

MTech Thesis Kanan 2

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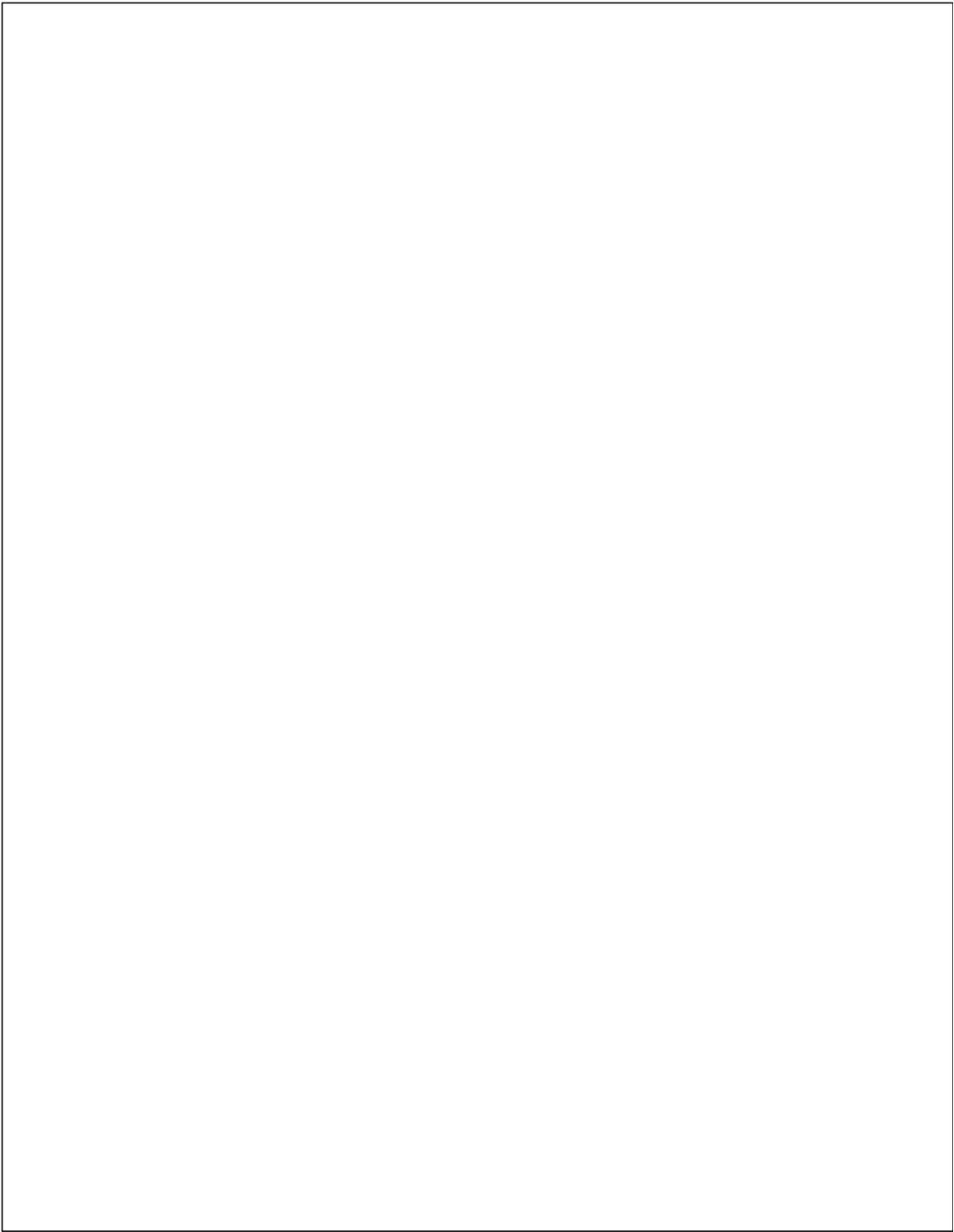
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ABSTRACT

Obesity is a multi-cellular metabolic syndrome characterised by dysregulated adipose tissue growth, mitochondrial bioenergetics and disrupted glucose and lipid homeostasis. Although transcriptome-scale profiling provides a thorough understanding of these changes, the accuracy of conventional statistical methods is limited by the nonlinear and high-dimensional structure of gene expression data. To address these challenges, an integrated ³⁶ machine-learning and explainable ¹³ artificial intelligence framework was developed to discover transcriptomic predictors of obesity in human skeletal-muscle and adipose tissue. Ensemble classification models i.e. Random Forest and Extreme Gradient Boosting were applied to normalised gene expression matrices derived from publicly available datasets(GSE22039 and GSE103766) with systematic hyperparameter optimization. SHapley Additive exPlanations(SHAP) promote model interpretability, enabling quantitative measure of each gene contributions to classification outcomes. Key transcripts such as BDH1, MRPS31, RARS2, STOML2, AKAP10 and UCP3 were aligned with mitochondrial metabolic pathways involving ketone oxidation, ribosomes function, cristae stability and proton leak modulation. Network analysis identified NUCB2, SRSF1 and ACTG2 as the key topological nodes while Enrichment analysis using Enricher revealed significant alterations in oxidative phosphorylation interactions, reactive oxygen and nitrogen mediated signalling pathways. Overall this study shows the significance of an xAI enabled framework for identifying biologically significant transcriptomic signatures related to obesity.

CHAPTER 1¹⁶

INTRODUCTION

1.1 Background

Obesity is a chronic multifactorial metabolic condition with an excess fat accumulation that negatively influences the functioning of the body and promotes the development of various metabolic and cardiovascular diseases [1,2]. BMI is widely used to diagnose obesity in adults (BMI > 30 kg/m²) [5]. BMI is an easy to measure epidemiological indicator but also does not reflect the distribution of body fat or metabolic differences between individuals [8]. Increased adiposity of various body compartments, especially abdominal fat, has been reported to have a stronger relationship with metabolic dysfunction and insulin resistance than with total body weight [9]. Therefore obesity is no longer viewed as simply over accumulation of body fat, but is now considered to be a systemic metabolic disease with a large amount of physiological and molecular abnormalities [10]. The biological mechanisms underlying obesity involve dysregulation of energy homeostasis controlled by neuroendocrine, metabolic and molecular signaling networks [11]. Ghrelin are hormones that are important for hunger, satiety and glucose metabolism [13]. Adipose tissue produces a hormone called leptin, which under normal physiological conditions signals fullness to the hypothalamus which in turn inhibits food intake [14]. In most cases, however, increased levels of leptin do not seem to provide sufficient satiety to curb appetite and lead to ongoing energy intake and weight gain (leptin resistance) [15]. The biologically active molecules produced by adipose tissue are called adipokines, which include leptin, adiponectin, inflammatory cytokines and resistin, all of which are responsible for the regulation of insulin sensitivity, lipid metabolism, and systemic inflammation [17].

Another important metabolic tissue that is also tightly coupled to obesity-associated pathophysiology is skeletal muscle, which in humans represents around 70-80% of insulin mediated glucose uptake. Glucose and fatty acids are effectively used by skeletal muscle under physiological conditions for energy homeostasis [21]. Obesity however, has a negative effect on insulin signalling pathways in skeletal muscle, resulting in decreased glucose uptake and mitochondrial oxidative efficiency [22]. These deficits lead to metabolic inflexibility, build up of intracellular lipid metabolites and are linked to ²⁰insulin resistance and type 2 diabetes mellitus [23]. Mitochondrial dysfunction ^{has been} well recognized in metabolic abnormalities of obesity [24]. The resulting elevated oxidative stress then triggers inflammatory pathways and leads to further damage, poor insulin signaling and metabolic disorders [27].

These cytokines alter the insulin receptor signaling pathways and worsen insulin resistance by triggering stress-related signaling pathways [31]. There is also an association with endothelial dysfunction, metabolic syndrome and elevated cardiovascular risk [32] that persists because of the inflammatory signalling. In particular, the techniques of transcriptomics have allowed genome-scale analysis ⁵of changes in gene expression that are linked to obesity and metabolic disorders [34]. ¹⁵Large scale gene expression repositories like the Gene Expression Omnibus (GEO) offer a collection of data that can be used to systematically study molecular signatures associated with obesity [35]. Complex gene-gene interaction and tissue-specific molecular relationships are not captured by conventional statistical methods [39]. Machine learning methods have thus become highly effective tools to address high-dimensional omics data analysis and to discover predictive molecular signatures [40]. Machine learning methods have impressive predictive power but suffer from low interpretability, which limits their use in the biological and clinical contexts [42]. Predictive models have therefore attracted significant interest in methods of making AI models

explainable (XAI) [43]. Cooperative game theory-based explainability framework, SHapley Additive exPlanations (SHAP) [44] allows quantitative interpretations of feature importance and individual prediction results. These help identify biologically relevant biomarkers and boost confidence in machine learning predictions [45]. Besides the predictive modelling, another contribution of system biology to the understanding of mechanistic changes in obesity-related changes in molecular pathways has been made [46]. The above mentioned databases and tools such as STRING, Cytoscape, KEGG, Reactome and Enrichr have therefore become essential tools for interpretation of transcriptomic data in relation to metabolic diseases [48]. Here, we use an integrated computational framework to uncover obesity-associated transcriptomic signatures in public human adipose tissue and skeletal muscle cohorts, GSE22309 and GSE103766 [35]. In addition, systems biology validation is conducted by protein interaction and pathway enrichment analysis to enhance biological interpretation of the identified biomarkers. This holistic strategy is designed to enhance mechanistic understanding of obesity and to help identify trustworthy transcriptomic markers for precision medicine of obesity.

1.2 Literature Review

1.2.1 Molecular Basis and Pathophysiology of Obesity

The disease is a result of the combination of genetic susceptibility, environmental exposure, eating habits, sedentary lifestyle and molecular changes in the regulation of systemic metabolism [3,11]. While excess calories still play a key role, there is significant evidence that molecular and transcriptomic changes in metabolically active tissues strongly influence the progression of obesity [34]. In addition, over-expanded adipose tissue induces hypoxia, inflammatory activation and cellular stress, all of which contribute to worsen metabolic imbalance [29].

²⁹ **1.2.2 Adipose Tissue Dysfunction in Obesity**

Adipose tissue is important for systemic energy homeostasis in regulating lipid storage, endocrine signaling and metabolic regulation [16,17]. Obesity-induced adipose tissue expansion leads to significant molecular and physiological changes and results in adipose tissue becoming a metabolically protective organ to a dysfunctional inflammatory environment [18].

But in obesity excess energy balance is maintained and causes excessive adipocyte expansion, causing adipocyte hypertrophy and hyperplasia [18].

Another important adipokine in regulating metabolism and insulin sensitivity is adiponectin [51].

In contrast, the blood ⁷levels of adiponectin are markedly decreased in obese people ⁷[52].

The expansion of adipose tissue is often greater than the capacity of the vessels, resulting in areas of hypoxia that trigger inflammatory signaling pathways [54]. As a result, the fat tissue hypertrophied becomes infiltrated with macrophages, neutrophils and T lymphocytes that together exaggerate inflammatory processes [30].

In obese adipose tissue, macrophages are the most prominent inflammatory immune cell [30].

Alternatively activated (M2) macrophages are known to promote anti-inflammatory functions and tissue homeostasis under lean physiological conditions [56].

