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The Link Between Salivary Amylase Activity, Overweight, and Glucose Homeostasis

Doctoral Thesis – set of publications – for obtaining the scientific degree "Doctor of Science (*PhD*)"

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Abstract

This doctoral Thesis explores the role of salivary amylase activity (SAA) as a potential non-invasive biomarker of metabolic health by examining its associations with overweight status, visceral fat, and markers of glucose homeostasis, emphasising that current evidence is largely correlational and requires further investigation to establish causality.

In a well-characterised cohort of overweight women of reproductive age, the crosssectional study, integrated as a foundational component of the interventional study, analysed associations between salivary amylase activity, visceral fat, and the triglyceride glucose index to establish baseline metabolic relationships, while the subsequent 12-week dietary intervention, stratified by salivary amylase activity levels, evaluated differential metabolic responses including changes in insulin sensitivity, GLP1 secretion, adipokines, and glucose homeostasis under calorie restricted and low starch diets, highlighting the potential of personalised nutrition in optimising metabolic health.

The findings of this investigation were reported through three thematically aligned peer-reviewed original research articles as well as two comprehensive review articles. A robust inverse relationship was observed between SAA and visceral adiposity, independent of total body fat, suggesting that higher SAA may be indicative of a metabolically healthier fat distribution phenotype. While visceral fat was positively correlated with the triglyceride-glucose (TyG) index, a surrogate marker of insulin resistance, SAA exhibited no direct association with the TyG index. These findings support the hypothesis that SAA may influence glucose regulation indirectly, potentially through its modulation of fat accumulation and cephalic-phase insulin dynamics patterns rather than directly.

While variability in SAA activity, largely driven by AMY1 gene copy number variation (CNV), also has been regulated by adrenergic activity, which can be partially modulated through lifestyle interventions. This is particularly relevant, as impaired autonomic regulation is commonly observed in individuals with obesity and metabolic syndrome and may be improved through targeted lifestyle changes. This potential responsiveness further supports its relevance as a candidate biomarker for metabolic plasticity.

This Thesis proposes that SAA, as a non-invasive and easily measurable biomarker, could play a valuable role in the early identification of individuals at metabolic risk and in guiding personalised lifestyle interventions for the prevention of insulin resistance and obesityrelated complications.

Keywords: salivary amylase activity, overweight, visceral fat, glucose homeostasis, insulin resistance, triglyceride-glucose index, metabolic health, cephalic-phase insulin secretion, dietary intervention.

Anotācija

Siekalu amilāzes funkcionālās aktivitātes saistība ar virssvaru un ogļhidrātu vielmaiņas traucējumiem

Šī promocijas darba mērķis bija izpētīt siekalu amilāzes aktivitāti (SAA) kā potenciālu neinvazīvu biomarķieri metabolās veselības novērtēšanai, analizējot saistību ar virssvaru, viscerālajiem taukiem un glikozes homeostāzes marķieriem, akcentējot, ka pašreizējie pierādījumi pārsvarā ir korelatīvi un nepieciešami turpmāki pētījumi, lai noteiktu cēloņsakarību. Šķērsgriezuma pētījumā, kas tika integrēts kā elements intervences pētījumā, precīzi definētā reproduktīvā vecuma sieviešu kohortā ar virssvaru ĶMI 25–29,9 (kg/m²), analizēja asociāciju starp siekalu amilāzes aktivitāti, viscerālo tauku apjomu un triglicerīdu-glikozes indeksu. 12 nedēļu diētas intervencē, kur pētījuma dalībnieces tika stratificētas pēc siekalu amilāzes aktivitātes, tika novērotas atšķirīgas metabolas reakcijas, tostarp izmaiņas insulīna jutībā, GLP-1 sekrēcijā, adipokīnu līmeņos un glikozes homeostāzē, izmantojot kaloriju ierobežojumu un zema cietes satura diētu, tādējādi aktualizējot personalizētas uztura pieejas potenciālu metabolās veselības uzlabošanā.

Šī pētījuma rezultāti tika publicēti trīs tematiski saistītos, recenzētos zinātniskos rakstos, kā arī divos pārskata rakstos. Pētījumā tika konstatēta spēcīga inversa saistība starp SAA un viscerālo tauku daudzumu, neatkarīgi no kopējās ķermeņa tauku masas, kas liecina, ka augstāka SAA var norādīt uz metaboliski veselīgāka tauku sadalījuma fenotipu. Lai gan viscerālie tauki pozitīvi korelēja ar triglicerīdu-glikozes (TyG) indeksu, netiešu insulīna rezistences marķieri, SAA neuzrādīja tiešu saistību ar TyG indeksu. Šie rezultāti apstiprina hipotēzi, ka SAA var ietekmēt glikozes regulāciju netieši, iespējams, modulējot tauku akumulāciju un cefāliskās fāzes insulīna dinamikas paternu, nevis tieši. Lai gan siekalu amilāzes aktivitātes (SAA) variabilitāti lielā mērā nosaka AMY1 gēna kopiju skaita variācija (CNV), to regulē arī adrenerģiskās nervu sistēmas aktivitāte, kuru daļēji iespējams modulēt ar dzīvesveida intervencēm. Šī saistība ir īpaši nozīmīga, jo autonomās nervu sistēmas disbalanss bieži tiek novērots personām ar aptaukošanos un metabolo sindromu, savukārt mērķtiecīgas dzīvesveida izmaiņas var veicināt šīs regulācijas atjaunošanos.

Šī darba rezultāti izceļ SAA kā iespējamu neinvazīvu un praktiski pielietojamu agrīna skrīninga marķieri, lai identificētu indivīdus ar paaugstinātu metabolo risku, kā arī par noderīgu marķieri personalizētu dzīvesveida stratēģiju izstrādē, kas vērstas uz insulīna rezistences mazināšanu un ar aptaukošanos saistīto komplikāciju profilaksi.

Atslēgvārdi: siekalu amilāzes aktivitāte, virssvars, viscerālie tauki, glikozes homeostāze, insulīna rezistence, triglicerīdu-glikozes indekss, metabolā veselība, insulīna sekrēcijas cefāliskā fāze, diētas intervence.

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Abbreviations used in the Thesis

Akt (PKB)	Protein kinase B
AMPK	Adenosine monophosphate-activated protein kinase
AMY1	Amylase, alpha 1 gene
BMI	Body mass index
CTR	Control group
DAG	Diacylglycerol
FFA	Free fatty acid
fMRI	Functional magnetic resonance imaging
GDM	Gestational diabetes mellitus
GIP	Glucose-dependent insulinotropic polypeptide
GLP-1	Glucagon-like peptide-1
GLP-1 (7-37)	Active glucagon-like peptide-1
GLUT1	Glucose transporter type 1
GLUT2	Glucose transporter type 2
GPCRs	G protein-coupled receptors
HDL-C	High-density lipoprotein cholesterol
HOMA2-%B	Homeostatic model assessment 2 of beta-cell function
HOMA2-%S	Homeostatic model assessment 2 of insulin sensitivity percentage
HOMA2-IR	Homeostatic model assessment 2 of insulin resistance
HSA-CR	High-salivary-amylase calorie restriction group
HSA-LS	High-salivary-amylase low-starch group
IKKβ	IκB kinase beta
IR	insulin resistance
IRS	insulin receptor substrate
JNK	c-Jun N-terminal kinase
K-ATP	ATP-sensitive potassium channel
LDL-C	low-density lipoprotein cholesterol
LPS	Lipopolysaccharides
LSA-CR	Low-salivary-amylase calorie restriction group
LSA-LS	Low-salivary-amylase low-starch group
MET	Metabolic equivalent task
mTORC1	Mechanistic target of rapamycin
PCR	Polymerase chain reaction
PKCε	Protein kinase C epsilon isoform
PLCβ2	Phospholipase C beta 2
PYY	Peptide YY
RBP4	Retinol-binding protein 4
RNA	Ribonucleic acid
SAA	Salivary amylase activity
SCFA	Short-chain fatty acid
SEM	Structural equation modelling

SGLT1	Sodium-glucose cotransporter 1
SREBP-1c	Sterol regulatory element-binding protein 1c
T0	Baseline
T1	30 minutes after starch-containing muesli consumption
T2	12 weeks post-intervention
Tregs	Regulatory T cells
TyG	Triglyceride-glucose index
T1R2/T1R3	Taste receptor type 1, member 2/ Taste receptor type 1, member 3
VF	Visceral fat

Introduction

The growing global burden of overweight and obesity has led to a surge in metabolic disorders, including insulin resistance, type 2 diabetes, and cardiovascular disease. Although the pathogenesis of these conditions is multifactorial, there is increasing interest in early, noninvasive biomarkers that can identify individuals at risk and offer insights into underlying physiological mechanisms (Al Akl et al., 2022; Habobe et al.2025).

Salivary amylase activity (SAA), which catalyses the initial hydrolysis of α1,4glycosidic linkages in dietary starches, has traditionally been regarded as a digestive enzyme (Freitas et al 2017). However, accumulating evidence indicates that SAA has functions extending beyond starch digestion, potentially contributing to the regulation of glucose homeostasis, insulin secretion, and overall energy balance (Mandel et al., 2010; Mejía-Benítez et al., 2015). Recent studies further demonstrate that interindividual variations in SAA are associated with differences in postprandial glycaemic responses, insulin dynamics, and adiposity distribution (Mandel & Breslin, 2012; Sekine et al., 2024). Collectively, these findings suggest that SAA may serve as a proxy for broader neuroendocrine and metabolic regulatory pathways involved in energy homeostasis.

Visceral adiposity, a hallmark of metabolically unhealthy overweight, is strongly associated with insulin resistance and systemic inflammation (Er et al., 2016; Tsuchiya et al., 2025). Identifying upstream markers that correlate with visceral fat accumulation and impaired glucose metabolism is critical for developing preventive strategies. Several observational studies have suggested that individuals with lower SAA levels may be predisposed to greater visceral fat accumulation and blunted cephalic-phase insulin responses, although the mechanisms remain poorly understood (Al-Akl et al., 2022; Marquina et al., 2019).

Aim of the Thesis

This Thesis builds upon these hypotheses and aims to clarify the associations between SAA, overweight status, and glucose homeostasis. Unlike previous work focused on isolated aspects, this study takes an integrative approach using data from a single population cohort, examined through multiple analytical approaches. The results are presented across three published articles, each emphasising different dimensions of the metabolic network involving SAA.

Objectives of the Thesis

1 Conduct a comprehensive review of current scientific literature to elucidate the physiological role of salivary amylase activity (SAA) and its potential associations with metabolic health, focusing on its impact on glucose regulation, insulin response, and adipose tissue distribution.

2 Investigate the relationships between salivary amylase activity, visceral fat accumulation, and surrogate markers of insulin resistance, such as the triglyceride-glucose (TyG) index.

3 Conduct a Physician-Directed Decision-Making observational design with a crosssectional analysis component to evaluate the impact of dietary interventions on metabolic markers in a well-defined cohort of overweight women with varying salivary amylase activity (SAA) of reproductive age to evaluate differences in anthropometric and metabolic parameters in response to a 12-week low-starch and calorie-restriction diet intervention.

Study Markers and Parameters

1 **Anthropometric markers:**
   1) Body mass index (BMI)
   2) Visceral fat percentage (VF %)

2 **Carbohydrate metabolism markers:**
   1) Glucose
   2) C-peptide
   3) Active GLP-1
   4) Glucagon

3 **Insulin sensitivity and resistance markers:**
   1) HOMA2-%S (insulin sensitivity)
   2) HOMA2-IR (insulin resistance)
   3) HOMA2-%B (beta-cell function)
   4) TyG – triglyceride-glucose

4 **Lipid profile:**
   1) Total cholesterol
   2) Low-density lipoprotein (LDL)
   3) High-density lipoprotein (HDL)
   4) Triglycerides

5 **Circulating gut/metabolic markers:**
   1) GDF15
   2) Leptin
   3) Butyrate

Hypotheses of the Thesis

1. Salivary amylase activity (SAA) is inversely associated with visceral adiposity and may serve as a non-invasive biomarker for early metabolic dysregulation in overweight individuals.
2. SAA's role in maintaining glucose homeostasis is mediated indirectly through mechanisms such as visceral fat modulation, rather than exerting a direct effect on systemic glucose handling.
3. The effect of low-starch and calorie-restriction diet on metabolic parameters differs according to the level of salivary amylase activity (SAA).

Novelty of the Thesis

This Thesis introduces an innovative perspective by exploring salivary amylase activity (SAA) as a potential upstream modulator of metabolic health in overweight women of reproductive age. While previous research has primarily focused on SAA's role in digestive physiology and acute postprandial responses, this work extends its significance to chronic metabolic adaptations. Specifically, it examines the relationships between SAA, visceral adipose tissue distribution, and markers of glucose homeostasis.

Key Contributions of this Thesis include:

Inverse Association Between SAA and Visceral Fat: The study demonstrates a significant inverse relationship between SAA and visceral fat percentage, independent of total body fat mass. This finding aligns with evidence from the research, which highlights that higher SAA activity is associated with reduced visceral fat accumulation, a critical marker of metabolic risk. Mediation analysis further reveals that visceral fat mediates the relationship between SAA and the triglyceride-glucose (TyG) index, accounting for 45 % of the total effect. This positions SAA as a potential surrogate marker for metabolically unhealthy fat distribution.

Challenging the Direct Link Between SAA and TyG Index: Contrary to the assumption of a direct linear relationship between SAA and insulin resistance markers like the TyG index, this Thesis provides empirical evidence of their dissociation. The findings suggest that SAA's influence on metabolic health is primarily mediated through its impact on visceral fat, rather than directly affecting the TyG index. This challenges traditional views and calls for a deeper exploration of alternative regulatory pathways.

Salivary Diagnostics as a Non-Invasive Screening Tool: The research highlights the potential of salivary diagnostics as a cost-effective and non-invasive method for metabolic screening. By measuring SAA, it may be possible to identify individuals at risk for visceral obesity and insulin resistance early, enabling timely interventions.

Collectively, this Thesis proposes a paradigm shift from traditional glucose-centric models to a more integrative approach that emphasises the role of digestive-metabolic interactions. By leveraging salivary biomarkers, particularly SAA, this work contributes to refining the evaluation of metabolic health dynamics and underscores the importance of personalised nutrition strategies tailored to individual metabolic profiles.

Personal Contribution

The researcher was actively involved in all stages of the work presented in this Thesis. This included conceiving the research concept, defining the study objectives, and designing the experimental protocols investigating salivary amylase activity, visceral adiposity, and metabolic health parameters.

Responsibility was taken for quality control of samples and standardisation of data collection procedures. Alongside this, direct participation was provided in participant recruitment, collection of saliva and blood samples, and storage of samples.

All figures, tables, and visual materials were prepared by the researcher, in addition, five scientific publications were prepared as part of this work.

1 Literature Review

1.1 Salivary Amylase and Its Biological Role

Salivary α-amylase (SAA) is an enzyme predominantly secreted by the parotid glands, with additional contributions from the submandibular, sublingual, and numerous minor salivary glands distributed throughout the oral mucosa. While the parotid glands are the primary source of SAA during stimulated secretion, the baseline enzymatic activity, under resting conditions, is maintained by the continuous output of the submandibular and sublingual glands (Proctor & Carpenter, 2007). The enzyme is encoded by the AMY1 gene, which exhibits significant copy number variation (CNV) among individuals, influencing both the concentration and enzymatic activity of SAA (Carpenter al., 2017). Environmental factors, including psychological stress, circadian rhythm, and habitual dietary intake, further modulate SAA levels (Nater et al., 2007; Ali, N., & Nater 2020).

Functionally, SAA catalyses the hydrolysis of α-1,4-glycosidic bonds in dietary starch, initiating its degradation into maltose, maltotriose, and other linear oligosaccharides. These are subsequently processed by brush border enzymes in the small intestine to form absorbable monosaccharides (Butterworth et al., 2011). This early-stage digestion not only contributes to the mechanical breakdown of food but also has significant metabolic implications, influencing postprandial glucose excursions and hormonal signalling (Mandel et al., 2012; Peyrot et al., 2016).

1.2 Genetic Variability and Nutritional Adaptation

Evolutionary evidence suggests that populations traditionally consuming high-starch diets possess increased AMY1 gene copy numbers and exhibit correspondingly elevated SAA activity (Perry et al., 2007). This genetic adaptation is posited to enhance preabsorptive carbohydrate digestion, reflecting dietary selection pressures. The inter-individual variability in SAA levels is therefore considered a marker of metabolic adaptation, potentially contributing to differences in glycaemic regulation and metabolic flexibility (Pérez-Ros et al., 2021).

1.3 SAA and Postprandial Glycaemic Response

Salivary amylase activity (SAA) plays a critical role in the initial digestion of dietary starch, influencing postprandial glycaemic and insulinemic responses. Individuals with higher SAA exhibit enhanced starch hydrolysis in the oral cavity, leading to increased production of maltose and other oligosaccharides. These smaller carbohydrate molecules are rapidly absorbed in the small intestine, resulting in a faster and more pronounced rise in postprandial blood glucose levels (Mandel et al., 2010; Farrell et al., 2021; Sekine et al., 2024). This process is thought to be mediated by pre-absorptive signalling mechanisms, where the early breakdown of starch triggers the release of incretin hormones such as glucagon-like peptide-1 (GLP-1) and

glucose-dependent insulinotropic peptide (GIP). These hormones enhance insulin secretion and contribute to glucose homeostasis (Mandel et al., 2010; Sekine et al., 2024).

Furthermore, research suggests that individuals with low SAA may experience delayed starch digestion, leading to slower glucose absorption and a blunted glycaemic response. This variability in glycaemic response has been linked to differences in AMY1 gene copy number, which determines the level of salivary amylase production (Falchi et al., 2014). Studies have shown that individuals with a higher AMY1 copy number tend to have lower body mass index (BMI) and reduced risk of obesity, potentially due to their ability to efficiently metabolise starch and maintain better glycaemic control (Falchi et al., 2014; Mandel et al., 2010).

The relationship between SAA and postprandial glycaemic response is further supported by evidence from neuroendocrine studies. Enhanced oral starch hydrolysis has been shown to activate taste receptors and stimulate cephalic phase insulin release, which prepares the body for efficient glucose metabolism (Forde et al., 2013). This pre-absorptive signalling may also influence satiety and appetite regulation, highlighting the broader metabolic implications of SAA beyond glycaemic control (Figure 1.1).

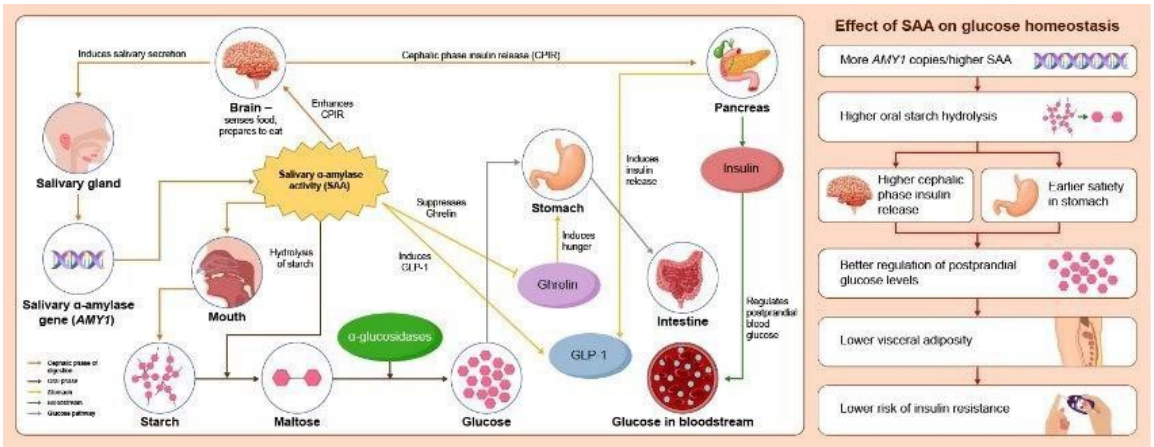

Figure 1.1 **Proposed mechanism by which salivary α-amylase (SAA) activity influences glucose homeostasis**

1.4 SAA and Carbohydrate Metabolism Efficiency

Individuals with higher SAA activity exhibit enhanced efficiency in starch digestion, which can influence postprandial glycaemic dynamics and insulin signalling. (Mandel et al., 2012; Sekine at al 2024).

In the oral cavity, sweet taste is detected by the T1R2/T1R3 heterodimer, a class C Gprotein-coupled receptor (GPCR) expressed on taste receptor cells (Perez-Aguilar et al., 2019) Upon activation by glucose or sweet compounds, T1R2/T1R3 initiate signalling via gustducin and phospholipase Cβ2 (PLCβ2), leading to intracellular Ca^{2+} release and

neurotransmittermediated activation of gustatory pathways, contributing to cephalic-phase insulin responses(Lee et al 2017) .

Beyond the tongue, T1R2/T1R3 are also expressed in the small intestine, particularly on enteroendocrine L-cells, where they function as luminal glucose sensors (Young et al., 2009). Unlike glucose transporters, these receptors trigger GLP-1 secretion via intracellular Ca^{2+}-dependent signalling, independent of glucose transport.

Following oral and gastric digestion, glucose reaches the small intestine and is absorbed primarily via sodium-glucose cotransporter 1 (SGLT1), located on the apical membrane of enterocytes and L-cells in the duodenum and jejunum. SGLT1 mediates Na^{+}coupled active glucose transport and also acts as a glucose sensor: electrogenic glucose uptake depolarises the membrane, induces Ca^{2+} influx, and stimulates GLP-1 release (Sun et al., 2017; Röder et al., 2014).

Glucose can also enter L-cells via GLUT2, a facilitative transporter on the basolateral membrane, enabling bidirectional transport depending on concentration gradients (Tagliavini et al.,2017). GLUT2 supports metabolic sensing by promoting intracellular ATP production and K-ATP channel regulation, indirectly enhancing GLP-1 synthesis and secretion.

Together, T1R2/T1R3, SGLT1, and GLUT2 coordinate oral and intestinal glucose sensing to regulate incretin hormone release and maintain postprandial glucose homeostasis (Parker et al., 2012).

Following oral and gastric digestion, glucose is absorbed in the small intestine primarily via the sodium-glucose cotransporter 1 (SGLT1), which is expressed on the apical membrane of enterocytes and enteroendocrine L-cells, particularly in the duodenum and jejunum. SGLT1 not only mediates active glucose uptake by coupling it with sodium ions but also acts as a glucose sensor. By generating an electrogenic current, SGLT1 induces membrane depolarisation, leading to calcium influx and subsequent glucagon-like peptide-1 (GLP-1) secretion from L-cells (Sun et al., 2017; Röder et al., 2014).

In addition to SGLT1, glucose can also enter L-cells via GLUT2, a facilitative transporter predominantly located on the basolateral membrane. GLUT2 allows bidirectional glucose movement depending on concentration gradients and contributes to metabolic sensing by promoting intracellular glucose metabolism, ATP production, and K_ATP channel modulation, indirectly supporting GLP-1 synthesis and secretion.

GLP-1 plays a central role in postprandial glucose homeostasis by enhancing glucose-stimulated insulin secretion, delaying gastric emptying, and suppressing glucagon release, thus contributing to the incretin effect and maintaining euglycemia.

Enhanced starch digestion due to higher SAA activity leads to a more rapid and efficient glucose delivery to the small intestine, which may augment early-phase GLP-1 secretion and improve insulinotropic responses. This could explain the association between elevated SAA and a more favourable metabolic profile, including lower visceral fat accumulation, as observed in our study. Efficient postprandial glycaemic regulation may limit compensatory hyperinsulinemia and reduce insulin-driven lipogenesis, especially in visceral depots (Dimitriadis et al., 2021 ; Uehara et al., 2023).

Moreover, recent evidence suggests that gut microbiota composition differs significantly between individuals with high and low SAA activity (Poole et al., 2019). HighSAA individuals tend to harbour more starch-utilising and butyrate-producing taxa, such as *Ruminococcaceae* and *Faecalibacterium prausnitzii,* while low-SAA individuals show an increased abundance of *Bacteroides,* which prefer simple sugars. Butyrate, a short-chain fatty acid produced via microbial fermentation of resistant starch, exerts beneficial metabolic effects, including improving insulin sensitivity, enhancing mitochondrial function in adipocytes, and reducing inflammation (Rampanelli, et al., 2025).

These microbial differences may act in synergy with host amylase activity: higher SAA could reduce starch delivery to the colon, but favour rapid glucose availability and early satiety signalling, while still supporting butyrate production from undigested fractions. On the other hand, in low-SAA individuals, the slower and less efficient hydrolysis of starch may lead to increased colonic fermentation of unabsorbed polysaccharides, possibly altering SCFA profiles and impacting enteroendocrine signalling.

Altogether, these data support the hypothesis that salivary amylase activity modulates carbohydrate metabolism not only through direct effects on glycaemic regulation via SGLT1 and GLP-1 pathways but also indirectly through shaping the gut microbiota and its metabolic products such as butyrate. These mechanisms jointly contribute to the observed variability in visceral fat deposition and systemic insulin sensitivity, positioning SAA as a physiologically integrative marker at the interface of diet, digestion, and metabolic health.

Salivary amylase activity is a key determinant of the rate of starch digestion and subsequent glucose absorption. In our study, elevated salivary amylase activity was found to facilitate rapid starch degradation, leading to faster glucose absorption and enhanced GLP-1 secretion. GLP-1, an incretin hormone, is critical for glucose homeostasis as it promotes insulin secretion, inhibits glucagon release, and slows gastric emptying. Conversely, individuals with lower salivary amylase activity experience slower starch digestion, altered glycaemic responses, and potentially impaired GLP-1 dynamics. This variability underscores the importance of salivary amylase activity in shaping post-prandial glucose metabolism.

1.5 SAA and Insulin Sensitivity

Recent studies have highlighted the potential link between SAA activity and insulin sensitivity, suggesting that variations in SAA activity may influence glucose metabolism and insulin dynamics, thereby impacting overall metabolic health.

SAA activity has been associated with markers of insulin sensitivity, such as fasting insulin levels and homeostatic model assessment indices, including HOMA2-IR (insulin resistance) and HOMA2-%S (insulin sensitivity). Elevated SAA activity has been correlated with lower fasting insulin levels and improved HOMA2-IR scores, indicating enhanced insulin sensitivity and reduced insulin resistance (Locia-Morales et al., 2022). This relationship may be explained by the enzyme's role in accelerating the breakdown of starch into glucose, which facilitates efficient glucose absorption and stimulates the secretion of incretin hormones, such as glucagon-like peptide-1 (GLP-1).4 GLP-1 enhances glucose-dependent insulin secretion, suppresses glucagon release, and improves beta-cell function, collectively contributing to better glycaemic control and insulin sensitivity (Holst et al., 2021; Yang et al., 2024).

Moreover, individuals with higher SAA activity may exhibit a metabolically favourable phenotype characterised by reduced postprandial glucose variability, improved beta-cell responsiveness, and lower circulating C-peptide levels, which are indicative of reduced pancreatic stress (Bonnefond et al., 2017; Goh et al., 2023). These findings suggest that SAA activity could serve as a biomarker for identifying individuals with a predisposition to better glucose homeostasis and insulin sensitivity. Additionally, the variability in SAA activity among individuals may be influenced by genetic factors, such as copy number variations in the AMY1 gene, which encodes salivary amylase. Higher AMY1 copy numbers have been linked to increased SAA activity and improved metabolic outcomes, including lower body mass index (BMI) and reduced risk of obesity-related insulin resistance (Carpenter et al., 2017; Al-Akl et al., 2022).

The mechanistic pathways linking SAA activity to insulin sensitivity are complex and multifactorial. Beyond its enzymatic role, SAA may influence the gut microbiota composition and the rate of carbohydrate digestion, both of which are critical determinants of postprandial glucose and insulin responses (Woolnough et al., 2010; Goh et al., 2023). Furthermore, the interplay between SAA activity and dietary composition, particularly the glycaemic index and starch content of consumed foods, may modulate the secretion of gut hormones and adipokines, further influencing insulin sensitivity and metabolic health (Sievenpiper et al., 2020; Steven et al., 2016).

1.6 Visceral Adiposity and the Glucose-Triglyceride Index

Visceral adiposity, characterised by the accumulation of visceral adipose tissue (VAT) around internal organs, is a well-established risk factor for metabolic syndrome, type 2 diabetes, cardiovascular diseases, and other metabolic disorders (Wang et al., 2025; Guldan et al 2025). VAT is metabolically active and contributes to systemic inflammation, dyslipidaemia, and insulin resistance through the secretion of pro-inflammatory cytokines and adipokines (Després et al., 2001, Niculescu et al 2025). Unlike subcutaneous fat, VAT is more strongly associated with adverse metabolic outcomes due to its proximity to vital organs and its role in hepatic lipid metabolism.

The triglyceride-glucose (TyG) index has gained attention as a reliable and costeffective surrogate marker for insulin resistance. It is calculated using fasting triglyceride and glucose levels, which are easily accessible in routine clinical practice (da Silva et al., 2019; Cabanillas-Lazo et al 2025). The TyG index has been shown to correlate strongly with hyperinsulinemic-euglycaemic clamp measurements, the gold standard for assessing insulin sensitivity (Simental-Mendía et al., 2008). This makes it a valuable tool for identifying individuals at risk of developing type 2 diabetes and cardiovascular diseases.

Several studies have demonstrated a strong positive association between VAT and the TyG index. For instance, research has shown that higher TyG index values are linked to increased VAT volume, independent of body mass index (BMI), and other anthropometric markers (Er et al., 2016). This suggests that the TyG index may be particularly useful in identifying individuals with metabolically unhealthy obesity, even in those with a normal BMI. Furthermore, the TyG index has been associated with other markers of metabolic dysfunction, including elevated liver enzymes, increased arterial stiffness, and a higher prevalence of nonalcoholic fatty liver disease (NAFLD) (Zhang et al., 2017).

The utility of the TyG index extends beyond its role as a marker of insulin resistance. It has been proposed as a predictor of cardiovascular events, with studies indicating that higher TyG index values are associated with an increased risk of coronary artery disease, myocardial infarction, and stroke (Samavarchitehrani et al., 2024). Additionally, the TyG index has been linked to the progression of atherosclerosis, as it reflects the interplay between dyslipidaemia and impaired glucose metabolism, both of which contribute to endothelial dysfunction and plaque formation (Zhao et al., 2022).

In the context of visceral adiposity, the TyG index provides a non-invasive and costeffective method for assessing metabolic health and identifying individuals at risk for cardiometabolic diseases. Its simplicity and strong correlation with insulin resistance make it a valuable tool for both clinical practice and epidemiological studies. Future research should

focus on refining the index, exploring its predictive value in diverse populations, and integrating it into personalised medicine approaches for the prevention and management of metabolic disorders.

1.7 SAA and Appetite Regulation

Recent studies suggest a novel role for SAA in appetite and energy intake regulation. Higher salivary amylase activity has been associated with greater satiety, reduced hedonic responses to food, and altered brain activation in regions related to reward and food motivation (Schlezingerová et al., 2024; Hartoon et al., 2008). These effects may be mediated through the oral-brain-gut axis, whereby early carbohydrate sensing in the oral cavity modulates the secretion of appetite-regulating hormones such as ghrelin and influences hypothalamic responsiveness to food stimuli (Raka et al., 2019). Oral glucose perception activates afferent sensory patways that transmit sensory signals to the brainstem nuclei, which modulate orexinproducing neirons in lateral hypothalamus to coordinate cephalic-phase insulin secretion (Sakurai et al., 1998). This adds a behavioural and neuroendocrine dimension to the metabolic significance of SAA.

1.7.1 SAA and Satiety

Higher SAA levels have been linked to enhanced satiety and reduced caloric intake. This may be attributed to the rapid hydrolysis of starches in the oral cavity, leading to faster glucose absorption in the small intestine and subsequent modulation of gut hormones such as glucagon-like peptide-1 (GLP-1) and peptide YY (PYY) (Forde et al., 2013). These hormones are known to promote satiety by slowing gastric emptying and signalling fullness to the brain. Additionally, individuals with high SAA activity exhibit lower postprandial glucose variability, which may contribute to sustained satiety and reduced hunger (Mandel et al., 2010).

1.7.2 SAA and Food Reward

SAA also appears to influence hedonic responses to food. Studies using functional magnetic resonance imaging (fMRI) have shown that individuals with higher SAA activity exhibit reduced activation in brain regions associated with food reward, such as the orbitofrontal cortex and striatum, when exposed to high-calorie foods (Schlezingerová et al., 2024). This suggests that SAA may dampen the reward-driven motivation to consume energy-dense foods, potentially reducing the risk of overeating and obesity.

1.7.3 Oral–Brain- Gut Axis

The oral–brain- gut axis provides a mechanistic framework for understanding how SAA influences appetite regulation. The enzymatic breakdown of starches in the oral cavity generates oligosaccharides that interact with taste receptors, particularly the sweet taste receptor (T1R2/T1R3). This interaction triggers neural signals via the vagus nerve to the hypothalamus, a key brain region involved in appetite control (Wang et al.,2024) Furthermore, the products of starch digestion in the gut stimulate the release of incretin hormones, which enhance insulin secretion and suppress appetite (Figure 1.2).

