Impact of BMI and Menstrual Cycle Phases on Salivary Amylase: A Physiological and Biochemical Perspective

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Abstract
Salivary amylase, a key enzyme in the initial digestion of dietary starch, also serves as a biomarker for stress, autonomic nervous system activity, and various physiological states. Its activity is influenced by several factors, including body mass index (BMI) and hormonal fluctuations associated with the menstrual cycle. This article explores the physiological and biochemical interactions between BMI, menstrual cycle phases, and salivary amylase activity, aiming to clarify their interconnected roles and potential clinical implications. Obesity and underweight conditions are associated with metabolic alterations that may affect the salivary glands' function and enzyme secretion. Individuals with higher BMI often exhibit altered sympathetic nervous system activity and systemic inflammation, which can modulate salivary amylase output. Conversely, low BMI may be linked to reduced glandular stimulation or nutritional deficiencies, affecting enzyme levels. These variations highlight BMI as a significant modifier of salivary biomarkers, including amylase. Moreover, the menstrual cycle introduces cyclic hormonal changes primarily in estrogen and progesterone that influence various physiological systems, including salivary gland function. Research suggests that salivary amylase activity fluctuates across the menstrual phases, with notable differences observed between the follicular and luteal phases, possibly due to the hormonal regulation of autonomic tone and glandular secretions. Understanding these interactions is vital for the appropriate interpretation of salivary amylase levels in both clinical and research settings. It also provides insight into broader metabolic and endocrine functions and supports the potential use of salivary amylase as a non-invasive biomarker for assessing physiological status. This article synthesizes current findings and identifies gaps in the literature, suggesting directions for future research to enhance our comprehension of how BMI and menstrual physiology jointly influence salivary biomarkers.

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1. Introduction

Saliva is a complex biological fluid secreted by the salivary glands, composed of various substances including water, electrolytes, mucus, and enzymes (Manjul, 2011). The functional units responsible for saliva production are known as acini. On average, the human body produces between 2 to 4 pints of saliva daily (Friedman, 2017). Saliva serves multiple essential roles, including lubrication and binding of food particles, solubilization of dry substances, maintenance of oral hygiene, and the initiation of starch digestion.

One of the key enzymes found in saliva is salivary alpha-amylase, which hydrolyzes complex polysaccharides such as starch and glycogen into simpler sugars like maltose and glucose (Pugh, 2000) [33]. In addition to its digestive function, salivary amylase is also considered a useful biomarker for assessing physiological stress and autonomic nervous system activity. Studies have shown that the activity of salivary alpha-amylase tends to be significantly lower in overweight and obese individuals compared to those with a normal body weight (Aldossari *et al*., 2019), suggesting a potential link between metabolic status and salivary enzyme function.

Body Mass Index (BMI) is a commonly used measure to classify individuals based on their weight relative to height. It is calculated by dividing body mass (in kilograms) by the square of height (in meters), and is expressed in kg/m^2. Research indicates that BMIs below 20.0 and above 25.0 are associated with increased mortality risk, with the risk rising as values deviate further from the 20.0–25.0 range (Di Angelantonio *et al*., 2016). Variations in BMI can influence metabolic and autonomic activity, which may, in turn, affect salivary amylase production.

Another important physiological factor that may influence salivary alpha-amylase activity is the menstrual cycle. The menstrual cycle consists of regular, natural changes in the female reproductive system that facilitate the possibility of pregnancy (Silverthorn and Dee 2013; Sherwood, 2013). These changes are driven by fluctuations in hormone levels, primarily estrogen and progesterone (Office of Women's Health, 2014). Women often experience symptoms such as irritability, fatigue, acne, breast tenderness, bloating, and mood changes during the menstrual phase (Office of Women's Health, 2014). Menarche, the onset of menstruation, typically occurs between the ages of 12 and 15 (Karapanou *et al*., 2010). The menstrual cycle is divided into distinct phases: the follicular phase, ovulatory phase, and luteal phase, each characterized by specific hormonal profiles and physiological changes.

The aim of this article is to investigate the effect of Body Mass Index (BMI) on salivary amylase activity and to determine whether different phases of the menstrual cycle influence salivary amylase levels. Salivary alpha-amylase is an important diagnostic tool, especially in studies related to acute stress response (Takai *et al*., 2004). However, the interpretation of its activity must account for various physiological factors. As hormonal fluctuations during the menstrual cycle are known to influence systemic physiology, it is important to determine whether these changes also affect salivary alpha-amylase activity. Understanding these relationships will enhance the reliability of using salivary biomarkers in both clinical and research contexts.

2. Methodology

Saliva samples were collected from 30 female adults at Bowen University, Iwo. The date of their last menstrual cycle, weight, and height were recorded. The phase of the menstrual cycle was determined based on the sample collection date and the cycle duration.

Reagents such as buffers, starch, and iodine were used, all of standard analytical grades. Other materials included tissue paper, masking tape, vortex mixer, gloves, micropipettes, volumetric flask, freezer, bathroom scale, measuring cylinder, beakers, spatula, test tubes, and sample collection tubes.

The study was conducted at Bowen University, Iwo, Osun State, Nigeria, and involved 30 female students. For height and weight measurements, subjects were asked to stand on a bathroom scale without footwear, and their weight was recorded. Height was measured using a doubled meter rule placed beside the subject.

Salivary amylase activity was measured using the starch-iodine method, where starch turns blue-black in the presence of iodine. At pH 6-7 and in the presence of chloride ions, α-amylase hydrolyzes starch to maltose and dextrins. The blue-black color indicates the presence of starch, while the disappearance of color, or the "achromic point," indicates complete hydrolysis of starch into maltose and dextrins.

