SUPPLEMENTAL MATERIAL

Altered branched-chain α-keto acid metabolism is a feature of NAFLD in individuals with severe obesity

Thomas Grenier-Larouche[1,2,3*], Lydia Coulter Kwee[1*], Yann Deleye[1], Paola Leon-Mimila[4,5], Jacqueline M. Walejko[1], Robert W. McGarrah[1], Simon Marceau[2], Sylvain Trahan[2], Christine Racine[2], André C Carpentier[3], Aldons J. Lusis[4], Olga Ilkayeva[1], Marie-Claude Vohl[6], Adriana Huertas-Vazquez[4], André Tchernof[2], Svati H. Shah[1], Christopher B Newgard[1], Phillip J White[1#].

1. Sarah W. Stedman Nutrition and Metabolism Center and Duke Molecular Physiology Institute, Duke University Medical Center, Divisions of Endocrinology and Cardiology, Department of Medicine, and Department of Pharmacology and Cancer Biology, Durham NC USA.
2. Institut universitaire de cardiologie et de pneumologie de Québec - Université Laval (IUCPQ-UL) Québec City, QC, Canada
3. Faculty of Medicine and Health Sciences, Centre de recherche du Centre hospitalier universitaire de Sherbrooke, Université de Sherbrooke, Sherbrooke, QC, Canada.
4. Department of Medicine/Division of Cardiology, University of California, Los Angeles, California.
5. Facultad de Química, UNAM/Instituto Nacional de Medicina Genómica (INMEGEN), Unidad de Genómica de Poblaciones Aplicada a la Salud, Mexico City, Mexico.
6. Centre de Nutrition, santé et société and Institute of Nutrition and Functional Foods (INAF), Université Laval, Québec City, QC, Canada

Address correspondence to PJW: (phillip.white@duke.edu)

Table S1. PCA Factors

PCA Factor	Metabolite Components
Factor 1 – Even chain acylcarnitines	C14:1, C12:1, C2, C16:1,C14:2,C4-OH, C16:2,C14:1-OH, C12, C18:1, C6-DC/C8-OH, C16:1-OH/C14:1-DC, C14, C12-OH/C10-DC, C18:1-OH/C16:1-DC, C16, C10:1, C10, C14-OH/C12-DC, C18:2, C8, C8:1-DC, C18:1-DC
Factor 2 – Glucosylceramides	Glucosylceramides d18:1/C24, d18:1/C16, d18:1/C24:1, d18:1/C22, d18:1/C23, d18:1/C18, d18:1/C20; ceramide C16
Factor 3 – Amino acid related	Phenylalanine, leucine/isoleucine, methionine, arginine, tyrosine, valine, ornithine, C3 acylcarinitine, proline, citrulline, KMV, histidine
Factor 4 – Ceramides	Ceramides C26:1, C20, C26, C25, C18; glucosylceramide d18:1/C26
Factor 5 – Branched-chain keto and amino acids	KIC, KMV, KIV, leucine/isoleucine, valine
Factor 6 – Medium chain OH/DC acylcarnitines	C8:1-OH/C6:1-DC, C10-OH/C8-DC, C8:1-DC
Factor 7 – Glycine related amino acids	Glycine, serine, histidine
Factor 8 – C18/C16 acylcarnitines	C18, C16, C18:2, C18:1
Factor 9 – Long chain ceramides	Ceramides C24:1, C16, C20:1, C18
Factor 10 – Long chain acylcarnitines	C20:4, C22, C18:2-OH
Factor 11 – Medium chain unsaturated acylcarnitines	C8:1, C10:3, C10:2
Factor 12 – Long chain ceramides	Ceramides C23, C22, C24
Factor 13 – Hydroxyisovaleryl/malonyl carnitine	C5-OH/C3-DC
Factor 14 – Alanine, proline	Alanine, proline
Factor 15 – C20 acylcarnitine	C20
Factor 16 – Short chain acylcarnitines	C4/Ci4, C3, C5
Factor 17 – Long chain dicarboxyl acylcarnitines	C20-OH/C18-DC, C18:1-DC
Factor 18 – Medium chain acylcarnitines	C8, C10, C10:1

PCA factors were created using 80 metabolites. Top 18 factors displayed above (each with eigenvalue >1) explain 73% of total variance.

Table S2. Baseline characteristics of the gene expression cohorts

Trait	QHLI (N = 60)	MOBES (N = 107)
Age (Y)	45.0 (40.0 - 51.0)	35.0 (28.0 - 44.5)
Female (%)	42 (70)	93 (86.9)
BMI (kg/m2)	49.7 (46.6 - 52.7)	43.2 (39.1 - 48.5)
Glucose (mg/dL)	117.0 (99.0 - 146.7)	91.0 (80.3 - 99.8)
TG (mg/dL)	129.3 (100.7 - 159.0)	124.0 (91.0 - 162.0)
TC (mg/dL)	181.3 (162.3 - 203.5)	172.0 (147.0 - 194.0)
HDL-C (mg/dL)	48.7 (42.4 - 58.8)	36.0 (31.0 - 43.0)
LDL-C (mg/dL)	106.0 (83.2 - 121.0)	105.0 (86.6 - 129.0)
ALT (UI/L)	29.5 (21.0 - 41.0)	27.0 (21.0 - 38.0)
AST (UI/L)	23.0 (18.0 - 31.0)	26.0 (21.0 - 32.0)
GGT (UI/L)	29.0 (22.5 - 47.0)	19.0 (14.0 - 26.0)

Data are shown as median (interquartile range) or n (%). Abbreviations: BMI, Body Mass Index; TG, Triglycerides; TC, Total Cholesterol; HDL-C, high-density lipoprotein cholesterol; LDL-C, low-density lipoprotein; ALT, alanine aminotransferase; AST, aspartate aminotransferase; GGT, gamma-glutamyl transferase.

Table S3. Primers used for qPCR

Target	Forward Primers	Reverse Primers
h***BCKDK***	5’ -TGAGAAGTGGGTGGACTTTGC- 3’	5’ -ATGGCATTCTTGAGCAGCTC- 3’
m***Bckdk***	5’ -ATCTGTACTCGTCTGTCGCC- 3’	5’ -TCCGTTGATGCGGACTCG- 3’
m***Fasn***	5’ -AGTCAGCTATGAAGCAATTGTGGA- 3’	5’ -CACCCAGACGCCAGTGTTC- 3’
RPLP0	5’ -TCTGCATTCTCGCTTCCTGG- 3’	5’-CCAGGACTCGTTTGTACCCG- 3’