Molecular Nutrition & Food Research

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RESEARCH ARTICLE OPEN ACCESS

Obesity Parameters in Women Is Associated With AMY1 Gene Copy Number, Nesfatin-1 Level, and Dietary Intake: A Case-Control Study

Fatma Tayhan[1] | Gizem Helvacı[2] | Nurcan Yabancı Ayhan[3]

[1]Health Sciences Faculty, Department of Nutrition and Dietetics, Çankırı Karatekin University, Çankırı, Turkey | [2]Health Sciences Faculty, Department of Nutrition and Dietetics, Mehmet Akif Ersoy University, Burdur, Turkey | [3]Health Sciences Faculty, Department of Nutrition and Dietetics, Ankara University, Ankara, Turkey

Correspondence: Fatma Tayhan (fatmatk@karatekin.edu.tr)

Received: 13 September 2024 | **Revised:** 11 March 2025 | **Accepted:** 12 March 2025

Funding: This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors.

Keywords: AMY1 gene copy number (GCN) | body composition | Nesfatin-1 | nutritional status | obesity

ABSTRACT

AMY1 gene copy number (GCN) variations and the satiety hormone Nesfatin-1 have recently emerged as potential contributors to obesity and related metabolic disturbances. This study evaluated the relationship between AMY1 GCN, Nesfatin-1 level, and nutritional status in obese/overweight and normal-weight women. Participants included 40 normal-weight and 45 overweight/obese women aged 19–50. Data were collected through a demographic and dietary habits questionnaire, a 3-day food recall, anthropometric measurements, and body composition analysis via bioelectrical impedance. Saliva samples were used to measure AMY1 GCN and Nesfatin-1 levels. The AMY1 GCN was significantly lower in overweight/obese participants compared to normal-weight participants. Increased AMY1 GCN was associated with a decrease in BMI (−0.154 units), while increased Nesfatin-1 level was linked to a rise in BMI (0.196 units) ($p < 0.05$). Women with low AMY1 GCN had higher daily intakes of energy, carbohydrate, protein, and fat ($p < 0.05$). This study highlights the significant roles of AMY1 GCN and Nesfatin-1 in the development of obesity. The findings suggest that lower AMY1 GCN and higher Nesfatin-1 levels are associated with unfavorable nutritional and metabolic profiles. Further comprehensive studies on genetic and hormonal factors, including AMY1 GCN and Nesfatin-1, are recommended to guide obesity prevention and treatment strategies.

1 | Introduction

Obesity is a treatable health condition that negatively impacts healthcare costs, quality of life, and work productivity by increasing the risk of various chronic diseases. Globally, the prevalence of obesity has doubled since 1980. Consequently, approximately one-third of the population worldwide is now classified as overweight or obese. Furthermore, the prevalence of obesity is higher in women than in men [1]. While excessive energy intake, sedentary lifestyle, socioeconomic status, fast food consumption, sugary beverages, junk food consumption, and easy access to such high-energy, low-nutrient foods are seen as the causes of the rapid increase in obesity prevalence [2], not everyone exposed to an obesogenic environment becomes obese. This can be attributed to factors such as fetal programming, gender, age, gut microbiome, and genetics [3].

Studies carried out on families, twins, and adoptees revealed that 40%–75% of body mass index (BMI) variations are influenced by genetic factors [4, 5]. There are many genes involved in the

body's energy homeostasis and contributing to the development of obesity [6]. Nowadays, new genetic variants associated with obesity and related comorbidities are widely investigated by using Genome-Wide Association Studies (GWAS) [7]. Copy number variations (CNVs) do not fall within the scope of GWAS but may represent the heritability of obesity [8]. CNVs are a significant component of genomic structural variants and they originate from the deletion or duplication of DNA segments ranging from one kilobase to several megabases [9].

The salivary amylase gene has a wide variation in copy numbers associated with obesity [10, 11]. Salivary and pancreatic amylases (AMY1 and AMY2, respectively) are the enzymes that are responsible for hydrolyzing alpha-1,4 glycosidic bonds facilitating the digestion of dietary starch [12]. Salivary alpha-amylase is a monomeric calcium-binding enzyme secreted by the salivary glands and it initiates the digestion of dietary starch. It hydrolyzes starch into maltose, maltotriose, and larger oligosaccharides. It is also the most abundant protein in saliva; it constitutes 40%–50% of the total salivary protein and rapidly alters the physical properties of starch. The amount and enzymatic activity of salivary amylase show significant individual differences. Salivary amylase levels are influenced by the CNVs of the AMY1 gene on the chromosome coding for salivary amylase [13]. In humans, the salivary amylase gene is located on chromosome 1p21.1, and the CNVs of the AMY1 gene, which was reported to vary between individuals, ranging from 1 to 27, with an average of six copies per person [14, 15]. It was stated that the average AMY1 gene copy number (GCN) in a population may be related to the starch content of that population's diet [16]. Starchy foods are one of the main components of the diet, particularly among agricultural societies [17]. In general, it is emphasized that populations with diets more reliant on starch may have higher salivary amylase concentrations and higher AMY1 GCNs when compared to populations consuming high-protein diets, and this variation is considered an adaptation to dietary habits [18].