For the experimental procedure, a starch solution of 2mg/ml was prepared, and phosphate buffered saline (PBS) was made using disodium hydrogen phosphate, sodium dihydrogen phosphate, and saline. Iodine solution was prepared with iodine crystals and potassium iodide. A mixture of 1960µl PBS and 40µl of saliva was mixed in a test tube, followed by the addition of 50µl of iodine to different spots on a white tile. Preheated starch solution (2ml) was added to the saliva mixture and stirred. The resulting solution was then placed on the iodine drops, and the color change from blue-black to reddish-brown was observed. The time taken for the color change was recorded, and amylase activity was calculated.

The menstrual cycle phase for each subject was determined based on the date of their last menstruation, cycle duration, and sample collection date. For a 28-day cycle, the first 13 days are the follicular phase, day 14 is ovulation, and days 15 to 28 are the luteal phase.

Amylase activity was calculated using the formula:

Salivary amylase activity (mg/ml/min) = (time × dilution factor)

Starch concentration = 4mg/ml

Enzyme volume = 2ml

Dilution factor = 1:50

Data analysis was conducted using GraphPad Prism 5.0. A correlation coefficient between body mass index (BMI) and amylase activity was determined, and Pearson's chi-square test was used to assess the significance of the correlation. One-way ANOVA was used to determine significant differences between amylase activity and BMI across different phases of the menstrual cycle.

2.1. Saliva

Saliva is a fluid substance secreted by the salivary glands into the oral cavity and plays a crucial role in both oral and systemic functions (Edgar *et al*., 2004). Its secretion, known as salivation, is primarily mediated by the autonomic nervous system. The parasympathetic nervous system stimulates the secretion of a waterier, enzyme-rich saliva, which facilitates digestion. This response is driven by the neurotransmitter acetylcholine, which binds to muscarinic receptors in the salivary glands to increase secretion. In contrast, stimulation by the sympathetic nervous system results in the production of a thicker, more viscous saliva, primarily to aid in respiration rather than digestion (Nosek, 2012).

Humans possess three pairs of major salivary glands—parotid, submandibular, and sublingual—along with numerous minor salivary glands distributed throughout the oral cavity (Edgar *et al*., 2012). These exocrine glands produce saliva through ductal systems and are classified based on the nature of their secretions as serous, mucous, or

seromucous (mixed). Serous secretions are rich in proteins such as alpha-amylase, an enzyme that initiates the breakdown of dietary starch into maltose and glucose (Martini *et al*., 2012). Mucous secretions, on the other hand, primarily contain mucins, which serve as lubricants and protect the oral tissues (Edgar *et al*., 2012). The average daily production of saliva in a healthy adult range between 0.5 and 1.5 liters (Young *et al*., 2011).

Saliva is composed of approximately 99.5% water, along with various electrolytes (including sodium, calcium, magnesium, chloride, bicarbonate, phosphate, and iodine), mucins, enzymes, antibacterial compounds, epidermal growth factors, opiorphin, haptocorrin, and exfoliated cells (Boron, 2003). These components contribute to its diverse biological functions.

Saliva performs several essential physiological roles. It provides protection by lubricating the soft and hard tissues of the oral cavity through mucins, which are the principal organic components of mucus and form a viscoelastic coating on mucosal surfaces (Tabak *et al*., 1982). Saliva also has a buffering function; a higher salivary flow rate correlates with increased buffering capacity and improved protection against dental caries. Conversely, individuals with low salivary flow and reduced buffer capacity are more susceptible to microbial growth and oral health issues (Comba, 2018).

The antimicrobial function of saliva is supported by several elements. For example, lactoferrin binds iron, which is essential for bacterial survival, thus inhibiting bacterial growth. Immunoglobulin A (IgA) aggregates oral bacteria such as *Streptococcus mutans*, preventing their colonization and plaque formation (Taylor, 2018). Saliva also aids in tissue repair by reducing clotting time and enhancing wound contraction, facilitating healing of the oral mucosa (Mandel, 1987).

In digestion, salivary amylase plays a critical role by breaking down starch into maltose and dextrins, enabling the digestive process to begin in the mouth before food reaches the stomach (Science Daily, 2018). Additionally, saliva contributes to taste perception by dissolving food particles, allowing them to interact with taste receptors located on the tongue within the foliate and circumvallate papillae, where minor salivary glands help in taste facilitation (Riley, 2017).

2.2. Salivary Alpha Amylase (PTYALIN)

Salivary alpha-amylase, also known as ptyalin, is an important digestive enzyme that hydrolyzes the alpha-1,4 glycosidic bonds of large, alpha-linked polysaccharides such as starch and glycogen, converting them into simpler sugars like maltose and glucose (Pugh, 2000) [33]. It is the predominant form of amylase in humans and other mammals (Voet *et al*., 2005), and it is also present in seeds that store starch as a food reserve. This calcium-dependent metalloenzyme plays a critical role in the early stages of digestion, operating within the oral cavity. It is responsible for approximately 40–50% of the total protein content secreted by the salivary glands (Gopal *et al*., 2014).

In saliva, alpha-amylase initiates the breakdown of dietary starch into smaller carbohydrates such as maltose and dextrins. It also helps convert insoluble starch into soluble dextrins like erythrodextrin, amylodextrin, and achrodextrin, which are further degraded into maltose. The enzyme specifically acts on linear α(1,4) glycosidic linkages. However, the hydrolysis of branched polysaccharides requires additional enzymes capable of cleaving α (1,6) linkages.