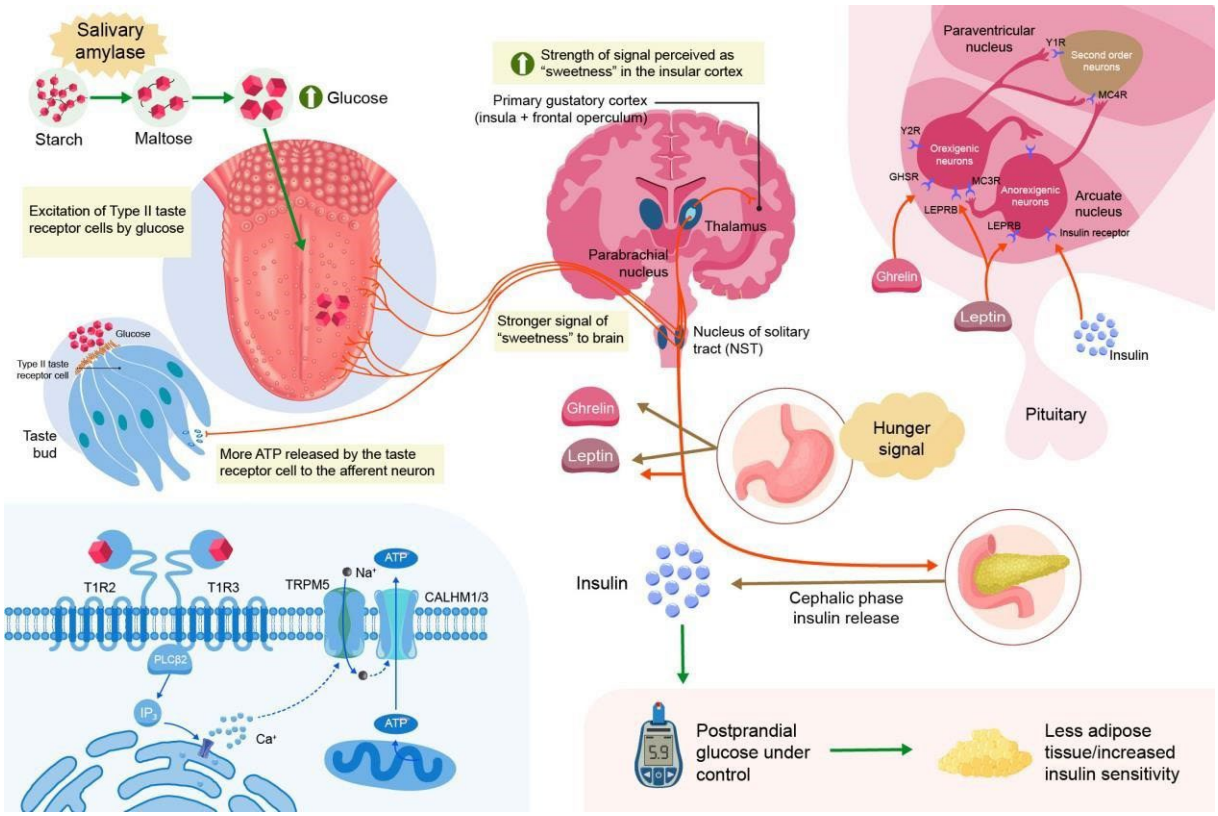

Figure 1.2 **SAA and appetite regulation**

1.7.4 Genetic Variability in SAA and Appetite

Genetic polymorphisms in the AMY1 gene, which encode salivary amylase, result in significant interindividual variability in SAA levels. Individuals with higher AMY1 copy numbers tend to have greater SAA activity, which has been associated with lower body mass index (BMI) and reduced risk of obesity (Falchi et al., 2014). This genetic predisposition may influence dietary preferences and metabolic responses, highlighting the potential for personalised nutrition strategies based on SAA levels.

1.8 Methodological Considerations in Measuring SAA

The assessment of salivary amylase activity (SAA) has garnered significant research interest due to its potential role in metabolic and appetite regulation. However, methodological variability remains a critical challenge in ensuring accurate and reliable measurements. Commonly employed techniques include enzymatic activity assays, protein quantification methods, and gene copy number estimation. While enzymatic assays are widely used for their ability to measure functional activity, they often face limitations in sensitivity and specificity, particularly when saliva samples are not standardised or contain interfering substances (Park et al., 2008). Protein quantification methods, such as immunoassays, provide insights into the concentration of salivary amylase but may not reflect its enzymatic activity, which is crucial for understanding its physiological role. Gene copy number estimation, typically performed using quantitative PCR, offers valuable information on genetic predisposition but does not account for environmental factors influencing SAA levels (Tangesart et al., 2019).

Additionally, variability in saliva collection methods further complicates the measurement of SAA. Factors such as the time of day, fasting state, hydration levels, and the use of stimulated versus unstimulated saliva can significantly influence SAA activity and concentration. For instance, stimulated saliva collection may yield higher volumes but dilute enzyme concentrations, whereas unstimulated saliva may provide more consistent results but lower sample volumes. These inconsistencies highlight the need for standardised protocols to minimise variability and improve the reproducibility of SAA measurements.

Recent advancements, such as mass spectrometry-based proteomics, have shown promise in providing detailed insights into enzyme structure and function, while microfluidic platforms offer potential for high-throughput, cost-effective, and rapid SAA measurement (Park et al., 2023; Tangesart et al., 2019). Additionally, integrating machine learning algorithms with these technologies could further improve data analysis and predictive modelling, paving the way for more precise and personalised applications in metabolic health research (Schlezingerová et al., 2024; Forde et al., 2013).

1.9 Summary and Knowledge Gaps

Cumulative evidence underscores the integrative role of salivary amylase in carbohydrate metabolism, insulin regulation, and adiposity. The associations between SAA, postprandial glucose dynamics, and insulin sensitivity position it as a potentially valuable biomarker for metabolic health. However, key mechanistic questions remain unresolved. Notably, the lack of direct correlation between SAA and the TyG index, despite shared associations with visceral fat, suggests differential or compartmentalised effects on glucose-lipid metabolism.

Importantly, most existing studies focus on general populations, with limited data on high-risk groups such as reproductive-aged women with overweight or obesity, individuals particularly susceptible to insulin resistance and metabolic syndrome and a heightened risk of developing gestational diabetes mellitus (GDM). Our research addresses this gap by investigating SAA as a potential non-invasive marker of metabolic health in this understudied cohort, with implications for early identification and personalised intervention strategies. This population is characterised by an increased susceptibility to insulin resistance, metabolic syndrome, and a heightened risk of developing gestational diabetes mellitus (GDM). The occurrence of GDM is of particular clinical relevance, as it not only compromises maternal metabolic health but also contributes to an adverse intrauterine environment capable of inducing epigenetic modifications that predispose the offspring to metabolic dysregulation.

2 Material and Methods

This PhD project is based on a single, well-characterised cohort of overweight women of reproductive age who participated in a structured 12-week dietary intervention trial. The primary objective of this trial was to investigate metabolic outcomes in response to dietary modifications, specifically in individuals with varying levels of salivary amylase activity, with a particular focus on glucose homeostasis and adipose tissue metabolism. The current Thesis integrates data previously published in the following peer-reviewed studies, each examining different yet complementary dimensions of the research focus:

- "Impact of a 12-Week Dietary Intervention on Adipose Tissue Metabolic Markers in Overweight Women of Reproductive Age",
- "The Link Between Salivary Amylase Activity, Overweight, and Glucose Homeostasis",
- "Decoding Metabolic Connections: The Role of Salivary Amylase Activity in Modulating Visceral Fat and Triglyceride Glucose Index".

The studies were interconnected within a broader research framework, each exploring specific aspects of the investigation while adhering to the same recruitment processes, sampling techniques, and ethical guidelines. The study was approved by the Research Ethics Committee of Rīga Stradiņš University (protocol number 22 − 2/479/2021), and informed written consent was obtained from all participants.

2.1 Study Design and Participants

The participants in this study were healthy volunteers, who were recruited through routine health check-up programs. Inclusion criteria ensured that all participants were free from any diagnosed chronic diseases or conditions that could interfere with the study outcomes. In total, 67 women aged 18–45 years were enrolled, of whom 60 were overweight (BMI 25–29.9 kg/m²) and 7 had normal body weight (BMI 18.5–24.9 kg/m²) and served as the control group (Table 2.1).

Table 2.1

Baseline Characteristics of Study Population

Characteristics	Mean ± SD / Median (IQR)	Range	n (%)
Age (years)	29.18 ± 3.58	18–45	–
Gender – (Female)	67	–	–
BMI (kg/m²)	27.8 ± 2.1	20.1–29.9	–
VF %	15.3 (12.1–18.5)	10.0–25.0	–
SAA (U/mL)	27.77 (10.64–56.24)	10.64–56.24	–

Table 2.1 continued

Characteristics	Mean ± SD / Median (IQR)	Range	n (%)
TyG Index	4.425	4.050–5.110	–
Physical activity	–	–	Sedentary:15 (22) Moderate: 35 (52) Vigorous: 17 (26)
Diet	–	–	Low starch: 30(50) Calorie restriction: 30(50)
Adherence to prescribed diet	85 ± 10	60–100	–

Exclusion criteria included diagnosed diabetes mellitus, endocrine disorders, use of insulin-sensitising or lipid-lowering medications, pregnancy or lactation, recent infections, or antibiotic use within the past 3 months. Participants underwent baseline and post-intervention assessments over a 12-week period during which dietary intake was controlled and monitored.

2.1.1 Justification for a Physician-Directed Decision-Making Observation Design

This study employed a physician-directed decision-making observational design with a cross-sectional analysis component to evaluate the impact of dietary interventions on metabolic markers in overweight women of reproductive age. Group allocation was determined by clinical decision-making, with the treating physician assigning participants to study groups based on individual characteristics and clinical considerations.

By using a physician-guided allocation, the study captured clinically relevant effects of dietary interventions while considering confounding factors such as baseline diet, physical activity, and individual metabolic profiles.

Through this design, the study provides meaningful insights into the role of SAA in metabolic health and the potential effectiveness of personalised nutrition strategies in routine clinical practice.

2.1.2 Sample size Calculation

The study included a total of 60 participants, distributed across four experimental groups with 15 participants each and one control group with 7 participants. The sample size was determined based on the availability of participants recruited through routine health check-up programs.

Statistical power calculations were performed to estimate the ideal sample size for detecting a medium effect size (Cohen's d = 0.5) with a power of 0.8 and an alpha level of 0.05. Using these parameters, the required sample size for a two-tailed test would be approximately 64 participants per group, as calculated using GPower software. However, due

to practical constraints, the study was conducted with a smaller sample size, which is acknowledged as a limitation.

The reduced sample size, particularly in the control group, may have implications for the statistical power of the study. With fewer participants, the ability to detect statistically significant differences is diminished, increasing the likelihood of Type II errors (failing to detect a true effect). This limitation should be considered when interpreting the results, as the findings may be less generalizable and require validation in future studies with larger and more balanced sample sizes. It should be clearly emphasised that this study has a pilot or exploratory nature, and the findings should be interpreted as preliminary, requiring validation in a larger cohort. The potential reduction in statistical power resulting from this correction was also taken into account when interpreting the results. Despite these constraints, the study provides valuable preliminary insights into the relationships between salivary amylase activity, dietary interventions, and metabolic outcomes.

2.2. Study Groups

Participants were classified into two groups based on baseline salivary amylase activity:

1 High Salivary Amylase (HSA)

2 Low Salivary Amylase (LSA)

Each group was further subdivided into two dietary intervention groups:

1 Calorie Restriction (CR): Daily caloric intake reduced by 500 kcal

2 Low-Starch Diet (LS): Daily starch intake limited to < 50 g This resulted in four intervention subgroups:

2.1 HSA-CR: High salary amylase, calorie restriction.

2.2 HSA-LS: High salivary amylase, low-starch diet

2.3 LSA-CR: Low salivary amylase, calorie restriction

2.4 LSA-LS: Low salivary amylase, low-starch diet

Additionally, a control group (CTR) of 7 normal-weight individuals was included, with no dietary intervention.

2.3 Dietary Interventions

Participants were randomly assigned to one of two dietary intervention groups for a duration of 12 weeks:

1 Low-Starch Diet Group (LS): Participants followed a diet emphasising lowglycaemic-index vegetables, proteins, and healthy fats, with daily starch intake limited to less than 50 g.

2 Caloric Restriction Group (CR): Participants reduced their daily caloric intake by 500 kcal from their estimated energy requirement, calculated based on the HarrisBenedict equation (Table 2.2).

Table 2.2

Macronutrient and Micronutrient Composition of Dietary Interventions

Nutrients	Low-Starch Diet (per day)	Calorie-Restricted Diet (per day)
Energy (kcal)	~1800	~1200
Carbohydrates (%)	25–30	40–45
Carbohydrates (g)	110–135	120–150
Starch (g)	< 50	70–90
Sugars (g)	40–50	45–55
Fibre (g)	25–35	20–30
Protein (%)	25–30	20–25
Protein (g)	110–135	70–90
Fat (%)	40–45	30–35
Fat (g)	80–100	40–60
Saturated fat (g)	15–20	10–15
Monounsaturated fat (g)	30–40	15–25
Polyunsaturated fat (g)	15–20	10–15
Omega-3 (g)	1.5–2.5	1.0–2.0
Omega-6 (g)	8–12	6–10
Cholesterol (mg)	< 300	< 200
Sodium (mg)	2000–2500	1500–2000
Potassium (mg)	3500–4000	3000–3500
Calcium (mg)	1000–1200	900–1100
Magnesium (mg)	350–450	300–400
Iron (mg)	12–15	10–12
Zinc (mg)	10–12	8–10
Vitamin C (mg)	75–100	60–80
Vitamin D (IU)	800–1000	600–800
Vitamin B12 (µg)	2.4–3.0	2.0–2.5

Dietary Intake Assessment

Dietary intake was evaluated using a Food Frequency Questionnaire (FFQ), which was administered to all participants. The FFQ collected detailed information on the frequency and quantity of food consumption over a study period. The macronutrient and micronutrient composition of the reported dietary intake was analysed using the Fineli database, a comprehensive nutritional database that provides accurate nutrient profiles for various food items.

Adherence to the diet can be measured by comparing the reported dietary intake with the prescribed dietary intervention (e. g. low-starch or calorie-restricted diet) and assessing how closely participants followed the recommended nutrient composition by endocrinologist.

2.4 Physical Activity Assessment

The physical activity levels of the participants were assessed using the International Physical Activity Questionnaire (IPAQ). The data collected through the questionnaire were expressed as metabolic equivalent task (MET) minutes per week, which is a standardised measure of energy expenditure. MET minutes were calculated based on the frequency, duration, and intensity of physical activities reported by the participants. This information was used to categorise participants into sedentary, moderate, or vigorous physical activity levels. Based on the IPAQ scoring protocol, participants were categorised into the following groups:

- Sedentary: < 600 MET-min/week
- Moderate: 600–3000 MET-min/week
- Vigorous: >3000 MET-min/week.

2.5 Salivary Amylase Activity Assessment

Unstimulated whole saliva samples were collected in the morning between 08:00 and 08:30 following an overnight fast (≥ 10 h) to minimise diurnal and dietary variability. Participants were instructed to avoid eating, drinking (except water), smoking, or oral hygiene activities for at least one hour prior to sampling. Saliva was allowed to accumulate in the mouth and then passively drooled into sterile polypropylene tubes over a 5-minute period. The supernatant was aliquoted and stored at −80 °C until analysis.

Salivary α-amylase activity was determined using the Salimetrics αAmylase Activity Assay Kit (Salimetrics LLC, State College, PA, USA; Catalog No. 1-1902), following the manufacturer's protocol. This kinetic colorimetric assay is based on the cleavage of a chromogenic substrate, 2-chloro-p-nitrophenol linked with maltotriose (CNPG3), by αamylase. The enzymatic activity is proportional to the rate of increase in absorbance at 405 nm, which was measured using a microplate spectrophotometer (e. g. BioTek Synergy HTX, USA). Results were expressed in units per millilitre (U/mL) of saliva. The inclusion of triplicate saliva collections ensured the robustness and reproducibility of the data, minimising potential variability in the measurement process. To account for interindividual variability, participants were stratified into high-salivary-amylase (HSA) and low-salivary-amylase (LSA) groups based on the median split of the measured amylase activity values.

2.6 Visceral Fat Measurement

Visceral adiposity was quantified using multifrequency bioelectrical impedance analysis (BIA) with an Omron BF511 device (Omron Healthcare, Kyoto, Japan). The BIA technique estimates body composition by measuring the resistance and reactance of body tissues to an alternating electrical current of low amplitude. The device integrates impedance data with anthropometric parameters (height, weight, age, and sex) to generate a visceral fat index, which reflects the relative accumulation of adipose tissue in the abdominal cavity.

The Omron BF511 device has been validated against gold-standard methods for body composition analysis, such as dual-energy X-ray absorptiometry (DXA) and magnetic resonance imaging (MRI), in previous studies (Kovács et al., 2025; Sanca et al., 2024). These validations have demonstrated that the device provides reliable and accurate estimates of visceral fat, making it suitable for use in clinical and research settings. While BIA may not achieve the precision of imaging techniques, it offers a practical, non-invasive, and costeffective alternative for large-scale studies, particularly in populations where accessibility to advanced imaging modalities is limited.

All measurements were performed under standardised conditions in the morning, following an overnight fast (minimum 10 hours), with participants barefoot and standing upright while maintaining light contact with both hand and foot electrodes. To minimise measurement variability, participants were instructed to avoid strenuous physical activity, alcohol consumption, and excessive fluid intake for at least 24 hours prior to assessment.

2.7 Biochemical and Hormonal Analyses

Fasting blood samples were collected at three time points: baseline (T0), 30 minutes after consuming starch-containing muesli (T1), and after the 12-week dietary intervention (T2). The following metabolic markers were analysed:

- Active GLP-1: Measured using a GLP-1 (Active) ELISA kit (Millipore, Billerica, MA, USA).
- C-peptide: Assessed using a chemiluminescent immunoassay kit (IMMULITE 2000, Siemens Healthineers, Erlangen, Germany).
- Glucagon: Measured using the Glucagon ELISA Kit (Cat. No. EZGLU-30K,
- Millipore Sigma, Burlington, MA, USA).
- Leptin: Measured using multiplex immunoassay (Luminex, Austin, TX, USA).
- GDF-15: Quantified using ELISA kits (R&D Systems, Minneapolis, MN, USA).
- Triglycerides: Measured using an enzymatic colorimetric method with a commercial kit (Roche Diagnostics, Basel, Switzerland).

Use of Starch-Containing Muesli (T1)

Purpose: A starch-containing muesli was used as a standardised test meal to evaluate the acute metabolic response to dietary starch intake. This test meal enabled the measurement of postprandial changes in metabolic markers, including glucose, active GLP-1, and leptin dynamics.

Composition: The muesli provided 50 grams of starch as the primary carbohydrate source, ensuring a controlled and consistent stimulus for assessing the effects of salivary amylase activity on starch digestion and subsequent metabolic responses.

2.8. Insulin Sensitivity, β-Cell Functional Capacity, and Insulin Resistance Assessment

Insulin sensitivity, β-cell functional Capacity, and insulin resistance were evaluated using the Homeostatic Model Assessment (HOMA), calculated from fasting plasma glucose and peptide concentrations. The indices were derived using the HOMA2 Calculator (version 2.2.3; Diabetes Trials Unit, University of Oxford, UK), which accounts for the nonlinear relationship between glucose and insulin to provide updated estimates of HOMA2-%S (insulin sensitivity), HOMA2-%B (β-cell function), and HOMA2-IR (insulin resistance).

2.8.1 Insulin Resistance Assessment. Triglyceride-glucose (TyG) index

The study utilised the triglyceride-glucose (TyG) index as a marker for insulin resistance instead of the Homeostasis Model Assessment of Insulin Resistance (HOMAIR) due to its established reliability and practicality in assessing insulin resistance in overweight individuals. The TyG index is calculated using fasting triglyceride and glucose levels, which are easily obtainable and less influenced by external factors such as stress or circadian rhythms compared to fasting insulin levels required for HOMA-IR.

Additionally, the TyG index has been validated as a robust surrogate marker for insulin resistance and has shown strong correlations with visceral fat, a key metabolic risk factor. In contrast, HOMA-IR, while widely used, requires fasting insulin/c-peptide measurements, which can be more variable and less accessible in certain settings. The choice of TyG index aligns with the study's focus on visceral adiposity and its role in mediating the relationship between salivary amylase activity (SAA) and metabolic health outcomes.

2.8.2 Triglyceride–Glucose Index Calculation

The triglyceride–glucose (TyG) index was calculated using a glucose–triglyceride calculator, with fasting glucose and triglyceride concentrations entered in mmol/L, corresponding to the measurement units used in the Health Centre 4 (Veselības Centrs 4) laboratory.

This formula is an adaptation of the original calculation based on mg/dl units:

TyG index = ln (fasting triglycerides (mg/dl) × fasting glucose (mg/dl)/2 refer to Guerrero Romero et al.

To ensure consistency with the original method, we utilised direct calculations in mmol/L, thereby avoiding unit conversion.

2.9 Statistical Analysis

All statistical analyses were conducted using GraphPrism 10 and R software (version v4.4.2). Normality of continuous variables was assessed using the Shapiro-Wilk test. Non-parametric tests were employed due to the non-normal distribution of data:

- Descriptive Statistics: Median and interquartile range (IQR) calculated for each variable.
- Group Comparisons: Kruskal–Wallis H test with Dunn's post hoc test for pairwise comparisons.
- Within-Group Comparisons: Wilcoxon signed rank test for changes over time.
- Correlation Analysis: Spearman's rank correlation coefficients to assess relationships between salivary amylase activity and metabolic marker
- Multivariate linear regression models were adjusted for potential confounders, including age, BMI, and dietary intake. A two-tailed p-value < 0.05 was considered statistically significant.

2.9.1 Impact of a 12-Week Dietary Intervention on Adipose Tissue Metabolic Markers in Overweight Women of Reproductive Age

Statistical analyses were conducted using non-parametric methods, which were appropriate due to the relatively small sample size and the non-normal distribution of several variables, as confirmed by preliminary data exploration.

- Within-Group Comparisons: The Wilcoxon signed-rank test was employed to evaluate changes within groups over time. This test is robust for paired data and does not require the assumption of normality, making it suitable for assessing the effects of dietary interventions on metabolic markers within each group.
- Between-Group Comparisons: The Kruskal–Wallis H test was used to compare differences across multiple independent groups. This test is suitable for nonnormally distributed data and allows for the comparison of dietary intervention effects between groups classified by salivary amylase activity and dietary strategy.
- Post Hoc Analyses: Where significant differences were identified in the Kruskal–Wallis H test, Dunn's test with Bonferroni correction was applied to

control for Type I errors associated with multiple comparisons. This ensured the reliability and validity of the results.

- Correlation Analysis: Spearman's rank correlation coefficients were calculated to assess the relationships between salivary amylase activity and relevant metabolic markers. This method was chosen for its ability to evaluate monotonic relationships without requiring normality in the data.
- A p-value < 0.05 was considered statistically significant for all analyses. These statistical methods were selected to ensure robust and reliable evaluation of the effects of dietary interventions on adipose tissue metabolic markers and their associations with salivary amylase activity.

2.9.2 The Link between Salivary Amylase Activity, Overweight, and Glucose Homeostasis

- Normality Assessment: The Shapiro–Wilk test was used to assess the normality of continuous variables, including salivary amylase activity and butyrate concentration. Significant deviations from normality were observed for these variables, necessitating the use of non-parametric statistical methods.
- Correlation Analysis: Spearman's rank correlation coefficient (ρ) was employed to evaluate the association between salivary amylase activity and butyrate levels. This method was chosen due to its suitability for non-normally distributed data and its ability to assess monotonic relationships.
- Data Presentation: Continuous variables were summarised as medians with interquartile ranges (IQR) to account for non-normal distributions.
- Group Comparisons: Differences in baseline salivary amylase activity and butyrate concentrations between groups were analysed using the Mann–Whitney U test, a nonparametric test appropriate for comparing two independent groups.
- Regression Analysis: To investigate the relationship between salivary amylase activity and insulin sensitivity, linear regression analysis was performed with HOMA-2%S (Homeostatic Model Assessment of Insulin Sensitivity) as the dependent variable. This approach allowed for the evaluation of predictive relationships while accounting for continuous data characteristics.
- Multiple Comparisons: For group comparisons involving more than two groups, the Kruskal–Wallis H test was applied. Post hoc pairwise comparisons were conducted using Dunn's test with Bonferroni correction to control for Type I errors.
- Significance Threshold: A p-value < 0.05 was considered statistically significant for all analyses.

2.9.3 Decoding Metabolic Connections: The Role of Salivary Amylase Activity in Modulating Visceral Fat and Triglyceride Glucose Index

To examine the associations between salivary amylase activity (SAA), visceral fat percentage (VF %), and the triglyceride-glucose (TyG) index, multivariable linear regression models were employed. These models were adjusted for potential confounders, including age, body mass index (BMI), physical activity, and dietary intake.

Regression Model Specification

The regression model was defined as:

$Y = \beta_0 + \beta_1 X + \beta_2 C + \varepsilon$

- **Y** represents the dependent variable (VF % or TyG index).
- **X** is the independent variable (SAA).
- **C** represents covariates (age, BMI, physical activity, and dietary intake).
- **ε** is the error term.

The strength of association was reported using standardised regression coefficients (β), 95 % confidence intervals (CI), and p-values. Effect sizes were interpreted according to Cohen's guidelines:

- **β ≥ 0.1**: Small effect.
- **β ≥ 0.3**: Moderate effect.
- **β ≥ 0.5**: Large effect.

Mediation Analysis

To assess whether VF % mediates the relationship between SAA and the TyG index, structural equation modelling (SEM) was conducted using the lavaan package in R. The mediation model estimated the following effects:

- **Total Effect (c)**: The overall relationship between SAA and the TyG index.
- **Direct Effect (c′)**: The direct association between SAA and the TyG index, independent of VF %.
- **Indirect Effect (a × b)**: The mediated effect of SAA on the TyG index through VF %.
- Bootstrapping with 5,000 resamples was used to derive bias-corrected 95 % confidence intervals (BCa 95 % CI). A mediation effect was considered statistically significant if the 95 % CI of the indirect effect did not include zero.

The proportion of mediation was calculated as:

Proportion of Mediation = (a × b) / c

- **a** represents the effect of SAA on VF %.
- **b** represents the effect of VF % on the TyG index.
- **c** represents the total effect of SAA on the TyG index

2.10 Visualisation of Study Design

This study was an observational study with a cross-sectional analysis component, designed to evaluate the impact of dietary interventions on metabolic markers in overweight women of reproductive age. Group allocation was based on clinical decision-making, with the treating physician determining the assignment of participants to study groups.

The study design is summarised in Figure 2.1.

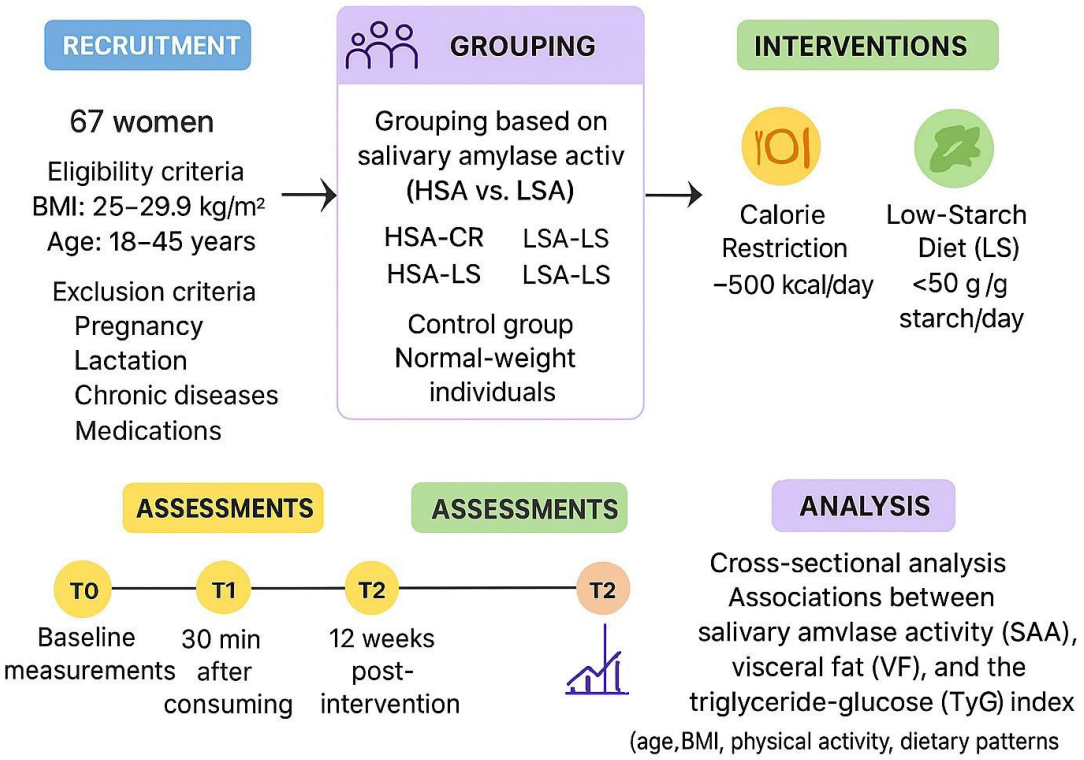

Figure 2.1 **Flowchart of Study Design**

Participants (n = 67; age 18–45 years), including 60 overweight women (BMI 25–29.9 kg/m²) and 7 normal-weight women (BMI 18.5–24.9 kg/m²), were classified according to salivary amylase activity into high (HSA) and low (LSA) SAA groups. Each SAA group was further subdivided into a calorie restriction (CR; −500 kcal/day based on the Harris– Benedict equation) or a low-starch (LS; < 50 g starch/day) dietary intervention subgroup. The seven normal-weight participants served as the control group (CTR). Assessments were performed at three time points: T0 (baseline), T1 (30 min after starch-containing meal), and T2 (12 weeks post-intervention). The cross-sectional analysis examined associations between SAA, visceral fat, and the TyG index at T2, adjusted for age, BMI, physical activity, and diet.

3 Discussion

3.1 Overview of Key Findings

This study provides novel insights into the role of salivary amylase activity (SAA) as a potential biomarker for metabolic health in overweight women of reproductive age. The findings revealed significant associations between SAA, visceral fat percentage (VF %), and glucose homeostasis, highlighting the importance of SAA in modulating metabolic outcomes. Specifically, higher SAA was associated with lower VF %, suggesting a protective role against visceral fat accumulation. Furthermore, mediation analysis demonstrated that VF % partially mediates the relationship between SAA and the triglyceride-glucose (TyG) index, accounting for 45 % of the total effect.

The study also showed that tailored dietary interventions based on SAA levels, calorie restriction (CR) for high SAA individuals, and low-starch (LS) diets for low SAA individuals resulted in significant improvements in metabolic markers. These findings underscore the importance of personalised nutrition strategies in addressing metabolic health challenges, particularly in populations at risk for obesity-related disorders.

3.1.1 Impact on Glucose Homeostasis

The study highlights the differential effects of dietary interventions on glucose homeostasis based on salivary amylase activity:

- **High Salivary Amylase Activity (HSA):** Participants with HSA showed superior improvements in insulin sensitivity when following calorie-restricted diets. This suggests that individuals with higher enzymatic activity may benefit more from interventions targeting overall caloric intake reduction.
- **Low Salivary Amylase Activity (LSA):** Participants with LSA exhibited better glycaemic control on low-starch diets. This is likely due to reduced post-prandial glucose spikes and hyperinsulinemia, which collectively contribute to improved glucose regulation

These findings pave the way for future research to further explore the mechanistic pathways underlying these associations and to validate the use of SAA as a biomarker in diverse populations. While the study reported associations between salivary amylase activity, overweight, and glucose homeostasis, the evidence does not establish direct causality.

3.1.2 Assessment of Post-Prandial Metabolic Biomarker Modulation Induced by Starch-Containing Muesli

The study provided detailed insights into the acute changes in active GLP-1 and leptin levels following the consumption of starch-containing muesli (T1) in individuals with high salivary amylase activity (HSA) and low salivary amylase activity (LSA). These changes highlight the role of enzymatic variability in modulating post-prandial metabolic responses. The acute changes in active GLP-1 and leptin levels from T0 to T1 demonstrate the influence of salivary amylase activity on post-prandial metabolic responses. Elevated salivary amylase activity facilitates rapid starch degradation and glucose absorption, potentially increasing GLP1 release and enhancing metabolic outcomes. HSA individuals exhibited quicker but less sustained increases in these markers, while LSA individuals showed slower but more prolonged elevations, potentially enhancing satiety and glycaemic control. These findings underscore the importance of tailoring dietary interventions based on enzymatic activity to optimise metabolic outcomes

3.2 Role of Salivary Amylase Activity in Metabolic Health

Salivary amylase activity (SAA) has emerged as a potential upstream modulator of metabolic health, influencing key processes such as visceral fat distribution and glucose-insulin homeostasis. This study demonstrated significant associations between SAA and visceral fat percentage (VF %), with higher SAA linked to lower VF %. This finding aligns with the hypothesis that individuals with elevated SAA activity may exhibit enhanced carbohydrate metabolism efficiency, which reduces postprandial glycaemic spikes and insulin demand, thereby mitigating lipogenesis and visceral fat accumulation.

The mediating role of VF % in the relationship between SAA and the triglyceride-glucose (TyG) index further underscores the metabolic significance of SAA. Mediation analysis revealed that VF % accounted for 45 % of the total effect of SAA on the TyG index, highlighting its critical role in linking digestive enzyme activity to markers of insulin resistance. This finding suggests that SAA may indirectly influence glucose-insulin homeostasis by modulating visceral fat distribution, a key determinant of metabolic health.

The study also explored the differential impact of dietary interventions tailored to SAA levels. Participants with high SAA activity responded more favourably to calorie-restricted diets, which were associated with reductions in VF % and improvements in insulin sensitivity. Conversely, participants with low SAA activity benefited more from low-starch diets, which improved glycaemic control and increased GLP-1 levels. These results emphasise the importance of personalised nutrition strategies that account for individual enzymatic

profiles, as SAA appears to play a pivotal role in determining the metabolic response to dietary composition.

Beyond its direct metabolic effects, SAA may also influence gut microbiota composition and short-chain fatty acid production, such as butyrate, which are known to impact glucose metabolism and insulin sensitivity. Although this study did not include direct microbiome profiling, the study highlights that salivary amylase activity influences butyrate production, with low-starch diets significantly increasing butyrate levels in women with low salivary amylase activity, thereby enhancing insulin sensitivity and supporting metabolic health, future research should investigate the interplay between salivary amylase activity, gut microbiota, and metabolic health.

3.3 SAA and Visceral Adiposity

The inverse association between salivary α-amylase activity (SAA) and visceral adiposity observed in our study aligns with previous evidence suggesting that individuals with higher SAA exhibit more favourable body fat distribution profiles (Al-Akl et al., 2022; Bonnefond et al., 2017). SAA plays a critical role in the initiation of starch digestion, hydrolysing α-1,4-glycosidic linkages in dietary polysaccharides to release maltose and maltotriose, thereby facilitating rapid glucose availability during the early postprandial phase (Mandel et al., 2010; Butterworth et al., 2011; Komeine et al., 2013).

Higher SAA levels have been associated with more efficient carbohydrate digestion, improved early-phase glycaemic control, and a more rapid onset of postprandial satiety, which may collectively contribute to reduced total energy intake and lower risk of excessive adiposity (Foster-Powell et al., 2002; Mandel & Breslin, 2012). From a mechanistic perspective, efficient pre-digestive starch hydrolysis may attenuate postprandial glycaemic excursions and insulin demand, thereby reducing chronic hyperinsulinemia, a major driver of de novo lipogenesis and visceral fat deposition (Jenkins et al., 1981; Elder, et al., 2018).

Chronic hyperinsulinemia activates the PI3K–Akt–mTORC1–SREBP-1c signalling axis, enhancing the transcription of lipogenic enzymes such as acetyl-CoA carboxylase and fatty acid synthase, ultimately promoting triglyceride synthesis and storage in visceral adipocytes (Porstmann et al., 2008; Kwon et al., 2020; Nakagawa et al., 2025).

3.4 Visceral Fat and TyG Index

The observed strong positive correlation between visceral adiposity and the triglyceride–glucose (TyG) index in our cohort aligns with previous findings, which identify central fat accumulation as a key determinant of insulin resistance and metabolic syndrome (Simental-Mendía et al., 2008; Guerrero-Romero et al., 2010).Visceral adipose

tissue (VAT) is highly metabolically active and functions as an endocrine organ, secreting free fatty acids (FFAs), proinflammatory cytokines, and adipokines. These secretions collectively impair insulin signalling and disrupt glucose homeostasis (Wajchenberg, 2000). The TyG index, which primarily reflects hepatic insulin resistance, has been validated as a reliable surrogate marker for assessing insulin resistance (Guerrero-Romero et al., 2010).

