Profound Perturbation of the Metabolome in Obesity Is Associated with Health Risk

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Introduction

Obesity is one of the most widespread problems in our society's health today.

- Increase in risk for conditions like diabetes mellitus and CVD **(Hales et al., 2017; Whitlock et al., 2009)**
- Tripled prevalence of obesity since 1975
- 39% of the world's adults being overweight, and 13% being obese **(WHO, 2018)**
- **Main cause**: increasing consumption of hypercaloric foods and sedentary lifestyles **(WHO, 2018)**

Previous Studies

Identified metabolic signatures associated with obesity, but have some limitations.

- Identified increased levels of branched-chain and aromatic amino acids as well as glycerol and glycerophosphocholines.

  (Butte et al., 2015; Chen et al., 2015; Ho et al., 2016; Menni et al., 2017; Park et al., 2015; Piening et al., 2018)

- However, these studies have limitations on:
  - Relativley small number of metabolites
  - Small sample size
  - Small number of obesity phenotypes

Why Important?

Important to understand the relationship between metabolic perturbations and the obese state.

- Characterization of the metabolites associated with obesity gives us many insights.
  - **Longitudinal assessment of weight gain and weight loss over time**
  - **Maybe we could predict future weight change using current metabolomic change?**
  - To identify

    1) whether there are metabolomic changes that cause obesity.

    2) or whether all metabolite changes associated with obesity are a consequences of weight changes.

- Yanovski and Yanovski (2018) tried to understand factors making people susceptible to (or protected from) obesity.

Dataset

Dataset includes 2396 individuals' 3 time-point BMI, anthropomorphic data, whole-body DEXA scans, and metabolome with baseline genetic risk.

- Identified almost ⅓ of metabolome are assoicated with BMI,
- Metabolite levels can predict obesity status with ~80%–90% specificity and sensitivity.
- Metabolome profile is a strong indicator of metabolic health compared to the polygenic risk assessment and anthropomorphic measurements of BMI.

Result **Profound perturbation of metabolome by obesity metabolites associated with BMI**

Compared the levels of individual metabolites to the BMIs of 832, 882, and 861 unrelated people of European ancestry in the TwinsUK cohort at 3 time points (8-18 years) (Moayyeri et al., 2013)

- 110 metabolites were significantly associated with BMI at all 3 time points.
  - 284 metabolites were significantly associated ($p<0.001$) with BMI at one or more time points
  - 307 metabolites were significantly associated with BMI in at least one cohort and time point

- 83 metabolites that showed directions of effect that were consistent between the two cohorts, of which **49 were statistically significant replications.**
  - The most significantly associated metabolite was **urate (uric acid)**

Lipids (n=23)	Amino acids (n=14)	Nucleotids (n=3)	Others (n=6)

Table 1. Metabolite Signature Associated with BMI

Super Pathway	Metabolite	Subpathway (Correlated Blood Lipids[c])	Direction of Effect (Rank[a])	BMI r^{2}[b]
Nucleotide	urate	Pur.Met.	↑ (1)	16.4%
	N2,N2-dimethylguanosine	Pur.Met.	↑ (6)	8.8%
	N6-carbamoylthreonyladenosine	Pur.Met.	↑ (28)	7.3%
Amino acid	glutamate	Glu.Met.	↑ (2)	11.5%
	N-acetylglycine	Gly.Met.	↓ (9)	9.0%
	5-methylthioadenosine (MTA)	Poly.Met.	↑ (10)	7.5%
	valine	Leu.Met.	↑ (11)	8.8%
	aspartate	Ala.Met.	↑ (16)	7.0%
	N-acetylvaline	Leu.Met.	↑ (18)	7.3%
	kynurenate	Try.Met.	↑ (19)	6.0%
	alanine	Ala.Met.	↑ (23)	5.3%
	asparagine	Ala.Met.	↓ (26)	3.7%
	N-acetylalanine	Ala.Met.	↑ (31)	6.6%
	tyrosine	Phe.Met.	↑ (34)	1.8%
	leucine	Leu.Met.	↑ (37)	6.8%
	N-acetyltyrosine	Phe.Met.	↑ (40)	4.2%
	2-methylbutyrylcarnitine (C5)	Leu.Met.	↑ (41)	8.3%

