Association between salivary amylase enzyme activity and obesity in Saudi Arabia

Norah Mubarak Aldossari, MD<sup>a,b,∗</sup>, Eman E. El Gabry, PhD<sup>b,c</sup>, Gihan E.H. Gawish, PhD<sup>d,e</sup>

Abstract

Obesity is a significant public health concern that predisposes individuals to a high risk of premature mortality. Previous studies also reported that low serum concentrations of AMY1 have been associated with obesity. The aim of the study was to assess the relationship between salivary amylase (AMY1) activity and body mass index (BMI) in Saudi male and female adults in Riyadh. This study included a total of 200 (100 individuals who were overweight and obese and 100 who had normal body weight [control individuals]) Saudi participants aged 20 to 50 years old. They were recruited from physical fitness clubs and were school employees in Riyadh City. The dietary food intake was assessed using a 24-hour dietary recall. The activity of the AMY1 was measured using a microplate fluorescence reader. A significant $P < .05$ increase was observed in the incidence of hypertension, dyslipidemia, diabetes mellitus (DM), and family history of overweight and obesity in overweight and obese individuals than in the control individuals, and these were in parallel to the significant increase in weight, waist circumference (WC), hip circumference (HC), systolic blood pressure (SBP), diastolic blood pressure (DBP), and BMI. A significant $P < .05$ increase was also observed in the carbohydrate and total fat dietary intake of overweight and obese individuals in relation to the respective dietary reference intake (DRI) values. AMY1 activity was significantly lower than the reference values in the overweight and obese group. Furthermore, AMY1 activity was significantly $P < .05$ reverse with weight, WC, HC, and BMI in both males and females in the overweight and obese group. In conclusion, the Saudi overweight and obese population seems to be at risk of low AMY1, which correlates with their obesity.

Abbreviations: AMY1 = salivary amylase, AMY2 = pancreatic amylase, BMI = body mass index, CNVs = copy number variations, DBP = diastolic blood pressure, DM = diabetes mellitus, DQ = quenching starch, DRI = dietary reference intake, HC = hip circumference, SBP = systolic blood pressure, SPSS = Statistical Package for the Social Sciences, T2DM = type 2 diabetes mellitus, WC = waist circumference, WHO = World Health Organization.

Keywords: body mass index, obesity, overweight, salivary amylase, Saudi adult

1. Introduction

Obesity is a significant public health concern that predisposes individuals to a high risk of premature mortality, through an increased risk of chronic diseases, including type 2 diabetes mellitus (T2DM), cardiovascular diseases, hypertension, and cancer. The prevalence of obesity is increasing worldwide at an alarming rate in both developing and developed countries. Globally, about 2.1 billion people, nearly 30% of the world’s population, are either overweight or obese. By 2030, an estimated 38% of the world’s adult population will be overweight, and another 20% will be obese. In Saudi Arabia, the prevalence of obesity in the general population is approximately 28% in males and 44% in females.

AMY1 is an enzyme produced and excreted from the norepinephrine-responsive salivary gland cells. It is responsible for starch hydrolysis, which begins in the oral cavity, to produce maltose, maltotriose, and dextrin, accounting for at least 50% of salivary protein. The amount of AMY1 varies among individuals. This variation is due to a number of environmental factors, including stress levels, and circadian rhythms. Additionally, there is evidence that populations with a long history of eating starch-rich foods have higher concentrations of AMY1 than that of the populations who consume protein-rich foods.

For the last decade, early clinical studies have reported the low serum amylase in both obese mice and humans. Previous studies also reported that low serum concentrations of AMY1 have been associated with increased body mass index (BMI) and waist circumference (WC). In contrast, another study suggested that AMY1 activities were higher in overweight individuals than that of the control individuals. The clinical relevance of AMY1 and the underlying mechanisms have not been fully elucidated, therefore highlighting contrasting findings in this field. To date, no large-scale epidemiological
studies in Saudi Arabia have been conducted to study the relationship between low AMY1 levels and obesity. This study aimed to assess the relationship between AMY1 activity and BMI in 200 Saudi male and female adults in Riyadh.

