Abdelilah Arredouani,[1,2] Matteo Stocchero,[3] Nicola Culeddu,[4] Julia El-Sayed Moustafa,[5] D.E.S.I.R. Study Group,* Jean Tichet,[6] Beverley Balkau,[7,8] Thierry Brousseau,[9] Marco Manca,[10,11] and Mario Falchi[2,5]

Metabolomic Profile of Low–Copy Number Carriers at the Salivary α-Amylase Gene Suggests a Metabolic Shift Toward Lipid-Based Energy Production

Diabetes 2016;65:3362–3368 | DOI: 10.2337/db16-0315

Low serum salivary amylase levels have been associated with a range of metabolic abnormalities, including obesity and insulin resistance. We recently suggested that a low copy number at the *AMY1* gene, associated with lower enzyme levels, also increases susceptibility to obesity. To advance our understanding of the effect of *AMY1* copy number variation on metabolism, we compared the metabolomic signatures of high– and low–copy number carriers. We analyzed, using mass spectrometry and nuclear magnetic resonance (NMR), the sera of healthy normal-weight women carrying either low–*AMY1* copies (LAs: four or fewer copies; $n = 50$) or high–*AMY1* copies (HAs: eight or more copies; $n = 50$). Best-fitting multivariate models (empirical $P < 1 \times 10^{-3}$) of mass spectrometry and NMR data were concordant in showing differences in lipid metabolism between the two groups. In particular, LA carriers showed lower levels of long- and medium-chain fatty acids, and higher levels of dicarboxylic fatty acids and 2-hydroxybutyrate (a known marker of glucose malabsorption). Taken together, these observations suggest increased metabolic reliance on fatty acids in LA carriers through β- and ω-oxidation and reduced cellular glucose uptake with consequent diversion of acetyl-CoA into ketogenesis. Our observations are in line with previously reported delayed glucose uptake in LA carriers after starch consumption. Further functional studies are needed to extrapolate from our findings to implications for biochemical pathways.

Salivary α-amylase (sAA), encoded by the *AMY1* gene, is produced under the control of the autonomic nervous system by the salivary glands. It initiates starch digestion in the mouth, yielding a mixture of maltose, isomaltose, and glucose. Therefore, it was expected that individuals with high levels of sAA would show higher postprandial glycaemia, compared with low sAA counterparts. A recent study (1) showed, surprisingly, the opposite effect. Lower serum amylase levels have been associated with susceptibility to metabolic abnormalities, including nonalcoholic fatty liver disease, obesity, and diabetes (2–4).

Salivary amylase level is strongly correlated with the copy number (CN) at the *AMY1* gene (5). Higher *AMY1* CN and

[1]Hamad Ben Khalifa University, Qatar Biomedical Research Institute, Diabetes Research Centre, Qatar Foundation, Doha, Qatar
[2]Department of Genomics of Common Disease, Imperial College London, U.K.
[3]S-IN Soluzioni Informatiche, Vicenza, Italy
[4]Istituto di Chimica Biomolecolare, Laboratory of Nuclear Magnetic Resonance, Consiglio Nazionale delle Ricerche, Sassari, Italy
[5]Department of Twin Research & Genetic Epidemiology, King's College London, St. Thomas' Hospital Campus, London, U.K.
[6]Institut Inter-Régional Pour la Santé, La Riche, France
[7]Centre de Recherche en Epidémiologie et Santé des Populations, Le Centre de Recherche en Epidémiologie et Santé des Populations, INSERM U1018, Renal and Cardiovascular Epidemiology, Villejuif, France
[8]Universities Versailles-St. Quentin and Paris-Sud, UMRS 1018, Villejuif, France
[9]UF8832, Biochimie Automatisée, Pôle de Biologie Pathologie Génétique, Centre Hospitalier Régional Universitaire, Lille, France
[10]Experimental Vascular Pathology, Cardiovascular Research Institute Maastricht, University of Maastricht, Maastricht, the Netherlands
[11]Accelerators & Technology Sector, CERN, Geneva, Switzerland

Corresponding authors: Abdelilah Arredouani, aarredouani@qf.org.qa, and Mario Falchi, mario.falchi@kcl.ac.uk.