Excessive collagen deposition decreases tissue compliance and adipocyte distension, and thus lipid storage capacity [59]. The fibrotic adipose tissue therefore encourages ectopic fat deposition in metabolically active tissues and helps to develop systemic insulin resistance [60].

Visceral fat has been found to be more active from an endocrine and inflammatory standpoint than ³⁸other fat depots [61]. Visceral adipose tissue, due to its anatomical location, directly affects the metabolic function of the liver by secreting inflammation mediators and free fatty acid, which is why it affects liver metabolism [62].

¹⁹ **1.2.3 Mitochondrial Dysfunction and Oxidative Stress in Obesity**

Mitochondria are vital cellular organelles that play a key role in energy production, control of oxidative phosphorylation, fatty acid oxidation and maintenance of metabolic homeostasis [24,25]. As obesity is defined as a chronic state of over-consumption of nutrients and altered energy metabolism, mitochondrial dysfunction has become one of the major molecular mechanisms of obesity-associated metabolic disorders [24]. The ability to use glucose and fatty acids in an appropriate manner to meet cellular energy requirements is related to efficient mitochondrial respiration [22]. Obesity disturbs mitochondrial bioenergetics in a way that involves a decreased activity of the electron transport chain, a decreased oxidative capacity, and impaired substrate oxidation [26]. There have been several reports of decreased mitochondrial density and diminished mitochondrial biogenesis in obese people [64]. Thus, defects in FAMO lead to increased lipid storage in the cell and hence insulin resistance [23]. Impaired mitochondrial function is well linked to the production of ROS [26]. In normal physiological conditions, naturally occurring ROS are found in mitochondria and are involved in redox homeostasis and cell signaling in a controlled manner [67]. But when the electron transport chain is overloaded by excess mitochondria, due to obesity, the amount of ROS produced is over the limit [68]. These pathways are activated and lead to impaired insulin receptor substrate signaling and have a significant role in metabolic dysfunction [57]. Mitochondrial respiration and the activity of oxidative enzymes are often impaired in the skeletal muscle tissues of obese people [72]. Changes in genes related to oxidative phosphorylation and ATP synthesis and to the organization of the mitochondrial membrane in obesity have been identified by transcriptomic studies [36]. Mitochondrial metabolic genes are decreased in skeletal muscle which reduce the ability to use glucose and fatty acids leading to reduced metabolic

flexibility and systemic insulin resistance [22,72]. Likewise, during the onset of obesity, there are significant abnormalities in the mitochondria in adipose tissue [18].

Impaired mitochondria function has also been associated with changes in adipokines and inflammatory mediators secretion, which worsens obesity-related endocrine disturbances [17,18]. Some mitochondrial-associated genes have emerged as being involved in the pathogenesis of obesity, based on transcriptomic data [36]. In obesity, the genes that regulate oxidative phosphorylation, mitochondrial ribosomal function, and fatty acid oxidation and proton leak regulation are often dysregulated in obese tissues [73]. Thus, the study of mitochondrial metabolism has become an important therapeutic avenue to obesity management and intervention in metabolic diseases [74]. Transcriptomic technologies offer genome-wide analysis of coordinated molecular changes associated with disease progression as opposed to conventional molecular technologies that target individual genes [33]. There are extreme changes of gene expression in the various metabolically active ²¹ tissues of obese animals, including adipose tissue, skeletal muscle, liver and pancreatic tissue [17,20]. These tissues are involved in glucose metabolism, lipid homeostasis and endocrine signaling, and as such, transcriptomic studies offer insightful mechanistic information regarding metabolic dysfunction in obesity [36]. ²⁶ A large number of transcriptomic technologies have been developed to study disease-related molecular changes in a systematic manner, including high throughput microarray analysis and the RNA sequencing (RNA-seq) approach. Transcript levels across biological samples can be quantified in thousands of genes simultaneously using microarray based studies [75]. The improvements in transcriptomic resolution of RNA sequencing technologies further enhance the capability to identify changes in gene expression in an unbiased way, as well as transcript variants and novel molecular signatures [76].

1.2.5 Transcriptomics and Biomarker Discovery in Obesity

Transcriptomic obesity studies have benefited from the use of publicly available repositories [35]. The existence of transcriptomic datasets related to obesity makes it possible to carry out integrative analysis and to obtain reproducible obesity-related biomarkers in different cohorts [77]. Given the data set used for obesity, the adipose tissue and skeletal muscle sets are important since these tissues are central to energy metabolism and insulin sensitivity [18,20]. Transcriptomic analysis of these skeletal muscle tissues has shown differences ²³ in the expression of genes that control mitochondrial oxidative metabolism, glucose transport and ATP production in obese people [22,36]. In order to find systemic obesity-associated molecular signatures, cross-tissue transcriptomic analysis has become an important strategy [37]. Integrative transcriptomics studies give a biological overview superior to single tissue studies as obesity impacts a multitude of interconnected metabolic tissues [38]. The analysis of the simultaneous adipose and skeletal muscle data allows the identification of conserved molecular pathways involved in metabolic dysfunction, inflammation and mitochondrial impairment [28]. Although the use of transcriptomics to discover biomarkers has made significant progress, it is difficult to discover them because of its high dimensionality and heterogeneity of gene expression data [38]. Often biological variation, technical noise and non-linear interactions between genes make the identification of candidate biomarkers using traditional statistical approaches less reproducible [39]. As a result, biomarker identification in transcriptomics has been increasingly assisted by advanced computational and machine learning methods to enhance the accuracy of biomarker identification [40].

1.2.6 Machine Learning Approaches in Obesity and Transcriptomic Research

However, conventional statistical techniques, which are used mainly to detect linear relationships, can be limited in their ability to provide insights into these types of datasets and may not be able to detect complex molecular interactions [39]. As a result, machine learning techniques have become important computational tools that allow the extraction of biologically relevant information from omics-scale data, and the discovery of molecular signatures that are predictive of complex diseases, such as obesity [40].

Supervised learning models are commonly applied in the field of obesity research for classification of obese and non-obese samples, for prediction of disease susceptibility and for the discovery of molecular biomarkers linked to metabolic dysregulation [40]. Unlike supervised learning, the unsupervised learning methods discover hidden patterns or subgroups of molecules without any specific labels and are especially suited for clustering transcriptomic profiles and detecting the heterogeneity of diseases [82].

Transcriptomic obesity studies have shown that several supervised machine learning techniques have shown to perform well [41]. Logistic Regression is among the simpler classification methods and models used to describe relationship between predictor variables, and categorical outcomes [83]. Logistic Regression, interpretable and computationally efficient, has been found to have an inferior performance in high dimensional and highly non-linear transcriptomic datasets [39]. But the performance of SVM can be degraded in very heterogenous biological data sets when the number of features becomes too high [85].

The interpretability and capability of modelling nonlinear interactions have made Decision Tree based algorithms extremely important in the field of transcriptomic biomarker discovery [86]. Decision Trees are a classification model that splits the observations sequentially along features

and hence offer a clear prediction path [87]. Single decision trees can suffer from overfitting and loss of predictive stability, however [88]. To address these drawbacks, ensemble learning methods were later proposed to combine the predictions of the aggregates of multiple models [89].

Moreover, Random Forest is very useful to deal with high-dimensional data, such as transcriptomic data including thousands of variables related to the expression of genes [92].

Another highly popular ensemble machine learning method is Extreme Gradient Boosting (XGBoost) which is often used in the study of omics [93]. ³² The prediction performance of XGBoost models is incrementally enhanced by using gradient boosting, which sequentially uses models to reduce the error of the previous models [94]. There are several transcriptomic studies that have successfully applied XGBoost to find gene signatures associated with disease and enhance the discovery of biomarkers [96].

While machine learning methods overcome these disadvantages, they can identify informative subsets of genes that are linked to disease phenotypes and exclude redundant features [97]. Thus, computational approaches that integrate transcriptomics and machine learning have grown in use to discover obesity-associated biomarkers and therapeutic targets [98].

Though machine learning models are often highly predictive, they are often “black-box” systems that are not very interpretable [42]. This opacity often inhibits biological confidence and clinical translation as the molecular basis of predictions cannot be easily identified [43]. Thus, an important challenge in computational biology and precision medicine is to make the predictions of machine learning more interpretable [44].

1.2.7 Explainable Artificial Intelligence (XAI) and SHAP in Biomarker Discovery

Explainable Artificial Intelligence (XAI) denotes a set of computational techniques that have been created to make ML predictions more interpretable and transparent [43]. In contrast to traditional black-box algorithms, XAI methods enable researchers to gain insights into the contribution of specific features to prediction results [44]. Interpretability is a crucial property in biomedical research, as beyond clinical trust, there is a need for biologically meaningful explanations for therapeutic decisions and mechanistic understanding.

SHapley Additive exPlanations (SHAP) [44] is one of the most commonly used explainability frameworks in transcriptomic and biomedical research.

¹ To quantify the average contribution of each feature across all the predictions, the global SHAP analysis can be applied and it can help identify biologically important genes associated with disease phenotypes [102]. Combinations of genes explain the prediction outcomes at the individual sample level with the help of local interpretability approaches, such as SHAP force plots and waterfall plots [103].

³⁴ Ensemble machine learning algorithms, including Random Forest and XGBoost, have been integrated into SHAP, greatly enhancing the biological interpretability in omics research [96]. SHAP-based analyses could be used to prioritize molecular biomarkers and to also provide a biological explanation for computational predictions [44]. Such explainability frameworks are thus a step toward connecting predictive performance with mechanistic understanding, further improving the translatability of machine learning-based biomarker discovery [45].

Although machine learning has great potential in obesity-associated transcriptomic studies, explainable machine learning methods are relatively underutilized [98]. ¹ The majority of the previous studies have been conducted on predictive performance alone with limited biological validation of the biomarkers identified [39]. Thus, the potential for incorporating explainability

within transcriptomic machine learning applications is important for enhancing the reliability, reproducibility and mechanistic understanding of molecular signatures associated with obesity [44,98].

1.2.8 ¹Protein-Protein Interaction Networks and Pathway Enrichment Analysis in Obesity Research

These methods help in understanding the mechanisms by mapping molecular interactions, pathways of regulation, and the biological processes leading to the disease process.

Proteins do not normally act alone in biological systems, but rather are involved in highly coordinated networks of molecular interactions that control cellular homeostasis [104]. Protein-protein interaction networks provide a systematic representation of functional interactions among proteins and can identify central regulatory molecules that are associated with pathways of disease [105]. PPI network analysis has been widely used ³³in the study of obesity to discover the genes that are hub genes in inflammation, insulin signalling and lipid metabolism and mitochondrial function [106].

A hub gene is a highly interconnected molecular hub that often has significant regulatory effects on biological pathways [107]. The genetic signature of obesity has been determined by several obesity-related transcriptomic studies, which have been able to identify central hub genes that are related to mitochondrial respiration, oxidative phosphorylation and inflammatory signaling [108]. The presence of hub genes means that they control multiple downstream molecular mechanisms and may be suitable therapeutic targets and clinically relevant biomarkers [109].

STRING combines experimental data, computational predictions and literature-based evidence to construct detailed molecular interaction networks [111]. Machine learning or differential

expression analysis can then be applied to identify candidate genes from transcriptomes that can be mapped in STRING to discover functionally connected biological pathways [112].