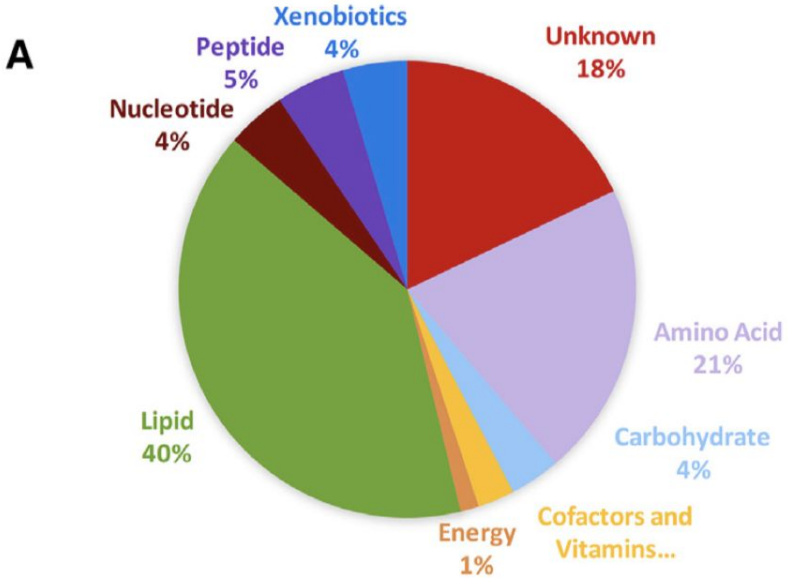
Super Pathway	Metabolite	Subpathway (Correlated Blood Lipids[c])	Direction of Effect (Rank[a])	BMI r^{2}[b]
Lipid (n=23)	1-(1-enyl-palmitoyl)-2-oleoyl-GPC	Plas. (HDL, TG)	↓ (3)	7.1%
	1-stearoyl-2-dihomo-linolenoyl-GPC	Phos.Met. (TG, Chol)	↑ (4)	9.8%
	1-eicosenoyl-GPC	Lysolipid	↓ (5)	6.2%
	1-arachidoyl-GPC	Lysolipid	↓ (7)	8.6%
	1-(1-enyl-stearoyl)-2-oleoyl-GPC	Phos.lip. (HDL)	↓ (8)	6.5%
	propionylcarnitine	BCAA.Met	↑ (12)	9.9%
	1-nonadecanoyl-GPC	Lysolipid	↓ (14)	4.2%
	1-linoleoyl-GPC	Lysolipid	↓ (15)	4.9%
	sphingomyelin	Sph.Met. (Chol)	↑ (20)	6.8%
	1-palmitoyl-2-dihomo-linolenoyl-GPC	Phos.Met. (TG, Chol)	↑ (21)	5.1%
	1-(1-enyl-palmitoyl)-2-linoleoyl-GPC	Phos.Met. (HDL)	↓ (22)	5.7%
	1-palmitoyl-3-linoleoyl-glycerol	Phos.Met. (TG)	↑ (24)	7.6%
	1-oleoyl-2-linoleoyl-GPC	Phos.Met.	↓ (27)	5.6%
	1-(1-enyl-stearoyl)-2-docosahexaenoyl-GPC	Phos.Met.	↓ (29)	2.5%
	1-oleoyl-3-linoleoyl-glycerol	Di.gly. (TG, HDL)	↑ (30)	6.3%
	carnitine	Car.Met.	↑ (33)	7.5%
	1-palmitoyl-2-linoleoyl-glycerol	Phos.Met. (TG, HDL)	↑ (36)	7.2%
	1-oleoyl-2-linoleoyl-glycerol	Di.gly. (TG, HDL)	↑ (38)	5.9%
	1,2-dilinoleoyl-GPC	Phos.Met.	↓ (39)	4.2%
	1-palmitoleoyl-2-oleoyl-glycerol	Phos.lip. (TG)	↑ (42)	5.6%
	1-palmitoleoyl-3-oleoyl-glycerol	Phos.lip. (TG)	↑ (45)	6.0%
	1-palmitoyl-2-adrenoyl-GPC	Phos.Met. (TG)	↑ (47)	2.9%
	cortisone	Plas. (HDL, TG)	↓ (49)	2.5%