A communication between the gastrointestinal system, adipose tissue, and the satiety center in the brain is required to regulate food intake. Various peptides and hormones are involved in the pathophysiology of this communication [19]. Nesfatin-1 is one of the anorexigenic peptides that are thought to take part in the physiological regulation of feeding behavior and body weight by suppressing food intake and peristalsis [20]. Nesfatin-1 is predominantly localized in hypothalamic nuclei that play an important role in the regulation of food intake, such as the arcuate nucleus, paraventricular nucleus, and solitary nucleus [21]. It is also produced in peripheral tissues, such as gastric mucosal cells, pancreatic beta cells, and adipocytes, and in limited amounts in the liver, testes, and muscle tissues [22]. It was shown that Nesfatin-1 decreases energy intake by affecting the hedonic aspects of food intake and negatively modulating dopaminergic neuron activity. Nesfatin-1 was reported to be an inhibitory factor on appetite and a regulator of energy balance that reduces body weight gain [23].

Although both AMY1 GCN [11, 12] and Nesfatin-1 [20] have been studied independently in the metabolic processes of obesity, no research has yet investigated their combined effects. It will help us better understand how genetic and hormone factors interact in the control of energy balance. Therefore, this study was designed and carried out to compare AMY1 GCN and Nesfatin-1 levels in overweight/obese and non-obese adult women, and to determine the relationship between AMY1 GCN and women's BMI, energy and nutrient intake, biochemical parameters, and Nesfatin-1 levels.

2 | Method

2.1 | Participant Group

This study is a randomized case-control study. Ethical approval was obtained from Çankırı Karatekin University's Ethics Committee (No. 17/07.10.2020). This study involved women aged 19–50, who were categorized as overweight/obese (BMI $\geq$ 25.0 kg/m^2) and non-obese (BMI $<$ 25.0 kg/m^2). Considering similar studies [12, 15] and using the G*Power 3.1.9.2 program, a minimum sample size of 68 was determined to be sufficient with 95% power, 5% error margin, and an effect size of $d = 0.89288$ ($n_1 = 34$, $n_2 = 34$). Accordingly, this study was completed with 45 adult women in the overweight and obese group and 40 adult women with normal body weight in the control group. The women in the study and control groups were selected from among the staff working at Çankırı Karatekin University and their relatives. The study was conducted exclusively among women, as previous research has demonstrated a specific association between AMY1 GCN and BMI in women but not in men [24]. Furthermore, obesity prevalence is higher among women than men in Turkey [25], making this population more relevant for the study's objectives. Inclusion criteria included not being on a diet, not having chronic diseases, not using regular medication, not having undergone surgical obesity treatment, not having psychiatric disorders, not having been diagnosed with cancer in the last 5 years, not consuming excessive alcohol (defined as more than two standard alcoholic drinks per week for women, each containing 14 g of alcohol), not being in their menstrual period, and not having a pacemaker.

2.2 | Data Collection

A questionnaire was administered to the participants in a face-to-face interview, which included general characteristics and a food consumption record. The anthropometric measurements of the women were taken by the researcher during the interview by following standard methods. Body weight, height, waist circumference, neck circumference, and hip circumference of the individuals were measured. The BMI values of the women were calculated using the formula (Body weight [kg]/Height [m]^2). The WHO classification was used for the evaluation of BMI as follows: women with a BMI between 18.5 and 24.99 kg/m^2 were considered to have normal body weight, those with a BMI between 25.0 and 29.99 kg/m^2 were classified as overweight, and those with a BMI $\geq$ 30.0 kg/m^2 were classified as obese [26]. WHO-defined cut-off points for waist circumference and waist-hip ratio were used to assess metabolic risk. A waist–hip ratio higher than 0.85 indicates a significantly increased risk [27]. A waist–height ratio of 0.5 is globally recommended for determining the risk of cardiovascular and metabolic diseases [28]. Individuals with a ratio $\geq$ 0.5 were considered to have increased health risk. Moreover, body analyses

of the individuals were performed, and saliva samples were collected to determine salivary Nesfatin-1 levels and AMY1 GCN. Records of biochemical parameters (total cholesterol, triglycerides, HDL-cholesterol, LDL-cholesterol, insulin, blood glucose, CRP, HbA1c, T3, T4, TSH, WBC, hemoglobin, ferritin, transferrin, urea, and creatinine) from any health institution within the last 3 months were put into the questionnaire form.

2.3 | Bioelectrical Impedance Analysis

The body weight, fat percentage, and visceral fat ratio of the women were measured by using the TANITA MC 780 MA Bioelectrical Impedance Analysis (BIA) 22 device. Participants were asked to not eat for at least 2–4 h before the measurement, to avoid heavy physical activity for 1–2 days prior, to limit coffee, water, and tea consumption before the test, to not consume alcohol the day before, and to remove any metal objects [29]. Before the measurement, each participant's height was measured by the researcher, and personal information (name, surname, age, gender, and height) was entered into the computer connected to the device. Participants were then asked to step onto the device for measurement, and the results were recorded.

2.4 | Body Adiposity Index

This index is a practical measure that is used in order to estimate body fat percentage. The body adiposity index (BAI) value of the women was computed by using the height (m) and hip circumference (cm) values. The formula used is (hip circumference [cm]/height $[m]^{1.5} - 18$) [30].

2.5 | Lipid Accumulation Product Index

The lipid accumulation product (LAP) index was computed by using the formula ([waist circumference [cm] − 58] × serum triglyceride level [mmol/L]). An increase in this index is associated with increased cardiometabolic risk [31].