After network generation, Cytoscape is often used to visualize and analyze protein interaction networks [113]. These analyses help to prioritize biologically important genes that could be related to the pathogenesis of obesity [115].

The biological interpretation of transcriptomic data can be further improved using functional enrichment analysis, which can elucidate the pathways that are strongly correlated with candidate genes [47]. Transcriptomic analyses frequently lead to the identification of gene sets that are known or suspected to be involved in pathways of metabolism, oxidative stress, mitochondrial respiration, inflammatory signaling and glucose homeostasis [36]. Functional enrichment, in turn, can give mechanistic understanding of disease progression and biological validation of computational predictions [46].

There are multiple pathway databases available for enrichment analysis of obesity studies. Enrichment analyses based on KEGG databases have been consistently found to include insulin resistance, oxidative phosphorylation, fatty acid metabolism and inflammatory signaling pathways as obesity-related pathways [117]. Likewise, detailed pathway annotations on molecular interactions, cellular processes and metabolic pathways are provided by the Reactome database [118].

Another popular functional enrichment platform is Enrichr, which combines multiple pathway databases into a single platform for convenient biological interpretation of transcriptomic data [119]. Enrichr is able to identify pathways that are overrepresented in the set of candidate genes and statistically significant using enrichment scoring and statistical significance testing [120]. The pathways associated with mitochondrial dysfunction, reactive oxygen species signaling, cytokine-

mediated inflammation and glucose metabolism are frequently reported to be enriched in transcriptomic studies of obesity [36,121].

Most importantly, systems biology validation enhances the reliability of machine learning-based framework for biomarker discovery [46]. Occasionally, features that are statistically significant but of no biological relevance can be identified by computational predictions only [42]. Combining PPI network analysis and pathway enrichment thus increases the confidence of discovered biomarkers that they play a role in biologically relevant molecular pathways [47].

Although numerous molecular pathways have been explored in obesity, there are a number of critical considerations that are not addressed in existing obesity biomarker discovery strategies [39]. The molecular interactions within systemic networks that play a role in obesity pathogenesis have been largely neglected in most obesity-related transcriptomic studies, which have been studying adipose tissue or skeletal muscle alone so far [37]. Because obesity is a multisystem metabolic disease that impacts the interconnected tissues, studies involving a single tissue may not capture the conserved transcriptomic signatures associated with metabolic dysfunction in the system [122].

Current transcriptomic studies have mainly focused on using differential gene expression analysis and classical statistical methods to identify biomarkers [38]. These approaches yield meaningful biological information, but they do not well represent the nonlinear molecular interactions and complex gene–gene interactions found in high-dimensional biological data sets [39]. This means that the reproducibility and predictive power of the biomarkers identified are often limited [40].

The challenges this poses have been met with machine learning approaches, which are able to describe complex nonlinear relationships and process multidimensional transcriptomic datasets [41]. In previous machine learning studies on obesity, however, most studies have focused on

prediction accuracy at the expense of limited biological interpretability [42]. Several machine learning algorithms, however, are “black box” in nature, which reduce biological confidence and clinical applicability of findings from computing [43].

Additionally, there are only some limited studies that systematically applied the EAI concepts, such as SHAP, to the field of obesity transcriptomic studies [44]. Current computational programs do not usually give a detailed description of the mechanisms through which the individual genes give rise to the classification outcome for obesity [98].

1.2.9 Research Gap

Also, biological validation studies at the systems level, by combining machine learning predictions with protein interaction networks and pathway analysis, have not been extensively used in obesity transcriptomic studies [46]. Numerous studies report candidate biomarkers without adequate validation of their involvement in functionally relevant metabolic pathways [47]. This limitation can hinder the ability to understand the mechanism and can limit the translational value of identified biomarkers [109].

Thus, there is still a significant demand for the development of an integrated computational framework that integrates multi-tissue transcriptomic analysis, ensemble machine learning, explainable artificial intelligence and systems biology validation to identify biologically interpretable obesity-associated molecular signatures [37,44].

1.3 Objectives

² The objectives of the present study were to:

1. To acquire and preprocess publicly available transcriptomic data from skeletal muscle and adipose tissue of obese individuals GSE22309 and GSE103766 [35].
2. To apply SHAP to explainable AI in the identification of obesity classification outcomes with significant genes for application in a biological context [44].
3. To discover biologically meaningful transcriptomic biomarkers and molecular pathways involved in the progression of obesity and metabolic dysfunction [36].

MATERIALS AND METHODOLOGY**2.1 Dataset Acquisition**

The selection of two obesity-associated transcriptomic datasets (GSE22309 and GSE103766) for the present investigation was based on their biological relevance to metabolic dysfunction associated with obesity and tissue specific importance in energy homeostasis [35,37]. These datasets capture the metabolically active tissues that are closely linked to glucose metabolism, insulin sensitivity, and systemic metabolism, thus providing a suitable context for integrated obesity transcriptomic analysis [20,37].

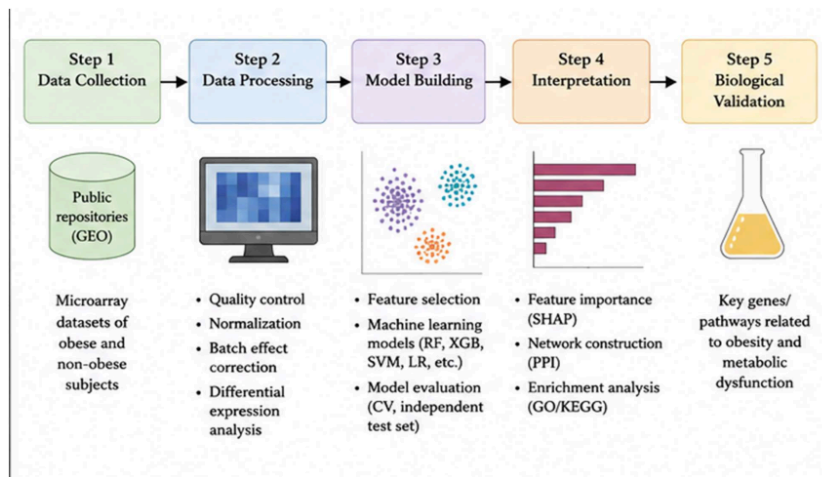
The GSE22309 data contained human skeletal muscle transcriptomic profiles [20]. Many molecular changes associated with obesity in skeletal muscle have been strongly correlated with mitochondrial dysfunction, decreased oxidative metabolism and insulin resistance [22,72]. Thus, transcriptomic analysis of skeletal muscle enabled the identification of molecular signatures associated with obesity that are related to reduced glucose utilization and impaired mitochondrial metabolic function [36].

The GSE103766 dataset was derived from transcriptomics data of human adipose tissue samples [17]. Adipose tissue acts as an endocrine and metabolic organ of lipid storage, adipokine signaling and systemic inflammatory response [16]. Obesity-induced adipose tissue remodeling has been correlated with increased inflammation, altered expression of adipokines and mitochondrial dysfunction, which all play a significant role in the development of insulin resistance and in the

progression of metabolic syndrome [18,29]. By incorporating the transcriptomic data from adipose tissue, the molecular changes linked to obesity and endocrine dysfunction and chronic inflammation were investigated [17].

For an extensive understanding of the molecular mechanisms involved in obesity, beyond tissue-specific biological changes, selection of cross-tissue transcriptomic datasets was performed [37]. As obesity is a multisystem metabolic disease that involves coordinated dysfunction of metabolically interconnected tissues, integrative analysis of adipose tissue and skeletal muscle datasets extends the identification of conserved transcriptomic biomarkers that are associated with systemic metabolic impairment [28,38]. Multi-tissue integration was supported by a number of previous transcriptomic studies which suggested an overlap between obesity-associated mitochondrial dysfunction, oxidative stress and inflammatory signaling in several tissues [24,36]. The datasets were carefully chosen for the following inclusion criteria: (i) obesity and control samples were available for both (ii) relevance to metabolically active human tissues, (iii) publicly accessible normalized gene expression matrix and metadata, and (iv) adequate sample representation for computational modeling and subsequent transcriptomic analysis [35,38]. Use of publicly available datasets further enhanced reproducibility, transparency and cost-effectiveness of the present computational framework [35].

After the selection of the datasets, raw transcriptomic matrices and platform annotation files were downloaded from GEO for further pre-processing, normalization and analysis for the identification of biomarkers using machine learning tools [35,40].



2.2 Data Preprocessing and Transcriptomic Integration

Transcriptomic datasets were subjected to preprocessing to enhance the biological consistency and minimize technical variation and to ensure the consistency of data from datasets collected under different experimental conditions and platforms [33,38]. Systematic pre-processing of the transcriptomic data is crucial to obtain reliable and biologically meaningful computational models, because many datasets are often contaminated with platform-specific effects, missing expression values and inter-sample variations. In the present study the preprocessing pipeline used was the gene filtering, missing value imputation, normalization and batch effect correction followed by machine learning implementation, all of which were done previously to the implementation of machine learning [35,40].

Transcriptomic expression matrices for GSE22309 and GSE103766 were downloaded from the GEO repository, as were the platform annotation files [35]. GEO platform annotation files include

detailed information on probe identifiers, gene symbols and transcript annotations necessary for the biological interpretation of expression profiles [35].

After the mapping of annotations, common genes between both transcriptomic sets were identified and kept for integration analysis [37]. The two GEMS GSE22309 and GSE103766 were collected from different tissue types and different experimental platforms, so direct integration without feature harmonization would create inconsistencies and bias based on these platforms [38]. Thus, the common genes that were represented in both sets were used to generate a common gene transcriptomic expression matrix [37]. Common genes were retained, leading to biological comparisons and the identification of conserved obesity-associated molecular signatures in adipose tissue and in skeletal muscle [28].

However, transcriptomic data often are missing expression values because of the variability of expression at the level of the probe, inconsistencies in the signal, or noise in the experiment [39]. When the data is missing, it can significantly influence the downstream statistical analysis and performance of machine learning models, leading to computational instability and distortion of features [97]. Thus, the gene expression values that were not present in the integrated dataset were imputed with median imputation, where gene expression values that were not present were replaced with median gene expression value from samples [40]. The advantage of median imputation was that it was relatively robust to outliers and maintained the biological distribution pattern [98].

After imputation, the transcriptomic data was normalized to reduce inter-sample variability and normalize the measurements of expression of genes [33]. The values of gene expression on transcriptomic platforms can vary by several orders of magnitude due to technical variability, batch effects and biological variability [39]. To overcome this, transcriptomic features were z-score-

normalized, which involves shifting all gene expression values [40]. Standardization was performed according to the following equation:

$$Z = (X - \mu) / \sigma$$

Where:

Z represents normalized gene expression value,

X denotes original expression value,

μ corresponds to mean gene expression and

σ represents standard deviation [78].