2. Methods

2.1. Study population and participants

This study included a total of 200 Saudi participants aged 20 to 50 years old. The participants had been divided into 2 groups; the first group included 100 individuals who were overweight and obese (50 males and 50 females), and the second group included 100 individuals who had normal body weight (control group) (50 males and 50 females). They were recruited from physical fitness clubs and were school employees in Riyadh City. They were randomly chosen from January 2017 to August 2017. All of the participants had signed the written informed consent, and their approved and signed consent forms were received. The inclusion criteria were based on the BMI values established by the International Obesity Task Force/World Health Organization (WHO) as follows: 18.5 to 24.9 kg/m² is considered normal, 25 to 29.9 kg/m² is considered overweight, and >30 kg/m² is considered obese.[23] Ethical approval was obtained from the Ethics Committee of the College of Science Research Center of King Saud University, Riyadh, Saudi Arabia.

2.2. Data collection

Dietary intake was assessed using a 24-hour dietary recall conducted by an interviewer using a pretested questionnaire. The following personal information was collected using a questionnaire: age, weight, height, sex, marital status, educational level, health history of parental obesity, and sports activity. The participants were instructed to use rulers, measuring cups, and spoons in their own household. The data were entered and analyzed using the United States Department of Agriculture Health Tech Software Search and Food Composition for the Middle East,[26] and by another local study for traditional food composition.[27] Dietary adequacy was assessed by comparing the participants' intake with the available dietary reference intake (DRI) value, instead of assessing the control group's nutritional status for diet evaluation.[28]

2.3. Anthropometric analysis

The participants' anthropometric characteristics including weight and height were determined using standardized conventional methods. BMI was calculated using the following formula: weight in kilograms (kg) divided by height in meter squared (m²). Blood pressure was measured following the recommendations of the Joint National Committee using a standard mercurial sphygmonommanometer with the cuff on the right upper arm.[29] Two blood pressure readings were taken 5 minutes apart, with the participants in seated position for 10 minutes. The average of the 2 readings was noted.

2.4. Sample preparation

At least 30 minutes prior to eating, drinking, or smoking, the saliva samples were collected from each participant using 15-ml sterile polypropylene containers for 3 minutes under the tongue. A total of 3 ml of saliva sample was collected and placed in the polypropylene container for the assessment of AMY1 activity. The samples were immediately transported to Abdulaziz City for Science and Technology and stored at –20°C until analysis.

2.5. Measuring AMY1 activity in the saliva

AMY1 activity was measured by a microplate fluorescence reader using EnzChek Ultra Amylase Assay Kit (E33651) purchased from Thermo Fisher Scientific, Inc. (Waltham, MA) including the dye quenching starch (DQ starch), a cornstarch derivative labeled with BODIPY Fluorescein dye, which was used as a substrate. This substrate was degraded by the AMY1, resulting in highly fluorescent fragments that were proportional to AMY1 activity. According to the manufacturer's instructions, briefly, reaction buffer, 1 mg/ml stock solution of the DQ starch substrate, amylase standard curve between 0 and 20 μM/ml, and several dilutions of the sample were prepared. 50 μl of the samples and 50 μl of AMY1 standard solution in duplicate were added to the wells. The DQ starch (1 mg/ml, 50 μl/well) substrate solution was then added. The plate was incubated at room temperature, protected from light, for 30 minutes. Fluorescence was recorded using a microplate fluorescence reader with excitation and emission wavelengths of 505 nm and 512 nm, respectively. The normal values range from 27 ± 3.8 to 1440 ± 160 U/ml.[30]

2.6. Statistical analysis

Data were analyzed using Statistical Package for the Social Sciences (SPSS) version 22 (Chicago, IL). Categorical data were presented as frequencies and percentages (%). Continuous data were presented as mean ± standard deviation (SD) for normal variables, and non-Gaussian variables were presented as median. All continuous variables were checked for normality using Kolmogorov-Smirnov test. Non-Gaussian variables were log transformed prior to performing parametric analysis. Differences between groups were evaluated using a Student's t test. The relationship between variables was determined using Spearman correlation. A P value of < .05 was considered statistically significant.

3. Results

Table 1 shows the general and clinical characteristics of participants. A total of 200 participants (100 control group [50 males and 50 females] and 100 overweight and obese [50 males and 50 females]) were included in the study aged 20 to 50 years. The mean age and educational level of the control and overweight and obese participants were not statistically different from one another. There was a significant increase in hypertension, dyslipidemia, DM, and family history of obese in the overweight and obese group than that in the control group (P ≤ .05). The physical activity was significantly lower in the overweight and obese group than that in the control group (P ≤ .05).