Received 15 March 2016 and accepted 6 July 2016.

This article contains Supplementary Data online at http://diabetes.diabetesjournals.org/lookup/suppl/doi:10.2337/db16-0315/-/DC1.

A.A. and M.S. contributed equally to this work.

*A complete list of the members of the D.E.S.I.R. Study Group can be found in the APPENDIX.

See accompanying article, p. 3240.

sAA protein levels were observed in populations traditionally consuming starch-rich diets compared with those consuming a low-starch diet (5). We recently confirmed *AMY1* gene expression and protein levels in serum to be inversely correlated with obesity and suggested CN variation at *AMY1* to be an important driver of this relationship, showing an association between low amylase CN and increased BMI and obesity risk in both adults (6) and children (7). On the other hand, a novel study has suggested that CNV might not be the main factor driving the association between amylase levels and obesity susceptibility (8). The mechanisms by which sAA contributes to an increased risk of metabolic diseases remain elusive (8,9). To further investigate the role of genetic variation at the *AMY1* gene on the metabolism, we used untargeted ^{1}H-nuclear magnetic resonance (NMR) and mass spectrometry (MS)–based metabolomics, and compared the metabolomic profiles of a carefully selected sample of 100 healthy and normal-weight, age-matched women carrying either low–*AMY1* CNs (LAs) or high–*AMY1* CNs (HAs).

RESEARCH DESIGN AND METHODS

Study Population and Design

Only women were included to provide a more homogenous study population to maximize power, given our small study population. The characteristics of the women carrying HAs (n = 50) or LAs (n = 50) are shown in Supplementary Table 1. The women were selected from the prospective D.E.S.I.R. (Data from an Epidemiological Study on the Insulin Resistance Syndrome) cohort (9) from among those for whom data on the inferred CN at the *AMY1* gene were already available (6). To control for the effects of potential confounders, we carefully selected two subsamples from the top and bottom 20% of the CN distribution (four or fewer CNs and eight or more CNs). Exclusion criteria included the following: *1*) BMI >25 or <18.5 kg/m^2; *2*) fasting glycemia >6 mmol/L; and *3*) development of any metabolic disorders during the 9-year follow-up period. Food and caloric intake were not significantly different between the HA and LA groups.

All participants signed a written informed consent, and the study was approved by the Ethics Committee for the Protection of Subjects for Biomedical Research of Bicêtre Hospital (Le Kremlin-Bicêtre, France).

Serum Amylase Levels

Serum pancreatic and total amylase levels were measured by enzymatic colorimetric assays with an auto-Roche/Hitachi Cobas 8000 c701 (AMY-P-20766623322 and AMYL2–03183742122; Hoffman-La Roche). The sAA levels were obtained by subtracting the pancreatic amylase level from the total amylase level.

MS

MS-based metabolomics profiling was carried out at Metabolon (Durham, NC). The processing and analysis of samples was performed as previously described (10) (Supplementary Data).

^{1}H-NMR

Spectra of serum samples were measured at 600.13 MHz on a Bruker Avance II 600-MHz spectrometer (Bruker BioSpin GmbH, Rheinstetten, Germany) following the study by Beckonert et al. (11). NMR spectra were phased and baseline corrected. Spectra were aligned using the CluPA algorithm (12), and the alanine signal was used as a reference (1.4784 parts per million [ppm]). Residue water signal was removed. Data were reduced to 513 bins by intelligent bucketing and were normalized by total sum normalization. Mean centering and Pareto scaling were applied prior to performing the data analysis.

Identification of Metabolomics Signatures

We used multivariate statistical data analyses based on projection methods. Principal component analysis was used for the initial exploratory data analysis. Metabolomics signatures distinguishing HA and LA women were identified through projection to latent structures discriminant analysis (PLS-DA), based on variable importance in projection (VIP) selection (13). Given the limited sample size, we used a careful and conservative approach to validate our results.

First, results were validated through N-fold full cross-validation, using different values of N (N = 6, 7, and 8; we report only results for sevenfold as the prediction error estimated by cross-validation [cross-validated R^2] [Q^2] and area under the receiver operating characteristic curve estimated by cross-validation [AUC_{CV}], two measures to estimate the predictive ability of the model), as well as through 1,000 permutation testing on the HA/LA classes (13).