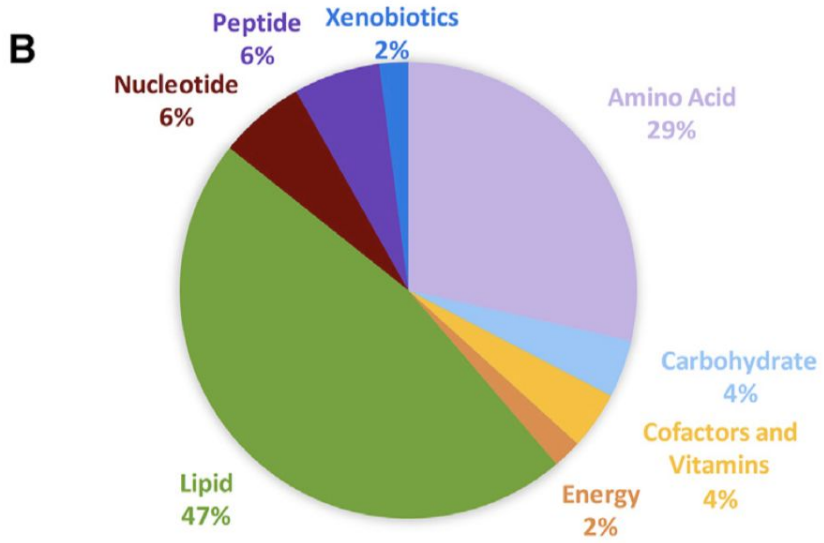
Super Pathway	Metabolite	Subpathway (Correlated Blood Lipids[c])	Direction of Effect (Rank[a])	BMI r^{2b}
Energy	succinylcarnitine	TCA	↑ (13)	9.8%
Carbohydrate	mannose	Fru.Met.	↑ (17)	6.6%
	glucose	Pyr.Met.	↑ (48)	6.3%
Xenobiotics	cinnamoylglycine	Food	↓ (43)	3.5%
Cofactors/ vitamins	gulonic acid	Asc.Met.	↑ (46)	3.2%
	quinolinate	Nic.Met.	↑ (44)	8.4%
Peptide	N-acetylcarnosine	Dipep.	↑ (25)	6.9%
	gamma-glutamylphenylalanine	Gam.	↑ (32)	6.0%
	gamma-glutamyltyrosine	Gam.	↑ (35)	4.6%

- Rank: order of significance of association with BMI
- r^2: the percent variation in BMI explained by each metabolite in univariate analysis for a combined analysis of the first time point of the TwinsUK cohort and the Health Nucleus data.

Result Patterns in Metabolite Change According to BMI

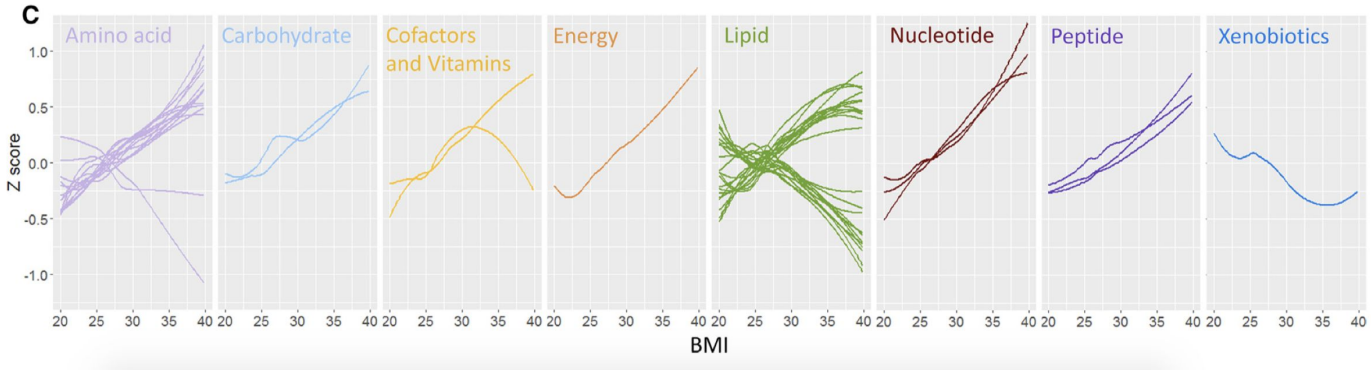
Pathway categories of (A) the 307 metabolites significantly associated with BMI and (B) the 49-metabolite signature.





Result Patterns in Metabolite Change According to BMI

Most metabolites change linearly (both proportionally and inversely) with BMI.



Values of each of the 49 BMI-associated metabolites are plotted with a Loess curve against the BMI for time point 1 in TwinsUK.

- Filters:
  - Only unrelated individuals of European ancestry are included.
  - Individuals with BMI < 20 (n = 31) or > 40 (n = 10) are removed to prvent graphs from being skewed.
- The apparent inversion of the relationship between one cofactor/vitamin metabolite and BMI at higher BMIs is an artifact that is corrected once morbidly obese individuals are included.

Result Patterns in Metabolite Change According to BMI

Other takeaways are ...

- Branched-chain and aromatic amino acids as well as metabolites related to nucleotide metabolism like urate had the most rapid increases.

