy expenditure by stimulating adrenergic thermogenesis, which in turn results in expending energy as heat.⁴¹ However, a chronic sympathetic stimulation has been suggested to result in desensitization of the beta-adrenergic signalling pathway, which has been considered a possible explanation for weight gain increases with age.⁴² Moreover, from our observations, at the end of the 18 weeks experiment OP animals did not present α -amylase levels augmented, even presenting higher body weights and higher plasmatic leptin levels. This also goes in accordance with the beta-adrenergic desensitization hypothesis.

Also, it was observed that while the OR animals adjusted food intake to fulfil their caloric needs the OP animals did not. Indeed the OP subgroup presented a significantly higher caloric intake compared to both OR and control subgroups, hence contributing to their augmented weight gain. The reasons for food consumption above their needs in the OP subgroup are unknown but an increased palatability, widely reported as a drive to consumption⁴³ due to the higher salivary α -amylase levels might be one contributing factor.

Another important finding was that α -amylase secretion is 3-fold increased after 18 weeks consuming the high fat diet in the subgroup resistant to weight gain. Since OR animals consumed lower amounts of starch than the other individuals, the increase in α -amylase secretion might be an adaptive response mechanism to increase digestive efficiency. Moreover, sympathetic nervous system activity increases with the consumption of high fat diet¹⁷ and, in the same line of thought described above, since OR animals continues to be sensible to adrenergic stimulation, it is not surprising a rise in α -amylase secretion.

Nonetheless, the use of salivary α -amylase as marker of sympathetic activity has some limitations⁴⁴ and further studies are important to elucidate the meaning of increases in this salivary protein expression induced by high fat diets only in animals resistant to weight gain.

Finally, from our knowledge, although saliva has been extensively explored as a source of biomarkers for diverse pathological conditions, there is a lack of studies comparing salivary protein composition according to individual propensity for obesity. Our results suggest that salivary α -amylase secretion differ between individuals with and without propensity to weight gain and that it may be meaningful in increasing understanding about obesity development. Moreover, a recent study demonstrated that salivary amylase gene (AMY1) copy number variations predisposes to obesity.⁴⁵ Although, no salivary protein expression was accessed in this study, it emphasises the importance of this salivary protein related to weight gain and BMI. Further studies elucidating the mechanisms which result in this observed higher secretion of this protein, and studies searching for the potential changes of

other salivary proteins in a pre-obesity state, can ultimately allow early dietary and behavioural interventions, avoiding the pathological state.

5. Conclusion

In summary, chronic consumption of high amount of sunflower oil differentially induced obesity in rats, according to individual propensity, without producing the negative effects usually attributed to saturated fat, namely insulin resistance and/or dyslipidaemia. It is concluded that saliva may be useful to evaluate susceptibility for obesity. Salivary α -amylase levels in young animals are related to the rate of future weight gain. Although the mechanisms responsible for the differential expression of salivary α -amylase according to obesity susceptibility need to be explored, the obtained results show that saliva composition relates to the amount of weight gain. Moreover, the consumption for several weeks of a high fat diet evoked an increase in salivary α -amylase levels in individuals resistant to obesity, possibly as an adaptation to optimize the usage of a poorer starch diet. Taking together these results reinforce the notion that salivary α -amylase levels are dependent on diet characteristics and emphasize the potential of saliva in studies about susceptibility for weight gain and obesity development.

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Conflict of interest statement

The authors have no potential conflicts of interest.

Ethical approval

All animal procedures were conformed to Portuguese law (PORTARIA 1005/92), which followed European Union Laboratory Animal Experimentation Regulations and were supervised by a scientist trained by the Federation of European Laboratory Animal Science Associations (FELASA).

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