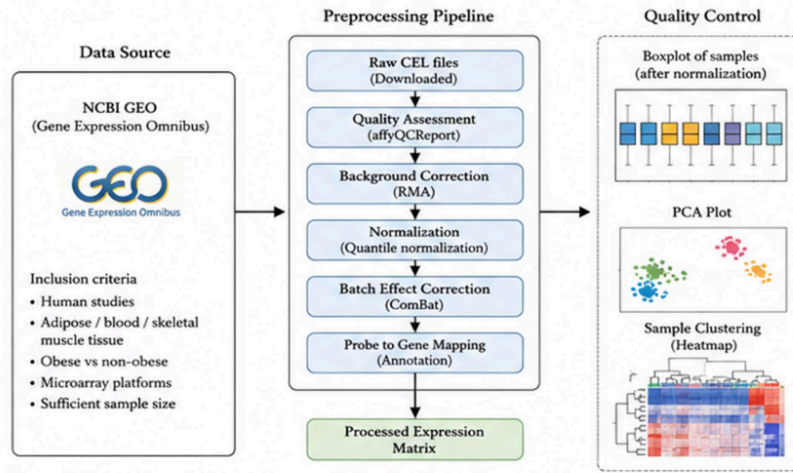
The idea of normalizing is especially relevant for transcriptomic data sets where there are thousands of molecular variables that are all heterogeneous [39].

The selected datasets were derived from various biological experiments and could be from various microarray platforms, so a batch effect correction was also performed to reduce non-biological technical variation [38]. Systematic differences that are observed in the experimental conditions, laboratory procedures, or platform-specific biases are considered batch effects and are not biological differences [99]. However, not correcting batch effects can cause artificial clustering and lead to poor biological representativeness of transcriptomic results [33].

To harmonize transcriptomic profiles across batches while retaining and focusing on biologically meaningful obesity-associated variation, an empirical Bayes-based batch correction method was used [100]. Empirical Bayes methods can be used to effectively adjust technical discrepancies by borrowing statistical information from other genes as well as minimize inter-batch variance while keeping the biological signals intact [101].

After preprocessing, normalization, and batch effect correction, an integrated transcriptomic matrix of the biologically comparable gene expression features was generated to be used for

downstream machine learning implementation and EAI analysis [40]. The resulting expression matrix was used as the basis for discovering obesity-associated transcriptomic biomarkers using an ensemble learning and systems biology approach [44].



2.3 Construction of Feature Matrix and Target Variable

The integrated gene expression data were then preprocessed and normalized for transcriptomics and converted into a machine learning-friendly format [40]. In classification problems, machine learning models need to be trained with a set of input features (predictor variables) and output labels (target variables) in a structured format of numbers [78]. For this reason, a feature matrix and target variable were created to enable supervised machine learning analysis of the obesity-associated transcriptomic profile [79].

In order to obtain a common feature matrix (X) between GSE22309 and GSE103766, the integrated transcriptomic expression matrix with normalized common genes was used [40]. Here

the rows represented biological samples and the columns gene expression features for fixed transcriptomic variables [33].

The target variable (Y) was outlined by obesity associated phenotype labels [40]. Samples of healthy, or metabolically normal individuals were classified in the control class (0), and samples of those with obesity-related phenotypes, such as insulin resistance or metabolically abnormal obesity states, were classified in the obesity class (1) [38]. A binary phenotyped assignment was used to support supervised classification modelling to differentiate obesity-associated transcriptomic signatures from control expression profiles [79].

The feature matrix and target variable were built and supervised ⁸machine learning algorithms were implemented to identify the nonlinear patterns of transcriptomic changes associated with obesity [41]. This type of structuring also enabled the downstream training, validation and SHAP-based explainability analysis of these models.

2.4 Machine Learning Model Development

After the preprocessing of the transcriptomic data and construction of the feature matrix, supervised machine learning models were applied to uncover obesity-related transcriptomic signatures and to classify biological samples from gene expression profiles [40,41]. Machine learning methods were chosen due to their effectiveness in handling high dimensional biological data and detecting complex nonlinear molecular interactions that are often missed by traditional statistical techniques [39]. There are thousands of genes in the obesity-associated transcriptomic data, and the relationships between these genes are highly complex, so that machine learning algorithms can be used to provide a computational framework for biomarker discovery and ¹⁷disease

Dataset splitting was used to train the model with 80% and to test it independently with 20% of the data samples [40]. In order to avoid sampling bias and ensure balanced class representation, stratified sampling was used, resulting in a training and testing set of the same size and balanced distribution of obesity and control phenotypes [81]. Furthermore, a fixed random state parameter was used to guarantee the computational results' reproducibility and consistency [78].

First, several supervised machine learning classifiers were tested for their classification performance and to find the best one for obesity transcriptomic analysis [41]. The algorithms chosen were due to their use in transcriptomic biomarker discovery and disease classification studies [41].

Logistic Regression is a method that estimates probabilities of classes using a sigmoid function and is widely used for biomedical classification problems that have binary classes [84]. In omics-scale analyses, however, transcriptomic data are often highly-dimensional and can have nonlinear relationships between genes, which can lead to poor predictive performance of Logistic Regression [39].

A Decision Tree (DT) classifier was also developed due to its easily explainable rule-based classification function [86]. The Decision Trees recursively partition features based on decision thresholds and offer explainable classification routes [87]. When the biological data are noisy or high dimensional, however, individual decision trees are prone to overfitting and instability [88].

Support Vector Machine (SVM) models were used because they optimise hyperplane parameters and transform the data into kernels, which are able to classify complex biological data sets [84]. SVM has proven to be very useful in transcriptomic classification problems, which involve the presence of high dimensional molecular variables [85]. When used with highly heterogeneous

transcriptomic data with large feature spaces, however, computational efficiency and interpretability can become a challenge [39].

³ K-Nearest Neighbors (KNN) algorithm as a distance-based classification method, where nearest transcriptomic profiles are used to classify the sample [82]. KNN has been used for disease classification; in these studies, dimensionality and sensitivity to irrelevant transcriptomic variables can affect model performance [83].

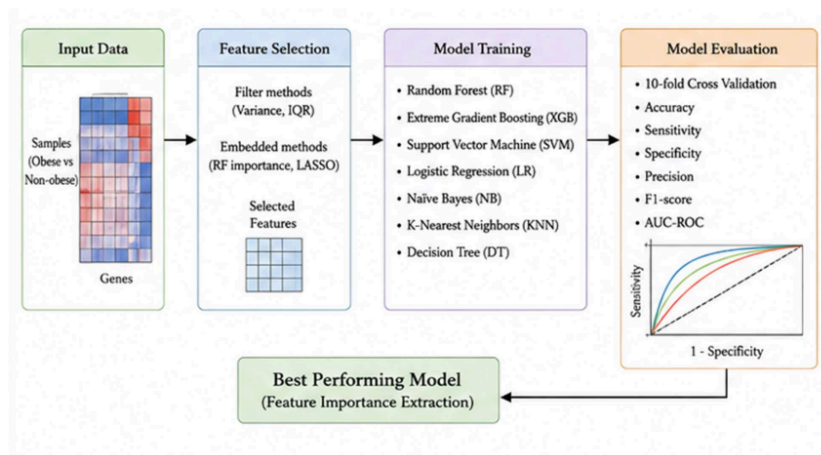
In addition, Gaussian Naïve Bayes classification was tested due to its use of a probabilistic learning method using Bayes' theorem [85]. Although it is simple and computationally efficient, Naïve Bayes makes the assumption of feature independence, which might limit the classification accuracy in transcriptomic datasets where biological variables are not independent [39].

Random Forest was chosen as the main classification algorithm because it is robust and can capture the non-linear relationships among the transcriptomes [90]. Random Forest is based on the principle of bootstrap aggregation (bagging) and random feature selection, which are used to generate a set of decision trees and minimize variance and enhance the stability of classification [91]. Random forest has proven to be useful in biomarker discovery studies because of its ability to rank the importance of transcriptomic features and its ability to efficiently process a large number of genes [92].

To enhance predictive robustness ¹ the number of decision-trees in the Random Forest classifier was optimized to 500 (n_estimators = 500) [91]. The more trees in the model, the more stable the model and the less variability in the classification, and the more consistent the prediction [90]. Random feature sampling at each split further reduced overfitting and improved the generalization of the models across transcriptomic features [89].

In parallel, XGBoost was used as an ensemble classifier based on gradient boosting to make a comparison in terms of predictive power [93]. XGBoost is iterative, and corrects classification errors made by previous learners in a sequential manner [94]. In omics-based disease classification problems, XGBoost often outperforms traditional algorithms in terms of accuracy and reliability [95]. Thus, the introduction of XGBoost allowed to perform a comparison of the performance of ensemble learning in obesity-associated transcriptomic classification [41].

Comprehensive performance comparison was enabled by implementing several supervised learning algorithms, and the most biologically valid and computation efficient framework for downstream explainability analysis and identification of obesity biomarker was selected for implementation [44].



2.5 Model Evaluation and Performance Assessment

To assess the predictive ability of machine learning classifiers to accurately classify obesity-associated transcriptomic profiles from control samples, performance of models was systematically evaluated after training. Model evaluation is a crucial part of supervised learning as sometimes the predictive accuracy of the model does not necessarily align with the biological reliability and classification robustness of the model, especially in biomedical datasets with class imbalance and transcriptomic heterogeneity [81]. Thus, several statistical performance measures were used to thoroughly evaluate classification ability and generalizability of the model [41].

Model performance was evaluated on the independent test dataset (20%) which was reserved during train–test splitting [40]. The use of an unseen test set allowed to objectively evaluate predictive generalization, and to reduce the risk of over-fitting, where models learned the training set and not the biologically meaningful transcriptomic patterns [88]. Transcriptomic datasets are characterized by high dimensional features and also by few biological samples and this is why independent validation ³¹ is of paramount importance in order to guarantee computational reliability [39].

These metrics give complementary information about classification behavior and taken together the information about the predictive performance in the context of obesity-associated transcriptomic analysis is enhanced [41].

While accuracy is a good estimate for overall model performance, use of accuracy may be misleading when dealing with biomedical datasets that are class-imbalanced [81]. So, other performance measures were used to get balanced evaluation.

Precision measures the percentage of correct predictions of obesity related samples out of all positive predictions [79].

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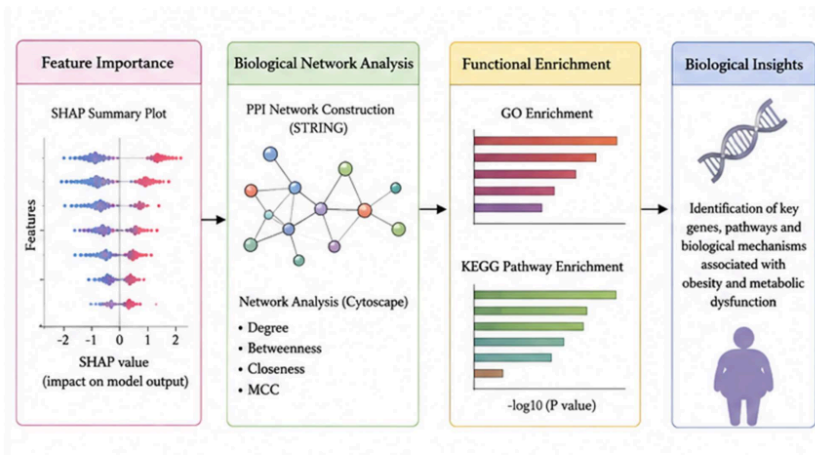
The higher the precision value, the more reliable the classification of obesity predictions are and the lower the number of false-positive predictions [81].