2.6 | Visceral Adiposity Index

An increase in visceral adiposity index (VAI) is related to an increased cardio-metabolic risk. It is calculated by using the formula (waist circumference [cm]/36.58 + [1.89 × BMI $[kg/m^2]$] × [Triglyceride [mmol/L]/0.81] × [1.52/HDL [mmol/L]]) [32].

2.7 | Determination of Nutritional Status

To determine the nutritional status of the participants, a 3-day food consumption record was taken, including 2 weekdays and 1 weekend day. The "Food and Nutrition Photo Catalogue for Portion Sizes and Quantities" book was used to accurately query the portions of consumed foods [33]. The "Standard Recipes" book was used to determine the amounts of ingredients in meals consumed outside the home [34]. The analysis of the food consumption records was conducted by using the "Computer-Aided Nutrition Program, Nutrition Information System (BeBIS) 7.0" package program. The women's daily average energy, macro, and micronutrient intakes were calculated by using the analysis results.

2.8 | Salivary AMY1 GCN Analysis

The salivary AMY1 GCN was evaluated by making use of a 96-well Quantitative Polymerase Chain Reaction (qPCR). Before collecting saliva samples, participants were asked to empty their mouths by swallowing all saliva and to rinse their mouths with water. Participants were instructed to spit approximately 2 mL of saliva (excluding bubbles) into Eppendorf tubes until the fill line was reached. The collected saliva samples were then immediately stored at +4°C in a refrigerator until DNA extraction [35]. For RNA isolation, the RiboEx kit (GeneAll, catalog number: 301-001) was used, followed by cDNA synthesis with the WizScript cDNA Synthesis Kit (catalog number: W2211). Real-time PCR analysis was performed using the WizPure qPCR Master (SYBR) Kit (catalog number: W1711, Wizbio, South Korea). The samples were then transported under appropriate conditions to Atlas Biotechnology Laboratory, located in Ankara, for analysis. In this study, the AMY1 GCN ranges from 2 to 36, and in accordance with previous studies [11, 36], values below the median are used to define the low AMY1 CNV group (< 5), while values above the median are used to define the high AMY1 CNV group (≥ 5). Previous studies have reported mixed findings regarding whether low or high AMY1 CNV is more beneficial. One study reported that low AMY1 CNV has no significant effect on obesity [36], while another study suggested that low AMY1 CNV may increase susceptibility to obesity [11].

2.9 | Nesfatin-1 Analysis

Saliva samples were collected from participants to assess Nesfatin-1 levels and were transported under suitable conditions to a laboratory at a specialized biotechnology firm for analysis. The saliva samples were centrifuged at 4000 rpm for 10 min (twice), and after being transferred into Eppendorf tubes, they were stored at −20°C until analysis [37]. For the analysis of Nesfatin-1 in saliva, Human Nesfatin-1 ELISA kits (BTLAB, catalog number: E3063Hu) were used in accordance with the protocols provided by the manufacturer, and the process was conducted in duplicate. For Nesfatin-1 analysis, Nesfatin antibodies present on the plates were incubated with Nesfatin standards and diluted saliva samples from individuals to form antigen-antibody complexes. Following the incubation, the color change proportional to the Nesfatin-1 concentrations in the wells was read and recorded at a wavelength of 450 nm [38]. No cutoff point has been specified for Nesfatin-1 levels. Salivary Nesfatin-1 levels are closely correlated with serum levels. The average concentration is 1.3 times higher in comparison to the serum. This relationship between salivary and serum Nesfatin-1 levels suggests the transport of this hormone from blood vessels to glandular cells [39].

2.10 | Statistical Analysis

The data analysis was conducted by using IBM SPSS 23.0 software. The Kolmogorov–Smirnov test was used to determine

whether the data were normally distributed. Numerical data are presented as mean and standard deviation ($\bar{x} \pm SD$), while categorical data are given as numbers and percentages. Pearson's chi-square test was used for the evaluation of categorical data. If the data were normally distributed, then the differences between independent variables were analyzed using the Independent Samples *t*-test; if not, the Mann–Whitney *U* test was implemented. To examine the relationship between the numerical data of the two groups, Pearson correlation analysis was used for normally distributed data and Spearman correlation analysis for non-normally distributed data. Multiple linear regression analysis was employed to examine the relationship of participants' BMI with salivary Nesfatin-1, AMY1 GCN, and daily dietary energy intake (kcal), evaluated within a 95% confidence interval. The statistical significance was set at $p < 0.05$.

3 | Results

3.1 | General Characteristics of the Women

The demographic, anthropometric, general nutritional, and genetic characteristics of the women are presented in Table 1. Education level, occupation, smoking status, alcohol consumption, and the prevalence of obesity in the family were similar across the groups. The metabolic risks, body fat percentages, visceral fat ratios, LAP index, BAI, and VAI based on waist/hip and waist/height ratios were significantly higher in the study group ($p < 0.001$). The average number of main meals was at a lower level in the study group (2.1 ± 0.3) when compared to the control group (2.4 ± 0.5) ($p < 0.05$), whereas the average number of snacks was higher in the study group ($p < 0.05$). Salivary Nesfatin-1 levels were higher, and AMY1 GCN was lower in the study group ($p < 0.05$).