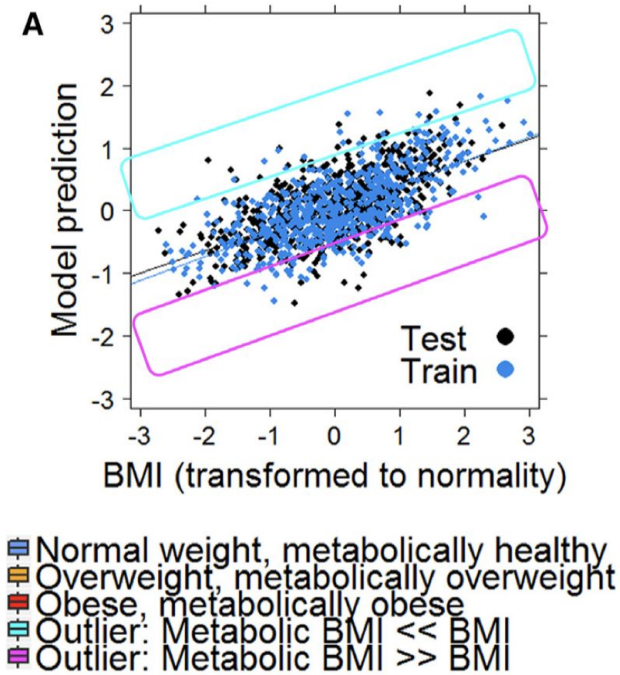
- The negatively associated lipids tended to reflect HDL (high-density lipoprotein) levels.
- The positively correlated lipids were more representative of triglyceride levels.

- Between BMI and cortisone, identified lower levels among the people with obesity.

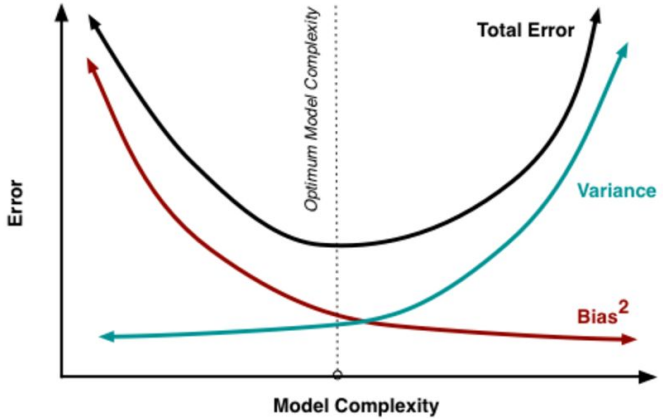
- PCA also conducted, 1st PC explains ~20% of the total variation in the levels of these 49 metabolites.

Result Modeling the Metabolome of Obesity

Ridge regression was used to predict BMI from the 49 BMI-associated metabolites.

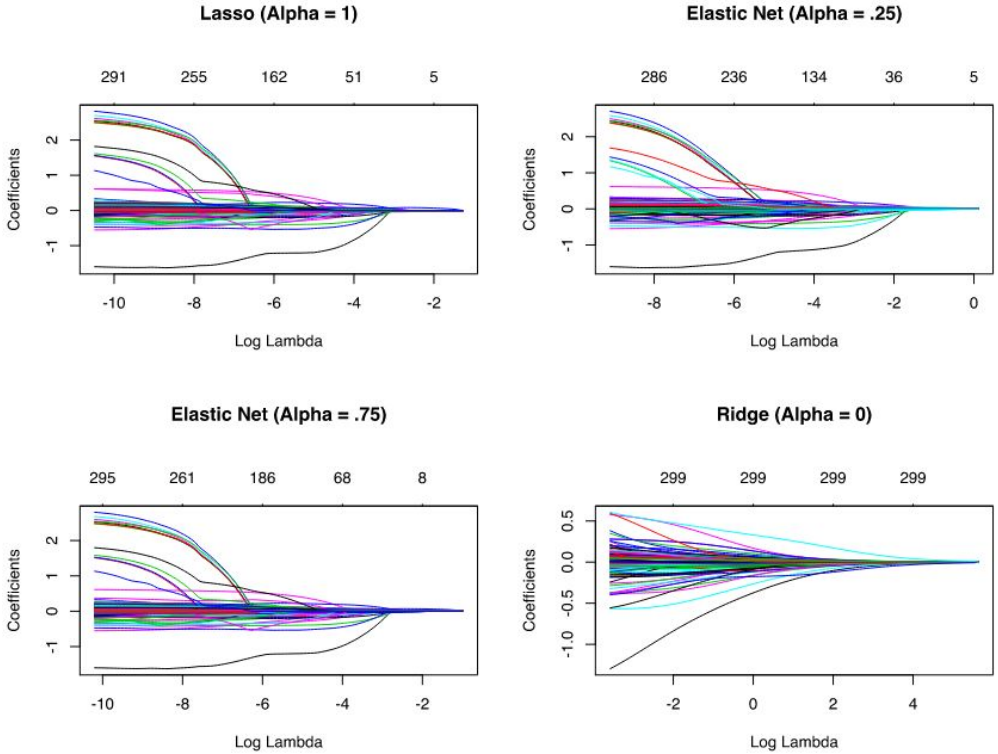


- **(Why Ridge?)** Allows us to 1) focus on the most stringently associated metabolites and 2) to remove effects of collinearity
- Similar result obtained from LASSO regression
- Trained with 10-fold cross-validation (Train-Test 1:1 random sampling)
- The model could explain 39.1% of the variation in BMI.
  - In predicting whether participants were obese (BMI>=30) or normal weight (BMI 18.5-25)
  - AUC of 0.922, specificity of 89.1%, and sensitivity of 80.2%
- Defined **mBMI (Metabolic BMI)**, the predicted BMI on the basis of metabolome.