Good recall values mean that there are fewer false negative classifications and that the obesity-associated transcriptomic profile is effectively identified [81].

AUC values close to 1 are indicative of good predictive discrimination and values close to 0.5 are similar to random classification [79].

Besides statistical indicators, a confusion matrix was produced to indicate the classification results visually and provide quantitative information on the classification errors [79].

Comparative evaluation of machine learning models helped identify the best algorithm for downstream explainability analysis and prioritizing transcriptomic biomarkers [44]. Ensemble learning methods often outperform traditional classifiers for high dimensional omics data sets, so special focus was given to these models: Random Forest and XGBoost [41].



2.6 Explainable Artificial Intelligence (XAI) Analysis with SHAP

Machine learning model assessment was followed by SHAP ⁴ analysis to gain insight into the interpretability of the predictive outcomes and to define the transcriptomic features that play significant role in obesity classification [44]. While ensemble machine learning models can achieve high performance in prediction, their decision-making process is still challenging to interpret due to their black-box nature [42]. For this reason, explainable AI techniques are growing in significance for biomedical research, enabling biological interpretation and enhancing the trust in computational forecasts [43].

SHAP offers an extra advantage over the conventional feature importance methods in that it offers global interpretability (average importance of the features for all the predictions) and local interpretability (explanation of the behavior of the model at a specific prediction level) [101]. This interpretability ²⁸ is especially useful in the context of transcriptomic obesity studies, where the ability to prioritize potential obesity biomarkers in a biologically sensible way becomes easier [45].

TreeExplainer efficiently computes exact Shapley values, and is also computationally efficient in high-dimensional biological datasets [103]. The thousands of gene expression variables in transcriptomic data made computational scalability an advantage of TreeExplainer that made it ideal for obesity-associated biomarker discovery [44].

Genes with high SHAP values were deemed to be biologically significant transcriptomic features that played a significant role in discriminating between the obesity and control phenotypes [45]. Global SHAP analysis allowed to prioritize obesity-associated molecular biomarkers for downstream biological interpretation [44].

To gain further insight into the contributions of transcriptomic features across biological samples, summary plots and beeswarm plots of SHAP values were created [103]. SHAP summary plots

show the distribution of feature importance by arranging transcriptomic variables in order of their 'global' predictive contribution [101]. Beeswarm plots also show the direction of gene expression change between samples (up or down), and whether this change has a positive or negative association with obesity classification [102].

For the local explanations, force plots and waterfall plots were used to produce local-level SHAP explanations [103]. These visualisations are used to elucidate prediction results for specific biological samples based on the cumulative contribution of transcriptomic features that inform the prediction [44]. These local interpretability methods help to promote understanding of the specific effects of genes on obesity-related transcriptomic predictions [101].

Biologically interpretable prioritization of transcriptomic biomarkers associated with obesity was achieved by integration with machine learning classification of SHAP [45]. The prioritized genes obtained by SHAP were then further analyzed using systems biology methods such as protein–protein interactions and pathway enrichment analyses for their biological significance [46].

⁴**2.7 Protein–Protein Interaction Network Analysis.**

The protein–protein interaction (PPI) network analysis was then conducted to explore functional interactions between the transcriptomic biomarkers identified by machine learning and SHAP analysis [46,104]. Genes do not always act alone but work together through molecular pathways, so PPI network analysis can be used to find functional relationships among candidate biomarkers [105]. These systems biology approaches help to deepen the understanding of the underlying mechanism of disease-associated transcriptomic signatures and aid in prioritizing important genes in biologic pathways that contribute to pathogenesis of obesity [106].

Downstream PPI network construction was performed after induction of the transcriptomic biomarkers using feature importance analysis based on SHAP [44,45]. SHAP selects genes that make a large contribution to the obesity classification, which allows for better confidence in their biological relevance and to interpret their functions through interaction network analysis [46].

STRING is broadly used for transcriptomic and systems biology research since it generates extensive protein interaction networks from known or predicted protein interactions [111]. The candidate genes were added to the STRING database to obtain networks and find proteins that are biologically connected to obesity [112].

Confidence thresholds for interaction were used to prune false-positives interactions and to guarantee biological reliability of interaction networks generation [111]. For downstream analysis, only statistically and biologically relevant interactions supported by experimental evidence, co-expression data and databases were considered [112]. This facilitated the confidence in identified obesity associated molecular interactions and minimized the inclusion of biologically irrelevant interactions [46].

The STRING interaction network was then exported and analyzed with Cytoscape for the in-depth visualization and topological analysis of the network [113]. Cytoscape is a popular systems biology tool to visualize molecular interaction networks and to detect central regulatory nodes [114]. PPI networks were visualized to examine connectivity patterns among obesity associated biomarkers, and to better interpret the molecular relationships [115].

The biologically important genes in interaction networks were identified by applying topological network analysis techniques based on centrality measures [114]. Genes with high interaction connectivity were deemed as hub genes as they often are involved in several biological pathways and significantly in the molecular mechanism associated with diseases [109].

Hub genes led to the prioritization of transcriptomic biomarkers likely to be linked to obesity-associated metabolic dysregulations, mitochondrial impairments and inflammatory signaling [106]. Hub genes also offer a mechanistic understanding of obesity pathophysiology, and may be targeted for therapeutic intervention or precision medicine applications [109]. This led to better biological validation of machine learning prioritized transcriptomic signatures [46].

2.8 Functional ¹¹Pathway Enrichment Analysis

Functional pathway enrichment analysis was conducted to assess the biological relevance of obesity-associated transcriptomic biomarkers that were discovered using machine learning and SHAP analysis [47]. Often, identification of a transcriptomic biomarker results in several potential target genes, which can have complicated relationships, which makes it important to use enrichment analysis for systematic interpretation of their role in molecular pathways and cellular processes [46]. Functional enrichment thus offers mechanistic insights of obesity-associated biological changes and confirms transcriptomic results in known metabolic contexts [116].

Candidate genes identified by SHAP and PPI network analysis were analyzed for pathway enrichment by the online functional annotation platform Enrichr [119] that integrates several biological pathway databases. Enrichr enables the identification of statistically enriched biological pathways, by comparing sets of candidate genes with curated molecular databases [120]. This strategy allowed the systematic study of molecular processes over-represented in obesity-associated transcriptomic biomarkers [121].

In addition to molecular pathway maps, KEGG offers curated maps related to metabolism, cellular signalling, disease progression and physiological regulation [116]. Reactome also provides

detailed pathway annotations for molecular interactions and biological reactions involved in cellular homeostasis as well as disease processes [118]. Integration of both databases resulted in biochemical and biological validation of the enrichment analysis [47].

Adjusted p values and enrichment scores were calculated using Enrichr [120] to determine statistical enrichment significance. Candidate genes that showed statistically significant enrichment in the pathways were selected for further biological interpretation [119]. This strategy allowed the identification of molecular pathways that could be involved in metabolic dysfunction and transcriptomic dysregulation associated with obesity [121].

Pathways expected to show significant enrichment based on previous obesity transcriptomic data included those involved in oxidative phosphorylation, reactive oxygen species signaling, insulin resistance, fatty acid metabolism, glucose homeostasis, and inflammatory signaling [36,117]. The identification of enrichment in these pathways would further support biological relevance of machine learning-prioritized biomarker [24,29].

Functional enrichment analysis also enabled the biological interpretation of transcriptomic signatures by connecting computational results with known mechanisms that are associated with obesity [47]. Genes that are enriched in the mitochondrial and metabolic pathways may be related to impaired oxidative metabolism, while genes in inflammatory pathways may be related to regulatory functions in chronic metabolic inflammation [121]. This translation of biological relevance and significance of transcriptomic biomarkers at the pathway level greatly enhances biological understanding and translational meaning of these biomarkers at the pathway level [46]. The integration of machine learning, SHAP explainability, protein interaction analysis and pathway enrichment led to the development of a biologically interpretable and systems-level computational framework for obesity-associated biomarker discovery, respectively [44,46]. This

integrative approach not only deepened the understanding of the mechanistic aspects of obesity progression but also boosted the reliability of transcriptomic biomarker prioritization [98].

CHAPTER 3

RESULTS

3.1 Implementation of Machine Learning Models and Comparative Performance Evaluation

Transcripts were preprocessed and models were built, followed by supervised machine learning algorithms for classification of obesity-associated transcriptomic profiles using integrated gene expression data from GSE22309 and GSE103766 [35,40]. Various classification models were tested to find the most reliable computational framework to discriminate an obesity-associated molecular signature from control transcriptomic profiles [41]. Comparative model assessment was necessary to find biologically sound predictive methods, due to the high dimensionality and nonlinear molecular interactions of obesity-associated transcriptomic datasets [39].

Systematic analysis of predictive performance using these classifiers compared their performance using traditional statistical classifiers, tree-based classifiers and ensemble learning classifiers [41]. The results of performance assessment revealed significant differences in performance across different classifiers implemented, which were attributed to varying capability of the classifiers to model the nonlinear transcriptomic relationships and high dimensional biological features [39]. The predictive performance of traditional statistical and probabilistic models like Logistic Regression and Gaussian Naïve Bayes was comparatively lower due to their limited capacity to capture the complex transcriptomic interactions [83,85]. Likewise, Decision Tree and KNN classifiers had moderate predictive power, but they were not as reliable or robust in highly heterogeneous biological datasets as compared to other classifiers [82,88].

Ensemble learning methods were the best performing algorithms across the group, showing that they are more suitable for obesity-associated transcriptomic analysis than the other evaluated algorithms [41]. The classifiers outperformed traditional classifiers on most statistical performance measures, as they were able to learn nonlinear relationships, with less overfitting and greater predictive stability [89,93].

Model	Accuracy	Precision	Recall	F1-score
Decision Tree	0.79	0.80	0.79	0.79
Logistic Regression	0.76	0.76	0.76	0.76
SVM	0.56	0.62	0.56	0.50
Naïve Bayes	0.67	0.70	0.67	0.67
Random Forest	0.74	0.75	0.75	0.75
KNN	0.62	0.63	0.63	0.62

TABLE I. CLASSIFICATION PERFORMANCE OF MACHINE LEARNING MODELS

The results observed here indicate that machine learning algorithms with the ability to model nonlinear feature interactions [39] are very useful for the classification of obesity, based on transcriptomic data. Ensemble learning methods, specifically Random Forest and XGBoost, outperformed other algorithms in finding obesity-associated transcriptomic signatures due to their noise resistance and their resistance to dimensionality issues [89].

3.2.1 Analysis of the Confusion Matrix of Random Forest Classifier

A machine learning model of random forest with 500 decision trees (n_estimators = 500) was chosen for detailed evaluation [90,91]. To evaluate the prediction outcomes and quantify the classification errors, further evaluation was carried out using the Random Forest, the balanced predictive performances with better classification stability was achieved, therefore the further evaluation was carried out using confusion matrix analysis [79].

The optimized Random Forest classifier showed overall classification accuracy of about 74.63% on the independent testing dataset, suggesting the classifier had good prediction accuracy for discriminating obesity-associated transcriptomic profile from control cases [40]. The classification accuracy observed indicates the ability of ensemble learning techniques to deal with the high level of transcriptomic complexity and with the nonlinear molecular interactions that are often encountered in obesity data [41].