3.2 | Associations of AMY1 GCNs and Nesfatin-1 Levels With Anthropometric Measurements and Dietary Intake

The relationship between women's anthropometric measurements, AMY1 GCN, and Nesfatin-1 levels is examined in Table 2. A significant inverse correlation is observed between the AMY1 GCN and body weight, BMI, body fat percentage, body fat mass, visceral fat percentage, waist circumference, hip circumference, neck circumference, and body adiposity index ($p < 0.05$). In addition, body weight, BMI, body fat (% and kg), visceral fat percentage, total fluid mass, lean body mass, waist and hip circumference, neck circumference, LAP index, body adiposity index, and visceral adiposity index were positively associated with Nesfatin-1 levels ($p < 0.05$).

Women with a low AMY1 GCN were found to have higher energy intake (2084.0 ± 610.2 kcal/day) when compared to those with a high AMY1 GCN (1764.5 ± 618.3 kcal/day) ($p < 0.05$). Moreover, daily intake levels of protein (g), carbohydrate (g), fat (g), saturated and monounsaturated fatty acids, and fiber are higher in the low AMY1 GCN group ($p < 0.05$). Daily intake of vitamin A, folate, carotene, vitamin B12, potassium, iron, magnesium, calcium, and phosphorus were found to be higher in the low AMY1 GCN group ($p < 0.05$). Nesfatin-1 levels in women were categorized into tertiles, and it was observed that daily energy intake was highest in women in the third tertile. In addition, the proportion of energy derived from dietary protein was higher in women in the first tertile. Total fat (g), saturated fat, dietary fiber, and vitamin E intakes were highest in the third tertile (Table 3).

Examining the factors influencing BMI in women, a one-unit increase in the AMY1 GCN causes a 0.154-unit decrease in BMI (95% CI: −0.275 to −0.011) ($p = 0.033$). A one-unit increase in salivary Nesfatin-1 levels results in a 0.196-unit increase in BMI (95% CI: 0.022–0.139) ($p = 0.007$). A one-unit increase in daily energy intake (kcal) leads to a 0.664-unit increase in BMI (95% CI: 0.006–0.010) ($p < 0.001$) (Table 4).

3.3 | Associations of AMY1 GCNs and Nesfatin-1 Levels With Biochemical Parameters

As shown in Table 5, which presents the relationship between women's biochemical parameters and AMY1 GCN, no correlation was found between the AMY1 GCN and any parameter, except for serum CRP levels ($p > 0.05$). There is an inverse relationship between AMY1 GCN and CRP levels ($p = 0.002$). Additionally, Nesfatin-1 levels showed a significant positive association with HbA1c, triglyceride, CRP, and uric acid levels (Table 5).

4 | Discussion

Recent studies have linked variations in the copy number of the AMY1 gene, which encodes the α-amylase enzyme taking part in the initial step of starch digestion, to obesity [36]. No study investigating the relationship between the AMY1 GCN, dietary habits, and Nesfatin-1 levels in the Turkish population could be found in the literature, and contradictory results were reported in the literature on this subject [11, 40].

The salivary amylase gene has a wide range of CNVs [10, 11]. In this study, the AMY1 GCN in women was determined to be at a lower level in the study group than in the control group. The mean AMY1 GCN among all participants was 8.0 ± 8.0, with a median value of 5.0. Marquina et al. [41] reported a median AMY1 GCN of 4.0 in a sample consisting of 57 adults, both male and female, whereas Pinho et al. [15] found a median value of 7.0 for both women and men. These differing results may be due to other genetic factors, differences in the ethnic backgrounds studied, and variations in the methods used to determine AMY1 GCN.

Even though the effect of AMY1 GCN variations on BMI is controversial, early studies in this field suggested a linear relationship between AMY1 GCN and BMI, showing that women with a lower AMY1 GCN have a significantly increased risk of obesity when compared to those with a higher copy number [11, 41, 42]. Similarly, in this study, the AMY1 GCN in the case group of women was found to be higher, and a one-unit change in AMY1 GCN resulted in a −0.154-unit change in BMI ($p < 0.05$). However, other studies have not supported this finding [41, 43, 44], suggesting that the relationship between AMY1 GCN and BMI may be influenced by additional factors such as dietary patterns, genetic background, or environmental

TABLE 1 | Demographic, anthropometric, and genetic characteristics of participants.