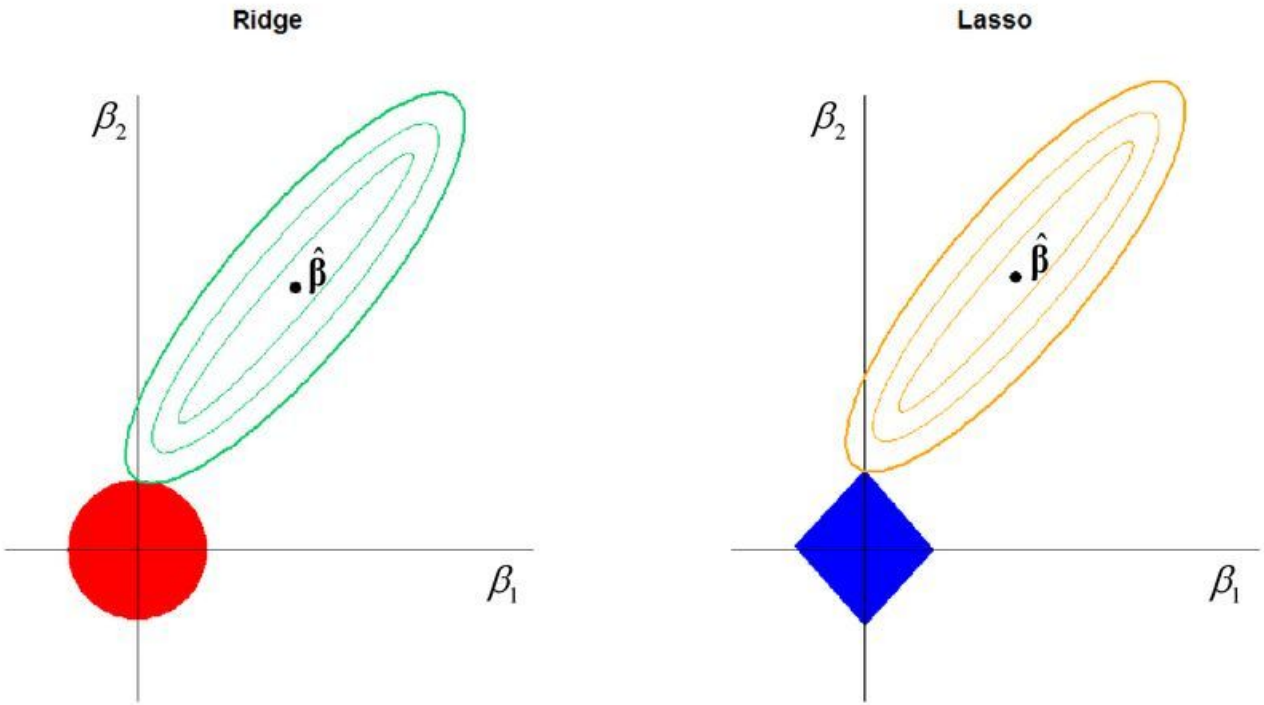


$$L_{ridge}(\hat{\beta}) = \sum_{i-1}^{n}(y_i - x_i'\hat{\beta})^2 + \lambda \sum_{j-1}^{m} \hat{\beta}_j^2$$

$$L_{lasso}(\hat{\beta}) = \sum_{i-1}^{n}(y_i - x_i'\hat{\beta})^2 + \lambda \sum_{j-1}^{m} |\hat{\beta}_j|.$$



- If λ = alpha, and alpha is from 0 to 1.
  - Alpha = 0 is Ridge regression: L1 Penalty
  - Alpha = 1 is LASSO: L2 Penalty
  - How about some of each? Elastic Net