The activity of salivary alpha-amylase is sensitive to pH. It becomes inactivated upon exposure to gastric acid in the stomach, where the low pH typically around 3.3 denatures the enzyme. At this pH, complete inactivation occurs within 20 minutes at 37 °C (Fried *et al*., 1987). However, its stability increases at slightly higher pH levels. At pH 4.3, 50% of enzymatic activity can persist for up to 150 minutes. Experiments have shown that when ptyalin is placed in buffer at pH 3.0, it becomes completely inactivated within 120 minutes. Interestingly, the presence of starch can preserve enzyme activity; 0.1% starch retained 10% of enzymatic activity after 120 minutes, while 1.0% starch preserved about 40% activity (Rosenblum *et al*., 1988).

The optimal conditions for salivary alpha-amylase include a neutral pH of around 7.0, along with the presence of specific anions such as chloride, bromide, sulfate, and phosphate, which serve as activators. Beyond its digestive role, ptyalin has emerged as a useful biomarker in clinical and psychological research. It is recognized for its rapid response to acute stress, making it a reliable indicator of sympathetic nervous system activation (Takai *et al*., 2004; Noto *et al*., 2005; Granger *et al*., 2007). Salivary amylase levels rise quickly in response to stress and return to baseline levels soon after the stressor is removed, allowing real-time assessment of stress responses (Nater *et al*., 2009).

In contrast to salivary alpha-amylase, pancreatic alpha-amylase functions in the small intestine and exhibits different substrate specificity and catalytic mechanisms. It randomly cleaves α(1,4) glycosidic linkages of amylose into smaller oligosaccharides such as dextrin, maltose, and maltotriose, using a double displacement mechanism that retains the anomeric configuration of the substrate. Additionally, it has been reported that tris(hydroxymethyl)aminomethane (TRIS) can inhibit some bacterial alpha-amylases (Ghalanbor *et al*., 2008; Aghajari *et al*., 1998).

Structurally, alpha-amylases share a conserved domain architecture as shown in figure 1. The catalytic domain consists of an eight-stranded alpha/beta barrel that forms the active site. This region is interrupted by a 70-amino acid calcium-binding loop between beta strand 3 and alpha helix 3. A carboxy-terminal Greek key beta-barrel domain is also present (Abe *et al*., 2005). Many alpha-amylases contain an additional beta-sheet domain at the C-terminus, typically arranged as a five-stranded antiparallel beta-sheet (Abe *et al*., 2005; Kadziola *et al*., 1998). Some variants also contain an all-beta domain located at the C-terminal end (Machius *et al*., 1995), contributing to the structural diversity and functional versatility of this enzyme family.

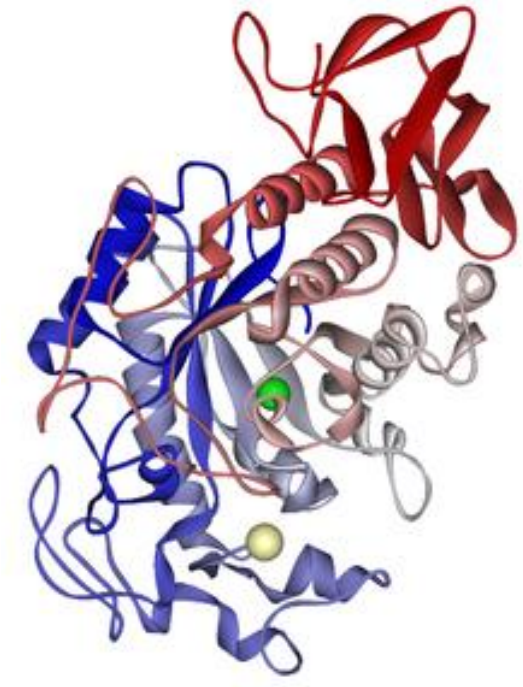

Fig 1: Ribbon diagram of human salivary alpha amylase (Wikipedia, 2007)
Chloride ion
Calcium ion

Food digestion begins in the mouth, where mechanical and chemical processes initiate the breakdown of food. The teeth cut and grind food into smaller pieces, while the salivary glands secrete saliva to moisten and mix with the ingested material. Saliva contains salivary alpha-amylase, an enzyme responsible for hydrolyzing dietary starch into simpler sugars such as maltose. Although this process begins in the oral cavity, the complete digestion of starch is finalized in the small intestine through the action of pancreatic amylase.

The enzymatic activity of salivary alpha-amylase is influenced by several environmental factors, particularly temperature and pH (Nedugandi *et al*., 2013). Like all enzymes, salivary alpha-amylase is a protein, and its functionality is highly dependent on its structural integrity. At temperatures lower than the enzyme's optimal range, its activity slows down significantly due to reduced molecular motion. At higher temperatures, the enzyme becomes denatured, losing its functional shape and rendering it inactive. The optimal temperature for salivary alpha-amylase activity lies between 32°C and 37°C, while its activity is most efficient at a pH of approximately 6.8. Upon entering the stomach, where the environment is highly acidic (with a pH around 3.0), the enzyme becomes denatured and loses its function. As a result, salivary amylase does not remain active in the stomach, and its role in digestion is limited to the oral cavity.

One of the classic methods for detecting the presence of starch is the iodine test. This test relies on a colorimetric reaction, where starch forms an intense blue-black complex when mixed with aqueous solutions of the triiodide ion (I_3^-). This occurs due to the formation of an intermolecular charge-transfer complex between starch and iodine. In the absence of starch, the solution remains a brownish color. This interaction also underpins iodometry, a common method in redox titrations. In these procedures, elemental iodine is dissolved in a potassium iodide solution to produce triiodide, which then interacts with starch to form the characteristic dark complex.