	Control group ($n = 40$)		Study group ($n = 45$)		Total ($n = 85$)		
	n	**%**	***n***	**%**	***n***	**%**	***p***
Demographic characteristics							
Educational level							
Secondary-high school	32	80.0	37	82.2	69	81.2	0.794[c]
Undergraduate-postgraduate	8	20.0	8	17.8	16	18.8	
Occupation							
Public servant	6	15.0	7	15.6	13	15.3	0.111[c]
Housewife	32	80.0	29	64.4	61	71.8	
Others	2	5.0	9	20.0	11	12.9	
Smoking							
Yes	9	22.5	6	13.3	15	17.6	0.411[c]
No	31	77.5	39	86.7	70	82.4	
Alcohol use							
Yes	3	7.5	1	2.2	4	4.7	0.265[c]
No	37	92.5	44	97.8	81	95.3	
Family history							
Obesity history in family							0.072[c]
Yes	25	62.5	37	82.2	62	72.9	
No	15	37.5	8	17.8	23	27.1	
Anthropometric characteristics							
Waist/Hip							
No risk (< 0.8)	39	97.5	34	75.6	73	85.9	0.003[c,*]
Risk (≥ 0.8)	1	2.5	11	24.4	12	14.1	
Waist/Height							
No risk (< 0.5)	38	95.0	1	2.2	39	45.9	< 0.001[c,**]
Risk (≥ 0.5)	2	5.0	44	97.8	46	54.1	
Body weight (kg) ($\bar{X} \pm SD$)	56.6 ± 6.6		85.0 ± 16.1		71.7 ± 18.9		< 0.001[a,**]
BMI (kg/m^2) ($\bar{X} \pm SD$)	21.7 ± 1.9		33.5 ± 5.9		27.9 ± 7.4		< 0.001[a,**]
Body fay percentage (%) ($\bar{X} \pm SD$)	20.7 ± 5.8		36.4 ± 5.8		29.0 ± 9.7		< 0.001[b,**]
Visceral fat ratio (%) ($\bar{X} \pm SD$)	13.6 ± 7.2		29.2 ± 6.5		21.9 ± 10.3		< 0.001[b,**]
Visceral adiposity index ($\bar{X} \pm SD$)	2.1 ± 1.2		4.7 ± 3.0		3.5 ± 2.6		< 0.001[a,**]
BAI ($\bar{X} \pm SD$)	28.9 ± 2.2		41.0 ± 6.7		35.3 ± 7.9		< 0.001[a,**]
LAP index ($\bar{X} \pm SD$)	10.0 ± 8.5		57.2 ± 39.1		35.0 ± 37.3		< 0.001[a,**]
Dietary habits							
Number of meals ($\bar{X} \pm SD$)	2.4 ± 0.5		2.1 ± 0.3		2.2 ± 0.4		0.005[a,*]
Number of snacks ($\bar{X} \pm SD$)	1.6 ± 0.7		2.0 ± 0.6		1.8 ± 0.7		0.018[a,*]
Skipping a meal							
Yes	36	90.0	43	95.6	79	92.9	0.283[c]
No	4	10.0	2	4.4	6	7.1	
Hormone and genome							
Nesfatin-1 (ng/mL) ($\bar{X} \pm SD$)	33.4 ± 15.7		45.5 ± 18.2		39.89 ± 18.0		0.002[b,*]
AMY1 gene copy number ($\bar{X} \pm SD$)	10.5 ± 10.0		5.7 ± 4.9		8.0 ± 8.0		0.011[a,*]

Abbreviations: BAI, body adiposity index; LAP, lipid accumulation product.

[a]Analyzed using Mann–Whitney *U* test.

[b]Analyzed using Independent Samples *t*-test.

[c]Analyzed using Pearson's chi-square test.

*$p < 0.05$.

**$p < 0.001$.

TABLE 2 | Relationship between participants' anthropometric measurements and AMY1 GCN and Nesfatin-1 levels.

	AMY1 GCN		Nesfatin-1 level	
	r	***p***	***r***	***p***
Body weight (kg)	−0.278	0.010[a,*]	0.357	0.001[a,*]
BMI (kg/m^2)	−0.298	0.006[a,*]	0.354	0.001[a,*]
Body fat (%)	−0.287	0.008[a,*]	0.326	0.002[*]
Body fat mass (kg)	−0.275	0.011[a,*]	0.349	0.001[a,*]
Visceral fat ratio (%)	−0.258	0.017[*]	0.296	0.006[*]
Total fluid mass (kg)	−0.184	0.092[a]	0.265	0.014[a,*]
Lean body mass (kg)	−0.173	0.114[a]	0.295	0.006[a,*]
Waist circumference (cm)	−0.237	0.029[a,*]	0.329	0.002[a,*]
Hip circumference (cm)	−0.245	0.024[a,*]	0.334	0.002[a,*]
Waist/hip	−0.157	0.150	0.203	0.062
Neck circumference (cm)	−0.272	0.012[*]	0.265	0.014[a,*]
LAP index	−0.168	0.124[a]	0.311	0.004[a,*]
Body adiposity index	−0.309	0.004[a,*]	0.336	0.002[a,*]
Visceral adiposity index	−0.037	0.733[a]	0.301	0.005[a,*]

[a]Spearman correlation analysis was used.

*$p < 0.05$, tested with Pearson correlation analysis.

exposures [45]. These discrepancies highlight the complexity of the AMY1–BMI interaction and underline the need for further investigation. Genetic adaptation provided by the AMY1 gene to improve the digestion of starchy foods may also affect the oral and gut microbiota profiles [46]. Changes in gut microbiota, interacting with dietary factors, were associated with obesity and related diseases in the host [47]. León-Mimila et al. [48] explained their result that a higher AMY1 GCN is associated with a lower BMI by the positive relationship between AMY1 GCN and the amount of "*prevotella bacteroides*" in the gut microbiota, which supports weight loss. Another explanation for this relationship, demonstrated in a previous study [13], is that since the AMY1 GCN is related with serum amylase levels, it may influence glucose metabolism by influencing carbohydrate digestion via salivary amylase. In the case of less salivary amylase, the digestion of starch-rich foods takes longer, reducing satiety and potentially increasing food intake. It can lead to increased anthropometric measurements and obesity [48]. Although this study revealed that AMY1 GCN is related to obesity, it does not explain the mechanism by which it takes part in the development of obesity. These mechanisms are also unclear in the literature, necessitating further studies in the future.