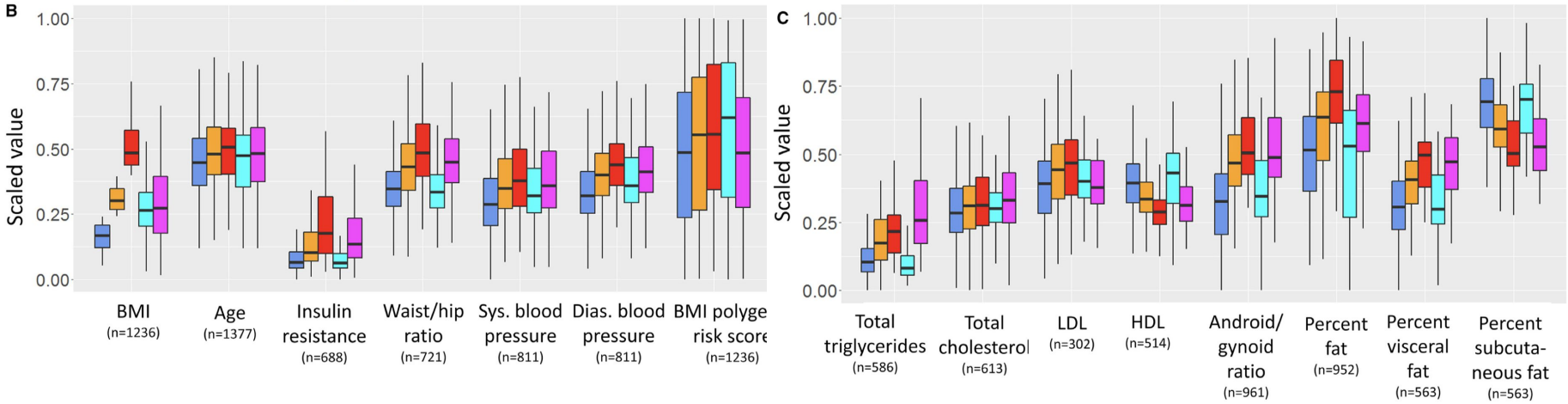


Solid areas represent the constraint regions $\beta_1^2 + \beta_2^2 \le t$ & $|\beta_1| + |\beta_2| \le t$

The ellipses represent the contours of the least square error function

Result Identification and Characterization of Metabolic BMI Outliers

Divided the participants into five groups, two groups were characterized as outliers. They had the same weight & age distribution but showed very different phenotypes.



Normal weight, metabolically healthy
Overweight, metabolically overweight
Obese, metabolically obese
Outlier: Metabolic BMI << BMI
Outlier: Metabolic BMI >> BMI

- People with mBMI << BMI: similar to normal-weight people
- People with mBMI >> BMI: similar to obese people
- **Obesity should be analyzed in the context of its metabolome perturbation rather than just on BMI alone!**

Result Identification and Characterization of Metabolic BMI Outliers

Other takeaways are ...

- Two outlier groups did have statistically significant differences in their metabolite levels for all of the 49 metabolites **but two: asparagine** and **cortisone**

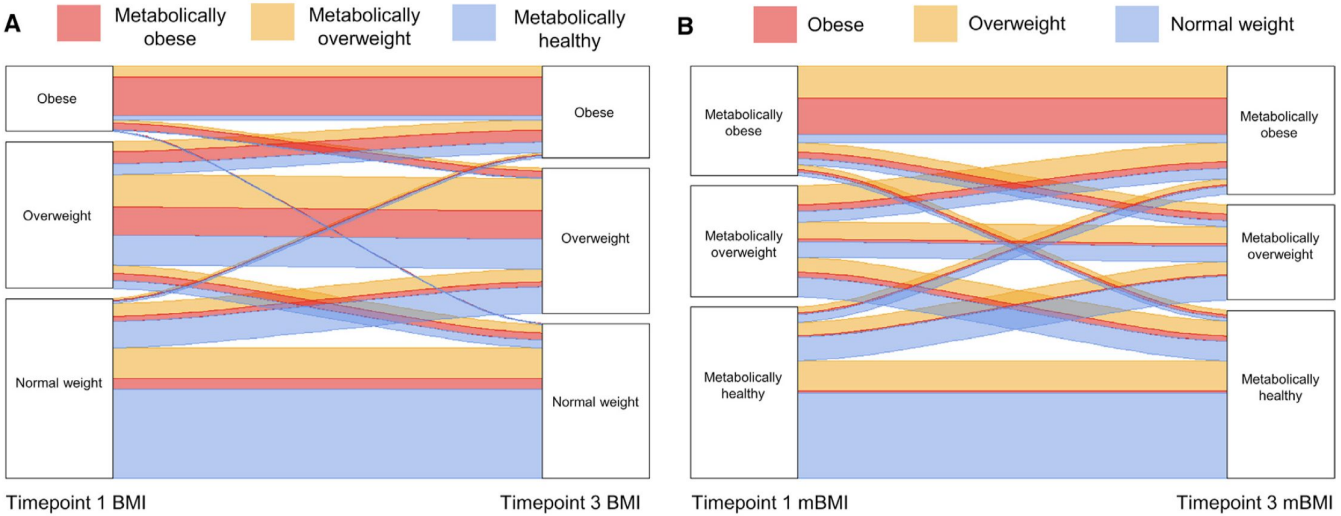
- This lack of association of cortisone with mBMI/BMI outliers may show that its correlation with BMI does not appear to extend to metabolic health.

- But, keep that in mind that cortisol levels change throughout the day, and the levels were not all measured at the same time.