The color intensity of the starch-triiodide complex diminishes with increasing temperature and in the presence of water-miscible organic solvents such as ethanol. Moreover, the iodine test is ineffective under strongly acidic conditions because starch undergoes hydrolysis at low pH levels (Brilliant Biology Student Master Biology Laboratories, 2015). It is now understood that the interaction between starch and the iodine-iodide mixture forms an extended polyiodide homopolymer, a concept supported by single crystal X-ray crystallography and Raman spectroscopy analyses (Madhu *et al*., 2016).

In analytical chemistry, starch is frequently used as an indicator in redox titrations involving iodine. The deep blue-black complex formed with triiodide can be visually detected at extremely low concentrations, as low as 0.00002 M at 20°C (Bertrand Gary, 2019). During such titrations, starch is typically added near the endpoint after most of the iodine has reacted with the titrant commonly thiosulfate to prevent premature complex formation, which could interfere with the reaction due to the starch-triiodide complex's relative insolubility. This principle is also used in practical applications, such as the Minor test, which detects moisture or perspiration using starch iodine color change.

2.3. Body Mass Index (BMI) and Salivary Amylase

Body Mass Index (BMI), also known as the Quetelet index, is a measure derived from an individual's mass (weight) and height. It is calculated by dividing body mass (in kilograms) by the square of height (in meters) and is universally expressed in units of kg/m². While BMI is useful for assessing obesity and body fat composition on a large scale, it has limitations when applied to individuals. Specifically, BMI is proportional to mass and inversely proportional to the square of height, meaning taller individuals may have a higher BMI that does not accurately reflect their body fat levels. For example, the Ponderal Index, which adjusts for

height by scaling mass to the third power of height, may provide a more accurate reflection of body fat in taller individuals. Despite these limitations, BMI remains a reliable tool for identifying obesity in large populations (The New York Times, 2010).

BMI values are categorized by the World Health Organization (WHO) as follows: a BMI under 18.5 is considered underweight, suggesting possible malnutrition or other health concerns; a BMI between 18.5 and 24.9 is considered normal; a BMI of 25 or above is classified as overweight, and a BMI above 30 is considered obese (WHO, 2006). However, while BMI is a useful screening tool, it does not differentiate between lean body mass and body fat. This means that individuals with high BMIs may still have relatively low-fat mass, making BMI less reliable for assessing body fat composition on an individual basis (Wellens *et al*., 1996; Strain and 1992). The limitations of BMI as a sole measure of obesity have been well-documented, with studies showing poor correlations between BMI and body fat percentage, particularly when using more accurate body composition methods like bioelectrical impedance (Romero-Corral *et al*., 2008).

The consequences of elevated BMI levels are substantial, with higher BMI ranges linked to an increased risk of various health conditions. These include sleep apnea, stroke, coronary artery disease, gallbladder disease, hypertension, type 2 diabetes, and osteoarthritis. For individuals who have never smoked, being overweight or obese is associated with a 51% higher risk of mortality compared to those with a normal BMI (Stokes and Preston, 2015). Therefore, while BMI is a helpful tool in public health and epidemiological studies, it is important to consider additional methods for assessing body fat and health risks in individuals.

Salivary alpha-amylase activity tends to be significantly lower in overweight and obese individuals compared to those with normal body weight (Aldossari *et al*., 2019). Both salivary and pancreatic alpha-amylase enzymatic activities are closely associated with lower body mass index (BMI) levels (Amelie *et al*., 2017). A bidirectional relationship has been observed between salivary alpha-amylase plasma enzymatic activity and adiposity, suggesting that changes in body fat may influence enzyme activity and vice versa as shown in figure 2 (Amelie *et al*., 2017). Factors such as genetics and environmental influences, like stress, contribute to variations in amylase production. For instance, copy number variation (CNV6) in the AMY1 gene, which codes for salivary amylase, has been shown to positively correlate with salivary amylase concentrations. Individuals can carry between 1 and 15 copies of the AMY1 gene, and this genetic variation impacts enzyme levels (Perry *et al*., 2007; Mandel *et al*., 2010; Abigail *et al*., 2012).

Although food only stays in the mouth for a short time, the initial amylolytic digestion of starch is considered to play a minimal role in digestion due to the presence of pancreatic amylase further down the digestive tract. However, emerging evidence suggests that salivary amylase may have more practical and clinical significance than previously thought. Studies have shown that considerable starch hydrolysis occurs within seconds in the oral cavity (Hoebler *et al*., 1998), and this process can continue even after swallowing. The partially digested starch can protect salivary amylase from inactivation by stomach acid (Rosenblum *et al*., 1988). Further research has demonstrated that bypassing oral digestion by directly introducing starch into the small intestine results in significantly less starch digestion and glucose absorption (Fogel *et al*., 1973). Individuals with higher levels of salivary amylase also tend to have lower postprandial blood glucose responses after starch consumption, suggesting that increased oral amylase activity plays a role in regulating blood sugar levels (Abigail *et al*., 2012).