In the present study, a significant negative relationship was determined between AMY1 GCN in women and body fat amount (kg and %), visceral fat ratio, waist circumference, hip circumference, neck circumference, and body adiposity index. Similarly, Viljakainen et al. [24] mentioned a significant inverse relationship between body fat percentage and AMY1 GCN. Barber et al. [10] reported an inverse correlation between visceral fat volume and AMY1 GCN. Marcovecchio et al. [42] determined that waist circumference was determined to be at a significantly higher level in the group with a low AMY1 GCN in comparison to the group with a high AMY1 GCN. Al-Akl et al. [36] demonstrated that salivary alpha-amylase levels, known to be positively related with AMY1 GCN, were significantly negatively related to body adiposity index in a population of both men and women. Based on our findings, AMY1 GCN could be considered related to abdominal obesity and increased cardiovascular risk.

There are studies providing evidence that individuals' food consumption patterns can be influenced by genetic factors [49, 50]. One of the main focuses of the present study is to investigate the relationship between AMY1 GCN and energy and nutrient intake in women. Accordingly, when examining energy and macronutrient intake based on the AMY1 GCN levels in women, those with a low AMY1 GCN were found to have a higher daily intake of energy, protein (g), and carbohydrates (g) compared to those with a high AMY1 GCN ($p < 0.05$). Tarragon et al. [51] found a significant negative relationship between salivary alpha-amylase enzyme concentration and reported preference for high-sugar foods. In this case, individuals with low salivary alpha-amylase enzyme concentration may have an increased desire to consume high-sugar foods, leading to higher consumption of such foods. Contrary to the present findings, Barber et al. [10] did not find a significant relationship between AMY1 GCN and individuals' macronutrient intake. In the present study, individuals with a low AMY1 GCN were found to have significantly higher total fat, saturated fat, and monounsaturated fatty acid intake from their daily diet compared to others ($p < 0.05$). Barber et al. [10] did not find a significant relationship between AMY1 GCN and the intake of monounsaturated fatty acids, polyunsaturated fatty acids, and saturated fatty acids from the daily diet. In fact, this study is the first to report a significant difference in dietary fat intake between low and high AMY1 GCN groups.

TABLE 3 | Energy, macronutrient, and micronutrient intake of participants by AMY1 GCN and Nesfatin-1 levels.

	AMY1 gene copy number			Nesfatin-1 level			
	Low (< 5) ($n = 36$) X ± SD	High (≥ 5) ($n = 49$) X ± SD	p	Tertile 1 (9.6–31.3) ($n = 28$) X ± SD	Tertile 2 (32.5–47.2) ($n = 29$) X ± SD	Tertile 3 (47.2–85.7) ($n = 28$) X ± SD	p
Energy (kcal)	2084.0 ± 610.2	1764.5 ± 618.3	0.011*	1687.4 ± 484.3	1904.2 ± 719.6	2107.6 ± 614.6	0.043*
Protein (g)	65.6 ± 22.9	54.0 ± 15.6	0.015[a,*]	55.9 ± 14.9	58.3 ± 22.5	62.5 ± 21.1	0.467[b]
Protein (Energy %)	12.9 ± 2.3	13.0 ± 3.2	0.712	14.1 ± 3.8	12.6 ± 2.1	12.2 ± 2.1	0.044*
Carbohydrate (g)	229.6 ± 72.0	193.3 ± 86.2	0.043[a,*]	185.0 ± 62.4	207.3 ± 98.7	233.8 ± 75.5	0.050[b]
Carbohydrate (Energy %)	45.1 ± 4.6	44.1 ± 7.0	0.430	44.7 ± 5.6	43.4 ± 6.7	45.4 ± 5.8	0.467
Fat (g)	98.2 ± 29.9	84.2 ± 29.4	0.034[a,*]	78.3 ± 25.6	91.6 ± 30.3	100.4 ± 31.3	0.036[b,*]
Fat (Energy %)	42.0 ± 4.1	42.9 ± 7.0	0.502	41.1 ± 6.5	43.9 ± 5.9	42.4 ± 5.2	0.225
Saturated fatty acid (g)	29.2 ± 9.7	24.3 ± 8.5	0.022*	22.9 ± 6.6	26.1 ± 9.2	30.1 ± 10.5	0.014*
PUFA (g)	30.0 ± 9.6	32.0 ± 12.1	0.232	25.0 ± 13.0	27.1 ± 10.0	32.2 ± 12.8	0.078
MUFA (g)	32.0 ± 12.1	27.1 ± 12.3	0.035[a,*]	24.4 ± 9.0	32.0 ± 15.0	31.1 ± 11.2	0.056[b]
Fiber (g)	24.4 ± 9.0	18.9 ± 8.8	0.003[a,*]	18.0 ± 8.4	22.2 ± 9.8	23.4 ± 9.0	0.046[b,*]
Cholesterol (mg)	305.9 ± 145.1	242.5 ± 101.6	0.057	260.8 ± 120.8	267.8 ± 126.6	279.5 ± 131.7	0.856
Vitamin A (mcg)	1892.5 ± 1916.1	1172.3 ± 1349.9	0.002[a,*]	1039.2 ± 1263.5	1726.5 ± 1997.2	1657.4 ± 1537.9	0.012[b,*]
Carotene (mg)	2.8 ± 1.6	2.0 ± 2.1	0.002[a,*]	2.0 ± 2.4	2.4 ± 1.6	2.5 ± 1.7	0.137[b]
Vitamin E (mg)	29.7 ± 10.1	24.8 ± 12.4	0.058	22.7 ± 12.1	27.3 ± 11.0	30.8 ± 10.8	0.033*
Vitamin B_{12} (mcg)	4.7 ± 5.1	3.2 ± 3.2	0.030[a,*]	3.3 ± 2.3	4.8 ± 6.2	3.35 ± 2.5	0.909[b]
Folate (mcg)	324.7 ± 111.5	248.6 ± 98.0	0.002[a,*]	244.5 ± 102.9	288.7 ± 120.7	309.1 ± 98.4	0.065[b]
Potassium (mg)	2522.8 ± 857.1	1944.8 ± 659.1	0.001*	1998.6 ± 736.0	2252.3 ± 824.9	2315.8 ± 821.9	0.292
Calcium (mg)	570.7 ± 220.3	463.0 ± 170.3	0.013*	474.7 ± 172.4	504.3 ± 193.1	546.9 ± 228.6	0.400
Magnesium (mg)	287.3 ± 105.8	239.0 ± 99.7	0.027*	239.5 ± 102.1	258.7 ± 92.7	280.2 ± 117.4	0.350
Phosphor (mg)	1137.5 ± 386.6	919.3 ± 277.9	0.003[a,*]	940.0 ± 290.9	1005.7 ± 341.7	1089.6 ± 387.6	0.302[b]
Iron (mg)	12.8 ± 4.3	10.5 ± 4.0	0.003[a,*]	10.2 ± 4.0	11.4 ± 4.5	12.1 ± 4.3	0.278[b]