Result Evolution of Obesity and Metabolome Clinical Profiles

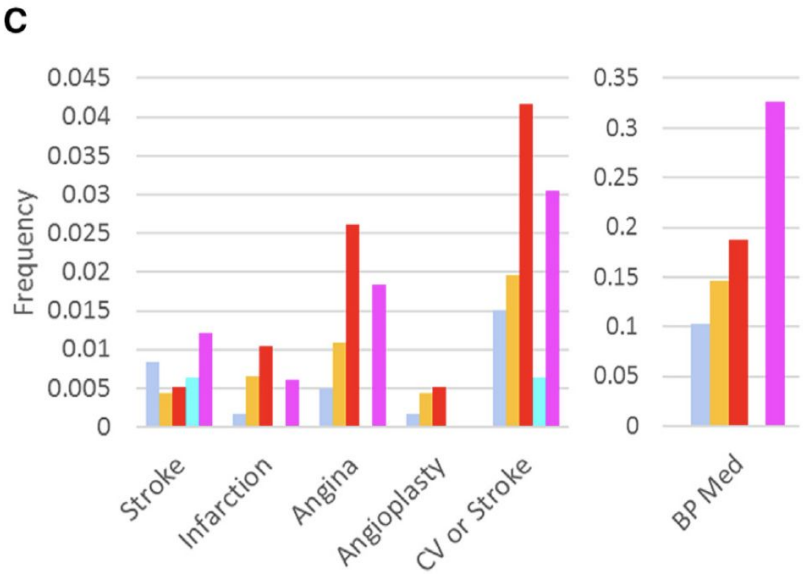
Are the outlier groups more likely to become obese over time?

- [mBMI >> BMI] group were marginally more likely to gain weight and convert to an obese phenotype (BMI > 30) over the 8–18 years of follow up.
- The mBMI states of the people remained fairly stable with time and were a function of BMI changes.



Result Cardiovascular Disease Outcomes

[mBMI << BMI] had no history of cardiovascular events, while [mBMI >> BMI] had a proportion of historical events similar to the overweight/obese group.



- Obtained a hazard ratio of at least 1.5 for differences in CVD outcomes between the mBMI/BMI groups.
  - People with healthy metabolome had 2.6 events per 100 people.
  - People with obese metabolome had 3.4 (normal/overweight BMI) and 4.4 events (in obese individuals) per 100 people.
- People with healthier metabolomes to have fewer/later cardiac events ($p = 0.003$)

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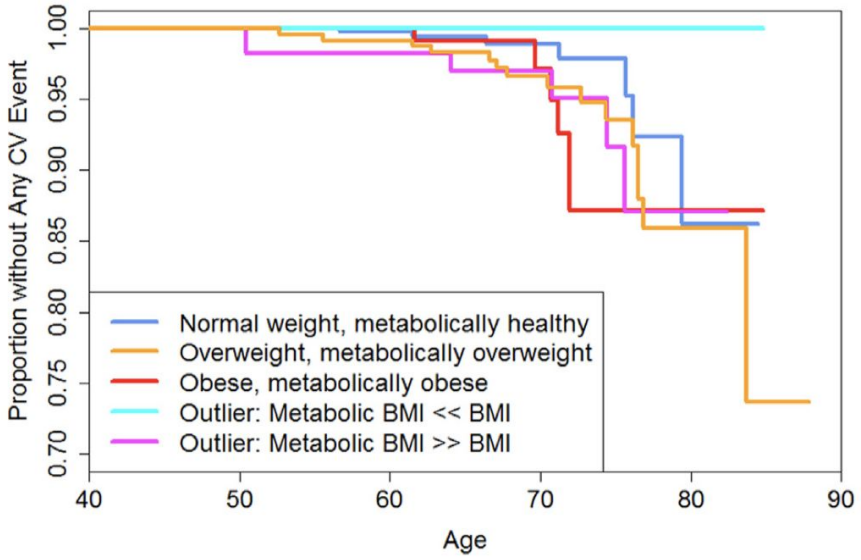

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Result Correlations between Twins

Reassessed the BMI model predictions and obesity status of 350 sets of twins: both normal BMI (n = 244); both obese (n = 67); either one obese (n = 39).

- Individuals with BMIs between 25 and 30 (overweight) and their twins were excluded.

- The correlations between metabolite-based obesity predictions are higher in monozygotic twins than the dizygotic twins.