Second, to avoid overfitting we performed stability selection by Monte Carlo subsampling: real differences should be present consistently and therefore should be found even under perturbation of the data by subsampling (14). We generated 500 random data sets by Monte Carlo subsampling (with prior probability of 0.70), and then VIP-based PLS-DA was applied to each subsample, obtaining a set of 500 discriminant models. Each model was additionally validated by N-fold full cross-validation and 1,000 permutations of the class response. Metabolites discriminating between HA and LA classes were identified as those that were selected by >50% of all the models.

Both in the original and in the randomly generated data sets, the VIP threshold for the selection of metabolites discriminating between HA and LA classes was determined by maximizing Q^2, whereas the number of latent variables of the PLS model was calculated by maximizing Q^2 under the constraint to pass the permutation test. To clarify model interpretation, PLS-DA VIP-based models were post-transformed into the equivalent orthogonal PLS-DA models (OPLS-DA), where the predictive and the orthogonal parts of the models were identified.

RESULTS

Serum Amylase Levels

HA subjects showed significantly higher levels of serum sAA compared with LA counterparts (Wilcoxon test,

$P = 6.04 \times 10^{-8}$). A smaller difference was detected for the pancreatic amylase levels (Wilcoxon test, $P = 0.025$), which is likely to be a reflection of both physiological and structural correlations between the two enzymes (15) (Supplementary Table 1 and Supplementary Fig. 1).

Model Fitting and Validation

The analysis of the NMR and MS data sets by principal component analysis detected the presence of 15 outliers that were excluded from further analysis. The major patterns within the original data were captured by a small number of components, three for both the NMR and MS data sets. The score scatter plots in Fig. 1*A* and *B* show the separation of the HA and LA samples based on the PLS-DA analyses of the MS and NMR data. The goodness of fit and the predictive ability estimated by sevenfold cross-validation were $R^2 = 0.45$ and $AUC_{CV} = 0.79$ for the NMR data set and $R^2 = 0.71$ and $AUC_{CV} = 0.92$ for the MS data set. Figure 1*C* and *D* compare the R^2 and Q^2 obtained through random permutation of the class response with what was observed in the original data set (empirical $P < 0.001$ for both R^2 and Q^2).

Stability Selection and Identification of the HA/LA Metabolomics Signature

All data sets generated through Monte Carlo sampling successfully passed both permutation testing and *N*-fold full cross-validation, thus suggesting that model fitting in the data was not driven by a small number of observations. On the basis of the stability selection, we identified two signatures from MS and NMR, encompassing 41 metabolites and 15 signals, respectively (Tables 1 and 2), which provided the largest contribution to the discrimination of the HA and LA groups. To investigate the role of each metabolite in the discrimination of the two groups, we plotted the median of the VIP calculated by stability selection versus the correlation between the predictive latent variable of the OPLS-DA model and the measured