[a]Mann–Whitney U test was used.

[b]Kruskal–Wallis test was used.

*$p < 0.05$, analyzed using Independent Samples t-test for AMY1 gene copy number groups, One Way ANOVA for Nesfatin-1 level tertiles.

TABLE 4 | Effect of changes in AMY1 GCN, Nesfatin-1 levels, and energy intake on BMI.

	Coefficient		Standardized coefficient			95% Confidence interval	
	B	**SE**	**Beta**	***t***	***p***[a]	**Lower**	**Upper**
AMY1 GCN	−0.143	0.066	−0.154	−2.163	0.033*	−0.275	−0.011
Nesfatin-1 (ng/mL)	0.081	0.029	0.196	2.751	0.007*	0.022	0.139
Energy intake (kcal)	0.008	0.001	0.664	9.040	< 0.001**	0.006	0.010

[a]Multivariable regression analysis was used.
*$p < 0.05$.
**$p < 0.001$.

TABLE 5 | Relationship between women's biochemical and hormonal parameters and AMY1 GCN.

	AMY1 gene copy number		Nesfatin-1 level (ng/mL)	
Biochemical parameters	***r***	***p***	***r***	***p***
Glucose (mg/dL)	0.064	0.559	0.172	0.116
Fasting insulin (μIU/mL)	0.039	0.726	−0.030	0.786
HbA1c (%)	0.040	0.718	0.318	0.003*
Total cholesterol (mg/dL)	−0.149	0.173[a]	0.190	0.082[a]
LDL-cholesterol (mg/dL)	−0.134	0.239[a]	0.140	0.203[a]
HDL-cholesterol (mg/dL)	−0.054	0.626	−0.120	0.274
Triglyceride (mg/dL)	−0.068	0.537[a]	0.260	0.016[a,*]
CRP (mg/L)	−0.350	0.002[a,*]	0.263	0.022[a,*]
Vitamin B_{12} (pg/mL)	0.046	0.677[a]	−0.052	0.636[a]
TSH (μIU/mL)	0.067	0.540[a]	0.226	0.037[a,*]
T3 (pg/mL)	0.020	0.856	0.200	0.066
T4 (ng/mL)	0.075	0.494[a]	−0.151	0.168[a]
Urea (mg/dL)	−0.060	0.585	−0.116	0.292
Uric acid (mg/dL)	−0.152	0.171	0.286	0.009*
Creatinine (mg/dL)	−0.119	0.277	−0.136	0.215
WBC	−0.209	0.055	0.051	0.644
AST (U/L)	−0.026	0.816	−0.005	0.963
ALT (U/L)	−0.099	0.367	0.034	0.761
Hemoglobin (g/dL)	−0.174	0.112	−0.176	0.107
Ferritin (ng/mL)	0.015	0.893[a]	0.029	0.792[a]
Transferrin saturation	0.038	0.729	−0.100	0.362
Iron (μg/dL)	−0.030	0.782	−0.097	0.376
HOMA-IR	0.069	0.528	0.043	0.694
Nesfatin-1	−0.065	0.557[a]		

Abbreviation: HOMA-IR, insulin resistance.
[a]Spearman correlation analysis was used.
*$p < 0.05$, tested With Pearson correlation analysis.

Since food preferences are largely dependent on taste perception, changes in factors affecting taste and other oral sensations may play a significant role in weight gain [52]. Therefore, the AMY1 GCN, which is reported to affect taste perception, may contribute to obesity by influencing individuals' food preferences [13, 53].

Women with a low AMY1 GCN were found to have significantly higher daily intake levels of vitamins A and B_{12}, carotene, folate, potassium, calcium, magnesium, phosphorus, and iron in comparison to individuals with a high AMY1 GCN. This outcome may be due to the higher total energy intake observed in the group with a low AMY1 GCN. To our knowledge, no other study in

the literature has examined the relationship between AMY1 GCN and daily micronutrient intake. There are limited studies in the literature that investigate nutrient intake based on variations in AMY1 GCN, and the present study expands the knowledge in this area by suggesting that AMY1 GCN may contribute to obesity by affecting dietary preferences and satiety.