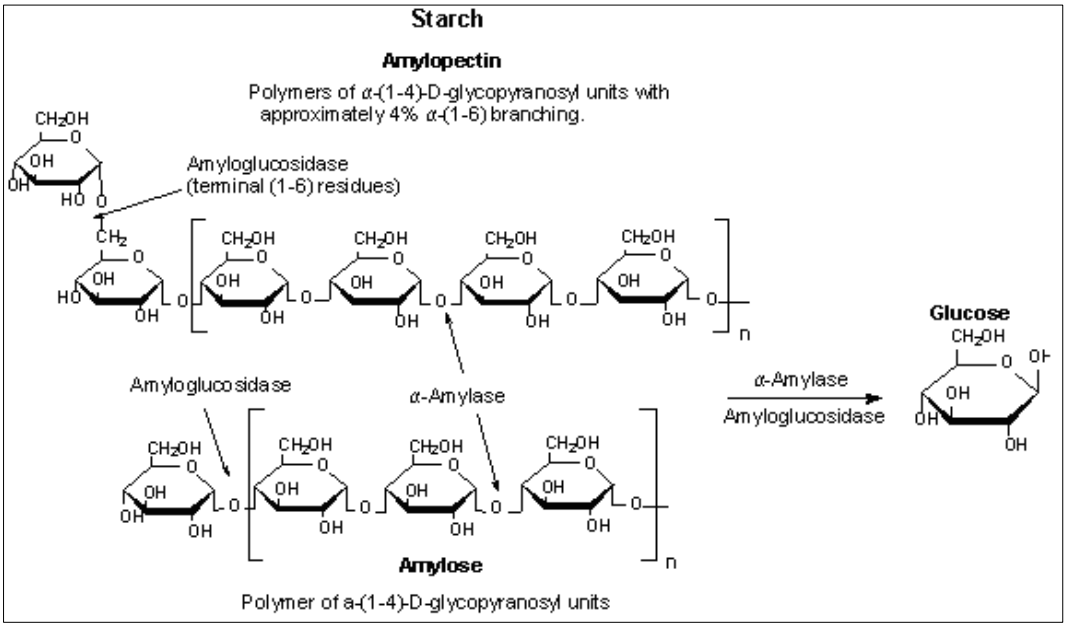

Fig 2: Alpha-amylase catalyzing the hydrolysis of starch to glucose

2.4. Menstrual Cycle Phases and Salivary Amylase Activity

The menstrual cycle is a series of natural changes in a female's reproductive system that enable the possibility of pregnancy. It begins at puberty, typically between ages 12 and 15, and involves hormonal fluctuations. Menstruation, the shedding of the uterine lining, marks the start of the cycle, which usually lasts 28 days, though it can range from 20 to 40 days. The cycle includes three phases: the follicular phase,

ovulatory phase, and luteal phase.

During the follicular phase, from day 1 to day 13, ovarian follicles mature and the ovum develops. Menstruation occurs, lasting 4-5 days, during which about 35ml of blood and fluid are expelled. Ovulation occurs on day 14 of a typical cycle, when the mature follicle bursts and releases the ovum into the abdominal cavity. This process is triggered by a surge in luteinizing hormone (LH), which stimulates the final stages of follicle growth and ovum release. The ovum is viable for 1-2 days after ovulation.

The luteal phase, from day 15 to day 28, follows ovulation. The remnants of the follicle form the corpus luteum, which produces progesterone, essential for maintaining the uterine lining for potential implantation.

The menstrual cycle is regulated by hormones from the hypothalamus, pituitary gland, and ovaries. Gonadotropin-releasing hormone (GnRH) from the hypothalamus stimulates the release of follicle-stimulating hormone (FSH) and luteinizing hormone (LH) from the pituitary, which in turn influence the ovarian hormones estrogen and progesterone. Estrogen peaks during the follicular and luteal phases, while progesterone rises after ovulation.

Hormonal fluctuations throughout the menstrual cycle also affect weight and body composition. Women tend to consume more high-carbohydrate and high-fat foods during the luteal phase, leading to increased energy intake and potential weight gain. Energy expenditure is also higher during this phase, which may contribute to weight changes, although results across studies are inconsistent. These hormonal influences contribute to variations in body mass index (BMI) throughout the cycle.

While salivary alpha-amylase activity is generally unaffected by the menstrual cycle, some studies suggest a slightly higher activity during the luteal phase compared to the follicular phase. However, the impact of menstrual cycle phases on salivary alpha-amylase activity, especially following exercise, remains unclear.

2.5. Results and Interpretation

The study involved 30 female students, each above 18 years of age, to investigate the relationship between BMI, menstrual cycle phases, and salivary amylase activity. The BMI of the participants ranged from 14.71 kg/m² to 41.4 kg/m², while salivary amylase activity ranged from 36.76 U/ml to 500 U/ml. A positive correlation was observed between BMI and amylase activity ($r = 0.137$, $P = 0.471$), indicating that as BMI increases, salivary amylase activity tends to increase as well. However, the difference was not statistically significant, as the p-value was greater than 0.05.

The study also categorized the participants based on their menstrual cycle phases: 40% were in the follicular phase, 20% in the ovulatory phase, and 40% in the luteal phase. One-way ANOVA analysis, using Tukey's multiple comparison test, showed no significant difference in BMI across the three phases ($p = 0.3959$). Similarly, there was no significant difference in amylase activity across the phases ($p = 0.1654$). No significant differences were found when comparing individual phases (follicular vs. luteal, follicular vs. ovulatory, luteal vs. ovulatory) for both BMI and amylase activity.

Tables included in the article summarize the data, with measurements for height, weight, BMI interpretation, menstrual phase, and salivary amylase activity for each participant. Further analysis of amylase activity and BMI classifications according to menstrual cycle phases is also presented in the tables.