Our findings propose a mechanistic framework in which salivary amylase activity (SAA) impacts metabolic health indirectly, not by directly regulating glucose or triglyceride metabolism, but by influencing fat distribution–specifically promoting the accumulation of visceral adipose tissue. The expansion of VAT contributes to insulin resistance through several interconnected molecular mechanisms.

Firstly, increased FFA flux from visceral fat depots to the liver elevates intracellular diacylglycerol (DAG) concentrations, which activates protein kinase C epsilon (PKCε). This activation inhibits insulin receptor substrate (IRS)-dependent signalling pathways, thereby impairing insulin action (Shulman et al., 2000; Janssen et al., 2025).Secondly, the remodelling of visceral adipose tissue is associated with macrophage polarisation toward a proinflammatory M1 phenotype. This process leads to elevated secretion of proinflammatory mediators such as tumor necrosis factor-α (TNF-α), interleukin-6 (IL-6), and monocyte chemoattractant protein1 (MCP-1).These factors activate c-Jun N-terminal kinase (JNK) and IκB kinase β (IKKβ) signalling pathways, further exacerbating insulin resistance (Hotamisligil et al., 2006; Lumeng et al., 2007; Guria et al., 2023).Thirdly, altered secretion of adipokines, including reduced levels of adiponectin and increased levels of resisting and retinol-binding protein 4 (RBP4), further impairs systemic insulin sensitivity and contributes to metabolic inflexibility (Kadowaki et al., 2006; Rabiee et al., 2025).

These mechanisms collectively outline a plausible biological pathway through which elevated SAA may exert protective effects on metabolic health. By enhancing oral starch hydrolysis and reducing postprandial glucose excursions, higher SAA levels may mitigate compensatory hyperinsulinemia, thereby limiting hepatic lipogenesis and the subsequent accumulation of metabolically harmful visceral fat. Supporting this integrative model, our mediation analysis revealed that approximately 45 % of the relationship between SAA and the TyG index is mediated through visceral fat volume. This finding highlights a potential indirect pathway that connects oral carbohydrate metabolism to systemic insulin sensitivity. It suggests that individual variability in SAA may represent broader adaptive mechanisms linking dietary starch processing to adipose tissue distribution and metabolic risk.

3.5 Lack of Direct Association Between SAA and TyG

While previous studies have proposed a direct link between SAA and glucose regulation (Perry et al., 2016; Mandel & Breslin, 2012), the present Thesis provides evidence supporting an indirect association. Although visceral fat demonstrated a moderate positive correlation with the TyG index ($r = 0.36$, $p < 0.01$), SAA did not correlate directly with TyG ($r = -0.12$, $p = 0.19$). This dissociation was further clarified through mediation analysis, which revealed that the effect of SAA on TyG was mediated by visceral adiposity (indirect effect $\beta = -0.09$, 95 % CI: -0.16 to -0.03, $p = 0.004$).

This observation suggests that the link between SAA and insulin resistance is more nuanced and may be mediated by intermediate variables such as visceral adiposity. This lack of a direct association contrasts with some studies reporting that higher SAA is predictive of better glycaemic control and lower insulin resistance.

3.6 Targeting Visceral Adiposity: Implications for Prevention

Specifically, the finding that 45 % of the total effect of SAA on the TyG index is mediated by VF highlights the biological plausibility of SAA acting as an upstream determinant of adiposity-driven metabolic dysfunction.

From a physiological standpoint, VF represents a unique metabolic compartment with distinct endocrine and inflammatory properties, contributing disproportionately to the development of IR and cardiometabolic disease (Gugliucci et al., 2022; Finelli et al 2013; Adeva-Andany et al 2024). Adipocytes in the visceral depot exhibit enhanced sensitivity to lipolytic stimuli and reduced responsiveness to insulin-mediated suppression of lipolysis, resulting in greater free fatty acid flux into the portal circulation and promotion of hepatic insulin resistance (Lundgren et al .2008; Zhao et al 2020). Adipocytes secrete pro-inflammatory adipokines, while macrophages infiltrating hypertrophic adipose tissue produce elevated levels of cytokines such as interleukin-6 (IL-6) and tumor necrosis factor-alpha (TNF-α). These cytokines contribute to systemic inflammation and disrupt insulin signalling by interfering with insulin receptor substrate phosphorylation and downstream signalling cascades (Ali et al., 2020; Simons et al 2007; Weisberg et al., 2003). Consequently, interventions aimed at reducing VF mass hold significant potential for improving insulin sensitivity and mitigating metabolic risk.

The inverse association between SAA and VF suggests that individuals with higher enzymatic capacity for starch hydrolysis may experience a more favourable metabolic profile, potentially due to reduced postprandial insulin responses. (Choi et al., 2015). Therefore, SAA may exert indirect regulatory effects on adipose tissue distribution through its influence on

early-phase carbohydrate digestion and subsequent hormonal responses, particularly insulin dynamics.

These findings carry important implications for metabolic health strategies, particularly in the context of personalised nutrition and risk stratification. If SAA activity modulates visceral fat (VF) deposition via postprandial metabolic pathways, then enhancing SAA activity, either through dietary modulation or by targeting individuals with favourable AMY1 gene copy number profiles, could serve as a preventive strategy to reduce VF accumulation. Thus, SAA may represent not only a biomarker of metabolic risk but also a modifiable factor in interventions designed to prevent or reverse visceral adiposity and its associated complications.

3.7 Clinical and Translational Implications

The identification of non-invasive biomarkers for early detection of metabolic dysregulation is of great clinical importance, particularly in young women of reproductive age, in whom prevention of future metabolic disease is critical. Given that SAA can be easily measured in saliva and is responsive to both genetic and environmental factors, it may serve as a valuable tool for risk stratification, lifestyle intervention targeting, and personalised nutrition strategies (Perry et al., 2016; Carpenter et al., 2013). Additionally, since SAA is partially regulated by adrenergic activity, it may also serve as a surrogate marker of sympathetic nervous system (SNS) function. However, further longitudinal studies are required to establish causality and assess whether modulating SAA through dietary or behavioural means can influence metabolic trajectories.

3.8 Salivary Amylase Activity and Butyrate Production

The study demonstrated a weak but statistically significant correlation ($\rho = 0.0486$, $p < 0.05$) between salivary amylase activity and butyrate levels, suggesting that individuals with higher salivary amylase activity may exhibit enhanced starch digestion, leading to increased butyrate production. This aligns with previous research indicating that salivary amylase activity influences the availability of substrates for microbial fermentation in the gut, favouring the proliferation of butyrate-producing bacteria such as *Faecalibacterium prausnitzii* and *Roseburia spp.* (Flint et al., 2012; Khalil et al., 2024). Notably, women with high salivary amylase activity had significantly higher baseline butyrate levels compared to those with low activity (Mann–Whitney $U = 44$, $p < 0.05$). These findings suggest that salivary amylase activity plays a critical role in shaping gut microbiota composition and metabolic outcomes.

3.9 Rationale for Using Both HOMA2-IR and TyG Index

Insulin resistance is a multifaceted condition influenced by various metabolic pathways, including glucose regulation, lipid metabolism, and visceral adiposity. In this study, both the Homeostasis Model Assessment of Insulin Resistance (HOMA2-IR) and the triglycerideglucose (TyG) index were utilised to provide a comprehensive evaluation of insulin resistance and its relationship with salivary amylase activity (SAA) and dietary interventions. The inclusion of both markers was driven by their complementary strengths and their ability to capture distinct aspects of insulin resistance.

3.9.1 Complementary Roles of TyG Index and HOMA2-IR

The TyG index and HOMA2-IR represent two different approaches to assessing insulin resistance:

TyG Index: The TyG index, calculated using fasting triglyceride and glucose levels, is a reliable surrogate marker for insulin resistance, particularly in the context of lipid metabolism and visceral adiposity. It has been shown to correlate strongly with visceral fat, which is a central focus of this study. The TyG index is less influenced by short-term fluctuations in insulin levels, making it a stable and practical marker for large-scale studies.

HOMA2-IR: HOMA2-IR, derived from fasting glucose and insulin/ c-peptide levels, provides a direct measure of pancreatic beta-cell function and hepatic insulin sensitivity. It offers insights into the dynamics of insulin secretion and glucose regulation, which are critical for understanding the broader metabolic implications of SAA.

By incorporating both markers, the study was able to capture a more holistic view of insulin resistance, addressing both lipid-related and insulin-related pathways (Table 3.1).

Table 3.1

Comparison of Common Insulin Resistance Indices and Their Relevance to Salivary Amylase Physiology

Index	Measurement Type	Physiological Target	Data Required	Capture Postprandial Dynamics?	Relevance to SAA Physiology	Limitations	References
HOMA-IR	Static	Hepatic insulin resistance	Fasting glucose & insulin	✗	Low. Not reflecting postprandial starch digestion, CPIR, or incretin action	Overlooks peripheral IR and dynamic insulin responses	Muniyappa et al., 2008; Moriyama et al., 2025; Bonora et al., 2000
HOMA2-IR	Static	Peripheral and hepatic insulin resistance	Fasting glucose & fasting insulin or C-peptide levels	✗	Low. Not reflecting postprandial starch digestion, CPIR (cephalic phase insulin release), or incretin action	Overlooks dynamic insulin responses and postprandial glucose metabolism, and does not account for the role of salivary amylase activity in starch digestion and subsequent glucose regulation	Delanghe et al., 2023
TyG Index	Static	Hepatic insulin–lipid interaction	Fasting glucose & triglycerides	✗	Low–moderate. May indirectly reflect insulin resistance but not specific to SAA-linked mechanisms	Non-specific insulin signalling; influenced by lipid metabolism	Simental-Mendía et al., 2008; Guerrero-Romero et al., 2010
Matsuda Index (OGTT-based)	Dynamic	Whole-body IR (hepatic + peripheral)	Glucose & insulin at 0, 30, 60, 90, 120 min	✓	High. Reflects postprandial insulin action and is sensitive to SAA effects on early glucose and insulin kinetics	Requires OGTT; more labour-intensive	Matsuda and De Fronzo, 1999
Stumvoll Index	Dynamic	Peripheral insulin resistance	OGTT data	✓	High. Captures insulin sensitivity during glucose challenge, aligned with early starch hydrolysis and incretin response	Assumptions may vary by population	Stumvoll et al., 2000

Table 3.1 continued

Index	Measurement Type	Physiological Target	Data Required	Capture Postprandial Dynamics?	Relevance to SAA Physiology	Limitations	References
Gutt Index (Insulin sensitivity index (ISI)	Dynamic	Whole-body insulin resistance	OGTT data	✓	High. Sensitive to changes in post-load glucose handling, relevant for assessing SAA's influence on glucose disposal	Less widely used; requires accurate time-point sampling	Gutt et al., 2000
Hyperinsulinemic–euglycaemic clamp	Gold standard	Peripheral insulin resistance	Continuous insulin & glucose infusion	✓	Very High. Ideal for quantifying IR; could confirm SAA-related differences	Invasive, expensive, not feasible in large cohorts	De Fronzo et al., 1979

3.9.2 Validation and Robustness of Findings

The use of both markers allowed for cross-validation of the study's findings. Consistent trends observed across TyG index and HOMA2-IR strengthen the reliability of the results, while any discrepancies between the two markers provide valuable insights into the differential mechanisms of insulin resistance. For example, the TyG index may better reflect the lipid related effects of SAA on visceral fat and triglyceride metabolism, while HOMA2-IR may capture the insulin secretion and hepatic glucose regulation pathways influenced by SAA.

The dual use of TyG index and HOMA2-IR enabled the study to explore the complex interactions between SAA, visceral adiposity, and glucose homeostasis. The findings suggest that SAA may influence glucose regulation through indirect mechanisms, such as gut-brain signalling, insulin secretion dynamics, or differential postprandial glycaemic responses. While the TyG index provided insights into the lipid-related pathways, HOMA2-IR offered additional information on the role of SAA insulin secretion and hepatic glucose regulation.

The inclusion of HOMA2-IR ensures that the results are aligned with previous studies on insulin resistance, while the TyG index provides a novel perspective on the role of lipid metabolism in the context of SAA and visceral adiposity.

While the inclusion of both markers enhances the robustness of the study, certain limitations must be acknowledged:

The inclusion of both HOMA2-IR and TyG indexes in this study was essential for providing a comprehensive assessment of insulin resistance and exploring the metabolic role of salivary amylase activity. By leveraging the complementary strengths of these markers, the study was able to capture both lipid-related and insulin-related pathways, offering deeper insights into the complex mechanisms underlying glucose homeostasis and metabolic health. Future research should continue to explore the interplay between these markers to refine our understanding of insulin resistance and its relationship with SAA and dietary interventions.

3.10 Hormonal Influence and Study Population

The focus on women of reproductive age adds a unique dimension to the study, as hormonal fluctuations during the menstrual cycle can influence gut microbiota composition and butyrate production. Oestrogen, which is elevated during the follicular phase, has been shown to promote the growth of beneficial gut bacteria associated with butyrate production. Conversely, progesterone, which dominates the luteal phase, may attenuate these effects. While the study controlled for hormonal variability by measuring butyrate levels during the follicular phase, future research should explore the dynamic interactions between hormonal fluctuations, gut microbiota, and metabolic outcomes.

3.11 Personalised Nutrition Based on Salivary Amylase Activity (SAA)

This study highlights the potential of salivary amylase activity (SAA) as a biomarker for personalised nutrition strategies aimed at optimising metabolic health. Variability in SAA levels among individuals has been shown to influence dietary responses, particularly in relation to visceral fat accumulation, insulin sensitivity, and glucose homeostasis.

The findings from this study demonstrated that tailored dietary interventions based on SAA levels can significantly improve metabolic markers. Participants with high SAA activity (HSA) responded more favourably to calorie-restricted diets, which were associated with reductions in visceral fat percentage and enhanced insulin sensitivity. Conversely, participants with low SAA activity (LSA) benefited more from low-starch diets, which improved glycaemic control and increased GLP-1 levels, a key incretin hormone involved in glucose regulation.

Mechanistically, SAA influences the rate of starch digestion and glucose absorption, which in turn affects insulin dynamics and adipose tissue metabolism. Elevated SAA activity facilitates rapid starch hydrolysis, leading to improved glycaemic control and reduced insulin demand, which may attenuate lipogenesis and visceral fat accumulation. On the other hand, reduced SAA activity results in slower starch digestion, altered glycaemic responses, and potentially diminished GLP-1 secretion, which may impact satiety and glucose regulation.

The integration of SAA as a biomarker in personalised nutrition strategies offers several advantages. First, it provides a non-invasive and cost-effective method for assessing individual metabolic profiles. Second, it enables the development of tailored dietary recommendations that optimise metabolic outcomes based on enzymatic activity. For example, individuals with high SAA activity may benefit from calorie-restricted diets to reduce visceral fat, while those with low SAA activity may achieve better glycaemic control through low-starch diets.

These findings emphasise the need for further research to validate the use of SAA as a biomarker in diverse populations and to explore its long-term implications for metabolic health. Future studies should investigate the molecular mechanisms underlying the relationship between SAA, gut microbiota composition, and metabolic outcomes, as well as the potential for dietary interventions to modulate SAA activity.

Personalised nutrition strategies based on SAA represent a promising approach to improving metabolic health and preventing obesity-related disorders. By leveraging salivary diagnostics and tailored dietary interventions, this research contributes to the growing field of precision nutrition and highlights the importance of integrating individual enzymatic profiles into dietary planning.

3.12 Strengths and Limitations

This study has several notable strengths that contribute to its scientific rigor and the relevance of its findings

1. **Innovative Focus on Salivary Amylase Activity:** The study provides a novel perspective by investigating salivary amylase activity (SAA) as a potential upstream modulator of metabolic health. While previous research has primarily focused on SAA in the context of digestive physiology, this study extends its relevance to chronic metabolic adaptations, highlighting its association with visceral fat distribution and glucose-insulin homeostasis.
2. **Tailored Dietary Interventions:** The study design incorporated personalised dietary strategies based on SAA levels, such as low-starch diets for individuals with low SAA and calorie-restricted diets for those with high SAA. This approach allowed for the exploration of individualised nutrition interventions and their impact on metabolic health.
3. **Robust Statistical Analysis:** The study employed appropriate statistical methods, including non-parametric tests, multivariable linear regression, and structural equation modelling (SEM) for mediation analysis. Adjustments for potential confounders such as age, BMI, physical activity, and dietary intake enhanced the reliability of the findings. The use of bootstrapping for mediation analysis further strengthened the robustness of the results.
4. **Focus on Visceral Fat as a Mediator:** The study highlighted the mediating role of visceral fat percentage in the relationship between SAA and the TyG index, providing valuable insights into the mechanisms underlying metabolic health. This approach advances the understanding of how visceral fat distribution contributes to glucose-insulin homeostasis.
5. **Non-Invasive Biomarker Assessment:** The use of salivary diagnostics to measure SAA offers a cost-effective, non-invasive method for assessing metabolic health. This approach has the potential to be applied in large-scale population studies and clinical settings for early identification of individuals at risk for metabolic disorders.
6. **Comprehensive Dietary Monitoring:** Despite the limitations of self-reported food diaries, the study implemented weekly dietary monitoring, which allowed for detailed tracking of dietary adherence and provided valuable data on the impact of dietary interventions.
7. **Focus on Women of Reproductive Age**: By targeting overweight women of reproductive age, the study addresses a population that is particularly vulnerable to

metabolic disorders, such as insulin resistance and visceral obesity. This focus contributes to the understanding of metabolic health in a critical demographic group.

The study is limited by:

1 **Population Specificity**: The exclusive focus on overweight women of reproductive age limits the applicability of the results to other populations, such as men, postmenopausal women, or individuals with different health profiles. This narrow demographic scope may not capture the full spectrum of metabolic responses across diverse populations.

2 **Sample size and Statistical Power**: The relatively small sample size, particularly in the control group, may have reduced the statistical power of the study and increased.

3 **Bioimpedance Analysis**: Visceral fat percentage was measured using the Omron BF511 bioimpedance scale, which relies on proprietary algorithms. While bioimpedance analysis is a widely used and non-invasive method, it is less precise compared to gold-standard techniques such as dual-energy X-ray absorptiometry (DXA) or magnetic resonance imaging (MRI). This limitation may have introduced variability in the measurement of visceral fat, potentially affecting the accuracy of the results.

4 **Causality**: Although the study was designed as an observational Doctor Decision-Making trial, in which diet allocation was guided by clinical parameters, the findings provide insights into associations but do not establish direct causality between salivary amylase activity, visceral fat, and glucose homeostasis. While associations were observed, additional long-term studies and mechanistic investigations are needed to confirm and further elucidate these causal pathways.

5 **Microbiome Analysis**: The lack of direct microbiome profiling limits the ability to fully elucidate the relationship between salivary amylase activity, gut microbiota composition, and butyrate production. This gap restricts the mechanistic understanding of how dietary interventions may influence metabolic outcomes through microbiota-mediated pathways.

Potential Biases

- **Selection Bias**: The inclusion criteria focused on overweight women without chronic diseases or medications affecting metabolic outcomes, which may limit the applicability of findings to individuals with more complex health profiles.

- **Reporting Bias**: Dietary adherence was monitored through self-reported weekly food diaries, which are prone to inaccuracies, underreporting, or recall bias.

Future studies should aim to address these limitations by incorporating larger, more diverse populations, direct microbiome profiling, and more objective methods of dietary adherence monitoring, such as digital tracking tools or biomarkers of dietary intake. By mitigating these biases, the robustness and generalizability of the findings can be improved.

3.13 Important Parameters That Did Not Change and Their Implications

In this study, total cholesterol, body weight, and growth differentiation factor-15 (GDF15) remained unchanged following dietary intervention. Understanding the physiological and methodological factors underlying this stability is important for the interpretation of metabolic outcomes.

3.13.1 Total Cholesterol, LDL-C, and HDL-C

The stability of cholesterol parameters across intervention groups suggests that short-term modulation of carbohydrate intake has limited influence on plasma cholesterol homeostasis. Cholesterol levels are predominantly regulated by long-term dietary patterns, hepatic synthesis, and genetic determinants, making them less responsive to transient nutritional changes (Lecerf JM et al., 2011).

As the intervention primarily targeted carbohydrate restriction rather than fat composition, hepatic regulatory mechanisms, including cholesterol synthesis and LDL receptor activity, likely maintained circulating total cholesterol, LDL-C, and HDL-C within a stable range. This observation is consistent with the finding that the intervention primarily influenced lipid metabolism by reducing triglycerides, as evidenced by alterations in the TyG index, rather than significantly impacting total cholesterol fractions.

Furthermore, the relatively short intervention duration may have been insufficient for measurable alterations in cholesterol metabolism, which typically requires prolonged dietary exposure. Effective lipid modulation generally depends on the inclusion of cholesterol-lowering nutrients, such as soluble fibre, plant sterols, omega-3 fatty acids, and monounsaturated fats, which were not a focus of the current dietary design. The absence of these components likely contributed to the unaltered lipid profile despite improvements in other metabolic markers.

3.13.2 Body Weight

Reductions in visceral adiposity can occur independently of total weight loss, particularly when interventions enhance metabolic flexibility, insulin sensitivity, or mitochondrial efficiency (Holloszy JO et al., 2011). Redistribution of adipose tissue or modest increases in lean mass may offset reductions in fat mass, resulting in stable total body weight. The moderate caloric restriction and relatively short duration likely further limited measurable changes. These findings underscore the limitations of body weight as an isolated marker of metabolic improvement and highlight the importance of body composition assessment, including visceral and subcutaneous fat indices.

3.13.3 Growth Differentiation Factor-15 (GDF-15)

GDF-15 is a stress-responsive cytokine associated with mitochondrial homeostasis and inflammatory regulation. Elevated concentrations are typically observed in states of oxidative stress, mitochondrial dysfunction, or systemic inflammation. The absence of significant change may reflect the participants relatively preserved metabolic health and the moderate intensity of the intervention, which likely did not elicit a sufficient mitochondrial or inflammatory response to alter GDF-15 expression (Tsai VWW et al., 2018). These results suggest that GDF-15 is more indicative of chronic metabolic stress than short-term dietary modulation.

In this study, several metabolic and biochemical parameters, including total cholesterol, body weight, and growth differentiation factor-15 (GDF-15), did not demonstrate significant alterations following dietary interventions. Understanding the biological and methodological factors underlying this stability is essential for accurate interpretation of the results and for refining future experimental designs. The following sections discuss potential explanations for the lack of change and their broader implications.

3.14 Future Directions

To deepen understanding of salivary amylase activity (SAA) as a modulator of metabolic health, future research should employ integrative approaches combining genetic, physiological, and molecular analyses across diverse populations.

3.14.1 Postprandial Glucose–Insulin Dynamics

Future studies should examine how SAA influences postprandial glycaemic and insulinemic responses through modulation of starch hydrolysis and glucose absorption. Continuous glucose monitoring and standardised meal tests, stratified by SAA activity, would enable precise characterisation of glucose–insulin kinetics and enzymatic variability.

3.14.2 Genetic and Autonomic Regulation

Precise quantification of *AMY1* gene copy number through advanced genomic sequencing techniques is critical to unravel the genetic determinants of salivary amylase (SAA) production and its role in driving metabolic variability among individuals. Concurrently, the integration of autonomic regulatory assessments–utilising heart rate variability (HRV) and salivary cortisol levels as biomarkers–offers a unique opportunity to explore the influence of sympathetic nervous system activation on SAA secretion. This dual approach could provide novel insights into the interplay between genetic predisposition and autonomic control in shaping SAA-mediated metabolic pathways and their downstream physiological effects.

3.14.3 Microbiome and Gut–Oral Axis Interactions

Advanced microbiome profiling techniques, such as 16S rRNA sequencing and metagenomic analysis, should be employed to investigate the interplay between salivary amylase (SAA) activity, gut microbial composition, and the production of short-chain fatty acids (SCFAs). These studies would provide critical insights into how starch availability and microbial fermentation modulate systemic insulin sensitivity and inflammatory responses.

Furthermore, experimental studies focusing on the gut–oral axis, including the neural pathways that mediate bidirectional communication between the oral cavity and gastrointestinal tract, are essential to elucidate the mechanistic links underlying these interactions.

3.14.4 Interventional and Causal Designs

Causality should be tested through pharmacological and dietary interventions that modulate SAA secretion or activity. Controlled feeding studies with varying starch content, glycaemic load, or prebiotic supplementation would reveal metabolic outcomes associated with differing SAA levels and their effects on butyrate production and insulin sensitivity.

3.14.5 Validation in Diverse Populations

Replication of findings in larger, demographically diverse cohorts – including men and individuals of varying age and ethnicity, is needed to assess population-specific associations and improve translational relevance. Multicentre trials with stratified analyses are recommended.

3.14.6 Long-Term and Personalised Dietary Interventions

Longitudinal studies (≥ 1–5 years) are required to evaluate the durability and clinical significance of SAA-tailored dietary strategies, including effects on diabetes risk, cardiovascular health, and quality of life.

3.14.7 Molecular Mechanisms and Hormonal Pathways

Mechanistic research should elucidate molecular links between SAA activity, GLP-1 secretion, and glucose homeostasis. In vitro and in vivo models examining SAA-derived oligosaccharides and enteroendocrine signalling will provide mechanistic insight into SAAmediated regulation of insulin sensitivity and lipid metabolism.

Conclusions

This study provides significant insights into the role of salivary amylase activity (SAA) in modulating metabolic health, particularly in overweight women of reproductive age. The findings demonstrate that dietary interventions, specifically calorie-restricted (CR) and low-starch (LS) diets, lead to distinct improvements in metabolic markers, with the response being influenced by individual variations in SAA.

Participants with high salivary amylase activity (HSA) showed greater improvements in insulin sensitivity and reductions in visceral fat percentage when following a calorie-restricted diet. Conversely, those with low salivary amylase activity (LSA) exhibited enhanced glycaemic control and significant increases in active GLP-1 levels when adhering to a low-starch diet. These results highlight the differential metabolic responses to dietary interventions based on SAA levels, emphasising the potential of SAA as a biomarker for personalised nutrition strategies.

The study also revealed that visceral fat plays a critical mediating role in the relationship between SAA and the triglyceride-glucose (TyG) index, accounting for 45 % of the total effect. This underscores the importance of targeting visceral adiposity in metabolic health interventions. Furthermore, reductions in leptin levels and improvements in insulin sensitivity were observed, particularly in the HSA-CR group, while the LSA-LS group demonstrated significant changes in GLP-1 secretion and C-peptide levels.

These findings suggest that SAA could serve as a non-invasive biomarker for early identification of individuals at metabolic risk and for guiding personalised dietary interventions aimed at reducing insulin resistance and preventing obesity-related complications. The study also highlights the need for further research to explore the long-term effects of dietary interventions, the genetic and hormonal regulation of SAA activity, and the molecular mechanisms underlying its impact on metabolic health.

Although the evidence remains correlational, SAA activity holds promise as a biomarker for metabolic health and appetite regulation. Future studies should focus on elucidating causal mechanisms.

This research contributes to the growing body of evidence supporting the role of SAA in metabolic regulation and emphasises the importance of personalised nutrition strategies tailored to individual enzymatic profiles. By integrating SAA as a biomarker into clinical practice, it may be possible to optimise dietary interventions and improve metabolic health outcomes in vulnerable populations, such as overweight women of reproductive age.

Proposals

1. Personalised Dietary Interventions

Tailoring Diets Based on Salivary Amylase Activity: The studies propose personalised nutrition strategies based on individual salivary amylase activity levels. For example:

- Women with high salivary amylase activity may benefit more from calorie-restricted diets, as these improve insulin sensitivity and reduce visceral fat.
- Women with low salivary amylase activity may experience better glycaemic control and increased butyrate production on low-starch diets.

Dietary Fibre and Butyrate Production: Increasing dietary fibre intake is recommended to enhance butyrate production, which improves insulin sensitivity and metabolic health. Specific fibres like inulin, pectin, and resistant starch are highlighted.

2. Use of Salivary Amylase Activity as a Biomarker

- Predicting Metabolic Health: Salivary amylase activity is proposed as a potential biomarker for assessing metabolic health, including insulin sensitivity and visceral fat levels.
- Genetic Screening: The AMY1 gene copy number, which influences salivary amylase activity, could be used to identify individuals who may benefit from specific dietary interventions.

3. Targeting Visceral Fat for Metabolic Health

Reducing Visceral Fat: The studies emphasise the importance of targeting visceral fat as a mediator of metabolic health. Higher salivary amylase activity is associated with reduced visceral fat, which indirectly improves insulin sensitivity and reduces the triglyceride-glucose (TyG) index.

4. Long-Term and Mechanistic Studies

- Sustainability of Dietary Interventions: Future research should focus on the long-term effects of dietary interventions, including their impact on gene expression related to lipid metabolism, inflammation, and insulin signalling pathways.
- Exploring Mechanisms: More studies are needed to understand the molecular and cellular mechanisms through which salivary amylase activity and dietary interventions affect metabolic markers.

5. Clinical Applications

- Dietary Modifications: The findings suggest that dietary interventions, such as low-starch diets and calorie restriction, can be effective strategies for improving metabolic health in overweight women of reproductive age.
- Metabolic Risk Prediction: Measuring salivary amylase activity in clinical settings could help identify individuals at risk for metabolic disorders, enabling early intervention.

6. Future Research Directions

To advance the understanding of salivary amylase activity (SAA) as a critical modulator of metabolic health, future research should adopt integrative and multidisciplinary approaches. These should encompass genetic, physiological, molecular, and environmental analyses across diverse populations to uncover the complex interactions between SAA and metabolic processes. Below are specific areas for future exploration:

6.1. Postprandial Glucose–Insulin Dynamics

Future studies should focus on elucidating the role of SAA in postprandial glycaemic and insulinemic responses, particularly its impact on starch hydrolysis and glucose absorption. Employing continuous glucose monitoring systems and standardised meal tests, stratified by SAA activity levels, will allow for precise characterisation of glucose–insulin kinetics. This approach can help identify enzymatic variability and its implications for personalised dietary interventions aimed at optimising glycaemic control.

6.2. Genetic and Autonomic Regulation

A deeper investigation into the genetic determinants of SAA production is essential. Advanced genomic sequencing techniques should be utilised to quantify AMY1 gene copy number and explore its association with SAA variability and metabolic health. Additionally, the role of autonomic regulation in SAA secretion warrants further study. Biomarkers such as heart rate variability (HRV) and salivary cortisol levels can be used to assess the influence of sympathetic nervous system activation on SAA production. This dual approach could provide novel insights into the interplay between genetic predisposition and autonomic control in shaping SAA-mediated metabolic pathways and their downstream effects.

6.3. Microbiome and Gut–Oral Axis Interactions

Emerging evidence suggests that salivary amylase activity may influence the composition and function of the gut microbiome, which plays a pivotal role in metabolic health. Future research should investigate the bidirectional interactions between the oral and gut microbiomes, focusing on how SAA-mediated starch hydrolysis impacts microbial

diversity, short-chain fatty acid production, and overall gut health. Metagenomic and metabolomic analyses could provide valuable insights into these complex relationships.

6.4. Interventional and Causal Designs

To establish causality, future studies should employ randomised controlled trials and longitudinal designs to assess the direct impact of SAA on metabolic health outcomes. Interventional studies that manipulate SAA levels through dietary or pharmacological means could help clarify its role in metabolic regulation. Additionally, exploring the effects of SAA modulation on specific populations, such as individuals with metabolic syndrome or type 2 diabetes, could provide targeted therapeutic strategies.

6.5. Validation in Diverse Populations

Given the variability in SAA activity across different ethnicities, age groups, and health statuses, future research should prioritise studies with larger and more diverse cohorts. This will ensure the generalizability of findings and help identify population-specific patterns in SAA activity and its metabolic implications.

6.6. Molecular Mechanisms and Hormonal Pathways

Future research should delve into the molecular mechanisms underlying SAA's role in metabolic health. This includes investigating its influence on hormonal pathways, such as GLP-1 secretion, insulin signalling, and glucagon regulation. Techniques such as RNA sequencing and proteomics could be employed to identify key genes and proteins involved in these processes, providing a deeper understanding of how SAA modulates metabolic outcomes.

This expanded version provides a detailed roadmap for future research, emphasising the importance of personalised approaches, advanced methodologies, and diverse populations to fully understand the role of salivary amylase activity in metabolic health.