Nesfatin-1, identified as an anorexigenic peptide, takes a significant part in regulating energy balance in the body and was associated with obesity. While some studies have shown a negative relationship between Nesfatin-1 levels and obesity [54, 55], others support the existence of a positive association [56–58]. In the present study, salivary Nesfatin-1 levels in overweight and obese individuals were 45.5 ± 18.2 ng/mL, which is higher than in individuals with normal body weight (33.4 ± 15.7 ng/mL). Additionally, a one-unit change in salivary Nesfatin-1 levels led to a 0.196-unit increase in BMI. Numerous studies are consistent with the results achieved in this study. Ramanjaneya et al. [56] demonstrated that plasma Nesfatin-1 levels have a positive correlation with BMI. Similarly, Tan et al. [57] reported that circulating Nesfatin-1 levels are elevated in obese adults. Ozkan et al. [58] found that serum Nesfatin-1 levels progressively increase from low body weight to the overweight group. It was also observed in the same study that serum Nesfatin-1 levels are higher in morbidly obese individuals compared to obese individuals. In another study, it was observed that circulating Nesfatin-1 levels decreased after weight loss following bariatric surgery [59]. It was suggested that the increase in Nesfatin-1 levels in obese individuals might originate from increasing production of Nesfatin-1 in the stomach, and, similar to leptin, high levels of Nesfatin-1 in obese individuals may be useful in reducing the food intake and counteracting the weight gain [60, 61]. Accordingly, it has been concluded that salivary Nesfatin-1 levels are positively associated with BMI. However, due to the inconsistencies in the literature, further studies are needed to fully understand this relationship.

Both central and peripheral administration of Nesfatin-1 has been reported to reduce food intake. Kuyumcu et al. [62] reported a significant negative association between serum Nesfatin-1 levels and energy and fat intake in obese individuals. They also showed that the obese group with low Nesfatin-1 levels had higher energy, carbohydrate, and protein intake. Similarly, Mirzaei et al. [63] reported a negative relationship between Nesfatin-1 level and energy and fat intake in obese participants. However, contrary to what was expected in this study, daily dietary intake of energy, fat (g), saturated fat, and monounsaturated fat was significantly higher in the group with higher Nesfatin-1 levels (tertile 3). Consistent with this finding, a study by Anwar et al. [64], which examined fasting serum Nesfatin-1 and dietary habits and daily food intake, showed that there was a significant positive correlation between the percentage of dietary energy from carbohydrate and saturated fat and Nesfatin-1. An experimental study also showed that mice fed a high-fat diet (40% fat, 45% carbohydrate, and 15% protein) had higher Nesfatin-1 levels than mice fed a low-fat diet (10% fat, 70% carbohydrate, and 20% protein) [56]. Mirzaei et al. [63] showed that obese individuals with higher body fat percentage had higher Nesfatin-1 concentrations. The conflicting results in the literature suggest that Nesfatin-1 may have varying effects depending on individual differences and dietary habits.

No significant association between AMY1 GCN and Nesfatin-1 levels was found in this study. AMY1 GCN and Nesfatin-1 are separately associated with obesity and dietary intake, the fact that our findings do not show a direct relationship suggests that their effects might be mediated through independent or more complex pathways. Highlighting a gap in the current literature, as far as we are aware, no previous studies have directly examined the interaction between AMY1 GCN and Nesfatin-1. Our findings contribute to this emerging field by providing preliminary evidence and highlighting the need for further research to explore potential indirect mechanisms or moderating factors that may influence this relationship. Future studies should include a larger number of participants and consider gender differences by including male subjects.

Limitations of the study include an all-female sample, a limited sample size, and a cross-sectional design that does not allow causality to be established. In addition, factors such as dietary reporting bias, genetic, and environmental influences may affect the results.

4.1 | Conclusion and Suggestions

This study highlights the important role of genetic factors, particularly the copy number of the AMY1 gene, in the influence of obesity and dietary habits in women. The results suggest that the AMY1 GCN is associated with both obesity and dietary behaviors. This indicates the importance of considering genetic predisposition when assessing nutritional status for obesity management. In addition, the study highlights the importance of Nesfatin-1 as a critical biomarker in the prevention and treatment of obesity. Nesfatin-1 levels are closely associated with the regulation of food intake, and assessing these levels in obese individuals may provide valuable insights for the development of effective weight loss interventions. The AMY1 GCN and Nesfatin-1 levels were found as individual determinants of obesity in women, but no significant associations were observed between them. A possible reason for this lack of association could be that these two factors affect obesity through different mechanisms. In addition, any potential correlation between these two parameters may be obscured by individual variability in genetic factors, diet, and other environmental influences.

In conclusion, while further research is necessary to comprehend the complex gene–diet interactions involved in obesity formation, the involvement of a multidisciplinary team comprising physicians, dieticians, genetic specialists, physiotherapists, psychologists, and social workers in the prevention and treatment of obesity will enhance success.

Acknowledgments

The authors would like to thank all participants who agreed to participate in this study.

Ethics Statement

Ethical approval was obtained from Çankırı Karatekin University's Ethics Committee (No. 17/07.10.2020). This study was derived from the doctoral thesis of the first author.

Conflicts of Interest

The authors declare no conflicts of interest.

Data Availability Statement

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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