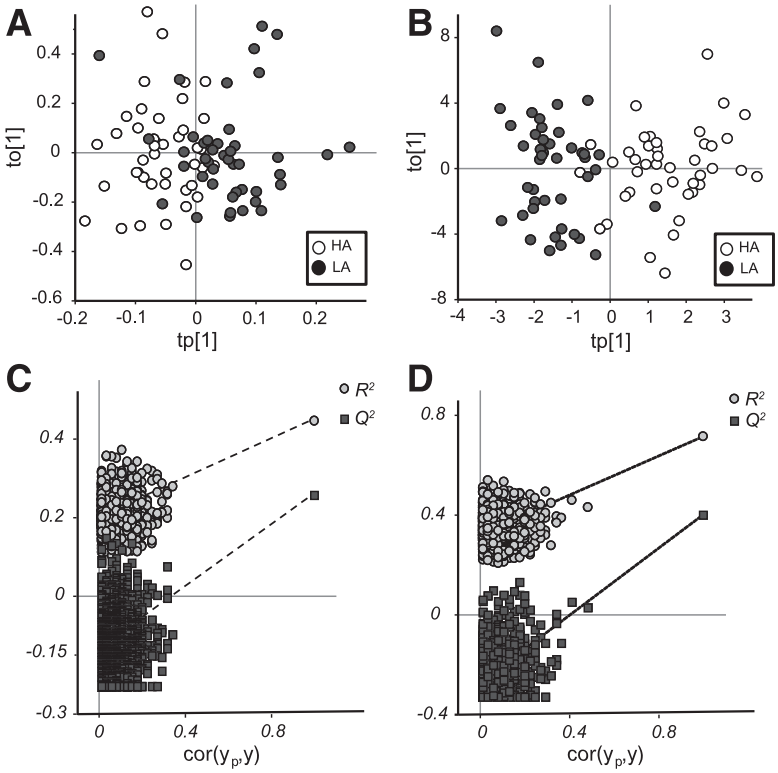

Figure 1—PLS-DA VIP-based models for MS and NMR data sets. The goodness of fit and predictive ability estimated by sevenfold full cross-validation were as follows: $R^2 = 0.45$, $Q^2 = 0.25$ ($AUC_{CV} = 0.79$) for the MS data set; and $R^2 = 0.71$, $Q^2 = 0.40$ ($AUC_{CV} = 0.92$) for the NMR data set. Score scatter plots obtained after the transformation of the PLS-DA VIP-based models into OPLS-DA models for the MS (*A*) and NMR (*B*) data sets. The samples are colored according to the class, as follows: HA, gray dots; and LA, black dots. The *x*-axis represents the predictive latent variable to providing a clear class separation of LA against HA, whereas the *y*-axis is the first orthogonal latent variable to 1 that is not related to the class samples. Plot for the response permutation test (1,000 random permutations) of the models built for MS (*C*) and NMR (*D*). The *x*-axis represents the correlation between the class response Y and the random permutated class response Yp, whereas the *y*-axis reports the values of R^2 and Q^2 for the calculated PLS-DA VIP-based models. Most of the models having low correlation between the permutated response and the class response shows negative Q^2, and the related R^2 is always lower than that calculated for the reference model (i.e., the model corresponding to cor(Yp,Y) = 1). This behavior proves the absence of overfitting in the reported models. cor(Yp,Y), the absolute value of the Pearson's correlation between Y permutated (i.e., the class response randomly permutated) and Y (i.e., the class response); to, nonpredictive score; tp, predictive score.

Table 1—Metabolites identified by stability selection applied to MS data set

Metabolite	Class	Pathway	LA/HA
N6-acetyllysine		Lysine metabolism	↑
Histidine		Histidine metabolism	↓
2-Hydroxybutyrate (AHB)	Amino acid	Cysteine, methionine, *S*-adenosylmethionine, taurine metabolism	↑
Xylitol	Carbohydrate	Nucleotide sugars, pentose metabolism	↑
Ribose			↑
Pyridoxate	Cofactors and vitamins	Pyridoxal metabolism	↓
Succinylcarnitine	Energy	Krebs cycle	↓
Sebacate (decanedioate)	Lipid	FA, dicarboxylate	↑
Azelate (nonanedioate)			↑
Suberate (octanedioate)			↑
Dodecanedioate			↑
3-Carboxy-4-methyl-5-propyl-2-furanpropanoate (CMPF)			↓
2-Linoleoylglycerol (2-monolinolein)		Monoacylglycerol	↑
1,2-Propanediol		Ketone bodies	↑
7-β-Hydroxycholesterol		Sterol/steroid	↓
Campesterol			↓
Cortisone			↑
Taurolithocholate 3-sulfate		Bile acid metabolism	↓
Oleamide		FA, amide	↓
10-Heptadecenoate (17:1n7)		Long-chain FA	↓
Arachidate (20:0)			↓
Myristoleate (14:1n5)			↓
Palmitate (16:0)			↓
Myristate (14:0)			↓
1-Linoleoylglycerol (1-monolinolein)		Monoacylglycerol	↑
2-Linoleoylglycerophosphocholine		Lysolipid	↑
1-Oleoylglycerophosphoethanolamine			↓
2-Oleoylglycerophosphoethanolamine			↑
Pelargonate (9:0)		Medium-chain FA	↑
Caprate (10:0)			↓
Laurate (12:0)			↓
Glycerol		Glycerolipid metabolism	↓
Linolenate (α or γ; [18:3n3 or 6])		Essential FA	↓
Stearoyl sphingomyelin		Sphingolipid	↓
13-Methylmyristic acid		FA, branched	↓
N-Acetylcarnosine	Peptide	Dipeptide derivative	↓
Leucylglycine		Dipeptide	↓
Leucylphenylalanine			↓
γ-Glutamylglutamate		γ-Glutamyl	↓
HWESASXX		Polypeptide	↑
Inosine	Nucleotide	Purine metabolism	↑