List of publications and reports on topics of Doctoral Thesis

Publications:

1. Erta, G., Gersone, G., Jurka, A., & Tretjakovs, P. (2024). Impact of a 12-Week Dietary Intervention on Adipose Tissue Metabolic Markers in Overweight Women of Reproductive Age. International journal of molecular sciences, 25(15), 8512. https://doi.org/10.3390/ijms25158512
2. Erta, G., Gersone, G., Jurka, A., & Tretjakovs, P. (2024). The Link between Salivary Amylase Activity, Overweight, and Glucose Homeostasis. International journal of molecular sciences, 25(18), 9956. https://doi.org/10.3390/ijms25189956
3. Erta, G., Gersone, G., Jurka, A., & Tretjakovs, P. (2025). Decoding metabolic connections: the role of salivary amylase activity in modulating visceral fat and triglyceride glucose index. Lipids in health and disease, 24(1), 98. https://doi.org/10.1186/s12944-025-02524-7
4. Erta, G., Gersone, G., Jurka, A., & Tretjakovs, P. (2025). Salivary α-Amylase as a Metabolic Biomarker: Analytical Tools, Challenges, and Clinical Perspectives. International Journal of Molecular Sciences, 26(15), 7365. https://doi.org/10.3390/ijms26157365
5. Erta, G., Gersone, G., Jurka, A., Tretjakovs, P. Salivary amylase activity: A potential modulator of glucose homeostasis, insulin secretion, and appetite regulation. J Nutr Biochem. 2025 Oct 21, 148:110154. https://doi.org/10.1016/j.jnutbio.2025.110154

Reports and theses at international congresses and conferences:

1. Erta, G., Gersone, G., & Tretjakovs, P. (2023). Telehealth and metabolic health: Unraveling the effects of a 12-week low-starch diet on HOMA2-B and insulin resistance in overweight women. *Journal of the Endocrine Society*, 7(Supplement_1), bvad114.127. https://doi.org/10.1210/jendso/bvad114.127 Research output: Contribution to journal › Meeting abstract › Peer-reviewed
2. Erta, G., Gersone, G., & Tretjakovs, P. (2023). What is found from placing a continuous glucose monitor in overweight non-diabetic reproductive-age women with insulin resistance? Medicina (Kaunas), 59(Suppl. 2), 46. Research output: Contribution to journal Meeting abstract Peerreviewed
3. Erta, G., Gersone, G., & Tretjakovs, P. (2022). Does phase angle analysis in overweight women be a surrogate marker of insulin resistance? Endocrine Abstracts, 83, DOMNP1. Research output: Contribution to journal › Meeting abstract › Peer-reviewed

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2. Al-Akl, N., Thompson, R. I., & Arredouani, A. (2022). Elevated levels of salivary α- amylase activity in saliva associated with reduced odds of obesity in adult Qatari citizens: A crosssectional study. *PloS One*, 17(3), e0264692. https://doi.org/10.1371/journal.pone.0264692

3. Ali, M. M., Phillips, S. A., & Mahmoud, A. M. (2020). HIF1α/TET1 Pathway Mediates Hypoxia-Induced Adipocytokine Promoter Hypomethylation in Human Adipocytes. *Cells*, 9(1), 134. https://doi.org/10.3390/cells9010134

4. Ali, N., & Nater, U. M. (2020). Salivary Alpha-Amylase as a Biomarker of Stress in Behavioral Medicine. *International Journal of Behavioral Medicine*, 27(3), 337–342. https://doi.org/10.1007/s12529-019-09843-x

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Annexes

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Article

Impact of a 12-Week Dietary Intervention on Adipose Tissue Metabolic Markers in Overweight Women of Reproductive Age

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Abstract: The prevalence of overweight and obesity in women of reproductive age leads to significant health risks, including adverse metabolic and reproductive outcomes. Effective dietary interventions are critical to improving health outcomes in this population. This study investigates the impact of a 12-week diet intervention on metabolic markers of adipose tissue in overweight women of reproductive age, determining whether calorie restriction or low-starch diets are more effective, while also accounting for salivary amylase activity. A total of 67 overweight women of reproductive age were enrolled in a randomized controlled trial (RCT). Participants were divided into high-salivary-amylase (HSA) and low-salivary-amylase (LSA) groups based on baseline salivary amylase activity measured using a spectrophotometric method. Each group was further subdivided into two dietary intervention groups: calorie restriction (CR) and low starch (LS), resulting in four subgroups (HSA-CR, HSA-LS, LSA-CR, LSA-LS), along with a control group (CTR) of normal-weight individuals (no intervention). Participants were assigned to a calorie-restricted diet or a low-starch diet for 12 weeks. Key metabolic markers of adipose tissue, including insulin sensitivity, adipokines, cytokines, and lipid profiles, were measured at baseline (T0), 30 min after consuming starch-containing muesli (T1), and 12 weeks after intervention (T2). Active GLP-1, glucagon, and C-peptide levels were assessed to clarify the hormonal mechanisms underlying the dietary effects. Salivary amylase activity was also measured to examine its role in modulating glucose and GLP-1 responses. Both diet interventions led to significant improvements in metabolic markers of adipose tissue, though different ones. Calorie restriction improved insulin sensitivity by effectively reducing visceral fat mass and enhancing insulin signaling pathways. In contrast, the low-starch diet was linked to a reduction in the coefficient of glucose variation influenced partly by changes in GLP-1 levels. Our findings highlight the importance of personalized diet strategies to optimize metabolic health in this demographic.

Keywords: salivary amylase activity; overweight; diet intervention; adipose tissue; metabolic markers; GLP-1

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1. Introduction

Adipose tissue plays a crucial role in regulating metabolic homeostasis, functioning not only as a site for energy storage but also as an active endocrine organ. It secretes various adipokines and cytokines that influence systemic metabolism and insulin sensitivity [1–3]. In the context of overweight and obesity, adipose tissue undergoes functional changes that can lead to metabolic dysfunctions, including insulin resistance, chronic inflammation, and dyslipidemia [4]. These changes are particularly critical for women of reproductive age, as they impact not only metabolic health but also reproductive outcomes [5–7].

Calorie restriction and adjustments in macronutrient composition, such as low-starch diets, have shown promising results in improving metabolic markers [8]. Calorie restriction, characterized by a reduction in total caloric intake without inducing nutrient deficiencies, has been linked to enhanced insulin sensitivity, improved lipid profiles, and reduced inflammatory markers [9,10]. Similarly, low-starch diets, which limit the intake of rapidly

digestible carbohydrates, have been shown to positively influence glycemic control and decrease adiposity [11].

Dietary interventions impose their effects primarily through the modulation of gut hormones, such as glucagon-like peptide-1 (GLP-1). GLP-1, an incretin hormone, is essential for glucose homeostasis by enhancing insulin secretion and inhibiting post-prandial glucagon release [12,13]. The secretion of GLP-1 is influenced by dietary composition and the rate of digestion and absorption of carbohydrates [14].

Salivary amylase, an enzyme that initiates starch hydrolysis in the oral cavity, has been identified as a significant contributor to this process [15]. Variability in salivary amylase activity among individuals can influence post-prandial glucose levels and subsequent GLP-1 secretion [16,17]. Elevated salivary amylase activity facilitates rapid starch degradation and glucose absorption, potentially increasing GLP-1 release and enhancing metabolic outcomes. Conversely, reduced salivary amylase activity results in slower starch digestion and altered glycemic responses [18,19] which may affect GLP-1 dynamics and adipose tissue metabolism.

Despite the growing body of evidence on the role of dietary interventions and salivary amylase activity in metabolic health, comprehensive studies are needed that focus on women of reproductive age, a demographic particularly vulnerable to the adverse effects of excess adiposity. Understanding how different diet patterns influence metabolic markers of adipose tissue and the underlying physiological mechanisms in this population is essential for developing targeted nutritional strategies.

This study aims to investigate the impact of a 12-week dietary intervention, comprising calorie restriction and low-starch intake, on adipose tissue metabolic markers in overweight reproductive-age women. We hypothesize that this intervention will improve key metabolic markers, potentially mediated by changes in GLP-1 levels and modulated by individual variations in salivary amylase activity. By clarifying these relationships, our research seeks to contribute to the development of personalized dietary recommendations that can improve metabolic health and reproductive outcomes in this population.

2. Results

In the LSA-LS participant group, the Wilcoxon signed rank test was used to compare active GLP-1 levels at baseline and post-intervention. The analysis revealed a statistically significant increase in active GLP-1 levels, with median values increasing from 12.65 at baseline to 60.38 after the low-starch diet ($W = 45$, $p = 0.001$) (Figure 1).

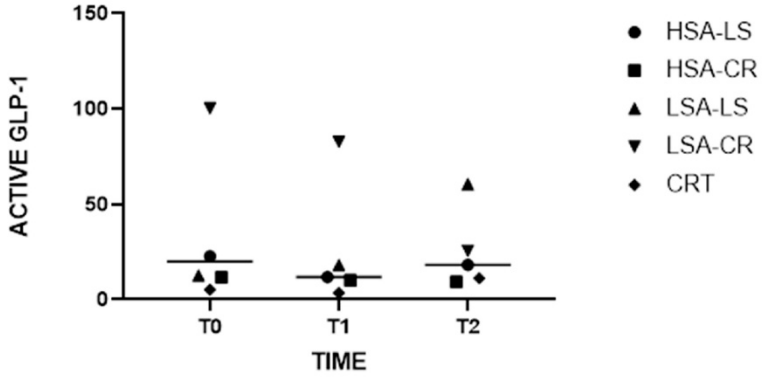

Figure 1. Medians of Active GLP-1 Levels at Baseline and Post-Intervention with Grand Median Across All Groups.

The Kruskal–Wallis H test demonstrated significant differences in glucagon levels between the dietary intervention groups ($H = 10.97$, $p = 0.0035$). Subsequent post hoc pair-

wise comparisons indicated that glucagon levels were significantly lower in the compared groups (Figure 2).

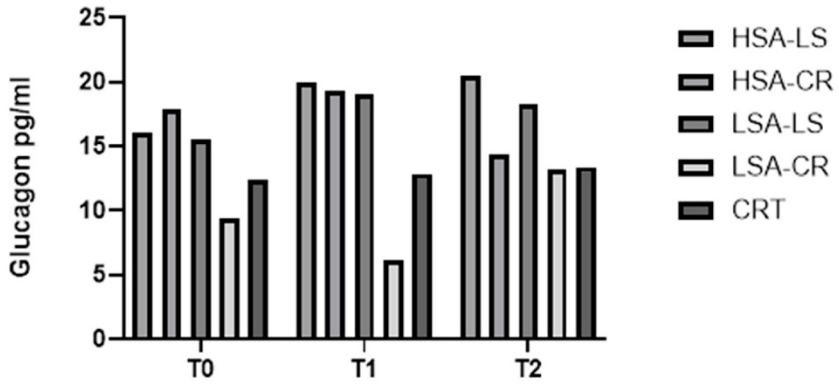

Figure 2. Glucagon.

These findings suggest that a combination of a low-amylase diet and calorie restriction is associated with a substantial reduction in glucagon levels, which may benefit the management of glucagon levels and improve fasting glucose levels.

Descriptive statistics indicated that the median glucagon level in the LSA-CR group before the intervention was 19 pg/mL (IQR: 9.107–63.90 pg/mL), which decreased to a median of 13.13 pg/mL (IQR: 11.3–25.71 pg/mL) after the intervention.

Additionally, the Kruskal–Wallis H test identified significant differences in triglyceride levels among the five groups at baseline, before the intervention ($H = 11.22$, $p = 0.0158$) (Figure 3), indicating initial variability in the metabolic profiles of the participants. However, post-intervention, these differences were no longer significant, suggesting that the dietary interventions normalized triglyceride levels across groups, effectively reducing the initial disparities. This normalization implies that both calorie-restricted and low-starch diets were equally effective in modulating triglyceride levels, irrespective of baseline differences.

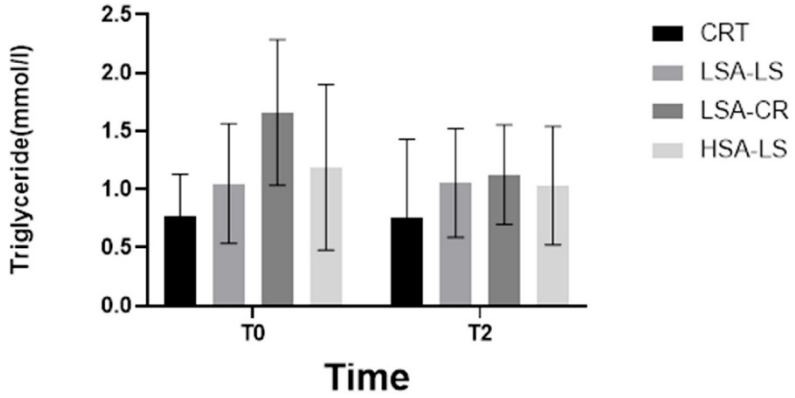

Figure 3. Triglyceride.

Furthermore, the Kruskal–Wallis H test revealed significant differences in C-peptide levels among the diet intervention groups ($H = 5.0$, $p = 0.0222$) (Figure 4) and in leptin levels ($H = 38.42$, $p = 0.0004$) (Figure 5). These significant differences underscore the impact of dietary interventions on these biomarkers.

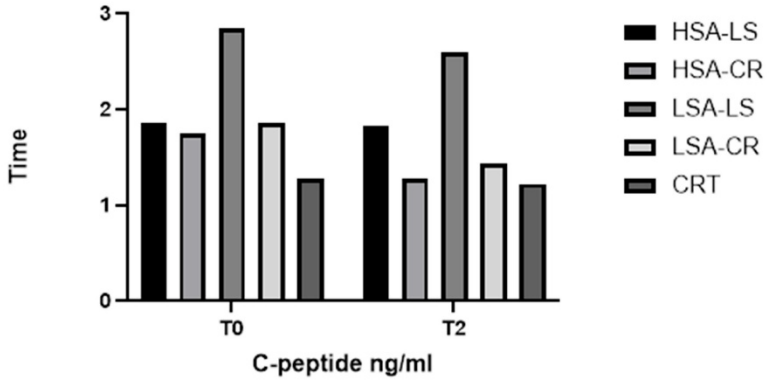

Figure 4. C-peptide.

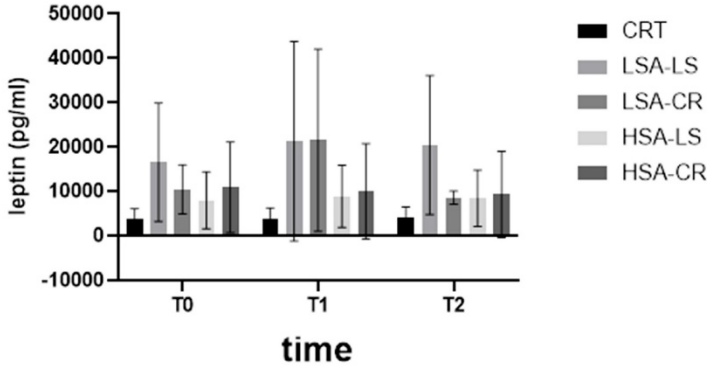

Figure 5. Leptin.

We measured Spearman's rank coefficient between salivary amylase activity and various adipose tissue markers in 67 women at three time points: baseline (T0), 30 minutes after consuming starch-containing muesli (T1), and 12 weeks after the diet intervention (T2) (Figure 6).

The analysis highlights the relationships between enzyme activity and adipose tissue markers at different points in time, providing insights into the effects of dietary intervention. While most parameters were measured at all three time points, glucose, C-peptide, calculated parameters (HOMA2IR, HOMA2-%S, and HOMA2-%B), and triglycerides were measured at the start and after 12 weeks.

Measuring these biomarkers provides insight into how dietary interventions affect various aspects of glucose homeostasis, offering critical insights for developing effective nutritional strategies to manage and prevent metabolic diseases (Tables 1 and 2).

Table 1. Key statistical results. Descriptive statistics.

Biomarker	Group	Time Point	Median (IQR)
Active GLP-1	LSA-LS	T0	12.65 pg/mL (3.428–128.0)
Active GLP-1	LSA-LS	T2	60.38 pg/mL (7.93–224.3)
Glucagon	HSA-CR	T0	17.85 pg/mL (12.02–31.75)
Glucagon	HSA-CR	T2	14.40 pg/mL (8.33–34.28)
C-peptide	LSA-LS	T0	2.31 ng/mL (1.26–3.3)
C-peptide	LSA-LS	T2	2.1 ng/mL (0.9–2.6)
Leptin	HSA-CR	T0	7146 pg/mL (4879–13,123)
Leptin	HSA-CR	T2	5607 pg/mL (4452–10,536)

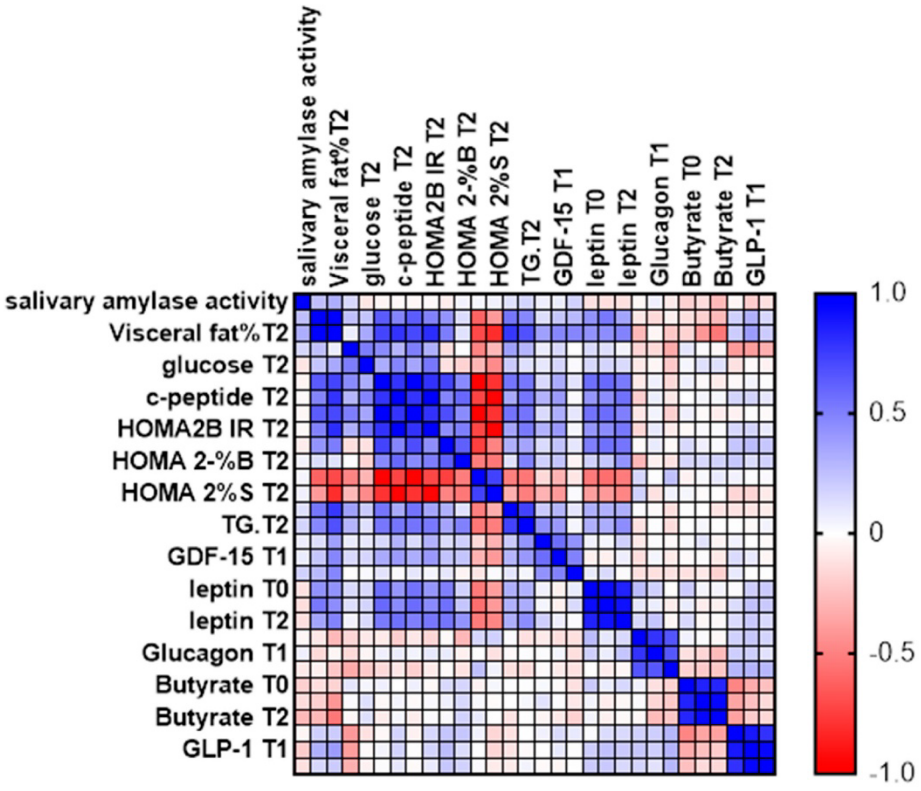

Figure 6. Spearman's Rank Coefficient.

Table 2. Key statistical results. Group comparisons.

Statistic/Analysis	Method	Result
Group comparisons	Kruskal–Wallis H test	Differences in glucagon levels between groups (H = 10.97, p = 0.0035). Glucagon levels significantly lower in high-amylase calorie restriction group
	Kruskal–Wallis H test	Differences in C-peptide levels (H = 5.0, p = 0.0222)
	Kruskal–Wallis H test	Differences in leptin levels (H = 38.42, p = 0.0004)
Within-group Comparisons	Wilcoxon signed rank test	Significant increase in active GLP-1 levels in LSA-LS group from 12.65 to 60.38 (W = 45, p = 0.001)
Dietary interventions	Friedman test followed by Dunn's test with Bonferroni correction	Post hoc analysis revealed a significant increase in active GLP-1 levels from T0 (median: 12.65) to T2 (median: 60.38), with a significant p-value (p = 0.001)

3. Discussion

In this study, we investigated the effects of different dietary interventions (low-starch diet and calorie restriction diet) on GLP-1, glucagon, C-peptide, and various metabolic markers in overweight women of reproductive age. Participants were categorized according to their salivary amylase activity (low and high) and compared to a control group with

normal weight. Our findings provide new insights into how dietary modifications and individual enzymatic differences impact key metabolic biomarkers.

3.1. Glucagon

- Role in Glucose Homeostasis: Glucagon, a hormone produced by pancreatic alpha cells, counterbalances insulin effects by stimulating hepatic glucose production through glycogenolysis and gluconeogenesis, raising blood glucose levels during fasting states or hypoglycemia [20].
- Relevance in Dietary Studies: Monitoring glucagon levels elucidates how different diets impact hepatic glucose production and overall glucose homeostasis. Diets that modulate glucagon secretion could influence fasting glucose levels and glucose variability, crucial components of metabolic health [21].

Our analysis showed significant differences in glucagon levels between the groups. Participants on a calorie restriction diet exhibited significantly lower glucagon levels after 12 weeks compared to those on a low-starch diet ($H = 10.97$, $p = 0.0035$) (Figure 2). This reduction in glucagon could contribute to improved glycemic control, as glucagon is known to raise blood glucose levels by stimulating hepatic glucose production [21,22].

3.2. C-Peptide

- Role in Glucose Homeostasis: C-peptide, co-secreted with insulin from pancreatic beta cells in equimolar amounts, serves as a marker of endogenous insulin production [23]. Unlike insulin, the C-peptide is not extracted by the liver, making it a reliable measure of beta-cell function.
- Relevance in Dietary Studies: Assessing C-peptide levels helps determine how dietary interventions affect insulin secretion and beta-cell function. This information is essential for understanding the long-term impacts of diets on insulin production and the risk of developing insulin resistance or type 2 diabetes.

C-peptide levels varied significantly between the groups (Figure 4). The control group with normal weight had significantly lower C-peptide levels compared to the overweight groups, both at fasting and post-prandially. Within the intervention groups, those on a calorie restriction diet with high salivary amylase activity had a more pronounced reduction in C-peptide levels over time.

3.3. GLP-1

- Role in Glucose Homeostasis: Glucagon-like peptide-1 (GLP-1) is an incretin hormone secreted by enteroendocrine L-cells in the intestine in response to food intake. It enhances glucose-dependent insulin secretion, inhibits glucagon release, and slows gastric emptying, collectively contributing to post-prandial glucose control [24,25].
- Relevance in Dietary Studies: Diet-induced changes in GLP-1 levels significantly affect post-prandial glucose metabolism. Measuring GLP-1 provides insights into how different dietary patterns influence incretin response, satiety, and insulin sensitivity. This is particularly relevant for developing dietary strategies to improve glucose regulation and manage diabetes.

The Kruskal–Wallis test revealed significant differences in GLP-1 levels among the different intervention groups at various time points ($H = 10.97$, $p = 0.0035$) (Figure 1). Notably, post-dietary intervention analysis (T2) indicated that GLP-1 levels increased significantly more in the low-starch diet group with low salivary amylase activity compared to the other groups. This suggests that individuals with lower salivary amylase activity may experience increased GLP-1 secretion in response to starch intake, potentially enhancing satiety and glucose homeostasis (Figure 1).

3.4. Leptin Levels

Leptin, a hormone produced primarily by adipocytes, regulates energy balance and body weight. Our study observed a significant decrease in leptin levels in the CR groups compared to the baseline and control groups ($H = 38.42$, $p = 0.0004$) (Figure 5). This is consistent with previous research that indicates that caloric restriction reduces leptin levels due to decreased fat mass and reduced adipocyte size [26]. However, the LS groups did not show a significant reduction in leptin levels, suggesting that macronutrient composition could play a lesser role than caloric intake in modulating leptin.

3.5. GDF-15 Levels

Growth differentiation factor 15 (GDF-15) is a stress-induced cytokine linked to metabolic regulation [27–29]. Our findings did not show a significant change. Overall, while our dietary interventions were successful in addressing some metabolic markers, the lack of significant differences in GDF-15 changes suggests that this cytokine's regulation is multifaceted and may not be easily modified by diet alone in the short term (Figure 7).

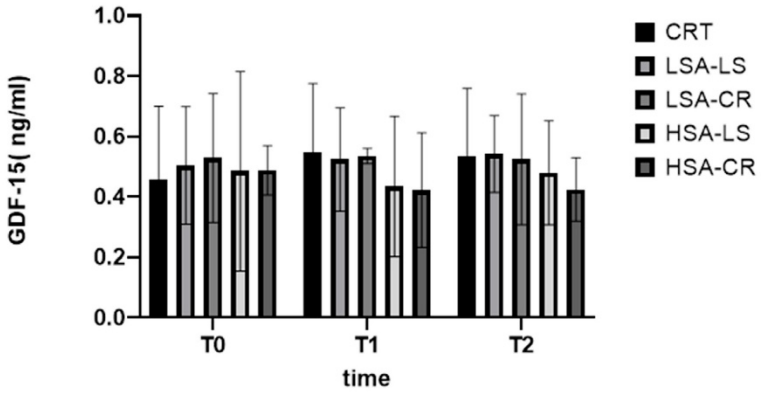

Figure 7. GDF-15.

3.6. Implications and Future Directions

- Long-term Effects and Sustainability: Future studies should investigate the long-term effects and sustainability of different diet interventions. This could include reviewing the maintenance of improvements of metabolic health and adherence to dietary regimens for extended periods.
- Personalized Nutrition Approaches: Given the variability in individual responses based on salivary amylase activity, future research should focus on developing personalized nutrition strategies. This includes exploring how genetic and phenotypic differences can guide personalized diet recommendations to improve metabolic health.
- Mechanistic Studies: More mechanistic studies are needed to elucidate the underlying pathways through which diet interventions affect metabolic markers. This involves exploring the molecular and cellular mechanisms in adipose tissue that respond to different dietary components.

Collectively, these findings underscore the importance of personalized dietary interventions in managing metabolic health. Future research should continue to explore these relationships to develop more effective and individualized dietary strategies for the prevention and management of metabolic diseases.

Elucidating the role of glucagon in different dietary contexts can open new avenues to improve glycemic control in overweight individuals. Future research should examine the long-term effects of these diet interventions and explore changes in the expression of genes involved in the lipid metabolism, inflammation, and insulin signaling pathways due

to dietary interventions. Techniques such as quantitative PCR and RNA sequencing are essential for identifying these alterations in gene expression.

Additionally, studies with larger and more diverse cohorts are necessary to validate these findings and determine their generalizability to broader populations. By tailoring dietary interventions to individual metabolic profiles, we can enhance the efficacy of treatments aimed at mitigating obesity-related metabolic disorders in reproductive-age women.

3.7. Limitations

The duration of the study was limited to 12 weeks, but larger, longer-term studies are required to confirm our findings. Additionally, we did not control other lifestyle factors, such as physical activity, that could influence metabolic outcomes. Despite these limitations, our findings provide valuable insights into the role of diet and enzymatic activity in metabolic regulation.

3.8. Key Findings

- Improvements in Metabolic Markers: Both calorie-restricted (CR) and low-starch (LS) diets led to significant improvements in metabolic markers, although of different kinds.
- Impact of Salivary Amylase Activity: Participants with a higher baseline salivary amylase activity (HSA) showed better responses to the CR diet in terms of insulin sensitivity, although this finding should be interpreted with caution due to variability in individual responses.

Individuals with low salivary amylase (LSA) activity exhibited superior glycemic control on low-starch diets. However, further research is needed to confirm these results and understand the underlying mechanisms.

3.9. Personalized Nutrition

The study underscores the importance of tailoring dietary interventions based on individual metabolic profiles, specifically salivary amylase activity, to optimize metabolic outcomes.

3.10. Detailed Findings

- Leptin Levels: Significant reductions in leptin levels were observed in the LSA-CR group from T0 to T2, indicating the effectiveness of calorie restriction diets in decreasing leptin levels in participants with low salivary amylase activity. Interestingly, a more pronounced increase in leptin levels was observed after consuming starch-containing muesli (T1) in both LSA groups compared to those with high salivary amylase activity.

It can be attributed to slower starch digestion and absorption of starch in LSA individuals [15]. This results in prolonged elevation of blood sugar and a sustained insulin response, which may influence further activation of the PI3K/Akt pathway [29], thereby enhancing the transcription and translation of leptin in adipocytes. Studies have shown that insulin can upregulate leptin mRNA expression and increase leptin secretion in adipose tissue [11].

3.11. Insulin Sensitivity (HOMA2-%S)

The LSA-CR group exhibited significant improvements in the HOMA2-%S score, reflecting enhanced insulin sensitivity (Figure 8).

- Physiological Mechanism: Calorie restriction can result in a more effectively reduced fat mass and enhanced insulin signaling pathways.
- Overall Metabolic Improvements: The 12-week dietary intervention resulted in significant metabolic improvements in overweight women of reproductive age, highlighting the importance of considering salivary amylase activity.

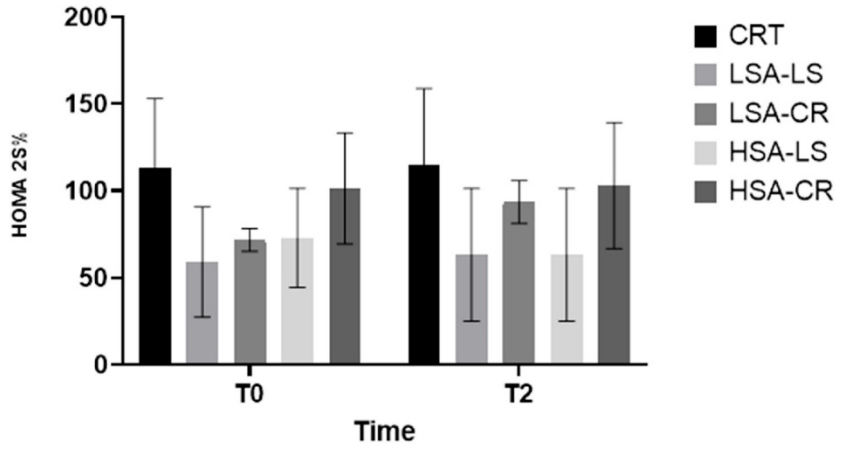

Figure 8. HOMA2-%S.

3.12. Mechanistic Insights

The observed changes in metabolic markers can be attributed to several mechanisms.

- Caloric Restriction: It likely reduces adipocyte size and fat mass, leading to lower leptin production and enhanced insulin sensitivity [26].
- Low-starch Diet: Reduces post-prandial glucose spikes and reduces hyperinsulinemia, which collectively contribute to better metabolic health [30,31]

3.13. Personalized Responses Based on Salivary Amylase Activity

Our study also highlights the importance of personalized nutrition, particularly the role of salivary amylase activity in modulating metabolic responses to diet interventions. There is the potential to use salivary amylase activity as a biomarker for personalized dietary recommendations.

3.14. Implications and Future Directions

- Personalized Nutrition Approaches: The findings highlight the potential of personalized nutrition strategies based on salivary amylase activity to enhance the efficacy of dietary interventions for metabolic health.
- Long-term Effects and Sustainability: Future studies should investigate the long-term effects and sustainability of different diet interventions, including gene expression profiling to understand changes in lipid metabolism, inflammation, and insulin signaling pathways.
- Larger, Diverse Cohorts: Studies with larger and more diverse cohorts are necessary to confirm these findings and determine their generalizability to broader populations.

4. Materials and Methods

4.1. Study Design and Participants

This study was conducted with 67 women of reproductive age (18–45 years) with a BMI between 25 and 29.9 kg/m^2 kg/m^2 (Table 3). Participants were recruited from a health center and screened for eligibility. Exclusion criteria include pregnancy, lactation, chronic diseases (e.g., diabetes, cardiovascular disease), and the use of medications that affect metabolism. The study protocol was approved by the Ethics Committee of Rigas Stradiņš University (Ethics Committee number: 22-2/479/2021), and all participants provided their written informed consent in accordance with the Declaration of Helsinki.

Table 3. Demographic Information.

Characteristic	
Age (years)	
Mean (SD)	29.5 ± 6.2
Range	18–45
BMI (kg/m^2)	
Mean (SD)	27.8 ± 2.1
Range	25–29.9
Adherence (%)	
Mean (SD)	85 ± 10
Range	60–100

4.2. Dietary Interventions

Participants were randomly assigned to one of two dietary intervention groups for a duration of 12 weeks.

- Low-starch Diet Group: This group followed a low-starch diet, emphasizing the consumption of low-glycemic-index vegetables, proteins, and healthy fats. Daily starch intake was limited to less than 50 g.
- Caloric Restriction Group: This group followed a caloric restriction diet, reducing their daily caloric intake by 500 kcal from their estimated energy requirement, calculated based on the Harris–Benedict equation.

Dietary adherence was monitored through weekly food diary and online consultations with an endocrinologist.

4.3. Evaluation of Salivary Amylase Activity

Salivary amylase activity was evaluated using the Salimetrics Amylase Activity Assay (Salimetrics, State College, PA, USA). Unstimulated saliva samples were collected in the morning after an overnight fast to ensure consistency. Salivary amylase activity was determined according to the manufacturer's protocol. Participants were classified into high- and low-salivary-amylase-activity groups based on the median split of amylase activity data.

4.4. Biochemical and Molecular Analysis

Fasting blood samples were collected at baseline (T0), after the consumption of starch-containing muesli (T1), and after the 12-week dietary intervention (T2). The following metabolic markers were analyzed:

- Active GLP-1: Measured using a GLP-1 (Active) ELISA kit (Millipore, Billerica, MA, USA).
- C-peptide: Assessed using a chemiluminescent immunoassay kit (IMMULITE 2000, Siemens Healthineers, Erlangen, Germany).
- Glucagon: Measured using the Glucagon ELISA Kit (Cat. No. EZGLU-30K, Millipore Sigma, Burlington, MA, USA).
- HOMA2-%S: Calculated using fasting insulin and glucose levels with the HOMA calculator (version 2.2.3, Diabetes Trials Unit, University of Oxford).
- Leptin: Measured using a multiplex immunoassay (Luminex, Austin, TX, USA).
- GDF-15: Quantified using ELISA kits (R&D Systems, Minneapolis, MN, USA).
- Triglycerides: Measured using an enzymatic colorimetric method with a commercial kit (Roche Diagnostics, Basel, Switzerland).
- Visceral fat: Measured using a bioimpedance scale (Omron BF511, Omron Healthcare, Kyoto, Japan).

4.5. Study Groups and Interventions

Participants were divided into two groups according to their baseline salivary amylase activity: High salivary amylase (HSA) and low salivary amylase (LSA). Each salivary amylase group was further subdivided into two dietary intervention groups.

- Calorie Restriction (CR) Group: Participants followed a calorie-restricted diet, reducing their daily caloric intake by 500 kcal.
- Low-Starch (LS) Diet Group: Participants followed a low-starch diet, limiting their daily intake of starch to less than 50 g.

This resulted in four subgroups: HSA-CR, HSA-LS, LSA-CR, LSA-LS. Additionally, a control group (CTR) of normal weight was included (Table 4).

Table 4. Distribution of participants in control and study groups.

Group	Number of Participants
Control Group	7
HSA-CR	15
HSA-LS	15
LSA-CR	15
LSA-LS	15
Total	67

4.6. Dietary Intervention

Dietary interventions were designed by registered dietitians and included meal plans and educational sessions to ensure adherence. Participants received weekly online counseling and were required to maintain a food diary. Compliance was monitored through regular check-ins and food diary reviews.

4.7. Sample Collection and Analysis

- Saliva Samples: Collected in the morning after an overnight fast. Salivary amylase activity was measured using an enzymatic assay kit (Salimetrics, State College, PA, USA) according to the manufacturer's instructions.
- Blood Samples: Fasting blood samples were analyzed for metabolic markers as described above.

4.8. Ethical Approval

The study was conducted in accordance with the Declaration of Helsinki and was approved by the Ethics Committee of Rigas Stradiņš University (Ethics Committee number: 22-2/479/2021). Written informed consent was obtained from all participants prior to their enrollment in the study.

This comprehensive methodology ensures a rigorous assessment of the dietary interventions' impact on metabolic health markers in the target population.

4.9. Statistical Analysis

Data Analysis

Statistical analyses were conducted using GraphPad Prism 10. Due to the non-normal distribution of the data, non-parametric tests were employed. Descriptive statistics were summarized as medians with interquartile ranges (IQRs) for each variable.

- Descriptive Statistics: Median and IQR were calculated for each variable (e.g., leptin, GDF-15, HOMA2-%B) at each time point (T0, T1, T2) across all groups (HSA-CR, HSA-LS, LSA-CR, LSA-LS, and control) (Table 1).

Spearman's rank correlation coefficients were calculated to assess the relationships between salivary amylase activity and metabolic markers. Statistical significance was set at $p < 0.05$ (Figure 6).

- Group Comparisons: The Kruskal–Wallis H test was used to assess differences between the five groups at each time point. For post hoc pairwise comparisons, Dunn's multiple comparison test with Bonferroni correction was used to determine which specific groups differ from each other (Table 2)
- Within-group Comparisons: Changes within each group over time (T0 to T1, T1 to T2, T0 to T2) were analyzed using the Wilcoxon signed rank test.
- Effect of Dietary Interventions: To evaluate the impact of diet interventions on the primary outcomes, the Friedman test was used to compare values at T0, T1, and T2 within each subgroup, followed by post hoc analysis with Dunn's test.