A confusion matrix was created to represent the prediction results and assess the discrimination of the model between obesity and control transcriptomic phenotypes [79]. The confusion matrix analysis indicated ³ that the Random Forest (RF) classifier had appropriate classification rates with a significant number of obesity and control samples as well [81].

The model generated:

Classification Outcome	Number of Samples
⁶ True Positive (TP)	25
True Negative (TN)	27
False Positive (FP)	7
False Negative (FN)	8

Table 2 Confusion Matrix Interpretation of Random Forest Model

These results suggest that misclassification rate is relatively low and the prediction capability is fairly balanced among transcriptomic classes [79].

The ²⁵precision, recall and F1-score values also helped to validate the Random Forest (RF) classifier [79]. This well-balanced predictive performance is especially relevant in biomedical classification applications where mispredictions could impact biological interpretation and biomarker prioritization [44].

Differential molecular patterns associated with obesity also proved to be reproducible when applied to the confusion matrix with machine learning models [39]. Random Forest correctly classified the transcriptomic profiles, which indicates the presence of biologically informative molecular signatures, as obesity is a coordinated dysregulation of mitochondrial metabolism, inflammatory signaling and glucose homeostasis pathways.

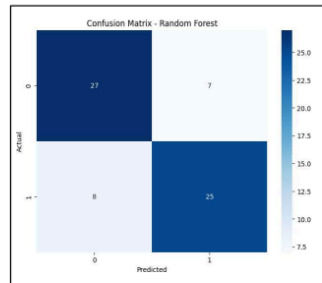


Fig 1. Random Forest confusion matrix showing robust classification performance between control and obesity samples

The observed predictive performance led us to choose the Random Forest model for downstream SHAP-based explainability analysis, from which we identified a number of transcriptomic biomarkers that were significant for the classification of obesity and biologically explained [44].

3.3 SHAP Based Global Feature Importance Analysis

SHAP was particularly beneficial since the ensemble models often operate as black-box classifiers. This explainability is especially valuable for transcriptomic studies as it can help with biologically relevant biomarker prioritization and increase the trust in computational predictions [45].

The initial aim of global SHAP analysis was to estimate the contribution of transcriptomic features on average over all the biological samples [102]. Genes with higher values of SHAP contribution were interpreted as having a greater effect on transcribing obesity-associated phenotype from a control phenotype [44].

A small number of transcriptomic biomarkers showed disproportionately high contributions to predictions of the model in the SHAP global importance analysis [102]. A number of high-ranking genes were linked to mainly mitochondrial metabolism, oxidative phosphorylation, RNA regulation, and cytoskeletal organization, indicating a significant role for these biological pathways in obesity-associated metabolic dysregulation [24,36].

Genes with strong predictive contribution to obesity classification among the top ranked transcriptomic biomarkers were the BDH1, MRPS31, RARS2, STOML2, AKAP10, UCP3, NUCB2, SRSF1 and ACTG2 genes [40]. These genes are involved in metabolic and mitochondrial functions that are important for obesity pathogenesis [24].

Likewise, RARS2 and SRSF1 are related to RNA processing and mitochondrial translational control, indicating possible changes in transcript levels that could impact cellular energy metabolism and protein production [123]. In addition, ACTG2, a cytoskeletal organisation-related gene, contributed significantly to classification results, signifying potential changes in structural remodeling and metabolic tissue organisation associated with obesity [125].

One of the key features of the global SHAP analysis was the sharp drop in feature importance after the top-ranked biomarkers, suggesting that there is a relatively clustered obesity-associated

transcriptomic signature [102]. This observation implies that although transcriptomic-obesity association datasets are highly dimensional, a limited subset of genes may have disproportionately large control over the classification results [38]. This focus of predictive importance greatly enhances the biological interpretability, and also helps prioritized biomarker identification [44]. The resulting SHAP feature importance bar plot showed ranked transcriptomic contributions which allowed visualization of the genes with the strongest effect on obesity classification outcomes [101]. This analysis also confirmed that machine learning models could detect biologically relevant transcriptomic signatures linked to metabolic dysfunction that is associated with obesity [45].

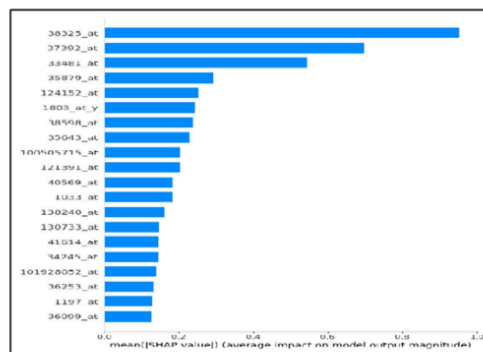


Fig 2. SHAP global feature importance bar plot highlighting the top 20 obesity-associated transcriptomic biomarkers.

The top-ranked transcriptomic biomarkers identified included genes associated with mitochondrial metabolism which further confirm the evidence supporting the role of mitochondrial dysfunction, oxidative stress and decreased metabolic flexibility as key mechanisms involved in obesity progression [24,28].

This individual can interpret the SHAP values that are reported at cohort level. This one person can interpret SHAP values reported at cohort-level. At the cohort level, feature importance can be calculated to evaluate the effect of the variation of a single gene's transcriptomic expression on the classification results of the obesity phenotype [102]. This kind of analysis can help shed light on the biological heterogeneity and molecular variability linked to obesity [38].

A beeswarm plot was created by the transcriptomic feature contribution to each sample in the study [103]. Each point in the beeswarm plots includes a single biological sample and the associated value of the SHAP contribution for a given gene [101]. Feature ranking is based on overall importance in prediction and is shown as color gradient, from low to high expression level in the transcript abundance [102].

The SHAP beeswarm analysis revealed distinct obesity and control phenotype expression states [44]. There was a substantial directionality effect for a number of top-ranked genes, indicating their key role in the molecular dysregulation involved in obesity [45]. High-ranking transcriptomic biomarkers showed wide distributions of SHAP values, indicating that these values contributed to the prediction heterogeneity of biological samples across a broad range [101].

Genes involved in metabolism of the mitochondria and oxidative phosphorylation proved to have different expression-dependent effects on obesity classification [73], such as genes BDH1, STOML2, MRPS31, and UCP3. Several mitochondrial-associated biomarkers are associated with the transcriptomic importance observed using SHAP analysis, and decreased mitochondrial oxidative capacity has been consistently linked to the progression of obesity [26].

Further, some transcriptomic biomarkers related to RNA processing and metabolic signaling, such as RARS2, SRSF1, and NUCB2, showed strong directionality of their influence on classification

predictions [123,124]. These observations highlight the complex reconfiguration of multiple molecular pathways that are associated with obesity, rather than a single disturbed pathway [36]. Furthermore, the wide spread of SHAP contribution values revealed the biological variability of the transcriptomic samples associated with obesity [102]. Obesity is highly phenotypically and molecularly heterogeneous, so the contribution of different transcriptomic features was expected to vary and further underscores the significance of interpretable machine learning models to discover obesity biomarkers [38].

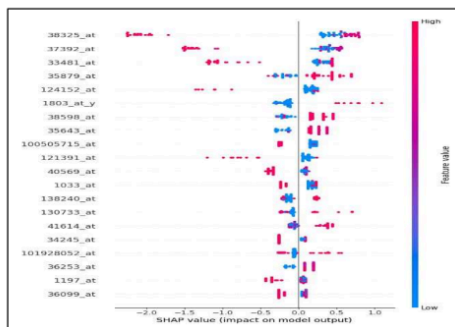


Fig 3. SHAP beeswarm plot illustrating cohort-level distribution and directional contribution of top-ranked transcriptomic features.

Overall, SHAP interpretation at the cohort level confirmed the relevance of the identified transcriptomic biomarkers and offered a mechanistic view of the role that the alterations in the expression of these genes, as a group, play in the classification results [44]. These results also helped downstream local interpretation analysis and systems biology validation of prioritized obesity-associated transcriptomic signatures [46].

3.5 Local Interpretability of Obesity Classification Using SHAP

Local SHAP analysis was carried out to explore the transcriptomic contributions affecting obesity classification at the individual sample level [101]. Global and cohort-level SHAP analyses ³⁰ give an overview of the importance of a transcriptomic data set and population-level trends, respectively, while local interpretability allows to study how combinations of genes affect the prediction results of specific biological samples [44]. This is especially relevant for obesity research, as obesity is a highly molecular- and metabolically heterogeneous disease [38].

Waterfall plots and force plots of SHAP values [103] were used to perform local interpretability analysis using the plots of cumulative contribution of transcriptomic features to the final model predictions. These plots help to quantify the positive and negative effects of individual genes on classification results and help to visualize the effect of the transcriptomic features on predictions as they shift toward the obesity or control phenotypes [101]. Genes that contribute positively to the classification of obesity tend to favor the prediction of obesity transcriptomic state, while genes that contribute negatively to obesity tend to favor the prediction of non-obesity transcriptomic state [102].

Several transcriptomic markers worked together to impact prediction results and demonstrated a multifactorial aspect of molecular obesity-related metabolic dysfunction [10,36]. These results further emphasize the value of ensemble learning frameworks in discovering non-linear transcriptomic relationships that are not captured by traditional statistical techniques [39].

Genes such as NUCB2 and SRSF1 showed significant and positive association with obesity classification in representative samples among the most influential local transcriptomic contributors [124]. These genes were found to have high SHAP values indicating their possible role in metabolic deregulation and disruptions in endocrine signaling during obesity [17]. NUCB2

has been previously associated with the regulation of appetite and metabolism, which further underscores the importance of its role in obesity-related mechanisms [124].

By contrast, a number of mitochondrial-associated genes, such as MRPS31, STOML2, BDH1, and UCP3, showed negative values in some biological samples, suggesting transcriptomic changes that may be linked to the disruption of mitochondrial metabolism and decrease in oxygen capacity [24,73]. These findings are also consistent with earlier findings linking mitochondrial dysfunction to the progression of obesity [24,26].

The overall contribution of the transcriptomic biomarkers produced unique prediction scores for each biological sample and demonstrated transcriptomic heterogeneity of obesity-associated molecular phenotypes [38]. Cumulative SHAP contributions for representative obesity associated predictions showed prediction shifts towards obesity class, whereas control samples showed opposing transcriptomic contributions [101]. These data suggest that obesity classification is enabled by an integrated molecular dysfunction rather than by single gene expression variations [36].

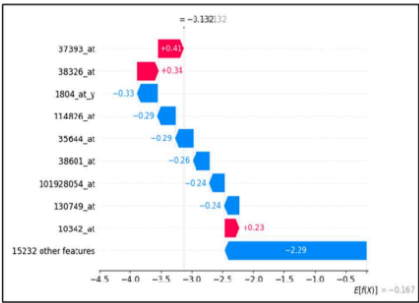


Fig 4. SHAP waterfall plot showing local feature contributions driving an individual obesity prediction.

The force plots from SHAP further depicted the directionality of the influence of the transcriptomic biomarkers on the prediction results by showing the cumulative effect on pushing towards obesity and control classifications [103]. These local explainability strategies greatly enhanced the interpretability of machine learning outcomes and allowed for a biologically meaningful understanding of obesity-associated transcriptomic heterogeneity [44].