Table 1: Showing Height, Weight, Menstrual Phase, Commencement Date of Last Menstrual Cycle and Amylase Activity

S/N	Height (M)	Weight (Kg)	BMI ($Kg/m^{2)}$	BMI Interpretation	Menstrual Phase	Date of Commencement of Last Menstrual Cycle	Salivary Amylase Activity (U/ml)
1.	1.79	85	26.53	Overweight	Follicular	APRIL 27TH	36.76
2.	1.7	69	23.88	Normal	Luteal	APRIL 11TH	58.14
3.	1.63	110	41.4	Obese	Follicular	APRIL 20TH	72.46
4.	1.7	98	33.91	Obese	Luteal	APRIL13TH	92.59
5.	1.65	75	27.55	Overweight	Follicular	APRIL 14TH	59.88
6.	1.67	87	31.2	Obese	Luteal	MARCH 24TH	52.08
7.	1.69	65	22.76	Normal	Follicular	APRIL 30TH	68.97
8.	1.72	54	18.25	Underweight	Luteal	APRIL 11TH	100
9.	1.69	46	16.11	Underweight	Luteal	APRIL 10TH	80
10.	1.56	45	18.49	Underweight	Follicular	APRIL 29TH	49.26
11.	1.78	68	21.46	Normal	Luteal	APRIL 10TH	75.17
12.	1.61	85	32.79	Obese	Follicular	APRIL 30TH	250
13.	1.63	67	25.22	Overweight	Luteal	APRIL 10TH	312.5
14.	1.67	55	19.72	Normal	Luteal	APRIL 17TH	312.5
15.	1.7	57	19.72	Normal	Follicular	APRIL 25TH	238.1
16.	1.54	58	24.46	Normal	Ovulation	APRIL 21ST	303.03
17.	1.72	55	18.59	Normal	Follicular	APRIL 19TH	86.96
18.	1.63	52	19.57	Normal	Luteal	APRIL 18TH	97.09
19.	1.71	57	19.49	Normal	Luteal	APRIL 15TH	97.09
20.	1.69	42	14.71	Underweight	Ovulation	APRIL 19TH	76.92
21.	1.71	81	27.7	Overweight	Ovulation	APRIL 18TH	263.15
22.	1.73	79	29.73	Overweight	Follicular	APRIL 11TH	250
23.	1.71	75	25.65	Overweight	Follicular	APRIL 28TH	250
24.	1.67	47	16.85	Underweight	Luteal	APRIL 14TH	121.95
25.	1.75	61	19.92	Normal	Follicular	MAY 1ST	172.41
26.	1.68	69	24.45	Normal	Luteal	APRIL 9TH	50
27.	1.68	78	27.64	Overweight	Follicular	APRIL 18TH	238.1
28.	1.61	60	23.15	Normal	Ovulation	APRIL 24TH	238.1
29.	1.58	58	23.23	Normal	Ovulation	APRIL 18TH	114.94
30.	1.67	84	30.12	Obese	Ovulation	APRIL 18TH	270.27

Table 2: Classification of Amylase Activity According to the Menstrual Cycle Phases

S/N	Follicular phase	Ovulation phase	Luteal phase
1.	36.76	303.3	58.14
2.	72.46	76.92	92.59
3.	59.88	263.15	52.08
4.	68.97	238.1	100
5.	49.26	114.94	80
6.	250	270.27	75.17
7.	238.1		312.5
8.	86.96		312.5
9.	250		97.09
10.	250		97.09
11.	172.41		121.95
12.	238.1		50

Table 3: Classification of BMI According to the Menstrual Cycle Phases

S/N	Follicular phase	Ovulatory phase	Luteal phase
1.	26.53	24.46	23.88
2.	41.4	14.71	33.91
3.	27.55	27.7	31.2
4.	22.76	23.15	18.25
5.	18.49	23.23	16.11
6.	32.79	30.12	21.46
7.	19.72		25.22
8.	18.59		19.72
9.	29.73		19.57
10.	25.65		19.49
11.	19.92		16.85
12.	27.64		24.45

Overall, this article indicates that there are no significant effects of menstrual cycle phases on either BMI or salivary amylase activity, although a positive correlation between BMI and amylase activity was observed.

The menstrual cycle in women is regulated by reproductive hormones that influence energy intake, expenditure, and fat storage (Poppitt *et al*., 1994). These hormonal fluctuations also affect various physiological processes. This study aimed to examine the impact of menstrual cycle phases on salivary α-amylase activity in 30 female adults from Bowen University, Iwo, Osun State. Forty percent of the participants were in the follicular phase, 20% were in the ovulatory phase, and 40% were in the luteal phase.

The results revealed a positive correlation between body mass index (BMI) and salivary α-amylase activity ($r = 0.137$, $P = 0.471$), suggesting that higher BMI was associated with higher amylase activity. However, this correlation was not statistically significant, as the p-value was greater than 0.05. This finding contrasts with a study by Aldossari *et al*. (2019) [3], which reported that overweight and obese individuals typically exhibit significantly lower salivary α-amylase activity compared to those with normal BMI.

Additionally, there was no significant difference in BMI across the menstrual phases ($p = 0.3959$), which contrasts with findings from Mohammad *et al*. (2014), who observed weight fluctuations during the menstrual cycle. This discrepancy may be due to differences in study methodology: while Mohammad *et al*. measured BMI daily throughout the cycle, this article only measured it once.