5. Conclusions

This study underscores the significant impact of dietary interventions on metabolic health markers in overweight women of reproductive age, with a particular focus on the roles of calorie restriction (CR) and low-starch (LS) diets. Both CR and LS diets led to notable improvements in metabolic markers, including reduced leptin levels and enhanced insulin sensitivity, as indicated by the HOMA2-%S scores.

A key finding of this study is the differential response to dietary interventions based on salivary amylase activity. Participants with higher baseline HSA exhibited superior improvements in insulin sensitivity when following the CR diet, while individuals with low salivary amylase activity (LSA) exhibited superior glycemic control on low-starch diets.

The study also suggests that variations in salivary amylase activity are associated with different compositions of the gut microbiota, which in turn influence metabolic health.

These findings emphasize the potential for the use of salivary amylase activity as a biomarker to tailor dietary recommendations, thus optimizing metabolic outcomes. Personalized diet interventions could enhance the effectiveness of treatments aimed at reducing obesity-related metabolic disorders in reproductive-age women.

Future research should focus on the long-term effects of these dietary interventions, including gene expression profiling, to understand changes in lipid metabolism, inflammation, and insulin signaling pathways. Larger and more diverse cohorts are needed to validate these findings and determine their generalizability. Additionally, further mechanistic studies are essential to elucidate the pathways through which dietary interventions affect metabolic markers.

Overall, this study provides valuable insights into the role of diet and enzymatic activity in metabolic regulation and underscores the importance of considering individual metabolic profiles when designing dietary interventions for metabolic health.

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Informed Consent Statement: All participants provided their written informed consent in accordance with the Declaration of Helsinki.

Data Availability Statement: Data are contained within the article.

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Conflicts of Interest: The authors declare no conflicts of interest.

Abbreviations

Akt (PKB)	Protein kinase B
AMY1	Alpha amylase 1
CTR	Control group
GLP-1	Glucagon-like peptide-1
HOMA2-%B	Homeostatic model assessment of beta-cell function
HOMA2-IR	Homeostatic model assessment of beta-cell function—insulin resistance
HOMA2-%S	Homeostatic model assessment of insulin sensitivity percentage
HSA-CR	High-salivary-amylase calorie restriction group
HSA-LS	High-salivary-amylase low-starch group
LSA-CR	Low-salivary-amylase calorie restriction group
LSA-LS	Low-salivary-amylase low-starch group
Quantitative PCR	Quantitative polymerase chain reaction
RCT	Randomized controlled trial
RNA sequencing	Ribonucleic acid sequencing
T0	Baseline
T1	30 min after starch-containing muesli consumption
T2	12 weeks post-intervention

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The Link between Salivary Amylase Activity, Overweight, and Glucose Homeostasis

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Abstract: Butyrate, a short-chain fatty acid (SCFA) produced by the fermentation of dietary fibers in the colon, plays a pivotal role in regulating metabolic health, particularly by enhancing insulin sensitivity. Given the rising incidence of metabolic disorders, understanding the factors that influence butyrate production is of significant interest. This study explores the link between salivary amylase activity and butyrate levels in overweight women of reproductive age. Participants were categorized into low (LSA) and high (HSA) salivary amylase activity groups and further divided into two subgroups: one followed a low-starch diet (LS), and the other underwent caloric restriction (CR). We assessed salivary amylase activity and measured serum butyrate concentrations to examine their associations. Our findings showed a significant, though weak, positive correlation ($\rho = 0.0486$, $p < 0.05$), suggesting a link between salivary amylase activity and butyrate levels. The statistical significance, despite the weak correlation, implies that this relationship is not random. Moreover, higher baseline butyrate levels were observed in women with elevated salivary amylase activity. Also, women with low salivary amylase activity on a low-starch diet experienced a more pronounced increase in butyrate levels compared to those on caloric restriction. These results suggest that salivary amylase activity and dietary intake interact to influence butyrate production, with potential implications for improving insulin sensitivity and metabolic health. The study underscores the potential of butyrate in enhancing insulin sensitivity and promoting overall metabolic well-being. Further research is necessary to clarify the mechanisms involved and to understand the long-term effects of butyrate on metabolic health across different populations.

Keywords: salivary amylase activity; butyrate; low-starch diet; insulin sensitivity; overweight

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1. Introduction

The gut microbiota's impact on human health has gained significant research attention in recent years, particularly concerning its metabolic products, such as short-chain fatty acids (SCFAs) [1–3]. Among these SCFAs, butyrate, primarily produced through the fermentation of dietary fibers by colon bacteria, stands out as a crucial metabolite with various physiological benefits [4–6]. Butyrate not only serves as a primary energy source for colonic epithelial cells but also exerts systemic effects, influencing metabolic health, immune responses, and inflammation regulation [5,6]. Recent studies highlighted butyrate's potential in modulating metabolic health, especially in the context of obesity and related metabolic disorders [7,8]. For instance, butyrate has been shown to activate the AMP-activated protein kinase (AMPK) pathway, improve oxidative metabolism, and reduce lipid synthesis in the liver. These metabolic effects are crucial for enhancing insulin sensitivity and reducing low-grade chronic inflammation, both of which are hallmark features of metabolic syndrome and type 2 diabetes [9].

In the present study, we investigated the relationship between salivary amylase activity, diet interventions, butyrate levels, and the influence of salivary amylase activity on insulin sensitivity in overweight women of reproductive age. The rationale for selecting this

demographic lies in their unique metabolic and hormonal profile, which may interact differently with butyrate production and metabolic outcomes compared to males and women beyond reproductive age. Furthermore, understanding these interactions in women of reproductive age could provide insights into targeted diet interventions for this specific group. Salivary amylase, an enzyme responsible for starch digestion, varies significantly between individuals, due to differences in the variation in the copy number of the AMY1 gene, and is also modulated by afferent signals that regulate enzyme activity.

Recent studies [10,11] indicate that variations in the AMY1 gene significantly impact salivary amylase activity, influencing starch digestion and glucose metabolism. Additionally, afferent stimuli, such as food sensory perception, play a role in modulating salivary amylase activity, which further contributes to individual differences, potentially influencing postprandial glycemic responses and subsequent metabolic results [12,13].

Participants were classified according to their levels of salivary amylase activity and subjected to a low-starch diet or a calorie reduction regimen. Additionally, a control group was formed of individuals of normal weight. Initial findings indicated that women with higher salivary amylase activity showed elevated levels of butyrate, suggesting a possible link between increased starch breakdown and butyrate production [5].

The justification for this design lies in the hypothesis that individuals with high salivary amylase activity may process starch differently, leading to variations in butyrate production and subsequent metabolic effects. This study aims to clarify the role of salivary amylase activity in butyrate production and to explore whether diet can modulate this process. Findings can provide information on personalized nutrition approaches, considering individual enzymatic profiles and their impact on the gut microbiota and metabolic health.

2. Results

The baseline demographic and biochemical characteristics of the study participants, stratified by group allocation, are presented in Table 1.

Table 1. Characteristics of the participants at baseline.

Group Allocation	Mean Age (Years) ± SD	Salivary Amylase (U/mL) Median (IQR)
Caloric Restriction (Low Salivary Amylase) (n = 15)	28.1 ± 3.9	15.3 ± 14.9
Low-Starch Diet (High Salivary Amylase) (n = 15)	29.4 ± 3.2	77.1 ± 50.5
Low-Starch Diet (Low Salivary Amylase) (n = 15)	28.5 ± 3.5	15.6 ± 19.7
Caloric Restriction (High Salivary Amylase) (n = 15)	30.1 ± 4.3	89.2 ± 47.4
Control Group (Normal Weight) (n = 7)	29.1 ± 3.2	31.7 ± 23.4

The descriptive statistics for the key baseline variables are summarized in Table 2, including salivary amylase activity and butyrate concentration.

Table 2. Summary statistics of baseline key variables.

Variable	Value
Salivary Amylase (U/mL)	Median (IQR): 27.77 (10.64–56.24)
Butyrate (µmol/L)	Median (IQR): 1.823 (0.373–6.985)

The correlation analysis supports a weak but statistically significant relationship ($\rho = 0.0486$, $p < 0.05$) between salivary amylase activity and butyrate levels (Figure 1).

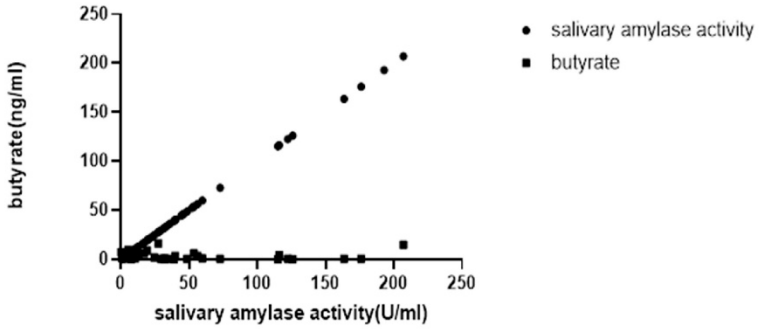

Figure 1. Correlation between salivary amylase activity and butyrate (n = 67). Spearman's rank correlation coefficient indicated a significant but weak correlation between salivary amylase activity and butyrate (ρ = 0.0486, $p < 0.05$).

2.1. Absence of a Positive Correlation in the Plot

Despite the overall significant positive correlation between salivary amylase activity and butyrate levels (ρ = 0.0486, $p < 0.05$), the plot may not visually demonstrate a clear positive correlation due to high variability. This variability can obscure the trend, making it less apparent in the scatter plot. Additionally, small sample sizes and outliers can further diminish the correlation's visual representation.

Additionally, we found that women with high salivary amylase activity had significantly higher baseline butyrate levels compared to those with low salivary amylase activity (Mann–Whitney U = 44, $p < 0.05$) (Figure 2).

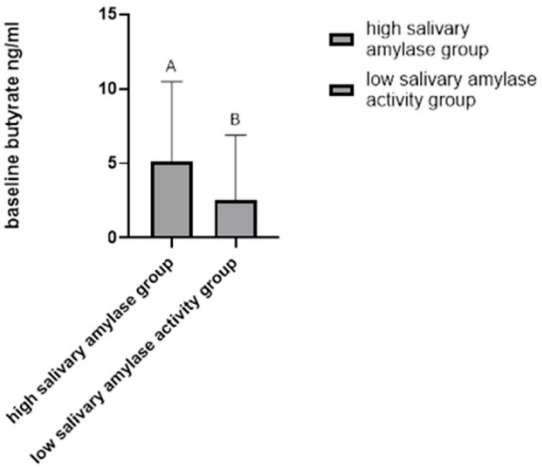

Figure 2. Basal butyrate level in participants with high vs. low salivary amylase activity. Women with high salivary amylase activity had significantly higher baseline butyrate levels compared to those with low salivary amylase activity (Mann–Whitney U = 44, $p < 0.05$). High salivary amylase (n = 30), low salivary amylase (n =30).

Participants with low salivary amylase activity on a low-starch diet showed a more pronounced increase in butyrate levels compared to those with caloric restriction (Mann–Whitney U = 59.50, $p < 0.05$) (Figure 3).

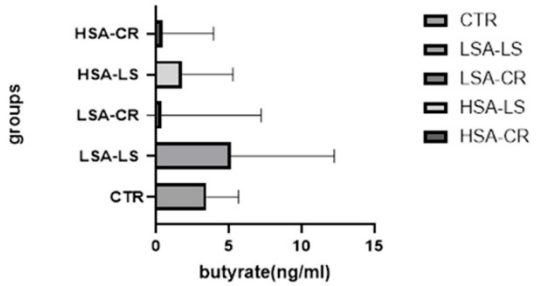

Figure 3. Butyrate levels after diet intervention by group. Low-starch diet with low salivary amylase activity (n = 15), low-starch diet with high salivary amylase activity (n = 15), calorie restriction diet with low salivary amylase activity (n = 15), calorie restriction diet with high salivary amylase activity (n = 15); control group (n = 7).

The statistical analysis identified significant differences between the intervention groups in butyrate concentrations, as outlined in Table 3, which presents key results including median values, interquartile ranges, and confidence intervals.

Table 3. Key statistical results.

Group	Median Butyrate Level (ng/mL) and 95% CI for Median Values	IQR (ng/mL)
Low-Starch Diet (Low Salivary Amylase) (n = 15)	5.140	0.7070–12.25
Caloric Restriction (Low Salivary Amylase) (n = 15)	3.90	0.246–7.225
Caloric Restriction (High Salivary Amylase) (n = 15)	0.47	0.29–3.955
Low-Starch Diet (High Salivary Amylase) (n = 15)	1.8	1.13–5.29
Control Group (Normal Weight) (n = 7)	3.47 (95% CI: 0.14–7.15)	-
Correlation	**ρ**	***p*-value**
Salivary Amylase Activity and Butyrate	0.0486	$p < 0.05$
Comparative Tests	**Mann–Whitney U**	***p*-value**
High vs Low Salivary Amylase Activity	44	$p < 0.05$
Low-Starch Diet vs Caloric Restriction (Low Salivary Amylase)	59.50	$p < 0.05$

In our study, we did not observe any significant effect of age on butyrate levels. However, it is worth noting that the women in our study had similar ages, which may have contributed to this lack of observed effect (Table 4).

Table 4. The effect of age on the lower and higher IQRs of butyrate levels in each group.

Group	Mean Age (Years) ± SD	Lower IQR Butyrate Level (ng/mL)	Higher IQR Butyrate Level (ng/mL)
Low-Starch Diet (Low Salivary Amylase)	28.8 ± 2.57	0.7070	12.25
Caloric Restriction (Low Salivary Amylase)	28.43 ± 3.59	0.246	7.225
Caloric Restriction (High Salivary Amylase)	30.2 ± 4.47	0.29	3.955
Low-Starch Diet (High Salivary Amylase)	29.33 ± 3.67	1.13	5.29
Control Group (Normal Weight)	28.57 ± 2.57	0.951	5.67

To further investigate the relationship between salivary amylase activity and insulin sensitivity, a linear regression analysis was conducted with salivary amylase activity as a

predictor and HOMA2-% S as the outcome variable. The regression analysis provided the following results (Table 5).

Table 5. A linear regression analysis between salivary amylase activity and insulin sensitivity.

Predictor	β	SE	*p*-Value (β)	95% CI	R^2	F (DF)	*p*-Value (Model)
Salivary Amylase Activity	0.435	0.12	<0.0003	[0.195, 0.675]	0.35	F (1, 34) = 5.5	0.0249

Coefficient (β): The estimated regression coefficient for salivary amylase activity was 0.435. This indicates that for each unit increase in salivary amylase activity, HOMA2-%S increases by 0.435 units.

Standard Error (SE): The standard error of the coefficient was 0.120.

p-Value (β): The *p*-value of the coefficient was less than 0.0003, indicating that the predictor is statistically significant.

95% Confidence Interval (CI): The 95% confidence interval for the coefficient was [0.195, 0.675].

The overall significance of the regression model was confirmed by an F-test.

F-Statistic: The F-statistic for the model was 5.5.

Degrees of Freedom (DF): The numerator degrees of freedom were 1, and the denominator degrees of freedom were 34.

p-Value (Model): The *p*-value for the F-test was 0.0249, indicating that the regression model is statistically significant.

The regression model explained a significant proportion of the variance in HOMA2-%S, with an R^2 of 0.35. This suggests that salivary amylase activity is a significant predictor of insulin sensitivity, accounting for 35% of the variability in HOMA2-%S (R^2 = 0.35), indicating a moderate fit among reproductive-age women.

2.2. Model Diagnostics

The residual analysis indicated that the assumptions of linearity, homoscedasticity, and normality of the residuals were reasonably met. There was no evidence of multicollinearity, as assessed by variance inflation factors (VIFs).

Furthermore, the results suggest that dietary modifications, specifically reducing dietary starch, could be an effective strategy to increase butyrate production and improve metabolic health in the low salivary amylase group.

3. Discussion

This study explores the link between salivary amylase activity, overweight, and glucose homeostasis, with an emphasis on how different diets impact these variables and the role of salivary amylase activity in these correlations.

The reason for selecting women of reproductive age as the study population was due to their unique hormonal profile, which can significantly influence metabolic processes and the composition of gut microbiota. Understanding this link in this demographic may offer insights into dietary strategies tailored to this specific group. However, it is crucial to recognize the broader applicability of these findings to males and women beyond reproductive age as well.

Butyrate's impact on metabolic health has been shown to be significant across different demographics. Studies have indicated that butyrate can improve insulin sensitivity, reduce inflammation, and enhance gut barrier function in both males and females, regardless of age [14]. For instance, research involving older adults has demonstrated that butyrate supplementation can improve metabolic markers and gut health, suggesting its beneficial effects extend beyond reproductive age [15]. Future studies should include these groups to provide a comprehensive understanding of butyrate's benefits across different demographics.

3.1. Butyrate and Adipocyte Health

Butyrate plays a crucial role in maintaining adipocyte health through multiple interconnected mechanisms. It exerts its effects primarily by modulating cellular processes that influence inflammation and metabolism. One key mechanism involves the inhibition of histone deacetylases (HDACs), which leads to increased histone acetylation and subsequent gene expression changes. This process results in the suppression of pro-inflammatory cytokines while enhancing the expression of anti-inflammatory genes [16].

Additionally, butyrate activates G protein-coupled receptors (GPCRs), such as GPR41 and GPR43, which play a significant role in immune regulation by modulating cytokine production [17]. This activation contributes to the overall anti-inflammatory environment. Butyrate also promotes the differentiation and function of regulatory T cells (Tregs), which are essential for maintaining immune tolerance and preventing excessive inflammatory responses [18].

Furthermore, butyrate strengthens the intestinal barrier by promoting the production of mucin and tight junction proteins. This enhancement reduces intestinal permeability and systemic inflammation, contributing to overall metabolic health [19]. Beyond these anti-inflammatory effects, butyrate stimulates adipogenesis, promotes lipid oxidation, and enhances mitochondrial function within adipocytes. These actions are crucial for maintaining a healthy balance between lipid storage and utilization, thereby preventing the excessive fat accumulation associated with obesity [4].

Overall, butyrate's multifaceted impact on inflammation, immune function, and metabolic processes underscores its importance in supporting adipocyte health and preventing metabolic disorders.

3.2. Enhancement of GIP Receptor Expression

GIP (Glucose-dependent Insulinotropic Polypeptide) is an incretin hormone produced by K-cells of the small intestine that stimulates insulin secretion in response to oral glucose intake. This plays a crucial role in glucose homeostasis, which could improve metabolic health by amplifying the gut response to GIP and its insulinotropic effects [20].

3.3. Enhancement of PYY Secretion

Peptide YY (PYY) is an anorexigenic hormone that suppresses appetite and is mainly secreted by L-cells in the distal ileum and colon in response to nutrient ingestion. The presence of butyrate in the gastrointestinal tract enhances the secretion of PYY, significantly regulating appetite and energy homeostasis [21].

3.4. Enhancement of GLP-1 Secretion

Butyrate stimulates GLP-1 (Glucagon-like Peptide-1) secretion primarily through the activation of GPCRs such as FFAR2 (GPR43) and FFAR3 (GPR41) on enteroendocrine L-cells in the intestine [22].

- Enhanced Insulin Sensitivity: GLP-1 receptors are present in adipose tissue. Activation of these receptors increases insulin sensitivity in adipocytes, improving glucose uptake and reducing blood glucose levels [23].
- Regulation of Lipolysis and Lipogenesis: GLP-1 signaling affects the balance between lipolysis and lipogenesis. It suppresses lipolysis, reducing the release of free fatty acids into circulation [24,25] This helps prevent lipid overload in tissues and organs, including adipocytes [26,27]
- Anti-inflammatory Effects: GLP-1 exhibits anti-inflammatory properties in adipocytes and adipose tissue by reducing the production of pro-inflammatory cytokines and promoting anti-inflammatory factors [28–30].
- Promotion of Adiponectin Release: GLP-1 can stimulate adiponectin secretion from adipocytes, enhancing insulin sensitivity and reducing inflammation [29].
- Indirect Effects on Body Weight: GLP-1 receptors, found in the pancreas, intestines, hypothalamus, and brainstem, are critical for regulating satiety and food intake. Acti-

vation of these receptors promotes feelings of fullness and reduces food consumption, contributing to weight loss [31].

3.5. Salivary Amylase Activity and Microbiome Composition

Salivary amylase activity critically shapes the composition of the gut microbiome. High activity enhances starch predigestion in the oral cavity, modifying the substrates available for microbial fermentation in the gut [32]. This alteration promotes the proliferation of butyrate-producing bacteria such as *Faecalibacterium prausnitzii* and *Roseburia* spp., adjusting the *Firmicutes*-to-*Bacteroidetes* ratio [33]. Our research indicates that individuals with higher salivary amylase activity exhibit elevated baseline butyrate levels, suggesting the potential for personalized diet strategies. For instance, individuals with low salivary amylase activity might benefit more from a low-starch diet, which favorably modifies their gut microbiota composition.

3.6. Influence of Hormonal Profile on Butyrate Production and Metabolic Outcomes

The hormonal profile of the study's demographic, particularly participants of reproductive age, likely played a significant role in modulating butyrate production and its subsequent effects on metabolic outcomes, and vice versa, short-chain fatty acids (SCFAs) may also influence female sex steroid hormone levels [34]. Estrogen and progesterone, two key hormones that fluctuate throughout the menstrual cycle, are known to influence gut microbiota composition and function, which may, in turn, impact butyrate levels.

In this study, butyrate levels were measured specifically during the follicular phase of the menstrual cycle, a period characterized by rising estrogen levels and relatively low progesterone.

Estrogen has been shown to promote the growth of beneficial gut bacteria, such as *Bifidobacteria* and *Lactobacilli*, which are associated with increased butyrate production. Higher estrogen levels, particularly during the follicular phase of the menstrual cycle, could enhance the abundance of these butyrate-producing bacteria, potentially leading to elevated butyrate concentrations. Butyrate, a short-chain fatty acid, plays a crucial role in maintaining gut health and has been linked to improved insulin sensitivity, reduced inflammation, and better overall metabolic profiles.

On the other hand, progesterone, which rises during the luteal phase, has been associated with changes in gut motility and a potential shift in the microbial community towards species that may not be as efficient in butyrate production. The interplay between estrogen and progesterone could therefore lead to fluctuations in butyrate levels throughout the menstrual cycle, which might partially explain the variability in metabolic outcomes observed in women of reproductive age.

Furthermore, the cyclical nature of these hormonal changes could result in periodic variations in gut microbiota composition and function, thereby influencing not only the production of butyrate but also its interaction with key metabolic pathways. For example, the protective effects of butyrate on glucose metabolism and lipid profiles might be more pronounced during phases of the menstrual cycle when estrogen levels are higher. Conversely, periods dominated by progesterone may attenuate these effects, leading to less consistent metabolic outcomes.

Given the complex and dynamic nature of hormone–microbiota interactions, it is important to consider the hormonal status of participants when interpreting the impact of butyrate on metabolic health. Future studies could benefit from more detailed monitoring of hormonal fluctuations and their direct impact on microbiota composition and butyrate production to better understand these interactions.

3.7. Dietary Strategies to Increase Butyrate

Increasing dietary fiber intake is a well-established method to elevate gut butyrate levels. Dietary fibers such as inulin, pectin, and resistant starch are fermented by gut bacteria and produce butyrate [15,35]. Recent research emphasizes the importance of specific diet

patterns, rich in fiber and polyphenols, in promoting butyrate production [36,37]. Diets rich in whole grains, fruits, and vegetables significantly boost butyrate production by providing substrates for microbial fermentation [38,39]. Moreover, dairy products like butter and milk can also contribute to butyrate levels. Butter contains butyrate directly, enhancing overall butyrate levels in the body [40]. Similarly, milk fat contains small amounts of butyrate and other SCFAs, which can increase gut butyrate levels upon consumption [41].

When formulating dietary recommendations, individual genetic variations, particularly those that affect salivary amylase activity, must be considered. Variations in the AMY1 gene, which encodes salivary amylase, lead to differences in enzyme activity between individuals. Those with higher salivary amylase activity tend to predigest starches more, altering the availability of substrate for fermentation and subsequently affecting butyrate production. Our study supports these observations, indicating that individuals with higher salivary amylase activity have elevated baseline butyrate levels. This suggests the potential for personalized dietary strategies, such as a low-starch diet for those with low salivary amylase activity, which favorably modify their gut microbiota composition. Personalized diet interventions based on genetic nuances ensure a more precise approach to enhancing health and metabolic outcomes.

The gut microbiome plays a crucial role in butyrate production. Butyrate-producing bacteria, such as *Faecalibacterium prausnitzii* and *Roseburia* spp., are essential for maintaining health and butyrate levels [42]. Recent advances in microbiome research highlight the importance of maintaining a diverse and balanced gut microbiota to support butyrate production [35].

3.8. Salivary Amylase Activity as a Predictor of Insulin Sensitivity

The regression coefficient (β = 0.435) indicates a positive relationship, where an increase in salivary amylase activity corresponds to an increase in HOMA2-% S. This finding aligns with previous research that highlights the metabolic implications of salivary amylase activity.

Higuchi R et al. (2020) reported that salivary amylase activity could affect postprandial glycemic responses [43].

The significance F-test (F = 5.5, p = 0.0249) confirms the overall model's validity, with an R^2 of 0.35 indicating that 35% of the variance in HOMA2-%S is explained by salivary amylase activity. This proportion of explained variance underscores the potential utility of salivary amylase as a biomarker for insulin sensitivity. While traditional methods like HOMA2-%S are valuable, they are invasive and can be influenced by external factors such as stress and circadian rhythms. Salivary amylase activity offers a less invasive alternative, which could enhance compliance and facilitate more frequent monitoring of insulin sensitivity.

Our study focused on reproductive-age women, a population with unique metabolic and hormonal profiles that can influence insulin sensitivity. Previous studies have shown that hormonal fluctuations during the menstrual cycle can impact insulin sensitivity and glucose metabolism.

3.9. Future Research Directions

Given the complex relationship between salivary amylase activity, overweight, and glucose homeostasis, future research should focus on several key areas to deepen our understanding and improve clinical outcomes:

- Salivary Amylase and Genetic Variability: Investigate the genetic factors influencing salivary amylase levels and their impact on glucose metabolism and obesity. Understanding individual genetic variability could lead to personalized dietary recommendations based on amylase activity.
- Longitudinal Studies on Glucose Homeostasis: Conduct long-term studies to assess how variations in salivary amylase activity affect glucose homeostasis over time. These studies should explore how early-life amylase activity levels may predict the development of metabolic disorders such as type 2 diabetes.

- Interventions Targeting Amylase Activity: Explore potential interventions that can modulate salivary amylase activity, such as dietary changes, medications, or lifestyle modifications. Understanding how these interventions affect glucose regulation and body weight could lead to new strategies for preventing and managing obesity and related metabolic conditions.
- Interactions with the Microbiome: Investigate the relationship between salivary amylase activity, the oral and gut microbiomes, and glucose metabolism. This research could reveal how microbial composition and function are influenced by amylase activity, potentially leading to microbiome-targeted therapies for metabolic health.
- Clinical Trials: Design and implement large-scale clinical trials to evaluate the effectiveness of amylase-based biomarkers in predicting metabolic risk and the impact of targeted interventions on glucose homeostasis and obesity. These trials should include diverse populations to ensure the broad applicability of findings.

3.10. Study Strengths and Limitations

The following are some strengths and limitations of this study:

- Innovative Focus: This study's exploration of the link between salivary amylase activity and metabolic health is relatively novel, particularly its emphasis on dietary impacts tailored to salivary enzyme activity. This innovative focus adds valuable knowledge to the field of personalized nutrition and metabolic health.
- Comprehensive Insights: The detailed discussion on butyrate's multiple mechanisms of action in maintaining adipocyte health and improving glucose homeostasis is a strength. It provides a thorough understanding of how butyrate influences metabolic processes, enhancing its therapeutic potential.
- Potential for Personalized Nutrition: By investigating the relationship between salivary amylase activity and gut microbiota composition, the study creates opportunities for the development of personalized dietary strategies. This aspect is crucial for developing more targeted interventions for metabolic disorders.
- Population Specificity: While the focus on women of reproductive age is valuable, it limits the generalizability of the findings. The study's results may not fully apply to males or individuals outside this age group, requiring careful consideration when generalizing these conclusions to broader populations.
- Cross-sectional Design: The study's cross-sectional nature limits the ability to infer causality. Longitudinal studies would be necessary to establish a clearer cause-and-effect relationship between salivary amylase activity, dietary patterns, and metabolic outcomes.
- Genetic Variability Considerations: While the study acknowledges the role of genetic variability in salivary amylase activity, it does not deeply explore the genetic factors that could influence these metabolic outcomes. Future research should explore the genetic influences on salivary amylase activity to enhance the personalization of dietary recommendations.
- Microbiome Analysis Limitations: Although the study discusses the impact of salivary amylase on gut microbiota, it lacks direct microbiome analysis. Future studies incorporating detailed microbiome profiling would strengthen the findings and provide more robust insights into the relationship between salivary amylase, diet, and metabolic health.

4. Materials and Methods

4.1. Study Design and Participants

This study was carried out with a cohort of 67 women of reproductive age (18–45 years) recruited from the multi-profile medical center, "Health Center 4". The sample size of 67 participants was determined based on a power analysis conducted prior to data collection.

Specifically, we aimed to detect a medium effect size (Cohen's d $\approx$ 0.5) with a power of 0.80 and an alpha level of 0.05. The choice of a medium effect size was informed by previous studies in this domain, which suggested that the expected effects would not be

large due to the complexity of the variables involved. Each experimental group consisted of 15 participants, while the control group included 7 participants. Despite the smaller sample size in the control group, we employed statistical methods, including sensitivity analyses, which are more resilient to differences in sample size.

Participants were selected by an endocrinologist based on specific inclusion criteria: body mass index (BMI) between 25.0 and 29.9 kg/m^2, no history of chronic diseases, and currently no medications that could affect metabolic outcomes. Additionally, a control group was formed consisting of individuals with normal weight, defined as having a BMI between 18.5 and 24.9 kg/m^2.

The study involved a 12-week dietary intervention period.

4.2. Dietary Interventions

Participants were randomly assigned to one of two dietary intervention groups for 12 weeks.

Low-Starch Diet Group (LS): Participants in this group followed a low-starch diet, focusing on the consumption of low-glycemic-index vegetables, proteins, and healthy fats. Daily starch intake was limited to less than 50 grams [44].

Caloric Restriction Group (CR): Participants in this group followed a caloric restriction diet, reducing their daily caloric intake by 500 kcal from their estimated energy requirement, calculated based on the Harris–Benedict equation [45].

Control Group with Normal Weight (CTR): This group consisted of participants with normal weight who maintained their usual dietary habits without any specific diet intervention. Their caloric intake was not restricted or modified, serving as a baseline to assess natural variation in butyrate levels and other metabolic markers [46].

Dietary adherence was monitored through a weekly food diary and weekly online consultations with an endocrinologist.

4.3. Evaluation of Salivary Amylase Activity

Salivary amylase activity was measured using the Salimetrics Amylase Activity Assay (Salimetrics, State College, PA, USA). Unstimulated saliva samples were collected in the morning after a fast overnight to ensure consistency. Saliva samples were collected at baseline. The activity of saliva amylase was determined according to the manufacturer's protocol. Participants were classified into high salivary amylase activity groups (HSA) and low salivary amylase activity (LSA) groups based on the median split of amylase activity data.

4.4. Measurement of Butyrate Levels

Blood samples were taken at the start of the study and at the end of the 12-week intervention period. Plasma butyrate concentrations were measured using the Butyric Acid ELISA Kit from Abbexa Ltd. Briefly, blood samples were centrifuged at 3000× *g* for 10 min at 4 ° C, and plasma was stored at −80 °C until analysis. Butyrate was extracted from plasma using an acidified water–ether solution (0.5 mL of 0.1 M hydrochloric acid in water mixed with 2.5 mL of ether). Butyrate levels were quantified based on calibration curves prepared using standard solutions [47].

4.5. Measurement of Insulin Sensitivity

Insulin sensitivity was assessed using the Homeostasis Model Assessment of Insulin Sensitivity (HOMA2-%S), which was calculated from fasting plasma glucose and C-peptide levels using the HOMA2 Calculator (Diabetes Trials Unit, University of Oxford).

4.6. Statistical Analysis

Data were analyzed using GraphPad Prism 10 software (GraphPad Software, San Diego, CA, USA). Given that the data did not follow a normal distribution, nonparametric tests were employed. The Shapiro–Wilk test was used to assess the normality of the

data distributions. The Mann–Whitney U test was used to compare differences in basal butyrate levels between the high and low salivary amylase groups. Correlations between salivary amylase activity and butyrate concentrations were evaluated using Spearman's rank correlation coefficient. A linear regression analysis was then performed to evaluate the predictive value of salivary amylase activity on the HOMA2-%S. The significance level was set at $p < 0.05$ for all analyses.

Descriptive statistics are presented as medians and interquartile ranges (IQRs) for continuous variables.

4.7. Ethics Statement

The study was carried out according to the Declaration of Helsinki and was approved by the Ethics Committee of Riga Stradiņš University (Ethical Committee number: 22-2/479/2021). Written informed consent was obtained from all participants prior to enrollment in the study.

5. Conclusions

This study demonstrates the link between salivary amylase activity, overweight, and glucose homeostasis. Baseline butyrate was higher in individuals with elevated salivary amylase activity, indicating a potential influence of this enzyme on gut microbiota metabolism. Post-intervention, butyrate production varied significantly across dietary groups, with low-starch diets and low salivary amylase activity showing increased butyrate levels, suggesting that salivary amylase activity influences gut microbiota composition, with differences observed depending on the type of diet. A weak but statistically significant correlation ($\rho = 0.0486$, $p < 0.05$) was found between salivary amylase activity and butyrate levels, supported by comparative tests showing differences in butyrate production across dietary patterns. Additionally, higher salivary amylase activity correlated with improved insulin sensitivity ($\beta = 0.435$, $R^2 = 0.35$), highlighting its potential as a biomarker for metabolic health.

Future research should explore the mechanisms underlying these findings and validate them in larger, diverse populations to enhance personalized nutrition strategies targeting gut and metabolic health.

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Institutional Review Board Statement: The study protocol was approved by the Ethics Committee of Riga Stradiņš University (Ethics Committee number: 22-2/479/2021).

Informed Consent Statement: All participants provided their written informed consent in accordance with the Declaration of Helsinki.

Data Availability Statement: Data are contained within the article.

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Conflicts of Interest: The authors declare no conflicts of interest.

Abbreviations

AMY1—alfa amylase 1; AMPK—adenosine monophosphate-activated protein kinase; CTR—control group; GIP—Glucose-dependent Insulinotropic Polypeptide; GLP-1—Glucagon-like Peptide-1; GLP-1(7-37)—Active Glucagon-like Peptide-1; GPCRs—G protein-coupled receptors; HSA-CR—High Salivary Amylase Activity Calorie Restriction Diet Group; HSA-LS—High Salivary Amylase Activity Low-Starch Diet Group; LPS—lipopolysaccharides; LSA-CR—Low Salivary Amylase Activity Calorie Restriction Diet group; LSA-LS—Low Salivary Amylase Activity Low-Starch Diet group; PYY—Peptide YY; SCFA—short-chain fatty acid; Tregs—regulatory T cells; xg—times the force of gravity.