Upward and downward arrows in the last column indicate that the LA/HA ratio of the metabolite level is increased (↑) or decreased (↓) in sera from LA compared with HA subjects (see also Fig. 2).

variables. Since the VIP and the correlation coefficient estimate the strength and the direction of the effect, respectively, metabolites in the right quadrant of the plot showed higher levels in the HA group, whereas those in the left quadrant showed higher levels in the LA group (Fig. 2*A* and *B*).

Metabolites from MS data were mapped into the Ingenuity Pathway Analysis Knowledge Base database (accessed October 2015; QIAGEN, Redwood City, CA). The reference set included the endogenous chemicals; we chose to focus only on experimentally observed interactions with no restrictions on cell type or species. The top five most significantly enriched molecular and cellular functions included carbohydrate metabolism, energy production, and lipid metabolism (Supplementary Table 2). Their most significant functional annotations were as follows: oxidation of glucose-6-phosphate ($P = 3.12 \times 10^{-8}$), oxidation of lipid ($P = 1.32 \times 10^{-6}$), and accumulation of acylglycerol ($P = 6.19 \times 10^{-8}$) and triacylglycerol ($P = 2.85 \times 10^{-6}$). Of the 41 MS compounds, 28 (68%) were fatty acids (FAs), 17 and 11, respectively, with higher and lower levels in LA sera, compared with HA sera. Similarly, 9 of the 15 NMR signals (60%) corresponded to FAs, and their levels were higher in LA sera.

Table 2—Metabolites identified by stability selection applied to NMR data set

Metabolite	Class	Pathway	LA/HA	From ppm	To ppm
Urea		Urea cycle	↑	5.25	5.29
D-glucose	Carbohydrate	Glycolysis	↑	3.39	3.39
Lactic acid			↓	1.33	1.34
Lactic acid			↓	1.32	1.33
Glutamate		Glutamate/glutamine metabolism	↓	2.03	2.07
FAs CH2-CH = CH-	Lipid	Lipid metabolism	↑	1.97	2.01
FAs -CH2-			↑	1.28	1.32
FAs -CH2-			↑	1.23	1.27
FAs -CH2-			↑	1.19	1.23
FAs -CH2-			↑	1.15	1.19
Linoleic chain			↑	0.88	0.92
FAs -CH3			↑	0.83	0.88
FAs -CH3			↑	0.75	0.79
FAs -CH3			↑	0.71	0.75

Upward and downward arrows in the last column indicate that the LA/HA ratio of the metabolite level is increased (↑) or decreased (↓) in sera from LA compared with HA subjects (see also Fig. 2). The signal of metabolites was defined with reference to alanine (1.4784 ppm).

Despite ^{1}H-NMR experiments being highly reproducible, different lipids cannot be easily distinguished from each other, but rather are assigned to multiple macrocategories, characterized by the abundance of specific aliphatic chains and their magnetic shielding. Therefore, since the signals of the lipids detected by NMR can be characterized by only the three main functional groups (CH2-CH = CH-, -CH2-, and -CH3), we investigated their