Table 2 shows the anthropometric characteristics and biochemical parameters of both groups. Weight, WC, SBP, DBP, and BMI were significantly increased in the overweight and obese group than that in the control group (P ≤ .05), whereas the mean AMY1 activity was significantly lower in the overweight and obese group than that in the control group (P ≤ .05).
Table 3 shows the dietary intake of both groups (control and the overweight and obese groups) and the DRI values. With regard to the control group, their mean energy intake was 1355.3 ± 78.3 kcal/day for males and 1173.7 ± 81.3 kcal/day for females. Intake of protein, carbohydrate, and total fat was not significant within group. With respect to the overweight and obese group, their mean energy intake was 2260 ± 390.1 kcal/day for males and 2460.2 ± 466.4 kcal/day for females, and there was a significant increase in protein, carbohydrate, and total fat intake more than the DRI values in both males and females (P < .05).

Table 4 shows the relationship between AMY1 activity and the anthropometric characteristics of the control group and the overweight and obese group. In the univariate correlation analysis, AMY1 activity was significantly reversed, which is associated with weight, WC, HC, and BMI in males ([r = −0.161, P ≤ .05], [r = −0.181, P ≤ .05], [r = −0.173, P ≤ .05], [r = −0.235, P ≤ .05]) and females ([r = −0.191, P ≤ .05], [r = −0.171, P ≤ .05], [r = −0.191, P ≤ .05], and [r = −0.291, P ≤ .05]), respectively, in the overweight and obese group.

4. Discussion
The present study reported that hypertension, dyslipidemia, DM, and family history of obese were increased in the overweight and obese group than that in the control group. These results were in agreement with the previous studies. [31–34] The rise of obesity in the Arabian Gulf is due to several factors, including significant growths in incomes resulting from the rich deposits of oil reserves and the resultant impact of rapid urbanization and improved living conditions. [35] The Arab countries in the Middle East as High-income countries such as Arab Gulf countries or Intermediate-income countries such as Egypt, Jordan, Iraq and Lebanon have been a drastic change in food consumption patterns in these countries as dietary habits and excessive consumption of fatty, salty and processed food and lack of exercise due to change in life style are important factors. [36,37] The total energy intake exceeds 3000 kcal/per capita in all Arab Gulf countries, and the fat represents 25% to 35% of total energy. However, animal fat represents 40% to 52% of the total energy intake in the region.
The average per capita calorie supply in the intermediate-income countries is between 2700 and 3000 kcal per capita. In agreement with these findings, the present study also reported that overweight and obese individuals had significantly increased carbohydrate and total fat intake greater than the DRI values. Furthermore, physical activity was significantly lower in the overweight and obese group than that of the control group.

Our study noted a significant increase in weight, WC, HC, and BMI in the overweight and obese group that is in agreement with previous observations of Viljakainen et al who reported the significant inverse relationship between BMI and WC and concentrations of amylase in the whole group, and separately in the obese, but not in the control group. To date, clinical studies have shown that obese individuals may have preferentially decreased AMY1 concentration, whereas others have revealed that pancreatic amylase (AMY2) level may preferentially decrease in insulin-dependent diabetic individuals. Despite this accumulated evidence, the clinical relevance of serum and salivary amylase and AMY2 and their underlying mechanisms have not been completely understood. Another important finding of our study was that SBP and DBP were increased among the overweight and obese group than that in the control group, and these findings were consistent with the previous studies that reported that SBP in both sexes and DBP in males were higher in the high BMI and high WC groups. Early studies reported that low copy number variations (CNVs) of AMY1, indicating low AMY1 activity, was associated with obesity. One of the main findings in this study was the significant decrease in AMY1 activity in the overweight and obese individuals than that in the control individuals. These observations are consistent with the previous studies that reported that AMY1 levels were significantly lower in overweight and obese individuals aged 30 to 79 years than that of normal body weight individuals. A similar observation was reported in Chinese, Japanese, and Finnish obese individuals.