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Decoding metabolic connections: the role of salivary amylase activity in modulating visceral fat and triglyceride glucose index

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Abstract

Background Salivary amylase activity (SAA) is recognized as a potential biomarker for metabolic health. Previous studies suggest an association between SAA and insulin sensitivity, but the mechanisms remain unclear. This study investigates the relationship between SAA, visceral fat (VF), and the triglyceride-glucose (TyG) index to clarify the pathways linking SAA to metabolic risk factors.

Methods This cross-sectional study analysed data from women of reproductive age who were classified as overweight. Linear regression models were used to assess associations between salivary amylase activity (SAA), visceral fat (VF) and the triglyceride-glucose (TyG) index, while adjusting for confounding variables such as age, body mass index (BMI), physical activity and dietary patterns. Mediation analysis was conducted to determine whether VF mediates the relationship between SAA and the TyG index.

Results Higher SAA was inversely associated with VF ($\beta = -0.45$, 95% CI: -0.65 to -0.25, $p < 0.001$). No direct association was observed between SAA and TyG index ($\beta = -0.10$, 95% CI: -0.25 to 0.05, $p = 0.18$) after adjustment for covariates. Mediation analysis revealed that visceral fat significantly mediated the relationship between SAA and the TyG index. The indirect effect of SAA on the TyG index through VF (A × B) was statistically significant ($\beta = -0.16$, 95% CI: -0.26 to -0.08), accounting for 45% of the total effect.

Conclusions These findings suggest that higher SAA may confer metabolic benefits by reducing VF, thereby indirectly influencing the TyG index. This highlights the critical role of VF in mediating the protective effects of SAA on metabolic health and provides insights into potential pathways for intervention.

Keywords Salivary amylase activity, Visceral fat, Triglyceride-glucose index, Metabolic health, Mediation analysis, Overweight, Biomarkers

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Background

Salivary amylase activity (SAA), an enzyme that initiates starch hydrolysis in the oral cavity, has attracted interest due to its potential influence on metabolic health. Emerging evidence suggests that variations in SAA activity can modulate key metabolic parameters, including visceral fat (VF) accumulation and insulin resistance (IR), thus contributing to individual differences in metabolic risk [1, 2].

Visceral adiposity, a well-established marker of metabolic risk, is strongly associated with IR and the development of cardiovascular diseases. The TyG index has emerged as a robust surrogate for assessing IR, with recent studies highlighting its strong correlation with visceral fat in diverse populations [3].

However, the relationship between SAA activity and metabolic health remains controversial. Some studies suggest that elevated SAA is associated with enhanced glycaemic control after starch ingestion, implying a protective metabolic role [2]. For example, a study in overweight and obese non-diabetic Qatari women demonstrated that higher SAA activity correlated with lower adiposity (BMI, waist, and hip circumference), elevated High-Density Lipoprotein cholesterol, and improved markers of systemic inflammation, such as reduced levels of CRP, TNF-α, and IL-6, as well as increased adiponectin and ghrelin levels [4]. On the contrary, other research has linked higher SAA activity with increased visceral adiposity and IR, indicating a potentially adverse metabolic impact [5].

These disparate findings suggest that SAA may exert sex- and adiposity-dependent effects on metabolic regulation, highlighting the complexity of its role in metabolic health.

This study aims to elucidate the associations between SAA activity, VF, and the TyG index in women of reproductive age classified as overweight. By investigating these interrelationships, we seek to clarify the mechanistic role of SAA in the modulation of metabolic risk and contribute to the identification of novel biomarkers for metabolic health.

Methods

Study design and participants

This cross-sectional study was conducted to evaluate the relationship between SAA, VF, and the TyG index. The study was carried out in Riga, Latvia, with participants recruited from a health centre during routine health check-ups. Importantly, these individuals were not patients but volunteers who agreed to participate in the study.

A total of 67 overweight women of reproductive age (18–45 years) with a BMI between 25 and 29.9 kg/m^2 were included in the study.

Exclusion criteria

Participants were excluded if they met any of the following criteria: pregnancy or lactation, a diagnosed chronic disease (e.g., diabetes, cardiovascular disease), or the use of medications that could affect metabolism.

The study protocol was approved by the Ethics Committee of Riga Stradiņš University (Ethics Committee number: 22 – 2/479/2021), and all participants provided written informed consent in accordance with the Declaration of Helsinki.

Participant assessments

Age Verified using the participant's identification card and recorded in years.

Body Mass Index (BMI): Calculated as weight (kg) divided by height squared (m^2), using measurements taken with a calibrated scale and stadiometer.

Physical activity Assessed using the International Physical Activity Questionnaire (IPAQ)] and expressed as metabolic equivalent task (MET) minutes per week.

Dietary intake: Evaluated through a 24-hour dietary recall or food frequency questionnaire (FFQ), with macronutrient composition analyzed using the Finelli database.

Salivary amylase activity measurement

Unstimulated saliva samples were collected from participants following overnight fasting. The samples were processed within one hour after collection and stored at -80 ° C until analysis. Salivary amylase activity was quantified using an enzymatic kinetic assay based on the hydrolysis of a chromogenic substrate, with results expressed in U/mL.

Classification of high vs. low salivary amylase activity

To classify participants into high and low salivary amylase activity groups, we used the median value of salivary amylase activity within the study population as a cut-off point. Participants with values above the median were classified as having high activity, while those below the median were classified as having low activity.

Visceral fat measurement

VF was estimated using a bioimpedance analysis scale (BIA) (Omron BF511, Omron Healthcare, Kyoto, Japan). Participants were instructed to stand barefoot on the device, ensuring proper electrode contact, and to hold the handgrips for accurate impedance measurement. The measurement was performed in a fasting state (e.g., after an overnight fast) and following the manufacturer's guidelines. The device generates a visceral fat score using proprietary algorithms, which serves as an indicator of visceral fat percentage.

Triglyceride-glucose index calculation

The TyG index is a widely recognized marker of insulin resistance. In this study, the TyG index was calculated using a glucose-triglyceride calculator, where fasting glucose and triglyceride concentrations were entered in mmol/L, as these were the measurement units used in our laboratory.

This formula is an adaptation of the original calculation based on mg/dl units:

TyG index = ln (fasting triglycerides (mg/dl) x fasting glucose (mg/dl)/2.

To ensure consistency with the original method, we utilized direct calculations in mmol/L, thereby avoiding unit conversion. For authoritative references on the TyG index, refer to Guerrero-Romero et al. [6].

Fasting blood samples were collected for triglyceride and glucose measurements using standardized enzymatic methods. All laboratory analyses were conducted by blinded personnel to prevent bias.

Dietary intervention and nutritional composition

Participants followed a diet intervention for a 12-week period prior to metabolic evaluations. The low-starch diet prioritized non-starchy vegetables, lean protein sources, nuts, seeds, and healthy fats while minimizing the intake of grains and starchy vegetables. The calorie-restricted diet maintained a balanced macronutrient distribution but with a controlled reduction in total energy intake. Both dietary regimens were designed to ensure adequate micronutrient intake primarily through whole foods, with supplementation provided when necessary to meet recommended daily allowances (Table 1).

Table 1 Macronutrient and micronutrient composition of dietary interventions

Nutrients	Low-Starch Diet (per day)	Calorie-Restricted Diet (per day)
Energy (kcal)	~1800	~1200
Carbohydrates (%)	25–30	40–45
Carbohydrates (g)	110–135	120–150
Starch (g)	< 50	70–90
Sugars (g)	40–50	45–55
Fiber (g)	25–35	20–30
Protein (%)	25–30	20–25
Protein (g)	110–135	70–90
Fat (%)	40–45	30–35
Fat (g)	80–100	40–60
Saturated fat (g)	15–20	10–15
Monounsaturated fat (g)	30–40	15–25
Polyunsaturated fat (g)	15–20	10–15
Omega-3 (g)	1.5–2.5	1.0–2.0
Omega-6 (g)	8–12	6–10
Cholesterol (mg)	< 300	< 200
Sodium (mg)	2000–2500	1500–2000
Potassium (mg)	3500–4000	3000–3500
Calcium (mg)	1000–1200	900–1100
Magnesium (mg)	350–450	300–400
Iron (mg)	12–15	10–12
Zinc (mg)	10–12	8–10
Vitamin C (mg)	75–100	60–80
Vitamin D (IU)	800–1000	600–800
Vitamin B12 (µg)	2.4–3.0	2.0–2.5

Statistical analysis

Data were analyzed using GraphPad Prism 10 and R v4.4.2. Data normality was assessed using the Shapiro-Wilk test and visual inspection of histograms and Q-Q plots. Continuous variables were expressed as mean ± standard deviation (SD) or median (interquartile range, IQR) based on data distribution. Categorical variables were presented as frequencies (percentages).

To examine the associations between salivary amylase activity (SAA), visceral fat (VF), and the triglyceride-glucose (TyG) index, we conducted multivariable linear regression models adjusted for potential confounders, including age, BMI, physical activity, and dietary intake. The regression model was specified as follows: $Y = \beta 0 + \beta 1 X + \beta 2 C + \varepsilon$.

Where *Y represents* the dependent variable (VF% or TyG index), *X* is the independent variable (SAA), *C* represents covariates, and ε is the error term. The strength of association was reported using standardized regression coefficients (β), 95% confidence intervals (CI), and p-values. Effect sizes were interpreted according to Cohen's guidelines, where $\beta \geq 0.1$ was considered a small effect, $\beta \geq 0.3$ a moderate effect, and $\beta \geq 0.5$ a large effect. We employed mediation analysis to assess whether VF% mediates the association between SAA and the TyG index, using structural equation modeling (SEM) with the lavaan package in R. The total effect (c), direct effect (c′), and indirect effect (a × b) were estimated using bootstrapping with 5,000 resamples to derive bias-corrected 95% confidence intervals (BCa 95% CI). A mediation effect was considered statistically significant if the 95% CI of the indirect effect did not include zero.

The proportion of mediation was calculated as $a \times b/c$, where a represents the effect of SAA on VF%, b represents the effet of VF% on TyG index, and c represents the total effect of SAA on TyG index.

Ethical considerations

All procedures were conducted in accordance with the Declaration of Helsinki. Data confidentiality was maintained by de-identifying participant information and securely storing it in a password-protected database. Participants were informed of their right to withdraw at any time without consequences.

Table 2 Baseline characteristics of the study population

Characteristic	Mean ± SD / Median (IQR)	Range	*n* (%)
Age (years)	29.18 ± 3.58	25–45	—
Gender -(Female)	67	—	100%
BMI (kg/m²)	27.8 ± 3.5	20.1–35.6	—
VF%	15.3 (12.1–18.5)	10.0–25.0	—
SAA (U/mL)	27.77 (10.64–56.24)	10.64–56.24	—
TyG Index	4.425	4.050–5.110	—
Physical activity	—	—	Sedentary:15 (22%) Moderate:35 (52%), Vigorous: 17 (26%)
Diet	-	-	Low starch: 30(50%) Calorie restriction:30(50%)

Table 3 Association between SAA, visceral fat, and TyG index (Multivariable linear Regression)

Predictor Variable	Outcome Variable	β Coefficient	95% CI	*p*-value
SAA	VF%	-0.45	-0.65 to -0.25	< 0.001
SAA	TyG Index	-0.10	-0.25 to 0.05	0.18
VF%	TyG Index	0.48	0.30 to 0.65	< 0.001

Results

Participant characteristics

A total of 67 participants were included in the analysis. The mean age was 29.18 ± 3.58 years, the mean BMI was 27.8 ± 3.5 kg/m².

There were no significant differences in baseline characteristics between participants with high and low salivary amylase activity (Table 2).

Correlation analyses

Salivary amylase activity (SAA) exhibited a significant inverse correlation with visceral fat percentage (VF%) (r = -0.301, 95% CI: -0.481 to -0.102, p = 0.026). However, no significant correlation was observed between SAA and the triglyceride-glucose (TyG) index (r = 0.125, 95% CI: -0.250 to 0.452, p = 0.438). In contrast, VF% demonstrated a significant positive correlation with the TyG index (r = 0.479, 95% CI: 0.215 to 0.679, p < 0.001).

Table 4 Mediation analysis: indirect effects of SAA on TyG index via visceral fat

Pathway	Effect Estimate (β)	95% CI	*p*-value	Proportion of Total Effect (%)
Direct Effect (SAA → TyG)	-0.10	-0.25 to 0.05	0.18	—
Indirect Effect (SAA → VF → TyG)	-0.16	-0.26 to -0.08	< 0.001	45%
Total Effect	-0.26	-0.37 to -0.15	< 0.001	100%

Multivariable regression

After adjusting for confounding variables, SAA remained a significant predictor of VF% (β = -0.45, 95% CI: -0.65 to -0.25, p < 0.001). In contrast, SAA was not a significant predictor of the TyG index (β = -0.10, 95% CI: -0.25 to 0.05, p = 0.18). VF% was independently associated with the TyG index (β = 0.48, 95% CI: 0.30 to 0.65, p < 0.001) (Table 3).

Regression Model Specifications:

The following linear regression models were used:

1. VF% as the dependent variable:
2. TyG index as the dependent variable:

Mediation analysis

A mediation analysis was conducted to examine whether VF% mediated the relationship between SAA and the TyG index. The indirect effect of SAA on the TyG index through VF% was statistically significant (β = -0.16, 95% CI: -0.26 to -0.08) (Table 4; Fig. 1).

We employed a bootstrapping approach with 5,000 resamples to estimate confidence intervals for indirect effects. The mediation model is represented as follows:

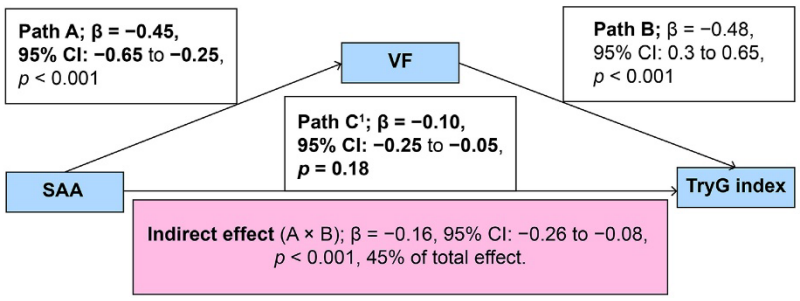

Fig. 1 Mediation analysis: indirect effects of SAA on TyG index via visceral fat

1. Effect of SAA on VF% (Path A):
2. Effect of VF% on TyG index (Path B):
3. Total effect of SAA on TyG index (Path C):
4. Direct effect of SAA on TyG index (Path C'):

Discussion

The findings of this study provide important insights into the role of SAA as a potential predictor of metabolic health. The inverse association between SAA and visceral fat, combined with the absence of a direct association between SAA and the TyG index, suggests a nuanced relationship in which VF mediates the effect of SAA on metabolic outcomes. Specifically, the mediation analysis revealed that 45% of the total effect of SAA on the TyG index could be attributed to VF, underscoring the critical role of adipose tissue distribution in linking SAA with IR.

The observed inverse association between SAA and VF aligns with emerging evidence suggesting that higher SAA is indicative of enhanced carbohydrate metabolism efficiency. SAA, an enzyme primarily responsible for starch digestion, has been hypothesized to contribute to glycemic regulation by modulating postprandial glucose responses. Previous studies have shown that individuals with higher SAA activity exhibit improved glucose tolerance, likely through a faster onset of carbohydrate digestion and absorption, which reduces prolonged postprandial hyperglycemia [7]. These effects may indirectly influence adiposity by reducing insulin demand and attenuating lipogenesis, mechanisms that warrant further investigation [8].

Contrary to the inverse association with VF%, no direct relationship between SAA and the TyG index was observed after adjusting for covariates. The lack of direct association in our study suggests that the influence of SAA on IR is primarily mediated by its effects on adiposity. This hypothesis is supported by the significant indirect effect of SAA on the TyG index through VF. Visceral adiposity, known for its endocrine activity and pro-inflammatory cytokine secretion, is a well-established mediator of metabolic dysfunction [9]. Our results extend this understanding by highlighting SAA as a potential upstream determinant of VF accumulation and, consequently, IR.

The mediation effect identified in this study underscores the importance of targeting visceral adiposity in metabolic health interventions. Given that SAA activity is influenced by dietary and genetic factors, it may represent a modifiable marker for predicting and managing visceral obesity. Emerging evidence suggests that dietary interventions targeting carbohydrate digestion, such as low-glycemic index diets, may modulate salivary amylase (SAA) activity and subsequently impact metabolic health outcomes [10].

From a mechanistic perspective, the link between SAA and VF may involve multiple pathways. Higher SAA activity facilitates efficient starch hydrolysis, leading to a rapid glucose supply and possibly influencing insulin dynamics [11]. Insulin, in turn, plays a critical role in regulating adipose tissue deposition. Additionally, SAA may exert systemic effects beyond digestion, as evidenced by its association with inflammatory markers and oxidative stress in studies examining stress physiology and metabolic health [12]. The interplay between these mechanisms and VF metabolism merits further exploration through experimental and longitudinal studies.

Potential mechanisms and unaccounted confounding variables

Several potential confounders could influence the observed relationships in our study. Genetic predisposition, particularly polymorphisms in the AMY1 gene, has been linked to interindividual variability in SAA activity and metabolic outcomes. Individuals with a higher AMY1 gene copy number tend to have elevated SAA activity, which may contribute to more efficient carbohydrate metabolism and lower risk of obesity and IR. Additionally, hormonal regulators such as insulin, cortisol, and catecholamines are known to modulate both SAA secretion and fat metabolism. Cortisol, for instance, is implicated in central fat accumulation, while catecholamines influence lipolysis. While our study adjusted for physical activity and dietary intake, these hormonal and genetic influences were not directly assessed, representing a limitation. Future studies incorporating genetic screening and endocrine profiling could provide further clarity on these mechanisms.

Clinical implications

Given the observed associations, SAA may serve as a novel biomarker for metabolic risk assessment. As SAA activity is influenced by dietary and genetic factors, it represents a potentially modifiable target for preventing or managing visceral obesity.

- Dietary interventions: Emerging evidence suggests that dietary modifications can influence salivary amylase (SAA) activity [10].
- Genetic screening: Assessing AMY1 gene copy number could help identify individuals with higher or lower SAA activity, enabling personalized dietary strategies.
- Metabolic risk prediction: Measuring SAA levels in clinical settings may improve early identification of individuals at risk for visceral obesity and insulin resistance.

In conclusion, this study contributes to the growing body of evidence on the metabolic relevance of SAA activity. The inverse association between SAA and VF%, along with the mediating role of visceral adiposity in the relationship between SAA and the TyG index, highlights the complex pathways linking carbohydrate digestion with systemic metabolic health. These findings support the potential utility of SAA as a biomarker for assessing metabolic risk and emphasize the need for targeted strategies to mitigate visceral adiposity in improving metabolic outcomes. Future research should aim to elucidate the molecular mechanisms underlying these associations and explore the translational potential of modulating SAA activity for metabolic health optimization.

Strengths and limitations

Strengths

Novel Insights: This study provides novel evidence linking SAA with VF% and the TyG index, contributing to the understanding of SAA as a potential biomarker for metabolic health.

Robust Methodology: The use of a mediation analysis allowed for a detailed investigation of the indirect effect of visceral fat, providing deeper insights into the pathways connecting SAA with IR.

Comprehensive Adjustment for Covariates: By controlling for a wide range of covariates, the study ensures that the observed associations are independent of confounding variables.

Clinical Relevance: The findings highlight the significance of VF as a mediator in metabolic dysfunction, suggesting potential avenues for targeted interventions and therapeutic strategies.

Limitations

Cross-Sectional Design: The cross-sectional nature of the study limits the ability to infer causality. Longitudinal studies are needed to confirm the directionality of the observed associations.

Unaccounted Confounders: While genetic and hormonal factors may influence SAA activity and metabolic outcomes, they were not directly assessed in this study. Future research should incorporate genetic profiling (e.g., AMY1 polymorphisms) and hormonal markers (e.g., insulin, cortisol, catecholamines) to better elucidate these relationships.

Lack of Mechanistic Data: Although mediation analysis suggests a pathway involving visceral fat, direct mechanistic studies were not performed to explore how SAA influences VF metabolism or IR.

Population Specificity: The study population may not be representative of broader demographics, such as different age groups, ethnicities, or individuals with pre-existing metabolic disorders, potentially limiting the generalizability of the findings.

Potential Measurement Bias: While SAA was assessed as a proxy for enzymatic function, factors such as hydration status, circadian variation, and acute stress, which can influence SAA, were not accounted for.

Limited Exploration of Dietary Influence: Although dietary factors were adjusted as covariates, the study did not comprehensively analyze how specific dietary patterns or macronutrient compositions might modulate SAA activity and its downstream effects.

Future directions Addressing these limitations in future research is essential for validating and expanding upon our findings. Longitudinal studies and interventional trials exploring the genetic and hormonal regulation of SAA activity could provide deeper mechanistic insights. Additionally, dietary intervention studies investigating the impact of carbohydrate intake patterns on SAA activity and metabolic outcomes would help translate these findings into practical applications for metabolic health optimization.

Conclusions

This study highlights the significant role of SAA in metabolic regulation, specifically its relationship with visceral adiposity and IR. Higher SAA was inversely associated with VF%, suggesting a potential protective role of enhanced carbohydrate digestion efficiency against visceral fat accumulation. While no direct association was observed between SAA and the TyG index, VF was found to mediate the relationship, accounting for a substantial proportion of the total effect. These findings underscore the complex interplay between enzymatic activity, adipose tissue distribution, and metabolic health.

The results suggest that SAA may serve as a novel biomarker for assessing VF-related metabolic risk and highlight visceral adiposity as a critical target for intervention. Future studies should explore the mechanistic pathways linking SAA with metabolic outcomes and investigate the potential of dietary and lifestyle modifications to optimize SAA activity for improving metabolic health. Ultimately, this research contributes to the broader understanding of metabolic regulation and offers insights into strategies for mitigating metabolic disorders through personalized approaches.

Abbreviations

BMI	Body Mass Index
IR	Insulin resistance
SAA	Salivary amylase activity
TyG	Triglyceride-glucose index
VF	Visceral fat

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Declarations

Ethics approval and consent to participate
The study protocol was approved by the Ethics Committee of Rigas Stradiņš University (Lthics Committee number: 22 – 2/479/2021).

Consent for publication
All participants provided their written informed consent in accordance with the Declaration of Helsinki.

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The authors declare no competing interests.

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Review

Salivary α-Amylase as a Metabolic Biomarker: Analytical Tools, Challenges, and Clinical Perspectives

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Abstract

Salivary α-amylase, primarily encoded by the AMY1 gene, initiates the enzymatic digestion of dietary starch in the oral cavity and has recently emerged as a potential biomarker in metabolic research. Variability in salivary amylase activity (SAA), driven largely by copy number variation of AMY1, has been associated with postprandial glycemic responses, insulin secretion dynamics, and susceptibility to obesity. This review critically examines current analytical approaches for quantifying SAA, including enzymatic assays, colorimetric techniques, immunoassays, and emerging biosensor technologies. The methodological limitations related to sample handling, intra-individual variability, assay standardization, and specificity are highlighted in the context of metabolic and clinical studies. Furthermore, the review explores the physiological relevance of SAA in energy homeostasis and its associations with visceral adiposity and insulin resistance. We discuss the potential integration of SAA measurements into obesity risk stratification and personalized dietary interventions, particularly in individuals with altered starch metabolism. Finally, the review identifies key research gaps and future directions necessary to validate SAA as a reliable metabolic biomarker in clinical practice. Understanding the diagnostic and prognostic value of salivary amylase may offer new insights into the prevention and management of obesity and related metabolic disorders.

Keywords: salivary α-amylase; *AMY1* gene; metabolic biomarker; glucose homeostasis; visceral adiposity; starch metabolism; enzymatic assay; biomarker standardization; personalized nutrition

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1. Introduction

Obesity is a complex, multifactorial disease characterized by a chronic state of metabolic dysregulation [1–3]. It is strongly associated with insulin resistance, impaired glucose homeostasis, low-grade inflammation, and increased cardiometabolic risk [4,5]. The global rise in obesity prevalence underscores the urgent need for reliable, non-invasive biomarkers that can facilitate early detection of metabolic disturbances and guide personalized intervention strategies [6,7].

Recent studies have drawn attention to salivary α-amylase (SAA)—an enzyme predominantly produced by the salivary glands and encoded by the AMY1 gene—as a potential metabolic biomarker [8,9]. Traditionally recognized for its role in the initial digestion of dietary starch in the oral cavity, SAA has emerged as a functionally relevant modulator of postprandial glycemia and insulin dynamics [10–12]. Interindividual variability in SAA, largely driven by AMY1 gene copy number variation [13], has been associated with dif-

ferences in glycemic response, visceral adiposity, and susceptibility to obesity and related metabolic disorders [14].

This review aims to summarize the physiological and metabolic roles of salivary amylase in the context of obesity and glucose regulation, critically evaluate current analytical approaches for measuring SAA, highlight key methodological challenges that hinder its clinical implementation, and explore the translational potential of SAA as a biomarker for metabolic risk stratification and personalized nutritional interventions.

By integrating recent advances in physiology, analytical biochemistry, and clinical research, this review seeks to clarify the role of SAA at the interface between oral digestion and systemic metabolic regulation in obesity.

The relevance of SAA as a metabolic biomarker has gained attention due to its ease of measurement from non-invasive saliva samples, making it an attractive candidate for clinical and research applications. However, substantial heterogeneity exists in the methodologies used to quantify SAA, and questions remain regarding the biological interpretation, reproducibility, and standardization of these measurements across populations and study designs.

2. Biochemical and Physiological Background

2.1. Structure and Function of SAA

Salivary α-amylase (SAA) is a calcium-dependent endo-enzyme belonging to the glycoside hydrolase family 13 (GH13) and is classified under EC 3.2.1.1 in the BRENDA enzyme database [15]. It catalyzes the hydrolysis of α-1,4-glycosidic linkages in polysaccharides such as starch, glycogen, and related oligosaccharides, generating maltose, maltotriose, and limit dextrins [16]. Although EC 3.2.1.1 includes both salivary and pancreatic isoforms, SAA is encoded by the AMY1 gene and is synthesized and secreted primarily by the acinar cells of the parotid and submandibular glands [17]. The enzyme comprises multiple domains, including a catalytic (β/α)-barrel domain (Domain A), a small Domain B contributing to substrate binding and calcium coordination, and Domain C, which is implicated in structural stabilization and substrate specificity [18].

In humans, two isoforms of α-amylase are encoded by the AMY1 (salivary) and AMY2 (pancreatic) gene clusters located on chromosome 1p21 [19]. The SAA, a glycoprotein with a molecular mass of approximately 56–62 kDa, is the predominant isoenzyme in the oral cavity that serves as the first enzymatic step in dietary carbohydrate digestion [20]. Beyond its digestive role, emerging evidence suggests that SAA may influence postprandial glycemia, insulin dynamics, and orosensory signaling pathways.

2.2. Physiological Regulation of SAA Secretion

SAA is synthesized and secreted predominantly by the serous acinar cells of the parotid glands, with minor contributions from the submandibular glands [21]. Its expression and secretion are under dual autonomic regulation, involving both the sympathetic and parasympathetic branches of the autonomic nervous system.

The sympathetic–adreno-medullary (SAM) axis modulates protein-rich secretion, particularly SAA, via norepinephrine release from postganglionic sympathetic fibers. Binding of norepinephrine to β-adrenergic receptors—predominantly β1 and β2 subtypes—on acinar cells triggers exocytosis of amylase-containing secretory granules through cyclic AMP (cAMP)-mediated intracellular signaling cascades [22].

In contrast, the parasympathetic nervous system regulates fluid volume and ionic composition of saliva, which indirectly facilitates SAA function and delivery. Parasympathetic innervation of the parotid glands arises from the glossopharyngeal nerve (cranial nerve IX), which relays via the otic ganglion and postganglionic fibers traveling through

the auriculotemporal branch of the mandibular nerve (V3). The submandibular and sublingual glands are innervated by the facial nerve (cranial nerve VII) via the chorda tympani and the submandibular ganglion. Parasympathetic signals utilize acetylcholine acting on muscarinic M1 and M3 receptors located on acinar and ductal epithelial cells. While M3 receptors primarily mediate fluid secretion through intracellular calcium signaling, M1 receptors contribute to modulating glandular responsiveness and electrolyte transport.

Although parasympathetic stimulation does not directly initiate SAA exocytosis, it creates an optimal fluidic and ionic environment essential for SAA transport, dilution, and enzymatic action.

At the genomic level, AMY1, the gene encoding salivary amylase, exhibits pronounced interindividual copy number variation (CNV), which has been strongly associated with both basal and stimulated SAA output [23]. This genetic variation interacts with environmental modulators—including dietary starch intake, autonomic tone, and psychosocial stress—highlighting the importance of integrating both genomic and physiological variables when interpreting SAA activity in clinical or experimental contexts.

2.3. Factors Affecting SAA

SAA activity exhibits pronounced temporal and situational variability. One of the primary modulators is the circadian rhythm, wherein SAA concentrations demonstrate a diurnal pattern: levels are typically low upon awakening, followed by a sharp increase (the "morning surge") and gradual decline throughout the day, independent of salivary flow rate [24]. This profile must be accounted for when standardizing sampling protocols.

Acute and chronic psychological stress are robust inducers of SAA secretion. Activation of the sympathetic nervous system results in a rapid elevation of SAA levels, rendering it a valuable non-invasive surrogate marker of adrenergic reactivity. However, individual differences in stress responsiveness and habituation can affect its reliability as a biomarker [22].

Moreover, pathophysiological conditions influence SAA expression and activity. For instance, individuals with metabolic syndrome, diabetes mellitus, or obesity often display altered SAA profiles, potentially reflecting neuroendocrine dysregulation. Similarly, oral health status, including salivary gland inflammation, xerostomia, or periodontal disease, can affect both qualitative and quantitative aspects of SAA secretion [25].

Nutritional status, physical exercise, smoking, medication use (e.g., β-blockers, corticosteroids), and even hydration state further modulate SAA [26]. Therefore, interpretation of SAA activity requires consideration of a multifactorial framework, encompassing genetic, physiological, behavioral, and environmental variables.

2.4. Metabolic Relevance of Salivary Amylase in Obesity

2.4.1. Influence on Postprandial Glycemia and Insulin Dynamics

Salivary amylase plays a critical role in modulating the rate and extent of carbohydrate digestion in the cephalic phase, thereby shaping postprandial glycemic responses [27]. Individuals with higher salivary amylase activity (SAA), often reflecting increased AMY1 gene copy number, exhibit more efficient hydrolysis of dietary starch into oligosaccharides and disaccharides such as maltose [28]. This enhanced starch pre-digestion has been associated with altered glycemic excursions and insulin secretion patterns.

Paradoxically, elevated SAA has been linked both to improved glucose tolerance in some populations and to exaggerated early-phase insulin responses in others, suggesting a dual, context-dependent metabolic impact [29]. Recent studies indicate that SAA may influence not only the magnitude but also the kinetics of insulin secretion, particularly affecting the first-phase insulin response [24]. This altered insulin dynamic may contribute to varia-

tions in insulin sensitivity and β-cell stress, potentially predisposing certain individuals to compensatory hyperinsulinemia [30] (Figure 1).

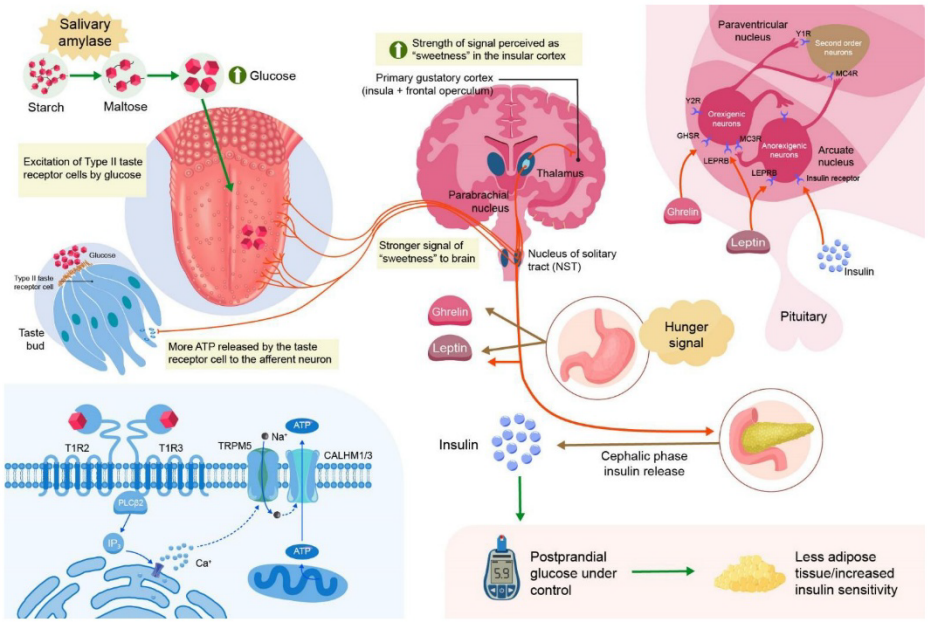

Figure 1. SAA influence on postprandial glycemia and insulin dynamics.

2.4.2. Association with Visceral Adiposity and Metabolic Phenotypes

A growing body of evidence supports a link between SAA and adipose tissue distribution, particularly visceral adiposity [31]. Low SAA levels have been associated with higher visceral fat accumulation, independent of total body mass index (BMI), indicating a specific association with metabolically adverse fat depots. Visceral adipose tissue is known to be more lipolytically active and pro-inflammatory, contributing to insulin resistance via adipokine secretion and ectopic lipid deposition [32].

Mechanistically, it is hypothesized that altered starch processing capacity, as indexed by SAA, may influence substrate availability and hormonal signaling pathways involved in energy storage and appetite regulation [4]. Individuals with low SAA may experience delayed starch digestion and prolonged glycemic load, potentially driving increased insulin secretion and fat accumulation over time.

2.4.3. Role in the Pathophysiology of Insulin Resistance and Glucose Intolerance

SAA may be implicated in the pathogenesis of insulin resistance and glucose intolerance through its modulation of postprandial insulin dynamics and nutrient sensing pathways. Chronic hyperinsulinemia, which may result from exaggerated insulin responses to rapidly absorbed starch in high-SAA individuals or from inefficient digestion and prolonged glucose absorption in low-SAA individuals, can downregulate insulin receptor sensitivity and disrupt metabolic homeostasis [33].

At the molecular level, salivary amylase-mediated differences in starch digestion may engage several intracellular signaling cascades, including the mechanistic target of rapamycin (mTOR) pathway, which integrates nutrient availability with cellular growth, metabolism, and insulin action [34]. Hyperactivation of mTOR complex 1 (mTORC1) in response to repeated postprandial hyperinsulinemia may contribute to negative feedback

inhibition of insulin receptor substrate (IRS) proteins, particularly IRS-1, impairing downstream insulin signaling via the PI3K-Akt pathway [35]. This molecular mechanism has been implicated in hepatic and skeletal muscle insulin resistance.