In aggregate, the local SHAP interpretability results showed that coordinated transcriptomic interactions that involve mitochondrial metabolism, regulation of RNA, remodeling of the cytoskeleton and/or endocrine signaling had an effect on obesity-associated classification outcomes [24,36]. These results further validated the machine learning predictions, and also provided biologically interpretable evidence to support identified obesity-related biomarkers [45].

²⁴ **3.6 Protein-Protein Interaction Network Analysis of Identified Biomarkers**

Many transcriptomic biomarkers are part of networks of genes that regulate various aspects of disease pathogenesis, and network analysis helps identify relevant genes for biological processes [104].

The top biomarkers identified using SHAP were loaded into the STRING database to obtain experimentally confirmed and predicted molecular interactions [110]. The interaction network they found revealed significant functional connectivity between the obesity-associated genes identified, suggesting co-ordinated molecular regulation of metabolic and inflammatory processes [111]. By topological network analysis, NUCB2, SRSF1 and ACTG2 were identified as well connected hub genes with high network connectivity, performed using Cytoscape software [113,114]. Hub genes are highly interconnected molecular nodes that are often targeted in the regulation of several downstream biological pathways and disease-associated processes [107].

Therefore, the identification of these genes suggests their possible role in obesity-related metabolic dysfunction and gene expression regulation.

NUCB2 was shown to be a hub gene with high connectivity and biological relevance because of its prior reported role in appetite regulation, endocrine signaling and metabolic homeostasis [124]. The dysregulation of NUCB2 might thus be implicated in the energy imbalance and endocrine disturbances associated with obesity [17]. Changing pathways involved in RNA processing could affect the stability of mitochondrial transcripts and metabolism involved in obesity [36].

ACTG2 is mainly involved in cytoskeletal organization and structural remodeling was also identified as a biologically important hub gene [125]. ACTG2 interactions indicated potential tissue remodeling and metabolic changes with obesity that could be addressed in skeletal muscle tissues [22].

Transcriptomic data also showed strong associations with the genes of mitochondrial metabolism and oxidative phosphorylation, which were further supported by network visualization [24,73], supporting a role of mitochondrial dysfunction in obesity progression. To confirm biological significance of identified transcriptomic biomarkers, observed network connectivity further confirmed substantial contribution of mitochondrial metabolic impairment to insulin resistance and oxidative stress [26].

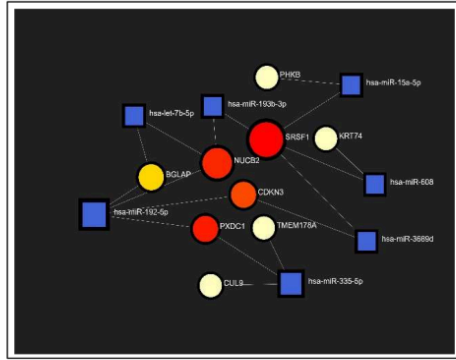


Fig 5. STRING-Cytoscape protein-protein interaction network identifying NUCB2, SRSF1, and ACTG2 as central hub genes.

PPI network analysis greatly enhanced biological confidence in machine learning-prioritized biomarkers by showing their involvement in coordinated molecular pathways involved in obesity pathophysiology [46].

3.7 Functional Pathway Enrichment Analysis of Obesity-Associated Transcriptomic Biomarkers

This systems biology approaches also boost the reliability of computational predictions by testing candidate genes in biologically meaningful pathways [46].

Priority transcriptomic biomarkers from the SHAP analysis and the protein-protein interaction network were then put through an enrichment analysis to determine the biological pathways that are statistically overrepresented [119]. Functional enrichment identified metabolic pathways that are heavily implicated in obesity-associated metabolic dysfunction including pathways for oxidative phosphorylation, reactive oxygen species (ROS) signaling, glycogen metabolism, insulin resistance and mitochondrial bioenergetics [24,36].

One of the most remarkable biological processes that appeared among the significantly enriched pathways was oxidative phosphorylation, which was among the identified transcriptomic biomarkers [117].

Other genes involved in insulin resistance pathways also showed significant enrichment [117]. Thus, the enrichment of insulin resistance pathways further confirmed their biological relevance of the machine learning prioritized transcriptomic biomarkers [31].

Other pathways related to glycogen metabolism and glucose homeostasis were also also significantly enriched [121]. Impaired mechanisms in energy regulation in obesity associated transcriptomic profiles are hence supported by dysregulated glycolytic metabolic pathways [22].

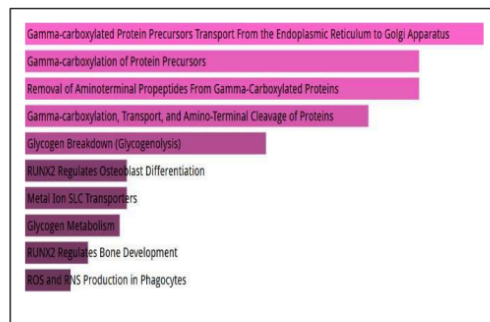


Fig 6. Enrichr KEGG/Reactome pathway enrichment analysis revealing mitochondrial dysfunction and metabolic dysregulation pathways in obesity.

Reactome-based enrichment also showed pathways related to mitochondrial organization, ATP synthesis, electron transport chain activity and cell stress response pathways [118]. The results are well correlated with transcriptomic data that shows mitochondrial metabolic impairment is a central mechanism of obesity [24]. The biological relevance of genes with high SHAP contribution values during transcriptomic classification [73] such as BDH1, MRPS31, STOML2 and UCP3 is further supported by the mitochondrial pathways they are enriched in.

Pathway enrichment analysis revealed strong relationships between obesity-associated transcriptomic biomarkers identified by machine learning and pathways of mitochondrial dysfunction, oxidative stress, insulin resistance and metabolic dysregulation [24,36].

Pathway	Biological Relevance in Obesity
Oxidative Phosphorylation	Reduced ATP production and mitochondrial dysfunction
ROS/RNS Signaling	Oxidative stress and inflammation
Glycogen Metabolism	Altered glucose utilization and insulin resistance
Insulin Resistance	Impaired glucose uptake and metabolic dysfunction
Mitochondrial Metabolism	Reduced fatty acid oxidation and metabolic flexibility

Table 3 Major Enriched Biological Pathways Identified in Obesity

3.8 Integrated interpretation of transcriptomic findings

The machine learning framework was able to achieve a balance predictive performance to distinguish obesity-associated molecular profiles from control samples, and successfully identify transcriptomic features capable of doing so [41].

The same explainability analysis methods used for SHAP were employed to further increase the interpretability of the transcriptomic predictions by highlighting the genes that have a significant contribution to the classification of obesity that are biologically relevant [44]. A number of biomarkers were identified, such as BDH1, MRPS31, RARS2, STOML2, AKAP10, UCP3, NUCB2, SRSF1 and ACTG2, which revealed significant role of mitochondrial metabolism, RNA

regulation and cytoskeletal remodeling in obesity pathophysiology [40]. The results confirm emerging evidence that points towards mitochondrial dysfunction and metabolic inflexibility as central players in the progression of obesity [24].

Protein-protein interaction analysis further confirmed the biological confidence of prioritized biomarkers by identifying highly connected hub genes, such as NUCB2, SRSF1, and ACTG2 [107]. The identified biomarkers are functionally connected, indicating coordinated molecular regulation in obesity associated metabolic dysfunctions, instead of gene-level alterations [46].

Pathway enrichment analysis also revealed high enrichment of oxidative phosphorylation, reactive oxygen species signaling, glycogen metabolism, insulin resistance and mitochondrial metabolic pathways [117,121].

Findings from predictive modelling combined with explainability analysis and systems biology validation add to the confidence of biomarkers identified and underpin the potential for obesity biomarker discovery and precision metabolic medicine[44,98].

Overall, the findings highlight the potential of EAI and transcriptomic analysis coupled with systems biology for generating a biologically interpretable framework to identify molecular signatures of obesity and decipher complex mechanisms of metabolic disease[45].

CHAPTER 4

DISCUSSION

4.1 Biological Interpretation of Obesity-Associated Transcriptomic Signatures

In the present study, a cross-tissue transcriptomics, ensemble machine learning, explainable artificial intelligence and systems biology validation-based computational pipeline was leveraged to uncover biologically relevant obesity-associated molecular signatures [40,44,46]. The study combined the transcriptomic data from both skeletal muscle (GSE22309) and adipose tissue (GSE103766) to identify the systemic molecular changes that lead to obesity instead of focusing only on tissue-specific dysregulations. Overall, these data indicate that mitochondrial dysfunction, oxidative stress, metabolic inflexibility and/or unbalanced regulation of transcription are major components of obesity-associated transcriptomic profiles, consistent with obesity being viewed as a multisystem disorder of metabolism [24,28].

This is one of the defining features of the current work, as this approach uses transcriptomics data from metabolically coupled tissues [37]. The cross-tissue approach enabled transcriptomic biomarkers that may be relevant for the regulation of metabolic processes at the systemic level to be identified, as opposed to studies on single tissues aimed at conventional obesity. In this way, integrative methods can help in the biological interpretation of the data, and in increasing the reproducibility of the biomarkers in several biomedical tissues related to obesity [122].

The machine learning analysis showed that the ensemble learning methods had better predictive performance compared to the traditional classifiers, ⁸such as Random Forest and XGBoost [41].

Ensemble learning offers significant advantages over traditional statistical modeling approaches because obesity-associated transcriptomic data is inherently heterogeneous and has complex gene–gene relationships. The use of ensemble learning for the discovery of obesity related biomarkers is also supported by previous transcriptomic studies that also report better performance with Random Forest and gradient boosting algorithms in predicting metabolic disorders [92,95].

The optimized Random Forest classifier exhibited good classification accuracy and showed almost equal misclassification rates for obesity-associated and control transcriptomic profiles, suggesting good discrimination between obesity-associated and control transcriptomic profiles [79]. The moderate, but not unrealistically high, predictive accuracy was still biologically meaningful in view of the fact that obesity is a very heterogeneous disease, and that there is significant inter-individual molecular variation. Furthermore, transcriptomic classification results often vary due to biological variability, tissue-specific differences, and the environment of obesity development [11]. The performance of the observed predictors is therefore supportive of

the existence of reproducible obesity-associated transcriptomic signatures as well as realistic biological heterogeneity [39].

Traditional machine learning models often operate as black boxes, limiting insights into biology and confidence in clinical application of computational results [42]. Of the prioritised transcriptomic biomarkers, genes such as BDH1, MRPS31, RARS2, STOML2, AKAP10, UCP3, NUCB2, SRSF1 and ACTG2 showed significant predictive value [40]. Some of these genes are directly linked to the metabolic pathways of mitochondria and oxidative phosphorylation, highlighting the role of mitochondrial dysfunction in obesity pathogenesis [24,73]. The roles of the mitochondrial transcriptomic biomarkers identified in the present study thus reinforce the previous evidence that impaired mitochondrial bioenergetics is a hallmark mechanism of obesity progression [28]. Decreased metabolic flexibility and insulin resistance associated with obesity may be linked to dys regulations of UCP3, which affects mitochondrial proton transport and mitochondrial oxidative efficiency [22,126]. Likewise, STOML2, which acts in the organization of the mitochondrial membrane, and MRPS31, which is a component of the mitochondrial ribosome, also highlight the significance of a dysregulated mitochondrial integrity in obesity [73].