In the present study, we determined the relationship between AMY1 activity and the anthropometric characteristics including weight, WC, HC, and BMI in the control and overweight and obese groups. Data obtained showed significantly inverse relationship with weight, WC, HC, and BMI in male and female adults in the overweight and obese group, which is in agreement with the previous observations of Viljakainen et al who reported the significant inverse relationship between BMI and WC and concentrations of amylase in the whole group, and separately in the obese, but not in the control group. To date, clinical studies have shown that obese individuals may have preferentially decreased AMY1 concentration, whereas others have revealed that pancreatic amylase (AMY2) level may preferentially decrease in insulin-dependent diabetic individuals. Despite this accumulated evidence, the clinical relevance of serum and salivary amylase and AMY2 and their underlying mechanisms have not been completely understood.

This study acknowledges a few limitations. Small sample size and cross-sectional-based study cannot suggest any causal decrease in AMY1 activity in overweight and obese individuals. Large-scale prospective studies are required to determine the exact predictive value of these findings.

### 5. Conclusions

In conclusion, our study indicates that hypertension, dyslipidemia, DM, and family history of obesity were significantly increase in overweight and obese individuals than that in the control individuals, and these were in parallel to the significant increase in

### Table 3

Daily nutrient intake of the study participants based on DRI.

| Parameters         | Control group |          | Overweight and obese group |          |
|--------------------|---------------|----------|----------------------------|----------|
|                    | Males         | Females  | Males                      | Females  |
|                    | Mean ± SD     | DRI      | Efficiency % (mean/DRI)    | Mean ± SD | DRI  | Efficiency % (mean/DRI) |
| Energy (kcal)      | 1355.3 ± 78.3 | 1180     | 114.8 ± 6.8                | 1173.7 ± 81.3 | 1180 | 99.4 ± 6.8                |
| Protein (g/day)    | 62.6 ± 2.1    | 56       | 111.7 ± 3.7                | 53.2 ± 5.2 | 46   | 115.6 ± 11.3              |
| Total fat (g/day)  | 40.7 ± 4.1    | 35.5     | 114.8 ± 11.5               | 29.3 ± 5.1 | 35.5 | 82.6 ± 14.3               |
| Carbohydrates (g/day) | 159.3 ± 8.8 | 130      | 116.4 ± 6.8                | 140.2 ± 5.8 | 130  | 114.7 ± 4.4               |

Data were presented as means ± standard deviation for continuous normal variables and medians (25th percentile, 75th percentile) for continuous non-normal variables; a column and at the same time point denoted with different superscripts that differ significantly in P values are shown in.

* P < 0.05.

### Table 4

Bivariate relationship between AMY1 activity and the anthropometric characteristics of the control and overweight and obese groups.

| Parameter         | Control group |          | Overweight and obese group |          |
|-------------------|---------------|----------|----------------------------|----------|
|                    | Males         | Females  | Males                      | Females  |
| Height (cm)       | −0.027        | −0.002   | −0.06                      | −0.003   |
| Weight (kg)       | −0.029        | −0.001   | −0.161                      | −0.093   |
| WC (cm)           | −0.035        | −0.036   | −0.181                      | −0.171   |
| HC (cm)           | −0.071        | −0.081   | −0.173                      | −0.191   |
| BMI (kg/m²)       | −0.006        | −0.006   | −0.235                      | −0.291   |

Data presented as coefficient (R) and at the same time point denoted with different superscripts that differ in significant P values are shown in.

* P < 0.05.
weight, WC, HC, and BMI. AMY1 activity was significantly lower in overweight and obese individuals than that in the normal individuals. Furthermore, AMY1 activity was significantly reverse, which was associated with weight, WC, HC, and BMI in males in the overweight and obese group. However, Saudi overweight and obese populations seem to be at risk of low AMY1 activity, which correlates with their obesity. Further larger-scale study is needed to confirm the present findings.

Acknowledgments

We would like to thank the assistance and cooperation of the physical fitness clubs and the school’s employees in Riyadh City and all the subjects in this study for their valuable participation.

Author contributions

Conceptualization: Gihan E.H. Gawish.

Data curation: Norah Mubarak Aldossari.

Formal analysis: Norah Mubarak Aldossari.

Investigation: Norah Mubarak Aldossari, Eman E. El Gabry.

Software: Norah Mubarak Aldossari, Eman E. El Gabry.

Writing – review & editing: Norah Mubarak Aldossari, Eman E. El Gabry, Gihan E. Gawish.

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