Additionally, frequent postprandial activation of mTOR may influence β-cell mass and function. While transient mTOR activation supports β-cell compensation during metabolic stress, chronic stimulation can lead to β-cell dysfunction, oxidative stress, and apoptosis, thereby exacerbating glucose intolerance. The interplay between SAA, insulin secretion, and mTOR signaling thus represents a potential mechanistic axis linking oral starch processing with systemic metabolic dysregulation.

Moreover, SAA-driven variations in glucose and insulin kinetics may impact other nutrient-sensing systems, such as the AMP-activated protein kinase (AMPK) pathway and gut-derived incretins (e.g., GLP-1), further modulating energy balance and metabolic health [36].

3. Methodological Considerations in the Assessment of Salivary Amylase Activity

Salivary amylase activity (SAA) assessment is characterized by considerable methodological heterogeneity, influenced by the analytical platform, reaction conditions, and sample collection strategy (Table 1).

Table 1. Overview of methodological heterogeneity in salivary amylase activity assessment.

Methodological Category	Analytical Technique	Analytical Range/Precision	Remarks
Dry Chemistry Platforms	Test strips with optical analyzers	0–200 kU/L	Fixed reaction time enhances reproducibility.
Electrochemical Biosensors	Enzyme-based electrodes; flow-injection systems	0–30 kU/L	Demonstrates high specificity and analytical sensitivity.
Portable Point-of-Care Devices	Saliva strip application or pipetting	Variable, method-dependent	Pipetting yields more consistent results than direct strip contact.
Spectrophotometric Assays	Colorimetric reaction with CNPG3 substrate	High precision, broad dynamic range	Allows differentiation of amylase isoforms (proteoforms).
Competitive or Product Inhibition Assays	Modified dry-chemistry platforms	Extended analytical range	Offers cost-effective high-throughput potential.
Sample Collection Devices	Salivette systems	Good intra-assay precision	Saliva recovery and analyte stability may vary with matrix.
Forensic Detection Methods	RSID™-Saliva immunochromatographic assay	High sensitivity, qualitative	Effective in detecting amylase in degraded or minute samples.

3.1. Colorimetric Assays

3.1.1. 2-Chloro-4-nitrophenyl-α-d-maltotrioside Substrate

This method utilizes the cleavage of 2-chloro-4-nitrophenyl-α-d-maltotrioside by salivary α-amylase, releasing a colored byproduct. The assay is optimized for concentrations between 20 and 500 µg/mL with a limit of detection (LOD) of 8 µg/mL.

A paper-based strip assay is proposed for rapid and specific detection, with minimal interference from saliva components. RGB analysis offers quantitative detection with an LOD of 11 µg/mL [37].

3.1.2. 3,5 Dinitrosalicylic Acid Assay

This method quantifies the reducing sugar released from soluble starch via α-amylase hydrolysis, producing a brick-red product measured at 525 nm. A hand-held device using

this method shows excellent correlation with commercial spectrophotometers and measures α-amylase activity in the range of 0.1–1.0 U/mL [38].

3.1.3. Cu/Au Nanoclusters

A novel technique using starch-stabilized Cu/Au nanoclusters that exhibit peroxidase-like activity. The presence of α-amylase leads to starch digestion, causing nanocluster aggregation and decreased peroxidase activity, detectable by a color change. This method has a detection limit of 0.04 U/mL and a linear range of 0.1–10 U/mL [38].

3.1.4. iPhone Imaging with Dinitrosalicylic Acid Assay Method

This approach employs iPhone imaging and Adobe Photoshop for colorimetric analysis. It provides comparable sensitivity and linearity to spectrophotometric methods, with better inter-day precision [39].

3.2. Spectrophotometric Assays

3.2.1. Phadebas Test

A widely used spectrophotometric method for detecting α-amylase activity. It involves a colorimetric endpoint assay and is benchmarked against newer methods for accuracy and reliability [40].

3.2.2. Automated Kinetic Spectrophotometric Method

This method uses standard reagents for pancreatic amylase activity and shows excellent correlation with manual colorimetric assays. It allows for standardized, center-independent analyses [41] (Table 2).

Table 2. Comparison of Colorimetric and Spectrophotometric Methods.

Method	Type	Detection Limit	Range	Advantages
2-Chloro-4-nitrophenyl-α-d-maltotrioside	Colorimetric	8 µg/mL	20–500 µg/mL	Rapid, specific, paper-based strip
Dinitrosalicylic Acid Assay (Hand-held)	Colorimetric	-	0.1–1.0 U/mL	Portable, cost-effective
Cu/Au Nanoclusters	Colorimetric	0.04 U/mL	0.1–10 U/mL	High selectivity, affordable
iPhone Imaging (Dinitrosalicylic Acid Assay)	Colorimetric	-	-	Field and lab use, high precision
Phadebas Test	Spectrophotometric	-	-	Standard method, reliable
Automated Kinetic Spectrophotometric	Spectrophotometric	-	-	Standardized, high correlation

3.3. Fluorometric Methods

Provide high sensitivity and lower detection limits compared to colorimetric assays; Often use 4-methylumbelliferyl-α-D-glucopyranoside as substrate; Require fluorescence detection instruments, limiting point-of-care use.

3.3.1. Nano CdS Doped Sol-Gel Matrix Method

This luminescence-based method utilizes a nano-CdS doped sol–gel matrix, where the emission intensity at 634 nm is quenched by maltose—the product of α-amylase-mediated starch hydrolysis. The luminescence quenching is proportional to maltose concentration, allowing indirect quantification of α-amylase activity. The method demonstrates a wide

linear calibration range (4.8×10^{-10} to 1.2×10^{-5} mol·L^{-1}) and an exceptionally low detection limit of 5.7×10^{-11} mol·L^{-1} [42]. This approach offers notable advantages, including high sensitivity and precision, making it suitable for trace-level detection. It has been successfully applied in the analysis of α-amylase activity in human saliva samples, highlighting its potential for clinical and biochemical diagnostics.

3.3.2. Starch–Iodine–Sodium Fluorescein Complex (SIF) Method

This method is based on the formation of a ternary starch–iodine–sodium fluorescein (SIF) complex, which exhibits low fluorescence under native conditions. Upon enzymatic hydrolysis of starch by α-amylase, the complex is disrupted, resulting in a measurable increase in fluorescence emission intensity. The method demonstrates a linear calibration range for α-amylase activity between 0.18 and 9.00 U/L [43]. It offers several advantages, including low cost, operational simplicity, and adequate sensitivity. This assay has been effectively applied to the detection of α-amylase activity in a range of biological matrices.

3.3.3. Aggregation-Induced Emission Luminogens (AIEgens) Method

AIEgens with D-π-A structures are used where the fluorescence emission is significantly enhanced upon interaction with α-amylase. The limit of detection (LOD) for this method is 0.1864 U/L, and it offers high specificity and selectivity [44].

3.3.4. Starch-Coated Fullerene C60 Complex Method

This method utilizes the quenching of Triphenylphosphine oxide (TPPOH) fluorescence by starch-coated fullerene C60. The analytical response shows a linear fluorescent response in α-amylase concentrations ranging from 0.001 to 0.1 Units/mL, with an LOD of 0.001 Units/mL [45]. This method provides high sensitivity and is applicable to clinical samples, making it suitable for caries detection and risk assessment.

3.3.5. Fluorescence Spectroscopy for Forensic Analysis

This method detects saliva stains on inanimate objects by comparing the fluorescence emission spectra of dried saliva samples to undiluted liquid saliva. The emission peak around 350 nm is used to identify the presence of amylase [46].

This method is simple and effective for forensic identification and is used for screening and selecting samples for subsequent DNA analysis (Table 3).

Table 3. Comparison of Fluorometric Methods.

Method	Principle	Sensitivity	Applications	Advantages
Nano CdS Doped Sol-Gel Matrix	Quenching of luminescence by maltose	5.7×10^{-11} mol L^{-1}	Human saliva samples	High sensitivity and precision
SIF Complex	Decomposition of SIF complex increases fluorescence	0.18–9.00 U/L	Biological samples	Inexpensive, easy to use
AIEgens	Enhanced fluorescence upon interaction with α-amylase	0.1864 U/L	Sensory experience assessment	Rapid, high reliability
Starch-Coated Fullerene C60	Quenching of TPPOH fluorescence	0.001 Units/mL	Caries detection	High sensitivity
Fluorescence Spectroscopy	Emission spectra comparison	N/A	Forensic analysis	Simple, effective

3.4. Emerging Technologies

3.4.1. Biosensors for Salivary Amylase Detection

Piezoresistive Microcantilever Biosensor

This biosensor detects salivary amylase activity by measuring the deflection of a microcantilever beam upon interaction with the enzyme. The deflection causes a change in resistance, which is measured using a Wheatstone Bridge circuit, converting the biochemical signal into a measurable voltage signal [47].

Flat-Chip Microanalytical Enzyme Sensor

Designed for wearable systems, this sensor incorporates enzymatic membranes on a small flow cell. It can measure amylase activity in a sample volume of 50 microliters with high sensitivity, making it suitable for continuous monitoring [48,49].

Amperometric Biosensor

This sensor uses salivary antibodies or antigens self-assembled onto an Au-electrode. The interaction between the immobilized antibody and salivary amylase is monitored via an electroactive indicator, providing analytical information based on current changes [49].

Smartphone-Based Potentiometric Biosensor

This system includes a smartphone app, a potentiometric reader, and a sensing chip. The saliva sample reacts with preloaded reagents on the chip, and the resulting potential is measured and converted into amylase concentration by the app [50].

Tri-Enzymatic Biosensor

Utilizing a screen-printed electrode modified with Prussian Blue, this biosensor co-immobilizes α-glucosidase, glucose oxidase, and mutarotase. It measures the maltose generated by the hydrolysis of maltopentose in the presence of salivary amylase [51].

3.4.2. Microfluidic Devices for Salivary Amylase Detection

Paper-Based Microfluidic Chip

This device isolates α-amylase from saliva using a starch-coated paper-based chip. The concentration of α-amylase is determined by comparing the enzyme concentration in different sections of the chip [52].

3.4.3. Lab-on-a-Chip (LOC) Devices

LOC devices integrate biosensors and microfluidics to analyze small sample quantities efficiently. These devices are particularly useful for non-invasive saliva analysis, offering high throughput, portability, and disposability [53,54].

Dual Microfluidic Paper-Based Analytical Devices (Dual-μPADs)

These devices combine colorimetric and electrochemical modules to detect salivary amylase along with other biomarkers. They are fabricated using a simple "do-it-yourself" protocol, making them versatile and user-friendly for point-of-care diagnostics [55] (Table 4).

Innovative platforms have emerged to enable portable, rapid, and minimally invasive testing.

Electrochemical biosensors show promise for real-time detection, with enzyme immobilization techniques enhancing stability.

Microfluidic paper-based analytical devices (μPADs) combine colorimetric readouts with saliva capillary flow, offering low-cost diagnostic potential.

Table 4. Comparison of Emerging Technologies.

Method	Principle	Sample Volume	Sensitivity	Application
Piezoresistive Microcantilever	Resistance change	Not specified	High	Stress detection
Flat-Chip Sensor	Enzymatic reaction	50 µL	High	Wearable systems
Amperometric Biosensor	Current change	Not specified	1.57 pg/mL	Real-time monitoring
Smartphone-Based Potentiometric	Potential measurement	Not specified	High	Point-of-care testing
Tri-Enzymatic Biosensor	Enzymatic reaction	Not specified	5 U/mL	Simple assays
Paper-Based Microfluidic Chip	Enzyme concentration	Not specified	High	Non-invasive diagnostics
Dual-µPADs	Colorimetric and Electrochemical	Not specified	High	Periodontal disease diagnosis

Integration with smartphone-based readers was reported in recent studies to improve field applicability.

Salivary amylase activity presents both physiological advantages and inherent limitations as a biomarker, particularly in the context of stress response and metabolic regulation (Table 5).

Table 5. Physiological Advantages and Limitations of Salivary Amylase Activity as a Biomarker.

Advantages	Limitations
Noninvasive, rapid sampling method—suitable for repeated measures and ambulatory settings.	High sensitivity to confounding variables—affected by circadian rhythm, diet, hydration, and oral hygiene.
Potential biomarker of autonomic nervous system (ANS) activation—particularly sympathetic–adrenal–medullary (SAM) axis responsiveness.	Insufficient large-scale clinical validation—limited normative data across populations and disease states.
Responsive to acute psychological and physiological stress—may reflect real-time stress-related physiological dynamics.	Marked inter-individual variability—influenced by genetic (AMY1 gene copy number), metabolic, and environmental factors.
Advances in enzyme detection technologies—allow for accurate, low-volume, and point-of-care measurements.	Comparative biomarker uncertainty—less standardized and validated compared to established stress or metabolic biomarkers.

4. Key Innovations and Developments

4.1. Miniaturization and Sensitivity

A flat-chip microanalytical enzyme sensor has been developed, incorporating a flow cell as small as a C battery. This sensor uses enzymatic membranes containing maltose phosphorylase and glucose oxidase immobilized on a planar surface, allowing for the detection of amylase activity in the range of 0–190 kU/L with a sample volume of 50 µL [49,56].

Another approach involves a smartphone-based potentiometric biosensor that uses a sensing chip with preloaded reagents. This system can quantitatively analyze salivary α-amylase within 5 min, correlating well with psychological states [57].

4.2. Wearable and Portable Systems

A completely automated hand-held monitor for salivary α-amylase activity has been developed, utilizing a dry-chemistry system with a disposable test strip. This device can measure amylase activity with high accuracy using only 30 µL of saliva, making it suitable for continuous monitoring and psychological research [52].

4.3. Microfluidic and Paper-Based Chips

A microfluidic starch-coated paper-based chip has been designed to isolate α-amylase from human saliva. This chip effectively concentrates α-amylase in specific sections, aiding in its detection using techniques like Western blotting and ELISA [58].

Another innovative design includes a polymer LOC with dried on-chip immunoassay reagents for detecting unbound cortisol in saliva, demonstrating the versatility of LOC systems for various biomarkers [59].

4.4. Integration with Modern Technologies

LOC systems often integrate microfluidic elements for fluid mixing, manipulation, and control, enabling the performance of conventional laboratory procedures on a miniaturized chip. These systems can be developed on paper or polymeric platforms using various fabrication techniques [60]

LOC systems for salivary amylase detection are particularly useful for non-invasive monitoring of the sympathetic nervous system and stress-related conditions [61–63]

They also hold promise for clinical diagnostics, forensic applications, and personalized health care by providing rapid, accurate, and cost-effective analysis of salivary biomarkers [43,64,65].

In summary, lab-on-a-chip systems for detecting salivary amylase activity represent a significant advancement in non-invasive diagnostics, offering high sensitivity, portability, and the potential for continuous monitoring in various health-related applications.

5. Discussion

This comprehensive review provides an in-depth analysis of analytical methods used for the detection of salivary amylase activity (SAA), encompassing both conventional enzymatic assays and novel biosensor technologies. The findings reveal considerable methodological heterogeneity across studies, which impacts analytical performance, data comparability, and the clinical applicability of SAA as a biomarker.

5.1. Analytical Performance Comparison

Analytical performance varied substantially across reported methodologies. Detection limits ranged from 0.01 to 50 U/mL, indicating significant differences in assay sensitivity. Intra-assay coefficients of variation ranged from 2% to 20%, reflecting inconsistencies in precision. Furthermore, calibration against recognized international reference standards, such as those of the International Federation of Clinical Chemistry (IFCC), was rare, limiting methodological comparability and the development of diagnostic cut-offs.

Traditional spectrophotometric assays (e.g., starch–iodine, dinitrosalicylic acid [DNS] methods) remain widely used due to their simplicity and low cost. However, these methods often exhibit suboptimal specificity and limited dynamic range in complex matrices like saliva [49,66,67]. Enzymatic assays using chromogenic or fluorogenic substrates demonstrate enhanced sensitivity and kinetic resolution but are hindered by a lack of standardized reagents and protocols [68].

Innovative biosensors and microfluidic platforms offer a promising future for real-time, point-of-care detection of SAA. These systems show potential for miniaturization, multiplexing, and integration with digital health tools. However, their clinical translation remains limited due to device fabrication complexity, sample matrix interference (e.g., saliva viscosity and contaminants) and a paucity of large-scale validation studies [69].

5.2. Pre-Analytical Considerations

Pre-analytical variability emerged as a major factor influencing SAA measurement reliability. Saliva collection methods differed considerably between studies, with both stimulated (e.g., chewing paraffin) and unstimulated (e.g., passive drool, swab) techniques employed. These variations directly affect enzyme concentration, volume, and sample composition [70–72].

Storage conditions were inconsistently reported—some studies applied immediate freezing or used preservatives, while others lacked detailed documentation. Additionally, physiological variables such as diurnal fluctuation, food intake, and acute stress were often uncontrolled, despite their known influence on SAA dynamics [73,74]. This pre-analytical inconsistency weakens inter-study comparisons and diminishes the biomarker's clinical and research validity.

5.3. Clinical and Research Applications

Beyond its classical digestive role, SAA has emerged as a promising biomarker across multiple physiological domains. In psychoneuroendocrinology, it serves as a non-invasive indicator of sympathetic nervous system activation in response to acute and chronic stressors [75,76]. It has also been explored as a surrogate marker for autonomic dysfunction in cardiovascular and psychiatric disorders [77,78].

Emerging evidence links SAA with metabolic outcomes, including obesity and insulin resistance, suggesting its potential as a biomarker of metabolic flexibility and resilience. Additionally, its role in oral health diagnostics is under investigation, particularly in relation to the oral microbiome and periodontal inflammation [79]. However, the clinical implementation of SAA remains limited by the absence of standardized protocols, validated thresholds, and reference ranges across populations.

5.4. Integration with Multi-Biomarker Panels

The integration of SAA into multiplex biomarker platforms offers a powerful strategy to enhance the diagnostic specificity of saliva-based assessments for both psychophysiological stress and metabolic disorders. While SAA serves as a dynamic biomarker of sympathetic–adrenal–medullary (SAM) axis activation, its diagnostic accuracy can be augmented by combining it with complementary salivary biomarkers representing distinct physiological axes, including hormonal, immunological, and metabolic pathways.

Notably, salivary cytokines—such as interleukin-6 (IL-6), tumor necrosis factor-alpha (TNF-α), and interleukin-1β (IL-1β)—can be co-analyzed with SAA to assess inflammatory states associated with chronic stress, metabolic syndrome, or low-grade systemic inflammation. These cytokines, when detected in saliva, offer insight into mucosal immune activation and neuroimmune crosstalk, contributing to a broader understanding of stress-related pathophysiology.

Moreover, the inclusion of salivary steroid hormones such as cortisol, dehydroepiandrosterone (DHEA), and testosterone further refines the interpretation of stress responses by capturing hypothalamic–pituitary–adrenal (HPA) axis activity. For example, the simultaneous measurement of SAA (SAM axis) and cortisol (HPA axis) provides a dual-axis framework to distinguish between acute and chronic stress, as well as dysregulations in stress reactivity.

Additionally, the incorporation of metabolic hormones and analytes detectable in saliva—such as insulin, leptin, ghrelin, and adiponectin—may facilitate early, non-invasive detection of metabolic impairments. This integrative approach holds promise for profiling metabolic flexibility, energy balance, and appetite regulation in conditions such as obesity, type 2 diabetes, or polycystic ovary syndrome (PCOS).

Emerging lab-on-chip technologies and electrochemical biosensors increasingly allow for the concurrent quantification of such panels from minimal sample volumes. The development of salivary multi-biomarker platforms may thus revolutionize point-of-care diagnostics by providing real-time, holistic insight into systemic physiological status.

5.5. Future Directions

The continuous evolution of analytical technologies for salivary α-amylase (SAA) detection expands the potential for clinical diagnostics, psychophysiological research, and personalized medicine. Despite significant advances in colorimetric, spectrophotometric, fluorometric, and biosensor-based methodologies, several challenges remain to be addressed to optimize the sensitivity, specificity, and field applicability of these techniques.

5.5.1. Integration and Miniaturization

Emerging lab-on-a-chip (LOC) systems and microfluidic paper-based analytical devices (μPADs) have demonstrated substantial promise for point-of-care (POC) diagnostics. Future research should focus on enhancing the integration of multistep assays (e.g., enzymatic reactions, signal amplification, and detection) within single miniaturized platforms. This would allow for fully automated, real-time monitoring of salivary biomarkers with minimal sample volume requirements and without the need for sophisticated laboratory infrastructure.

5.5.2. Multiplexed Detection and Biomarker Panels

Given the complex physiology of stress and metabolic regulation, future efforts should aim at developing platforms capable of simultaneous detection of SAA alongside complementary biomarkers, such as cortisol, glucose, and inflammatory cytokines. Dual-mode biosensors (e.g., combining colorimetric and electrochemical outputs) and multi-analyte LOC systems offer a promising strategy to improve diagnostic accuracy and broaden clinical utility.

5.5.3. Wearable Technologies and Remote Monitoring

The development of wearable biosensors that enable continuous, non-invasive monitoring of SAA in real-life environments is a critical frontier. Flexible, skin-mounted platforms or intraoral sensors integrated with wireless data transmission and smartphone interfaces could significantly enhance our ability to monitor stress, fatigue, and metabolic responses dynamically in ambulatory settings.

5.5.4. AI-Driven Signal Analysis and Decision Support

The integration of artificial intelligence (AI) and machine learning algorithms into biosensor platforms can transform raw data into actionable insights. Future systems could incorporate real-time pattern recognition to predict stress episodes or detect deviations in metabolic profiles, thus enabling preventive interventions and personalized health recommendations.

5.5.5. Standardization and Clinical Translation

To bridge the gap between laboratory innovation and clinical implementation, future studies should emphasize assay standardization, inter-laboratory validation, and regulatory approval. Harmonization of detection units, sample collection protocols, and calibration procedures are essential for the broad adoption of SAA-based diagnostics in clinical practice.

5.5.6. Expanding Diagnostic Applications

Although SAA is widely recognized as a surrogate marker of sympathetic nervous system activity, future research should explore its potential role in broader diagnostic contexts, including metabolic syndrome, oral diseases (e.g., caries risk), gastrointestinal disorders, and early detection of neuropsychiatric conditions. Novel assay designs and longitudinal studies are required to fully realize the biomarker potential of SAA in systemic health monitoring.

In conclusion, the future of salivary α-amylase detection lies in interdisciplinary innovation—combining biochemical engineering, materials science, data analytics, and clinical research—to develop robust, accessible, and informative diagnostic platforms for health care and beyond.

5.6. Limitations

This review is limited by the heterogeneity of included studies, which precluded meta-analytic synthesis. Moreover, potential publication bias may have favored the reporting of studies with positive or novel findings while underreporting those with null results or technical failures.

6. Concluding Recommendations and Best-Fit Methodologies

Given the increasing interest in salivary α-amylase (SAA) as a non-invasive biomarker at the intersection of metabolic, autonomic, and stress-related physiology, careful alignment of methodological approaches with specific research and clinical objectives is essential. Although a wide array of analytical platforms exists for measuring SAA, their applicability, reproducibility, and translational relevance vary significantly depending on the context of use.

To support researchers and clinicians in selecting appropriate methods, we summarize the current state of evidence regarding best-fit platforms for three key domains of application: (1) wearable stress monitoring, (2) metabolic phenotyping in obesity and insulin resistance, and (3) forensic or retrospective physiological assessments.

6.1. Wearable Stress Monitoring

For dynamic assessment of autonomic reactivity, particularly in ambulatory or ecologically valid conditions, real-time biosensing technologies based on enzymatic electrochemical detection of SAA are considered most suitable. These platforms offer high temporal resolution and direct assessment of enzymatic activity, correlating well with sympathetic nervous system activation. In contrast, immunoassays (e.g., ELISA) that quantify total SAA protein lack temporal resolution and functional specificity and are therefore not recommended for stress reactivity studies where rapid fluctuations are of interest [80].

6.2. Metabolic Phenotyping in Obesity

SAA activity shows emerging promise as a metabolic biomarker related to postprandial glucose regulation, visceral adiposity, and insulin sensitivity. In this context, kinetic enzymatic assays using colorimetric or fluorometric readouts remain the gold standard, particularly when combined with pre-analytical standardization (e.g., fasting state, collection time, flow rate normalization). While AMY1 gene copy number has been proposed as a genetic proxy for SAA levels, its application is limited by poor functional correlation, interindividual variability, and platform-dependent detection errors [81]. Thus, its use is acceptable only with caution and should ideally be supplemented by concurrent enzymatic activity measurement.

6.3. Forensic and Retrospective Assessment

In contexts where only archived or post hoc biological samples are available, the use of total protein quantification (e.g., Western blot, ELISA) may be pragmatically justified. However, these methods do not reflect enzymatic function and are susceptible to degradation and post-collection variability. Therefore, their use should be interpreted cautiously and ideally complemented by additional salivary biomarkers—such as cortisol (a marker of HPA axis activation), selected pro-inflammatory cytokines (e.g., IL-6, TNF-α) that reflect low-grade systemic inflammation, or adipokines like leptin and resistin, which are increasingly detectable in saliva and provide insight into the inflammatory–metabolic interface characteristic of obesity-related pathophysiology (Table 6).

Table 6. Summary of Methodological Suitability.

Application	Methodological Approach	Classification	Rationale
Wearable Stress Monitoring	Real-time enzymatic biosensors (electrochemical)	Recommended	High temporal resolution, functional specificity to sympathetic activity, real-time feedback.
	Point-sample enzymatic assay (e.g., spectrophotometric)	Conditionally Appropriate	Useful in lab settings; limited ecological validity and temporal resolution.
	Immunoassays (e.g., ELISA for total SAA protein)	Not Recommended	Low functional specificity; poor correlation with stress reactivity and enzyme activity.
Metabolic Phenotyping (e.g., Obesity, Insulin Sensitivity)	Enzymatic activity assay (e.g., kinetic, chromogenic)	Recommended	Reproducible in fasting/postprandial states; associated with glucose-insulin dynamics.
	AMY1 gene copy number estimation (e.g., qPCR, ddPCR)	Conditionally Appropriate	Moderate heritability; limited functional correlation due to CNV complexity.
	Total protein concentration (e.g., western blot)	Not Recommended	Does not reflect enzymatic function; poor metabolic specificity.
Forensic/Retrospective Analysis	Total SAA protein (e.g., western blot, ELISA)	Conditionally Appropriate	Useful when only preserved or archived saliva is available; low functional precision.
	Enzymatic activity (archived or stored samples)	Recommended	Preferred when integrity is preserved; enzyme stability is time- and storage-dependent.
	AMY1 copy number (retrospective genetic profiling)	Conditionally Appropriate	Stable DNA allows retrospective genotyping; functional extrapolation is uncertain.

7. Conclusions

Salivary amylase activity has emerged as a promising non-invasive biomarker with applications in stress physiology, metabolic health, and oral diagnostics. While traditional colorimetric and spectrophotometric methods remain widely used, recent advances in biosensors, microfluidics, and fluorometric assays offer improved sensitivity, portability, and diagnostic potential. However, significant variability in sample collection, assay protocols, and reporting standards continues to limit comparability across studies and hinders clinical translation. Future progress will depend on the development of standardized methodologies, validation in large cohorts, and integration of emerging technologies into clinical workflows. By addressing these challenges, SAA measurement could become a valuable component of precision health monitoring and personalized diagnostics.

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Abbreviations

AIEgens	Aggregation-induced emission luminogens
AMY1	Alfa amylase 1
cAMP	Cyclic adenosine monophosphate
Cu/Au	Copper/gold
CNPG3	2-chloro-4-nitrophenyl-β-D-maltotrioside substrate
CNV	Copy number variation
D-π-A	Donor–π bridge–acceptor
EC 3.2.1.1	Enzyme commission number for α-amylase
GH13	Glycoside hydrolase family 13
nCdS	Nano-cadmium sulfide
LOC	Lab on a chip (LOC)
LOD	Limit of detection
MPADs	Microfluidic paper-based analytical devices
RGB	Red, green, blue
SAA	Salivary amylase activity
SAM	Sympathetic–adreno-medullary SAM
SGM	Sol–gel matrix
SIF	Starch–iodine–sodium fluorescein complex
TPPOH	Triphenylphosphine oxid

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REVIEW ARTICLE

Salivary amylase activity: A potential modulator of glucose homeostasis, insulin secretion, and appetite regulation

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Abstract

This review examines the potential mechanisms by which SAA may influence glucose homeostasis and insulin sensitivity, emphasizing that current evidence is largely correlational and requires further investigation to establish causality. Salivary α-amylase (SAA), a key enzyme in the breakdown of dietary starch, has emerged as a potential regulator of glucose homeostasis, insulin secretion, and appetite control. Beyond its primary role in carbohydrate digestion, growing evidence highlights the influence of SAA on metabolic processes through its impact on early glucose release and its interaction with hormonal signaling pathways. This review examines the mechanisms by which SAA may affect insulin secretion and appetite regulation, focusing on its involvement in in incretin and other gut hormone-mediated pathways. Despite challenges posed by interindividual variability in SAA activity, its potential utility as a biomarker for metabolic health remains promising. Future research should prioritize uncovering the mechanistic links between SAA activity and metabolic outcomes, as well as establishing standardized protocols for its evaluation in both clinical and research contexts.

Keywords: Salivary α-amylase; Appetite regulation; Satiety hormones; Gut-brain axis; Incretins; Glucose homeostasis; Metabolic health.

1. Introduction

Appetite regulation is a complex biological process driven by dynamic interactions between the digestive system, hormonal pathways, and the central nervous system. Understanding the factors that modulate appetite is critical for addressing metabolic disorders such as obesity and type 2 diabetes. Among these factors, the role of digestive enzymes has received growing attention, particularly SAA, an enzyme traditionally recognized for its role in the breakdown of dietary starch.

SAA initiates the hydrolysis of complex carbohydrates into maltose and other oligosaccharides in the oral cavity, setting the stage for glucose availability early in digestion. Beyond this mechanical function, emerging evidence suggests that SAA activity may play a more nuanced role in appetite regulation through its impact on glucose dynamics and its interaction with hormonal signaling

Abbreviations: AC, adenylyl cyclase; AgRP, agouti-related peptide; AMPK, adenosine monophosphate-activated protein kinase; AMY1, alfa amylase 1; ATP, adenosine triphosphate; CALHM1/3, calcium homeostasis modulator 1 and 3; CART, cocaine- and amphetamine-regulated transcript; cAMP, cyclic adenosine monophosphate; CNVs, copy number variations; CPIR, cephalic phase insulin response; DAG, diacylglycerol; DNA, deoxyribonucleic acid; ddPCR, droplet digital polymerase chain reaction; FFAs, free fatty acids; GIP, glucose-dependent insulinotropic polypeptide; GLP-1, glucagon-like peptide-1; GLP-1, active glucagon-like peptide-1; G6Pase, glucose-6-phosphatase; GLUT, glucose transporters; GPCRs, G-protein-coupled receptors; GWAS, genome-wide association studies; HSL, hormone-sensitive lipase; HOMA, homeostatic model assessment; HOMA2-%S, homeostasis model assessment of insulin sensitivity; HPAx, hypothalamic–pituitary–adrenal axis; IP3, inositol trisphosphate; IS, insulin sensitivity; KATP, ATP-sensitive potassium channels; LPS, lipopolysaccharides; MPS, maltopolysaccharides; MOS, maltooligosaccharides; MFA, metabolic flux analysis; mTOR, mechanistic target of rapamycin; NST, nucleus of the solitary tract; OGTT, oral glucose tolerance test; PKA, protein kinase A; POMC, proopiomelanocortin; PI(4,5)P2, phosphatidylinositol 4,5-bisphosphate; PLC β2, phospholipase C beta 2; PYY, peptide YY; PCREB, phosphorylated cAMP response element-binding protein; PEPCK, phosphoenolpyruvate carboxykinase; SCFA, short-chain fatty acid; SAA, salivary α-amylase; SGLT1, sodium–glucose cotransporter 1; SNP, single nucleotide polymorphism SNP; TAS1R1, taste receptor type 1, member 1; TAS1R3, taste receptor type 1, member 3; T1R2+T1R3, heterodimeric complex formed by TAS1R1 and TAS1R3; TRPM5, transient receptor potential melastatin 5; TyG, the triglyceride/glucose index; 3B PDE3B, phosphodiesterase 3B; UCP1, uncoupling protein 1; AUC, area under the glucose curve; qPCR, quantitative polymerase chain reaction.

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pathways [1]. One notable mechanism involves the cephalic phase of insulin secretion, a process triggered by sensory and anticipatory signals before food reaches the stomach. Higher SAA activity has been linked to enhanced cephalic-phase insulin release, which may prime the body for glucose metabolism and influence satiety and hunger signals [2].

The rapid glucose release facilitated by SAA may also modulate the secretion of gut-derived hormones such as glucagon-like peptide-1 (GLP-1), peptide YY (PYY), and ghrelin, which are key mediators of the gut-brain axis. These hormones integrate peripheral signals to regulate food intake and energy balance, further underscoring the potential role of SAA in appetite control [3].

Despite these findings, the potential contribution of SAA to appetite control remains underexplored. Interindividual variability in SAA activity, influenced by genetic, environmental, and dietary factors, adds a layer of complexity to understanding its metabolic significance [4]. Additionally, the extent to which SAA interacts with other regulatory systems, such as insulin secretion and glucose homeostasis, requires further clarification.

While emerging evidence suggests a relationship between SAA activity and glucose homeostasis, the current data are primarily associative, and further research is needed to establish direct causal links.

This review aims to synthesize current knowledge on the role of SAA in appetite regulation, with a particular focus on its mechanistic links to hormonal signaling pathways and its potential as a biomarker for appetite and metabolic health. By exploring the interplay between digestive enzyme activity and appetite regulation, this review aims to uncover innovative directions for research and potential clinical applications in metabolic health management.

2. Current understanding

2.1. Overview of salivary amylase and AMY1 gene copy number variation

Salivary amylase, encoded by the AMY1 gene, plays an integral role in the initial stages of carbohydrate digestion, facilitating the breakdown of starches within the oral cavity. The AMY1 gene exhibits considerable *copy number variation* (CNV) among individuals and across populations, which directly influences salivary amylase levels and activity. Research shows that higher AMY1 CNV correlates with increased salivary amylase activity [5,4], which enhances starch hydrolysis at the onset of digestion. This variation is hypothesized to have evolved as an adaptive response to dietary carbohydrate availability, especially in regions characterized by high-starch diets, indicating that AMY1's CNV may play a role in population-specific metabolic adaptation [6]. Understanding AMY1 gene CNV's impact on salivary amylase activity lays the groundwork for exploring its implications for glucose metabolism and insulin response [7].

2.2. Cephalic phase insulin response

The cephalic phase insulin response (CPIR) is a rapid, anticipatory insulin release that occurs prior to a detectable rise in blood glucose, initiated by the sensory perception of food, particularly carbohydrates. This early-phase response prepares the body for nutrient assimilation and supports efficient postprandial glycemic regulation [8].