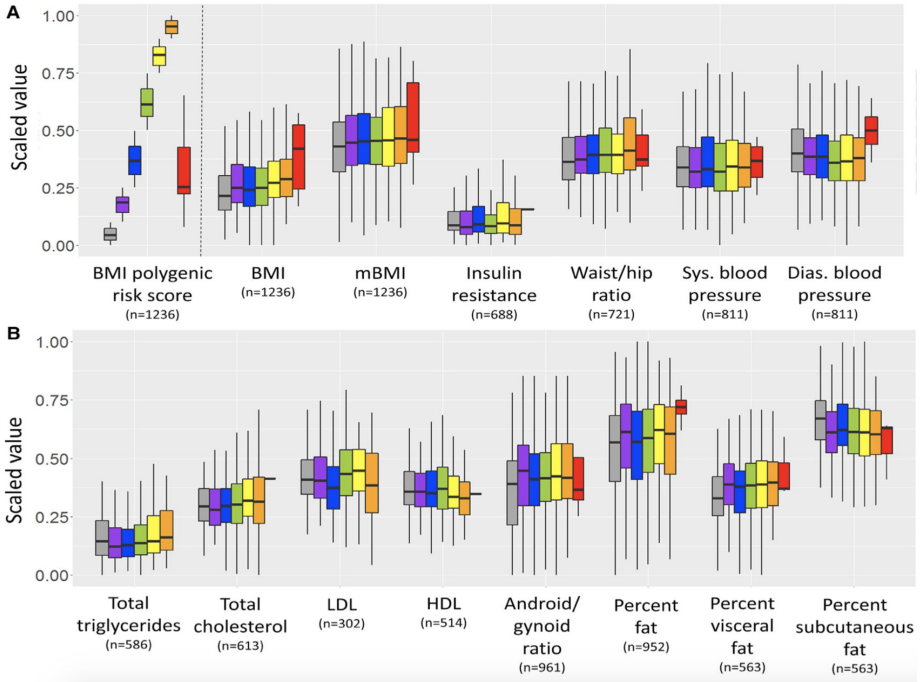
Genetic Analyses Known Genetics of Obesity

Investigated the known genetic factors contributing to high BMI

- Polygenic risk score only explained 1.2%–2.2% of the variation in BMI at each of the three TwinsUK time points and in Health Nucleus for unrelated participants of European ancestry.

Genetic Analyses Known Genetics of Obesity

Also investigated whether unique individuals with the highest polygenic risk scores would have a significant perturbation of the metabolome and anthropomorphic, insulin resistance, and DEXA measurements.



- Higher polygenic risk scores to be associated with a higher android/gynoid ratio ($p = 0.04$), waist/hip ratio ($p = 0.03$), and triglycerides ($p = 0.01$).
- But no association with mBMI ($p = 0.07$).
- MC4R carriers had significantly higher BMI ($p = 0.02$) than did non-carriers as well as not significant trends toward a higher dbp, insulin resistance, and percent body fat.

Genetic Analyses Genetics of the Metabolically Healthy Obese

Polygenic risk score for BMI may capture an anthropomorphic phenotype rather than a unique association with obesity as a disease trait.

- Individuals with mBMI << BMI had a higher polygenic risk score for BMI than did other groups.
- People whose mBMI >> BMI had low polygenic risk scores. ($p = 0.08$)

Genetic Analyses Genetics of Metabolome Differences

Do obese individuals with different genetic backgrounds have different metabolomes from other obese individuals?

- Metabolites are unlikely to be intermediate phenotypes that explain the underlying genetics of obesity.
  - No significant associations between any single metabolites and polygenic risk or MC4R carrier status in either the entire population or in only the obese individuals.
- To check for more specific signals beyond the compiled polygenic risk score, separate analyses were performed:
  - No evidence for any of these known GWAS variants to be more strongly associated with a metabolite than with BMI itself
- **Most metabolic perturbations that occur in the obese state are a response to obesity as opposed to shared genetic mechanisms.**

Discussion

- Metabolite levels did not provide predictive power for future weight changes.
- Metabolome perturbations appear as a consequence of changes in weight as opposed to being a contributing factor.
- There is a substantial loss of signal associated with the quest for a single biomarker.
- Future studies need to explore the role of rare functional variants in lipodystrophy genes on the metabolic traits in the general population.
- Limited access to longitudinal information on medication use → couldn't analzye how drug prescription influenced mBMI/BMI categories and vice versa.

Summary

- Obesity is a heterogeneous phenotype that is crudely measured by body mass index (BMI).
- Used non-targeted metabolomics and whole-genome sequencing to identify metabolic and genetic signatures of obesity.
- Highlighted the health risks of the perturbed metabolome.
  - Nearly ⅓ of the assayed metabolites associated with changes in BMI.
- A metabolome signature identifies the healthy obese and lean individuals with abnormal metabolomes these groups differ in health outcomes and underlying genetic risk.
- The methods used here can be applied to build similar models for insulin resistance, fat distribution, or any other number of clinical traits.
  - metabolic profiling could help select patients for clinical trials beyond genetic sequencing, thus expanding drug utility (Yanovski and Yanovski, 2018)

End of Document