The increased expression of NUCB2, a key transcriptomic player that has been reported to regulate appetite and systemic metabolic homeostasis [124], further supports its role in this pathway. NUCB2 is a gene that encodes nesfatin-1, a peptide that has been linked with appetite suppression and endocrine regulation of energy balance [127]. This condition of NUCB2 expression alteration might then participate in the process of obesity-related endocrine dysfunction and metabolic dysregulation [17]. Similarly, SRSF1, which plays a role in RNA

splicing and post-transcriptional regulation, underscores the potential role of transcriptomic regulatory mechanisms in obesity pathogenesis [123].

Protein-protein interaction network analysis further improved biological interpretation through identification of NUCB2, SRSF1 and ACTG2 as central hub genes [107]. Identification of hub genes indicates coordinated molecular regulation instead of isolated transcriptomic abnormalities, as they are often found to be controlling several downstream pathways.

Pathway enrichment analysis also confirmed transcriptomic results by identifying significantly enriched pathways related to oxidative phosphorylation, signalling by reactive oxygen species, insulin resistance, glycogen metabolism and mitochondrial organization [117,121]. The pathways represent known features of metabolic dysfunction associated with obesity and provide strong evidence for the relevance of identified transcriptomic biomarkers to the mechanisms of obesity-related metabolic dysfunction [24,36]. The high enrichment of oxidative phosphorylation and ROS signaling pathways further supports the hypothesis that mitochondrial dysfunction and oxidative stress are the key biological pathways involved in obesity progression [24,70].

4.2 Comparison with Previous Obesity Transcriptomic Studies

The analysis was compared with the results of previously published obesity transcriptomic studies. The present results confirm the molecular evidence and also provide a rationale machine-learning-based approach to biomarker prioritization [44].

Skeletal muscle dysfunction is a major factor in insulin resistance of obesity, due to reduced glucose uptake and metabolic inflexibility [20]. In the current study, several transcriptomic biomarkers, such as UCP3, BDH1 and STOML2, which have been related to mitochondrial metabolism, showed strong contribution towards obesity classification [73]. The results indicate

significant transcriptomic changes in the mitochondria function of the obesity-associated skeletal muscle tissues [24].

Most previous obesity transcriptomic studies have used single tissue analysis and differential gene expression for biomarker discovery [38] – a method that is not used in this study. While these approaches offer valuable biological insights, they often fail to capture coordinated and systemic transcriptomic dysregulation in metabolically coupled tissues [37]. However, obesity impacts the activity of several organs at the same time, so integrative cross-tissue transcriptomic analysis can yield a more holistic view of obesity-associated molecular mechanisms [122]. In the present study, therefore, an important methodological drawback of previous obesity studies is overcome, combining transcriptomic profiles of ⁵skeletal muscle and adipose tissue [37].

¹⁰The other significant difference of the present study is related to the use of ensemble machine learning methods, such as Random Forest [41] and XGBoost [42], in the identification of transcriptomic biomarkers. The traditional approach to obesity transcriptomic analysis is usually based on differential expression analysis and statistics, which may not be sufficiently informative to capture nonlinear interactions and complex molecular relationships [39]. Machine learning techniques, on the other hand, are efficient for modeling nonlinear relationships in the transcriptomic level and uncovering predictive molecular signatures from high-dimensional biological data [40]. The better prediction capabilities of the ensemble learning approaches reported in the present work thus aligns with previous reports highlighting the usefulness of machine learning in discovering omics-based biomarkers [92,95].

Although machine learning is being widely employed in biomedical research, explainability has not been adequately studied in obesity transcriptomic studies [44]. The majority of machine learning studies on obesity focus on prediction scores while lacking mechanistic insight into the

drivers of prediction results [42]. This is mainly due to the fact that the incorporation of SHAP-based explainability analysis in the present study allowed the prioritization of obesity-associated transcriptomic biomarkers with a biological interpretation [45], which constitutes an important contribution. The identification of the transcriptomic contributions at both the global and local levels greatly enhances biological confidence in computational predictions and in the translational relevance of identified biomarkers [101].

The present study also complements the increasing evidence underlining the validation of transcription markers in the context of systems biology [46]. Computational prediction of candidate genes can sometimes fail to have biological relevance if not supported by networks of interacting genes and/or pathway level interpretation [47]. The present study combined the construction of ¹protein-protein interaction networks and functional pathway enrichment analysis to provide strong biological evidence for prioritized transcriptomic biomarkers [109]. Integrative validation significantly increases the reliability and translational value of obesity biomarker discovery models [46].

4.3 Biological implications of mitochondrial dysfunction in obesity

A key finding from this current study was the identification of a set of transcriptomic biomarkers and gene pathways that are enriched for mitochondrial metabolism and oxidative phosphorylation [24]. Mitochondrial dysfunction has emerged as a key mechanism in obesity-associated metabolic disease, due to its role in ATP production, fatty acid oxidation and reactive oxygen species homeostasis [25].

The high transcriptomic priority of the mitochondrial-related genes BDH1, UCP3, MRPS31 and STOML2 reinforces the hypothesis that obesity progression is characterized by a broad

mitochondrial metabolic dysfunction [73]. A reduction in mitochondrial respiration impairs energy utilization and is associated with a build-up of intracellular lipid intermediates which increases metabolic inflexibility and insulin resistance [22].

Chronic low-level inflammation is then caused due to oxidative stress, which leads to endothelial dysfunction and development of metabolic disorders associated with obesity [29,70]. Thus, transcriptomic markers related to mitochondrial dysfunction could be clinically relevant molecular markers of metabolic dysfunction and obesity onset [24].

The observed enrichment of oxidative phosphorylation and ROS-associated pathway further underscores the therapeutic relevance of targeting the mitochondria for obesity management [74]. Several trials have explored the role of mitochondrial biogenesis enhancement, antioxidant therapy and metabolic modulators to enhance mitochondrial efficiency and decrease obesity-associated metabolic abnormalities [66,74]. Overall, the transcriptomic markers detected in this research can be useful targets for further research in therapeutic investigation and precision metabolic medicine [45].

18 **CHAPTER 5**

CONCLUSION, LIMITATIONS AND FUTURE SCOPE

5.1 Conclusion

Obesity is a multifactorial metabolic disorder characterized by coordinated dysregulations of adipose tissue function, skeletal muscle metabolism, mitochondrial bioenergetics, oxidative stress and inflammatory signaling pathways [10,24,29]. To this end, the present work sought to establish an integrated computational framework that leverages cross-tissue transcriptomics, ensemble machine learning, explainable artificial intelligence and systems biology validation to uncover

obesity-associated transcriptomic biomarkers and boost the understanding of obesity development mechanisms [40,44,46].

To examine molecular changes in metabolically interconnected tissues that are associated with obesity, the present investigation utilized publicly available transcriptomic data from two types of tissue: skeletal muscle (GSE22309) and adipose tissue (GSE103766) [35,37]. When using transcriptomic profiles from two tissues, their integration allowed to identify systemic molecular signatures, which were not restricted to tissue-specific dysregulation [122]. The coordinated dysregulation of multiple organs involved in glucose uptake, lipid metabolism, and endocrine homeostasis of obesity makes cross-tissue transcriptomic integration significantly enhance the biological comprehensiveness and biomarker discovery potential [20,37].

In this regard, the implementation of machine learning showed that the ensemble learning methods outperformed the conventional statistical and classification models, especially the Random Forest and the XGBoost method [41]. The optimized Random Forest (RF) classifier was among the evaluated algorithms that achieved equal levels of predictive performance and good discrimination between obesity-associated and control transcriptomic phenotypes [90,91]. The classification ability seen here demonstrates the success of ensemble learning methods in extracting biologically-relevant transcriptomic patterns from large datasets of obesity [89].

A significant gain in the interpretability of machine learning predictions arose from the integration of the explainable artificial intelligence and the capacity to prioritize obesity-associated transcriptomic biomarkers in a biologically relevant manner [44]. The genes BDH1, MRPS31, RARS2, STOML2, AKAP10, UCP3, NUCB2, SRSF1 and ACTG2 showed statistically significant associations with obesity classification outcomes [40]. The strong association of these biomarkers with mitochondrial metabolism, oxidative phosphorylation, transcriptomic regulation and

cytoskeletal organization, supported the hypothesis that obesity progression is accompanied by broad metabolic and molecular dysfunction [24,36].

Protein–protein interaction network analysis also revealed that NUCB2, SRSF1 and ACTG2 are highly connected hub genes which may have a coordinated regulation role in obesity-related molecular pathways. Biological relevance of identified transcriptomic biomarkers was further confirmed by functional pathway enrichment analysis which revealed significant roles of pathways related to oxidative phosphorylation, reactive oxygen species signaling, glycogen metabolism, insulin resistance and mitochondrial dysfunction [117,121].

Taken together, all ³⁵the results gained in the present study strongly suggest that mitochondrial dysfunction, oxidative stress, metabolic inflexibility and transcriptomic dysregulation are core molecular mechanisms involved in obesity progression [24,26]. The integrated computational framework was successfully demonstrated to be a powerful and biologically interpretable approach for discovering obesity-associated biomarkers using explainable machine learning, transcriptomics and systems biology [45,46].

This study therefore provides a contribution to the existing knowledge on the pathophysiology of obesity and points to the translational potential of EAI in precision metabolic medicine [44]. Biologically meaningful transcriptomic biomarkers can help for early diagnosis, molecular stratification and targeted therapeutic interventions to better manage obesity and its associated metabolic complications [98].

5.2 Limitations

The present study used publicly available transcriptomic data and computational prediction methods without experimental validation [35,109] to obtain biologically meaningful findings.

Even after preprocessing and batch effect correction, the biological heterogeneity and limited sample size and dimensionality of the transcriptome may affect the predictive results [38,99].

5.3 Future Scope

Experimental testing of prioritized transcriptomic biomarkers, including those related to mitochondrial metabolism and metabolic regulation [73,109] should be explored in future studies. Furthermore, the use of advanced explainable AI and deep learning approaches can improve predictive ability [44,98].

Future personalized diagnosis, machine learning and systems biology-based integrated transcriptomics approach can thus play a significant role in precision metabolic therapeutics and obesity diagnosis biomarker guided therapy[45,46].

An additional line of research that is being followed is the development of precision medicine frameworks to manage obesity [45]. The molecular obesity subgroups can be identified by the transcriptomic biomarkers discovered in the present study, which can help to guide personalized treatments [98]. Individual interventions for obesity that included mitochondrial dysfunction and/or oxidative stress or inflammatory signaling pathways may thus enhance therapeutic success and disease prevention [74].

Furthermore, larger and ethnically diverse patient cohorts might be included in future transcriptomic studies to increase biomarker replications and increase the applicability of the findings to patients in clinical settings [38]. Obesity is a highly variable phenotype, with genetic, environmental, and lifestyle factors contributing to the differences, so larger population-based studies are needed to confirm transcriptomic signatures across a wider range of populations [11].

In summary, future studies using multi-omics and explainable AI and systems biology could significantly ¹⁴contribute to the understanding of obesity-related metabolic dysfunction and assist in the design of precision metabolic medicine [45,46].

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