Recent evidence suggests that salivary amylase activity (SAA) modulates this process. Elevated SAA, often associated with increased AMY1 gene copy number, facilitates rapid oral-phase starch hydrolysis, producing simple sugars that may activate sweet taste receptors and prime metabolic pathways even before intestinal absorption occurs [7,9]. Individuals with higher SAA levels demonstrate more robust CPIR, likely due to both enhanced oral carbohydrate sensing and more efficient central integration of gustatory signals [8].

However, the magnitude and efficacy of CPIR are modulated by genetic variability (e.g., AMY1 CNV) and environmental influences such as dietary patterns and stress exposure [10].

At the molecular level, type II taste receptor cells express the T1R2+T1R3 heterodimer, which detects sweet stimuli, including sugars and starch-derived oligosaccharides. Activation of this G-protein-coupled receptor initiates a canonical signaling cascade: engagement of phospholipase C $\beta 2$ (PLC$\beta 2$) leads to the production of inositol 1,4,5-trisphosphate (IP3), which induces the release of intracellular Ca^{2+} stores. The resulting elevation in cytoplasmic Ca^{2+} activates the transient receptor potential cation channel subfamily M member 5 (TRPM5), permitting Na^{+} influx and depolarization of the taste cell. This depolarization drives ATP release through CALHM1/3 channels, which activates afferent gustatory sensory neurons, ultimately relaying taste information to the brainstem and higher centers [11].

These taste signals are first processed in the nucleus of the solitary tract (NST), transmitted to the parabrachial nucleus, and then relayed to the ventroposteromedial nucleus of the thalamus, which integrates sensory input. From the thalamus, information reaches the primary gustatory cortex in the insula and frontal operculum, allowing for conscious perception of taste—including discrimination of sweet, salty, sour, bitter, and umami—and evaluation of taste intensity [12].

Beyond conscious perception, gustatory pathways also project to the limbic system, particularly the amygdala and hypothalamus, facilitating the integration of taste with emotional, motivational, and metabolic signaling. The hypothalamus, a central regulator of energy balance, modulates feeding behavior, reward processing, and cravings. These pathways also influence key metabolic hormones, including insulin, leptin, and ghrelin, thereby linking taste perception to both hedonic and homeostatic control of food intake [13].

Together, these findings underscore a tightly coordinated oral-metabolic axis, wherein salivary amylase activity, via its impact on gustatory signaling and CPIR, may play a previously underappreciated role in regulating glucose homeostasis and energy balance.

2.3. Salivary amylase activity and glucose signaling pathways

Salivary amylase enzymatic activity not only facilitates digestion but also influences glucose-sensing mechanisms in the oral cavity and beyond [14].

Glucose activates at least two distinct signaling pathways. One pathway is activated by the binding of sugars or non-nutritive sweeteners to a G protein-coupled receptor, T1R2+T1R3. This heterodimer functions as a sweet taste receptor that detects a variety of natural and artificial sweeteners. Upon activation by sweet stimuli, this G protein–coupled receptor (GPCR) complex triggers a signaling cascade involving the G protein α-gustducin, which leads to the activation of phospholipase C $\beta 2$ (PLC$\beta 2$). This, in turn, results in the production of inositol 1,4,5-trisphosphate (IP_3), mobilization of intracellular Ca^{2-} stores, and the subsequent activation of the transient receptor potential cation channel M5 (TRPM5). The increase in intracellular Ca^{2+} ultimately contributes to downstream cellular responses, including insulin secretion and modulation of glucose metabolism (Fig. 1A and B).

Glucose uptake through sodium-glucose co-transporter 1 (SGLT1) or glucose transporters (GLUT) in the cell membrane leads to its metabolism and ATP production. ATP binds to ATP-sensitive

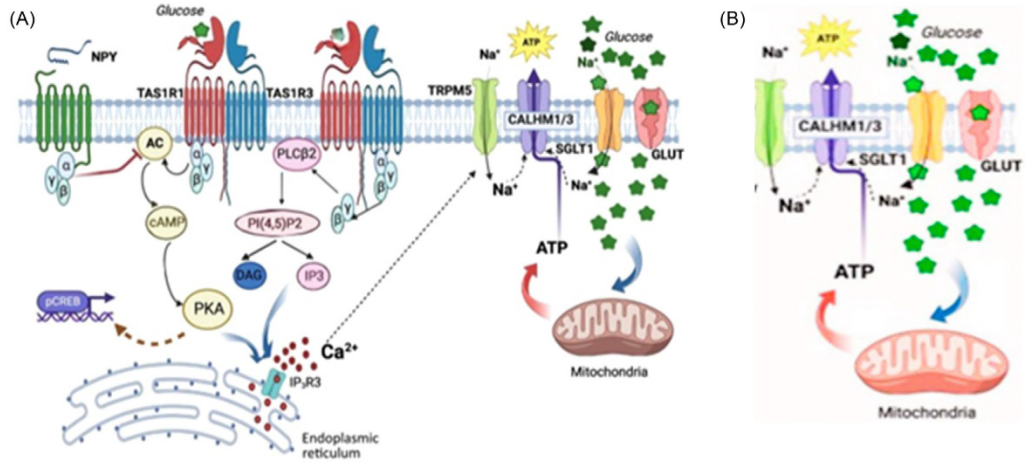

Fig. 1. (A) Glucose signaling pathways. Adapted and modified from Herzog et al. [15]. Shown below is another pathway identified in mice (B; [15]). and (B) Glucose signaling pathways. Adapted and modified from Herzog et al. [15].

potassium channels (KATP) channels, inhibiting K^+ efflux and causing membrane depolarization. This process triggers neurotransmitter release, transmitting signals to the NST in the brainstem.

Emerging studies further suggest that β-cell-derived signals, such as exosomes and microRNAs, play a significant role in linking insulin secretion to peripheral insulin sensitivity [16,17]. These signaling molecules, secreted in response to early-phase insulin release, modulate insulin action in target tissues, including skeletal muscle, liver, and adipose tissue, thereby influencing systemic glucose regulation.

Incretin hormones, particularly glucagon-like peptide-1 (GLP-1) and glucose-dependent insulinotropic peptide (GIP), are also proposed mediators of the relationship between salivary amylase activity and glucose metabolism. Rapid glucose digestion initiated by high salivary amylase activity may enhance incretin hormone release, further stimulating insulin secretion and improving glucose tolerance [18,19].

2.4. Salivary amylase activity and insulin resistance

Individuals with higher SAA demonstrate improved early-phase insulin responses and attenuated postprandial glucose excursions without inducing pathological hyperinsulinaemia, which can drive metabolic dysregulation [20]. In contrast, chronic hyperinsulinaemia, activates key lipogenic signaling pathways in hepatocytes and adipocytes:

- Phosphoinositide 3-kinase (PI3K) and mechanical target of rapamycin complex 1 (mTORC1) signaling converge on sterol regulatory element-binding protein 1c (SREBP-1c), a transcription factor that upregulates genes involved in *de novo* lipogenesis [21].
- This pathway leads to increased synthesis and storage of fatty acids, preferentially in visceral adipose tissue (VAT), which is more metabolically active and insulin-sensitive in the lipogenic context than subcutaneous fat [22].

Accumulation of VAT, in turn, contributes to the development and amplification of insulin resistance (IR) through multiple molecular pathways:

(1) Elevated free fatty acids (FFAs) released from visceral fat enter the liver via the portal vein, leading to diacylglycerol (DAG) accumulation. DAG activates protein kinase C epsilon (PKCε), which impairs insulin receptor substrate (IRS)-mediated signaling, particularly in hepatocytes [23].
(2) VAT expansion promotes infiltration by proinflammatory M1 macrophages, which secrete tumor necrosis factor-α (TNF-α), interleukin-6 (IL-6), and monocyte chemoattractant protein-1 (MCP-1). These cytokines activate stress kinases such as JNK and IKKβ, leading to serine phosphorylation and inhibition of IRS proteins, further blunting insulin signaling [14,24,25].
(3) The secretory profile of expanded VAT shifts toward reduced adiponectin and elevated levels of resistin and retinol-binding protein 4 (RBP4)—adipokines known to impair insulin action in skeletal muscle and liver [26,27].

Elevated salivary amylase activity is associated with enhanced cephalic-phase insulin secretion and diminished visceral adipose accumulation, which contribute to the attenuation of insulin resistance by preserving hepatic and peripheral insulin signaling pathways, minimizing lipotoxic metabolite accumulation (e.g., DAG, ceramides), and reducing proinflammatory cytokine-mediated inhibition of insulin receptor substrate (IRS) function (Table 1).

2.5. The role of AMY1A in salivary amylase secretion, satiety regulation, and oral sensory perception

Several studies have explored the relationship between *AMY1A* copy numbers, dietary starch intake, satiety, and oral perceptions of food texture. In the study by Perry et al. [36], AMY*1A* copy numbers were assessed across three populations with high-starch diets and one with low-starch diet. The proportion of individuals with at least six *AMY1A* copies was found to be twice as high in high-starch-consuming populations compared to low-starch-consuming populations (copy number variation=5.72). The authors propose a model suggesting directional selection for *AMY1A* in high-starch consumers, with their results indicating a potential correlation between *AMY1A* copy numbers and salivary amylase production.

Table 1
Mechanistic pathways and gene–diet interactions linking AMY1 CNV to insulin resistance.

Component	Mechanism	Effect on insulin resistance	Supporting evidence
AMY1 copy number variation (CNV)	Determines salivary amylase expression level	Low AMY1 CNV → reduced starch hydrolysis → delayed glucose appearance → impaired insulin dynamics	[28,29]
Salivary amylase activity (SAA)	Hydrolyzes starch in the oral phase → initiates cephalic phase insulin response (CPIR)	Low SAA → blunted CPIR → delayed insulin release → early insulin resistance	[4]
Cephalic phase insulin response	Parasympathetic-mediated insulin release triggered by taste/salivation	Absent/delayed CPIR → higher glucose peaks → β-cell stress	[30]
Rapid postoral glucose delivery	High SAA → faster glucose appearance in proximal intestine → early GLP-1, GIP secretion	Enhances first-phase insulin secretion; protective against insulin resistance	[31,32]
Gut microbiota composition	AMY1 CNV affects starch availability in colon → modifies SCFA production (esp. butyrate)	Butyrate ↑ GLP-1 and improves insulin sensitivity; low AMY1 CNV → lower SCFA → worsened metabolic profile	[33]
GLP-1 and GIP secretion	Incretin hormones enhanced by glucose absorption rate	Low AMY1 → slower digestion → weaker incretin response → impaired insulinotropic effects	[3]
Visceral adiposity	Low AMY1 CNV associated with increased visceral fat accumulation	Visceral adiposity promotes insulin resistance via inflammatory and lipotoxic pathways	[7,14]
Gene–diet interaction	Low AMY1 CNV + high-starch diet → mismatch between enzymatic capacity and substrate load	Exacerbates postprandial hyperglycemia and insulin resistance	[34]
Starch type (rapid vs. resistant)	High glycemic starches increase demand for early amylase activity	Individuals with low AMY1 CNV show poor metabolic tolerance to rapidly digestible starches	[35]

Another study by Mandel et al. [4] found a significant positive correlation (r=0.50) between *AMY1A* copy numbers and salivary amylase levels, suggesting that higher *AMY1A* copy numbers are associated with increased salivary amylase activity.

This study also assessed the oral perception of starch viscosity over time, revealing that viscosity decreased during mastication. Participants with higher salivary amylase concentrations were able to detect this decrease more quickly and to a greater extent than those with lower amylase levels. The above results suggest that individuals with higher salivary amylase concentrations experience enhanced texture (creaminess), and flavor (sweetness) perceptions of the same foods compared to those with low amylase levels. These differences in oral perception—such as viscosity, texture, and flavor—may influence dietary choices. Increased salivary amylase levels could, therefore, directly affect the duration of mastication and sensory perceptions of starches, influencing food preferences and satiety.

Supporting these findings, Harthoorn et al. [37,38] observed weak correlations between changes in salivary α-amylase concentration and food intake. Specifically, they reported a negative correlation (r^2=0.16) between test-meal intake and the percent change in salivary α-amylase before and after a preload meal, as well as a positive correlation (r^2=0.16) between test-meal intake and the percent change in salivary α-amylase concentration during the meal. However, these findings do not establish a direct relationship between baseline salivary amylase levels and meal size. Additionally, the study assessed only one aspect of subjective appetite ("how full are you") without analyzing its association with amylase activity. While the exact mechanisms remain unclear, these findings suggest that AMY1A copy number variations may influence oral food perception, potentially affecting dietary choices and metabolic risk.

2.5.1. Influence of salivary amylase activity on hypothalamic appetite centers

High salivary amylase activity may contribute to faster postprandial glucose rises, potentially resulting in the more rapid suppression of hunger signals. Salivary amylase activity (SAA) plays a multifaceted role in energy homeostasis that extends beyond digestion. Elevated SAA activity accelerates the breakdown of dietary polysaccharides into maltose and glucose, leading to an earlier and more pronounced rise in postprandial glycaemia. This rapid increase in circulating glucose facilitates an anticipatory cephalic-phase insulin response (CPIR) via vagally mediated activation of pancreatic β-cells, contributing to early satiety signaling before significant nutrient absorption occurs [39].

The rapid availability of glucose and the accompanying endocrine response influence central appetite-regulating circuits. Postingestive signals—including insulin, glucose, and gut-derived hormones—are integrated by hypothalamic nuclei such as the arcuate nucleus (ARC). Within the ARC, anorexigenic neurons expressing pro-opiomelanocortin (POMC) and cocaine- and amphetamine-regulated transcript (CART), and orexigenic neurons expressing neuropeptide Y (NPY) and agouti-related peptide (AgRP), transduce these signals to modulate feeding behavior and energy balance [40,41].

In parallel, SAA may influence appetite regulation via the gut–brain axis. By modulating the rate of starch digestion, SAA affects substrate availability for colonic fermentation, altering the composition and metabolic activity of the gut microbiota. Elevated SAA has been associated with increased basal levels of butyrate, a key short-chain fatty acid (SCFA) with endocrine activity [33]. Butyrate enhances the intestinal secretion of glucagon-like peptide-1 (GLP-1), peptide YY (PYY), and other incretins through G-protein coupled receptors (e.g., FFAR2/3), which engage vagal afferents and

act centrally to suppress appetite. These nutrient-derived and hormonal signals converge on hypothalamic energy-sensing pathways, including AMP-activated protein kinase (AMPK) and the mechanistic target of rapamycin (mTOR). AMPK is activated under conditions of energy deficit and promotes feeding, while mTOR activation signals nutrient sufficiency and suppresses food intake. Thus, changes in SAA-mediated glucose dynamics and gut-derived metabolites may shift the AMPK/mTOR balance in favor of satiety.

Downstream, hypothalamic integration of afferent metabolic signals modulates efferent autonomic pathways, regulating thermogenesis through uncoupling protein 1 (UCP1) expression in brown and beige adipocytes, lipolysis in white adipose tissue, and energy expenditure in skeletal muscle [42]. These processes contribute to the maintenance of the whole body's energy balance.

Overall, salivary amylase activity serves as a physiological modulator of central and peripheral pathways governing appetite regulation, thermogenic output, and energy expenditure, thereby linking oral carbohydrate sensing to long-term metabolic homeostasis.

2.5.2. Hormonal modulation of appetite: the role of insulin

Insulin, secreted by pancreatic β-cells in response to elevations in plasma glucose concentrations, exerts central anorexigenic effects through its action on the hypothalamus. Beyond its classical role in peripheral glucose uptake, insulin penetrates the blood–brain barrier via receptor-mediated transcytosis and binds to insulin receptors (IRs) expressed in the arcuate nucleus (ARC) of the hypothalamus. This initiates intracellular signaling cascades, notably the PI3K–Akt and MAPK pathways, modulating the activity of appetite-regulating neuropeptides.

Within the ARC, insulin inhibits the expression of orexigenic neuropeptides—neuropeptide Y (NPY) and agouti-related peptide (AgRP)—while concurrently stimulating the anorexigenic pro-opiomelanocortin (POMC) and cocaine- and amphetamine-regulated transcript (CART) neurons. This results in a suppressed appetite and reduced energy intake.

Individuals exhibiting high salivary amylase activity (SAA) display enhanced preabsorptive starch digestion, resulting in more rapid oral-phase glucose availability. This facilitates cephalic-phase insulin release (CPIR)—a parasympathetically mediated anticipatory insulin response via vagal efferents—which precedes measurable glycemia. This early insulin release contributes to central appetite suppression before substantial nutrient absorption occurs.

Therefore, high SAA may reinforce insulin's central anorexigenic signaling through both early hormonal release and potentiated hypothalamic action, leading to earlier satiety onset and reduced caloric consumption.

2.6. Incretin hormone response and the enteroinsular axis

The enteroinsular axis represents a highly regulated hormonal feedback loop between the gut and pancreatic islets, critically mediated by incretin hormones. Following carbohydrate ingestion, incretins—primarily glucagon-like peptide-1 (GLP-1) from L-cells in the distal ileum and colon, and glucose-dependent insulinotropic polypeptide (GIP) from K-cells in the proximal duodenum—potentiate glucose-stimulated insulin secretion (GSIS) in a glucose-dependent manner.

2.6.1. Modulation of GLP-1 and GIP by salivary amylase activity

Elevated SAA enhances the rate of oral-phase starch hydrolysis, providing a rapid increase in luminal glucose concentrations within the proximal small intestine. This stimulates sodium-glucose cotransporter 1 (SGLT1)-mediated glucose uptake by enteroendocrine cells, triggering the exocytosis of GLP-1 and GIP via intracellular cAMP and Ca^{2+} signaling pathways.

GLP-1 augments insulin biosynthesis and secretion from pancreatic β-cells via GLP-1 receptor (GLP1R)-mediated activation of adenylyl cyclase–cAMP–PKA signaling, while concurrently inhibiting glucagon secretion, slowing gastric emptying, and promoting satiety via hypothalamic GLP1R-expressing neurons.

In individuals with high AMY1 gene copy number (CNV), the elevated enzymatic efficiency of SAA leads to more effective starch digestion and accelerated glucose absorption, thereby amplifying incretin release and enteroinsular signaling. This cascade supports superior postprandial glycemic regulation, higher incretin-dependent insulinotropic effect, and a more physiologically efficient insulin response.

Thus, SAA functions not only as a digestive enzyme but as a regulator of metabolic homeostasis through its upstream modulation of the incretin axis [43] and downstream insulin dynamics.

3. Studies linking AMY1 CNV and salivary amylase activity with glycemic control and insulin dynamics

Salivary amylase, encoded by the AMY1 gene, has gained attention due to its role in metabolic processes, particularly its influence on glycemic control and insulin dynamics.

3.1. AMY1 CNV and glycemic regulation

Researchers have established a link between *AMY1* copy number variation (CNV) and glycemic regulation. Viljakainen et al., [29] demonstrated that low *AMY1* CNV is associated with early-onset obesity in Finnish females, suggesting that reduced salivary amylase activity may impair metabolic regulation. Similarly, Falchi et al. [28] reported a predisposition to obesity in individuals with lower *AMY1* CNV, implicating diminished salivary amylase activity in suboptimal starch digestion, altered glycemic responses, and disrupted insulin dynamics.

Bonnefond et al. [44] further supported these findings through a systems biology approach that revealed an inverse relationship between salivary amylase activity and body mass index (BMI). These findings highlight the potential of amylase in maintaining glycemic control via efficient carbohydrate metabolism.

3.2. Observational studies on AMY1A, insulin resistance, and type 2 diabetes

In several observational studies, researchers have investigated the relationship between AMY1 CNV, insulin resistance, and type 2 diabetes risk. Barber et al. [14] demonstrated that while AMY1 CNV correlates with glucose absorption and visceral fat volume, it does not directly correlate with insulin resistance. This finding emphasizes the complexity of metabolic pathways influenced by AMY1 activity.

Viljakainen et al. [29] reported that low AMY1 CNV is associated with early-onset female obesity, highlighting its potential link to metabolic dysregulation. Higuchi et al. (2014) observed similar associations in healthy young Japanese women, suggesting that reduced AMY1 CNV may contribute to impaired glucose metabolism.

Choi et al. [7] found a significant association between low AMY1 CNV and increased insulin resistance in asymptomatic Korean men, further supporting its role in glycemic control. Additionally, Liu et al. [34] revealed that AMY1 CNV modifies the risk of age-related type 2 diabetes, demonstrating a gene–environment interaction in metabolic health.

Marquina et al. [45] identified increased inflammation and cardiometabolic risk in individuals with low AMY1 CNV, suggesting a potential link between AMY1 activity and systemic inflammation. Atkinson et al. [46] summarized the phenotypic significance

Table 2
Summary of human observational studies on salivary amylase activity and glucose metabolism.

Reference	Population	Main findings	Implications for glucose metabolism
Barber et al. [14]	General population	AMY1 CNV correlates with glucose absorption and visceral fat, not directly with insulin resistance	Highlights complex metabolic pathways influenced by AMY1 activity
Viljakainen et al. [29]	Female children/adolescents	Low AMY1 CNV associated with early-onset obesity	Suggests a link between AMY1 and early metabolic dysregulation
Higuchi et al. [49]	Healthy young Japanese women	Low AMY1 CNV associated with impaired glucose metabolism	Supports role of AMY1 in glucose regulation in lean individuals
Choi et al. [7]	Asymptomatic Korean men	Low AMY1 CNV associated with increased insulin resistance	Indicates role of AMY1 in insulin sensitivity in men
Liu et al. [34]	General adult population	AMY1 CNV modifies age-related type 2 diabetes risk via gene–environment interaction	Shows AMY1's role in modulating T2D risk with aging
Marquina et al., 2020	Adults	Low AMY1 CNV linked to higher inflammation and cardiometabolic risk	Suggests AMY1 may influence systemic inflammation and metabolic syndrome
Atkinson et al. [46]	Literature review	AMY1 CNV impacts carbohydrate metabolism and overall metabolic health	Summarizes broad phenotypic consequences of AMY1 CNV
Mejía-Benítez et al. [35]	Mexican children	High AMY1 CNV associated with lower obesity risk	Protective effect attributed to better starch digestion and glycemic control
Marcovecchio et al. [48]	Prepubertal boys	Low AMY1 CNV associated with higher BMI and possibly reduced insulin sensitivity	Suggests AMY1 impacts early insulin release and glucose homeostasis

of AMY1 CNV, highlighting its impact on carbohydrate metabolism and its broader implications for metabolic health.

In contrast, high AMY1 CNV appears to confer protective effects against obesity and metabolic dysfunction. Mejia-Benitez et al. [35] found that Mexican children with higher AMY1 CNV exhibited a reduced risk of obesity. This protective effect is thought to result from enhanced starch hydrolysis, facilitating rapid glucose absorption, and favorable modulation of postprandial glycemic responses.

While studies have consistently reported associations between AMY1 CNV, SAA activity, and metabolic outcomes [47], the evidence does not establish direct causality.These findings should be interpreted as hypothesis-generating, and further experimental studies are required to confirm the mechanistic links.

Marcovecchio et al. [48] explored the relationship between AMY1 CNV and BMI in prepubertal boys, demonstrating that lower AMY1 CNV was associated with increased BMI and potentially decreased insulin sensitivity. These findings suggest that salivary amylase activity, modulated by AMY1 CNV, influences early insulin release and glucose homeostasis, underscoring its importance in metabolic health (Table 2).

4. Methodological considerations

4.1. Inconsistencies in measuring salivary amylase activity and AMY1 CNV

Despite growing interest in SAA and AMY1 gene copy number variation (CNV) as potential modulators of metabolic health, substantial methodological variability interferes with data comparability across studies. First, many investigations use AMY1 CNV as a proxy for enzymatic activity, assuming a linear relationship between gene dosage and salivary enzyme output. However, post-transcriptional and epigenetic regulation, autonomic nervous system activity, and dietary factors can significantly modulate enzyme expression independently of gene copy number [50,51].

Enzymatic activity assays also lack standardization. Studies utilize varied substrates, pH conditions, and buffer systems to quantify amylolytic activity, leading to divergent results even among individuals with similar AMY1 CNVs. Saliva collection protocols further complicate interpretation—stimulated versus unstimulated saliva, time of day, fasting status, and acute stress all influence SAA levels. As a result, cross-sectional studies often report inconsistent associations between SAA and metabolic phenotypes.

Future research must prioritize harmonized methodologies for both AMY1 genotyping (e.g., digital droplet PCR, whole genome sequencing) and salivary amylase activity quantification using physiologically relevant substrates under standardized conditions. Inclusion of both genotypic and phenotypic data in the same cohorts is essential to disentangle genetic predisposition from functional enzymatic capacity.

4.2. Dynamic vs. static measures of insulin resistance: implications for sensitivity to SAA

A critical methodological limitation in evaluating the metabolic effects of salivary amylase is the widespread reliance on static indices of insulin resistance, such as the Homeostatic Model Assessment of Insulin Resistance (HOMA-IR). These indices are derived from fasting glucose and insulin concentrations and may not capture early-phase insulin responses or glucose disposal dynamics influenced by cephalic or oral-phase stimuli.

Salivary amylase activity is hypothesized to modulate early postprandial insulin signaling, particularly through enhancement of the cephalic phase insulin response (CPIR) and incretin-mediated insulin secretion. These effects are acute, time-sensitive, and often not reflected in fasting measures. Therefore, studies utilizing only static indices may underestimate or entirely miss the metabolic influence of SAA.

Conversely, dynamic tests—including oral glucose tolerance tests (OGTT), mixed meal tolerance tests (MMTT), Matsuda index, insulinogenic index, or hyperinsulinemic-euglycemic clamps—offer

a more nuanced assessment of insulin sensitivity and β-cell function in response to glucose challenges [52]. For instance, the Matsuda index integrates fasting and postload glucose and insulin values, capturing whole-body insulin sensitivity, while the insulinogenic index reflects β-cell responsiveness within the first 30 minutes of glucose ingestion—a window closely aligned with the CPIR (Table 3).

Studies using these dynamic measures have more consistently observed associations between high SAA or AMY1 CNV and improved postprandial glucose control, insulin sensitivity, and lower glycemic excursions. These findings support the hypothesis that SAA exerts its metabolic effects primarily in the postingestive window, emphasizing the importance of using appropriate phenotyping tools.

Incorporating dynamic testing in future studies will likely enhance sensitivity to the modulatory role of SAA and better elucidate its physiological relevance.

5. Clinical and nutritional relevance

The modulation of glucose homeostasis, insulin secretion, and appetite regulation by salivary amylase activity has important implications for clinical diagnostics, metabolic disease prevention, and personalized nutrition strategies. Given its variability across individuals, which is primarily driven by AMY1 gene copy number variation, salivary amylase activity may serve as a functional, noninvasive biomarker for metabolic phenotyping.

Individuals with higher salivary amylase activity exhibit more efficient preabsorptive carbohydrate processing, potentially leading to attenuated postprandial glycemic excursions, enhanced insulin sensitivity, and improved satiety signaling. These traits support the use of salivary amylase profiling in guiding personalized dietary recommendations [53]. For example, those with lower enzymatic activity may benefit from low-glycemic-load diets to reduce the risk of insulin resistance. Furthermore, the association between salivary amylase and incretin-mediated satiety pathways underscores its relevance in obesity prevention and appetite control. Enhanced enzymatic activity may potentiate early-phase glucose signaling and gut–brain axis responses, influencing hunger regulation through modulation of hormones such as GLP-1 and insulin.

In the context of type 2 diabetes and insulin resistance, assessing salivary amylase activity could provide valuable insight into individual glucose–insulin dynamics. Integrating this parameter into clinical practice may help stratify patients by metabolic risk and support tailored nutritional interventions aimed at glycemic control.

Awareness of individual differences in carbohydrate metabolism could foster more targeted nutritional recommendations, ultimately contributing to the reduction of obesity and metabolic syndrome prevalence.

By linking oral-phase digestion to systemic endocrine and metabolic responses, salivary amylase represents a promising target for translational research and clinical innovation. Continued investigation into its mechanistic role and predictive capacity will be essential for advancing precision nutrition and improving outcomes in metabolic health [54].

6. Future directions and research gaps

Despite growing evidence supporting the role of AMY1 gene copy number variation (CNV) and salivary amylase activity in glycemic regulation and insulin dynamics, significant gaps remain in elucidating the precise mechanisms and clinical applicability of these findings. Addressing these limitations will be critical for advancing this field toward translational relevance.

6.1. Mechanistic studies and longitudinal human trials

While these studies provide valuable associations, mechanistic insights into how salivary amylase modulates glucose metabolism, insulin signaling pathways, and enteroinsular communication remain underexplored. Preclinical models and human interventional studies are needed to:

(1) Elucidate the molecular and neural pathways linking oral starch hydrolysis with pancreatic β-cell function and hypothalamic appetite regulation.
(2) Characterize the temporal dynamics of incretin hormone release in relation to salivary amylase-mediated glucose flux in the proximal small intestine.
(3) Assess how chronic modulation of salivary amylase activity, through diet or pharmacological agents, influences insulin sensitivity and adipose tissue metabolism over time.

Longitudinal cohort studies with detailed phenotyping and dietary tracking are essential to determine the long-term metabolic consequences of low versus high AMY1 CNV across diverse populations.

6.2. Standardization of salivary amylase activity assays

One of the major limitations in comparative studies is the lack of standardized protocols for the assessment of salivary amylase activity. Variations in assay temperature, pH conditions, substrate concentrations, and units of measurement hinder cross-study interpretation and meta-analytic synthesis [55]. To enable reproducibility and clinical translation, future efforts must prioritize:

(1) Development of harmonized protocols for enzymatic activity quantification, optimized for physiological relevance (e.g., near-neutral pH, human-specific starch substrates).
(2) Implementation of internal quality controls and calibration standards for enzymatic assays across laboratories.
(3) Integration of novel biosensor platforms and high-throughput microfluidic technologies to facilitate real-time, point-of-care amylase measurements with clinical diagnostic potential.

Such methodological advancements will allow for the robust evaluation of salivary amylase as a dynamic biomarker of glycemic adaptability and metabolic risk.

6.3. Gene–diet interactions and personalized intervention strategies

The interindividual variability in glycemic response to carbohydrate-rich meals is increasingly recognized as being modulated by host genetic factors, including AMY1 CNV. However, the field lacks comprehensive nutrigenomic studies investigating how specific dietary carbohydrate types (e.g., resistant starch, rapidly digestible starch) interact with salivary amylase activity to influence postprandial glucose dynamics and insulin response. Future research should aim to:

(1) Conduct controlled feeding trials stratified by AMY1 CNV to test differential metabolic responses to starch composition, glycemic index, and meal timing.
(2) Explore how amylase-related phenotypes modulate gut microbiota composition and short-chain fatty acid production (e.g., butyrate), with implications for insulin sensitivity.

Table 3
Comparison of common insulin resistance indices and their relevance to salivary amylase physiology.

Index	Type of measurement	Physiological target	Data required	Captures postprandial dynamics?	Relevance to SAA physiology	Limitations
HOMA-IR	Static	Hepatic insulin resistance	Fasting glucose & insulin	✗	Low. Does not reflect postprandial starch digestion, CPIR, or incretin action.	Overlooks peripheral IR and dynamic insulin responses.
TyG index	Static	Hepatic insulin–lipid interaction	Fasting glucose & triglycerides	✗	Low–moderate. May indirectly reflect insulin resistance but not specific to SAA-linked mechanisms.	Nonspecific to insulin signaling; influenced by lipid metabolism.
Matsuda index (OGTT-based)	Dynamic	Whole-body IR (hepatic + peripheral)	Glucose & insulin at 0, 30, 60, 90, 120 min	✓	High. Reflects postprandial insulin action and is sensitive to SAA effects on early glucose and insulin kinetics.	Requires OGTT; more labor-intensive.
Stumvoll index	Dynamic	Peripheral insulin resistance	OGTT data	✓	High. Captures insulin sensitivity during glucose challenge, aligned with early starch hydrolysis and incretin response.	Assumptions may vary by population.
Gutt index(Insulin sensitivity index(ISI)	Dynamic	Whole-body insulin resistance	OGTT data	✓	High. Sensitive to changes in postload glucose handling, relevant for assessing SAA's influence on glucose disposal.	Less widely used; requires accurate time-point sampling.
Hyperinsulinemic–euglycemic clamp	Gold standard	Peripheral insulin resistance	Continuous insulin & glucose infusion	✓✓	Very High. Ideal for quantifying IR; could confirm SAA-related differences.	Invasive, expensive, not feasible in large cohorts.

(3) Develop personalized dietary algorithms and digital decision-support tools that integrate AMY1 genotype, salivary amylase activity, and continuous glucose monitoring data to guide individualized carbohydrate recommendations.

Furthermore, integrating machine learning approaches to analyze large multiomic datasets may uncover previously unrecognized gene–environment interactions, supporting the implementation of precision nutrition frameworks aimed at preventing metabolic diseases.

Limitations of Current Evidence.

While numerous studies have reported associations between SAA activity and metabolic outcomes, the evidence remains largely correlational. The absence of randomized controlled trials and mechanistic studies limits the ability to draw definitive conclusions about causality. Future research should focus on experimental designs, such as interventional studies that manipulate SAA activity, to determine its direct impact on glucose homeostasis and insulin sensitivity.

7. Conclusion

Salivary α-amylase (SAA) has emerged as a potential modulator of appetite regulation through its influence on glucose availability, hormonal signaling, and cephalic-phase insulin secretion. Beyond its well-established role in starch digestion, SAA activity may interact with key metabolic pathways, including the gut-brain axis and the secretion of satiety-related hormones such as GLP-1, PYY, and ghrelin. These interactions suggest that SAA is not simply a digestive enzyme but also a contributor to the complex physiological mechanisms governing appetite and energy balance. Although the evidence remains correlational, SAA activity holds promise as a biomarker for metabolic health and appetite regulation. Future studies should focus on elucidating causal mechanisms.

Interindividual variability in SAA activity, driven by genetic, dietary, and environmental factors, highlights the need for further research to clarify its metabolic implications. Standardized methodologies for assessing SAA activity and its metabolic effects will be essential for advancing our understanding of its role in appetite control and metabolic health.

While the evidence is still emerging, SAA holds promise as a biomarker for appetite regulation and a potential target for therapeutic strategies aimed at improving metabolic outcomes. Future studies should focus on elucidating the mechanistic pathways connecting SAA activity to appetite and energy balance, with an emphasis on integration into personalized approaches for managing obesity, diabetes, and other metabolic disorders.

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CRediT authorship contribution statement

Gita Erta: Writing – original draft, Resources, Project administration, Investigation, Conceptualization. **Gita Gersone:** Supervision, Methodology, Data curation. **Antra Jurka:** Software, Investigation, Data curation. **Pēteris Tretjakovs:** Writing – review & editing, Validation, Conceptualization.

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Pētījumu ētikas komitejas
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Pētījumu ētikas komitejas sēdes datums: 30.09.2021.

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