Similarly, there was no significant difference in salivary amylase activity between the menstrual phases ($p = 0.1654$), which aligns with Yasuda *et al*. (2012) [48], who found no significant variation in amylase activity across the cycle. No significant differences were observed between the follicular and ovulatory phases, luteal and ovulatory phases, or follicular and luteal phases. However, this finding contradicts Yasuda *et al*. (2012) [48], who reported higher amylase activity during the luteal phase compared to the follicular phase. Differences in article conditions may contribute to these varying results.

3. Conclusion

In conclusion, this article provides valuable insights into the relationship between body mass index (BMI), menstrual cycle phases, and salivary amylase activity in adult women. While a positive correlation between BMI and amylase activity was observed, the results did not reach statistical significance, suggesting that other factors may contribute to variations in amylase secretion rather than body mass alone. The lack of significant differences in amylase activity across the menstrual phases further suggests that hormonal fluctuations associated with the menstrual cycle may not significantly influence salivary α-amylase activity. This contradicts some earlier studies that suggested higher amylase activity during the luteal phase, highlighting the complexity of physiological responses to hormonal changes. Additionally, the absence of notable BMI fluctuations across the menstrual phases aligns with some studies, while conflicting findings in previous research indicate that weight changes during the cycle may vary depending on the methods and timing of measurements.

These findings indicate that the relationship between BMI, menstrual cycle phases, and salivary amylase activity is more nuanced than previously thought, with various individual and methodological factors potentially influencing the outcomes. Future research should focus on a larger and more diverse sample population, with repeated measurements throughout the menstrual cycle to better understand the dynamic

interplay between hormonal, metabolic, and biochemical processes. Investigating other potential influencing factors, such as diet, lifestyle, and genetic predispositions, may provide a clearer understanding of how BMI and menstrual cycle phases affect salivary enzyme activity. Furthermore, exploring these relationships in clinical or therapeutic settings could yield significant implications for personalized healthcare, especially in the context of metabolic and hormonal disorders. Overall, this article contributes to the growing body of knowledge regarding the physiological and biochemical effects of BMI and menstrual cycle phases on salivary amylase, but further investigation is essential to draw more definitive conclusions.

4. References

1. Abigail L., Mandel AL, Breslin PAS. High endogenous salivary amylase activity is associated with improved glycemic homeostasis following starch ingestion in adults. The Journal of Nutrition. 2012;142(5):853–858.
2. Aghajari N, Feller G, Gerday C, Haser R. Crystal structure of the psychrophilic alpha-amylase from Alteromonas haloplanctis in its native form and complexed with an inhibitor. Protein Science. 1998;7(3):564–572.
3. Aldossari NM, El Gabry EE, Gawish GEH. Association between salivary amylase enzyme activity and obesity in Saudi Arabia. Medicine. 2019;98(23):e15978.
4. Bonnefond A, Yengo L, Dechaume A, Canouil M, Castelain M, Roger E, *et al*. Relationship between salivary amylase and body mass index: A system's approach. BMC Medicine. 2017;15:37.
5. Gary B. Iodine Clock Reaction. Missouri University of Science and Technology. 2019. [Retrieved 5 May 2019; Accessed 6 June 2019].
6. Boron WF. Medical Physiology: A Cellular and Molecular Approach. Elsevier/Saunders; 2003. p. 928.
7. Comba A. Saliva. Flipper e Nuvola. [Retrieved 25 February 2018; Accessed 4 April 2019].
8. Brilliant Biology Student. Iodine Test for Starch. Master Biology Labs. 2015. [Retrieved 1 December 2015; Accessed 3 April 2019].
9. Di Angelantonio E, Bhupathiraju SN. Body-mass index and all-cause mortality: individual-participant-data meta-analysis of 239 prospective studies in four continents. The Lancet. 2016;388(10046):776–786.
10. Edgar M, Dawes C, O'Mullane D. Saliva and Oral Health. British Dental Association; 2004.
11. Edgar M, Dawes C, O'Mullane D. Saliva and Oral Health. Duns Tew: Stephen Hancocks Limited; 2012.
12. Flegal KM, Shepherd JA, Looker AC, *et al*. Comparisons of percentage body fat, body mass index, waist circumference, and waist-stature ratio in adults. The American Journal of Clinical Nutrition. 2009;89(2):500–508.
13. Fogel MR, Gray GM. Starch hydrolysis in man: an intraluminal process not requiring membrane digestion. Journal of Applied Physiology. 1973;35:263–267.
14. Fried M, Abramson S, Meyer JH. Passage of salivary amylase through the stomach in humans. Digestive Diseases and Sciences. 1987;32(10):1097–1103.
15. Ghalanbor Z, Ghaemi N, Marashi SA, Amanlou M, Habibi-Rezaei M, Khajeh K, *et al*. Binding of Tris to Bacillus licheniformis alpha-amylase can affect its starch hydrolysis activity. Protein and Peptide Letters. 2008;15(2):212–214.
16. Sahu GK, Upadhyay S, Panna SM. Salivary alpha-amylase activity in human beings of different age groups subjected to psychological stress. Indian Journal of Clinical Biochemistry. 2014;29(4):485–490.
17. Granger DA, Kivlighan KT, el-Sheikh M, Gordis EB, Stroud LR. Salivary alpha-amylase in biobehavioral research: recent developments and applications. Annals of the New York Academy of Sciences. 2007;1098:122–144.
18. Hoebler C, Karinthi A, Devaux MF, Guillon F, Gallant DJ, Bouchet B, *et al*. Physical and chemical transformations of cereal food during oral digestion in human subjects. British Journal of Nutrition. 1998;80:429–436.
19. Kadziola A, Søgaard M, Svensson B, Haser R. Molecular structure of a barley alpha-amylase-inhibitor complex: implications for starch binding and catalysis. Journal of Molecular Biology. 1998;278(1):205–217.
20. Karapanou O, Papadimitriou A. Determinants of menarche. Reproductive Biology and Endocrinology. 2010;8:115.
21. Mandel ID. The functions of saliva. Journal of Dental Research. 1987;66(Spec No):623–627.
22. Madhu S, Evans HA, Doan-Nguyen VVT, Labram JG, Wu G, Chabinyc ML, *et al*. Infinite polyiodide chains in the pyrroloperylene-iodine complex: insights into the starch-iodine and perylene-iodine complexes. Angewandte Chemie International Edition. 2016;55(28):8032–8035.
23. Mandel AL, Peyrot des Gachons C, Plank KL, Alarcon S, Breslin PA. Individual differences in AMY1 gene copy number, salivary α-amylase levels, and the perception of oral starch. PLoS ONE. 2010;5:e13352.
24. Tiwari M. Science behind human saliva. Journal of Natural Science, Biology, and Medicine. 2011;2(1):53–58.
25. Martini FH, Nath JL, Bathlomew EF. Fundamentals of Anatomy and Physiology. San Francisco: Pearson Benjamin Cummings; 2012.
26. Hosseini MH, Karandish M, Ghoreishi M, Soroor F, Shirani F. Body weight changes during the menstrual cycle among university students in Ahvaz, Iran. Pakistan Journal of Biological Sciences. 2014;17(7):915–919.
27. Nedugandi P, Raman R, McGregor M. Action of salivary amylase activity in starch. Amrita Olabs. 2013. [Retrieved 15 June 2019; Accessed 15 June 2019].
28. Nater UM, Rohleder N. Salivary alpha-amylase as a non-invasive biomarker for the sympathetic nervous system: current state of research. Psychoneuroendocrinology. 2009;34(4):486–496.
29. Nosek TM. Essentials of Human Physiology. Panama: Jaypee Brothers Medical Publishers; 2012.
30. Noto Y, Sato T, Kudo M, Kurata K, Hirota K. The relationship between salivary biomarkers and state-trait anxiety inventory score under mental arithmetic stress: a pilot study. Anesthesia and Analgesia. 2005;101(6):1873–1876.
31. Perry GH, Dominy NJ, Claw KG, Lee AS, Fiegler H, Redon R, *et al*. Diet and the evolution of human amylase gene copy number variation. Nature Genetics.

2007;39(10):1256–1260.
32. Poppitt SD, Prentice AM, Goldberg GR, Whitehead RG. The energy-sparing strategies to protect human fetal growth. American Journal of Obstetrics and Gynecology. 1994;171(1):118–125.
33. Pugh MB. Stedman's Medical Dictionary. 27th ed. Baltimore: Lippincott Williams & Wilkins; 2000. p. 65.
34. Romero-Corral A, Somers VK, Sierra-Johnson J, *et al*. Accuracy of body mass index in diagnosing obesity in the adult general population. International Journal of Obesity. 2008;32(6):959–966.
35. Romero-Corral A, Somers VK, Sierra-Johnson J, *et al*. Normal weight obesity: a risk factor for cardiometabolic dysregulation and cardiovascular mortality. European Heart Journal. 2010;31(6):737–746.
36. Rosenblum JL, Irwin CL, Alpers DH. Starch and glucose oligosaccharides protect salivary-type amylase activity at acid pH. The American Journal of Physiology. 1988;254(5 Pt 1):G775–G780.
37. Riley P, Glenny AM, Hua F, Worthington HV. Cochrane Database of Systematic Reviews. 2017;7:CD012744.
38. Science Daily. Saliva. 2018. [Retrieved 24 February 2018; Accessed 1 April 2019].
39. Sherwood L. Human Physiology: From Cells to Systems. 8th ed. Belmont: Cengage; 2013. p. 735–794.
40. Silverthorn DU. Human Physiology: An Integrated Approach. 6th ed. Glenview: Pearson Education; 2013. p. 850–890.
41. Stokes A, Preston SH. Smoking and reverse causation create an obesity paradox in cardiovascular disease. Obesity. 2015;23(12):2485–2490.
42. Strain GW, Zumoff B. The relationship of weight-height indices of obesity to body fat content. Journal of the American College of Nutrition. 1992;11(6):715–718.
43. Takai N, Yamaguchi M, Aragaki T, Eto K, Uchihashi K, Nishikawa Y. Effect of psychological stress on the salivary cortisol and amylase levels in healthy young adults. Archives of Oral Biology. 2004;49(12):963–968.
44. Taylor J. Immunity in the oral cavity. British Society for Immunology. 2018. [Retrieved 25 February 2018].
45. The New York Times. Weight Index Doesn't Tell the Whole Truth. 31 August 2010. [Accessed 1 May 2017].
46. Wellens RI, Roche AF, Khamis HJ, Jackson AS, Pollock ML, Siervogel RM. Relationships between the body mass index and body composition. Obesity Research. 1996;4(1):35–44.
47. World Health Organization. BMI Classification. Global Database on Body Mass Index. 2006. [Retrieved 5 April 2019; Accessed 1 April 2019].
48. Yasuda N, Tanioka T, Iwashita N, Yamamoto K. Effects of menstrual cycle phase on salivary α-amylase activity following 2 hours of cycling exercise in recreationally active eumenorheic women. Journal of Science and Medicine in Sport. 2012;15(2):188–264.
49. Young AC, Ellis C, Sathasivam S, Pih N. Cochrane Database of Systematic Reviews. 2011;5:5–11.