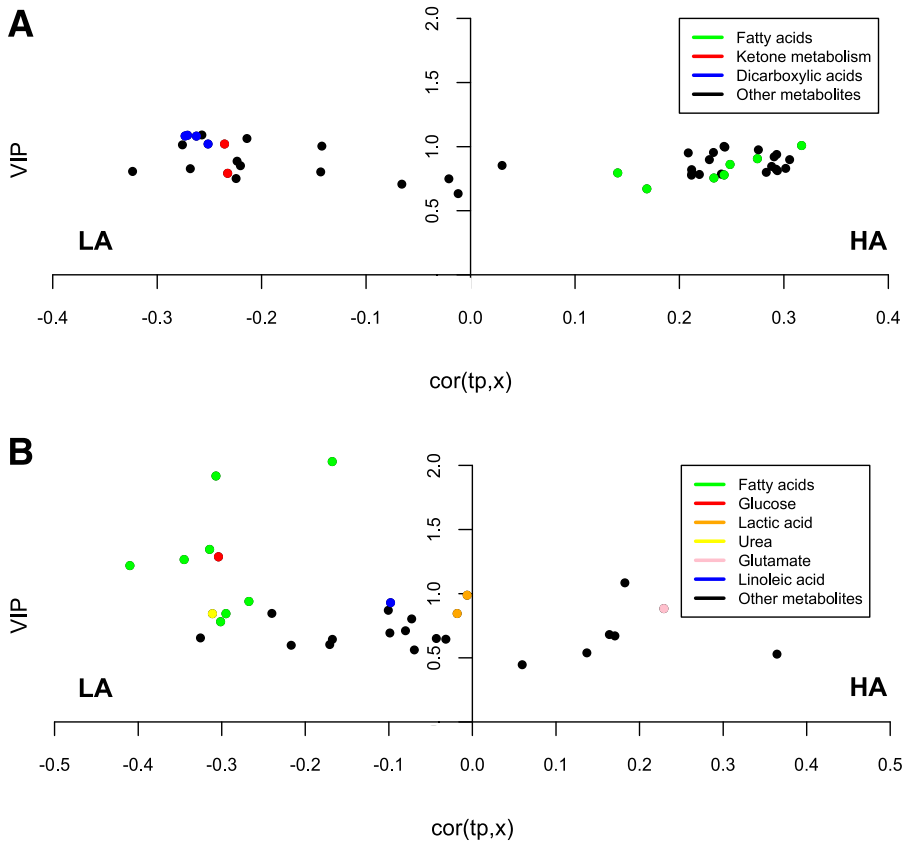

Figure 2—VIP vs. cor(tp,x) plot for the OPLS-DA models of the MS (*A*) and NMR (*B*) data sets reporting the metabolites identified by stability selection. The *x*-axis shows the direction of the effects of the metabolites on the model expressed as cor(tp,x), whereas the *y*-axis reports the strength of their contribution to the model (VIP), as follows: metabolites on the left of the origin are overrepresented in LA carriers (negative values for cor[tp,x]), while metabolites on the right of the origin are overrepresented in HA carriers (positive values for cor[tp,x]); the strength of the metabolite in the separation between LA and HA increases according to the VIP value. cor(tp,x), Pearson's correlation between tp and x; tp, predictive score.

correlation with the identified MS metabolites. The strongest correlations ($r > 0.7$) were observed among the four dicarboxylic FAs (DFAs) sebacate, azelate, suberate, and dodecanedioate, which were identified by MS and the -CH2- NMR bucket in the region from 1.15 and 1.19 ppm. Interestingly, the same DFAs were also strongly correlated ($r > 0.8$) with the glucose NMR bucket at 3.40 ppm. The BMI distribution was not different between the HA and LA groups (Wilcoxon test, $P = 0.986$). BMI was independent of HA/LA group definition and did not have any effect on the multivariable PLS models ($r^2 < 0.01$, $P > 0.20$). We further excluded the association between BMI and each single discriminant metabolite through linear regression by considering both BMI and HA/LA level as independent variables (P for BMI > 0.10).

DISCUSSION

In this study, we aimed to explore the effect of CN variation at *AMY1* on metabolism by comparing the metabolomic signature in carefully matched healthy women carrying LA or HA, as reflected in a significant difference in enzyme levels between the two groups ($P = 6.04 \times 10^{-8}$) (Supplementary Fig. 1). Although this study design facilitates the identification of discriminant metabolites associated with *AMY1* CN, the results might be affected by spectrum bias because women were selected from the two ends of the CN distribution.

The best-fitting models (empirical $P < 1 \times 10^{-3}$) identified through multivariate analyses of the MS and NMR metabolomics data concordantly highlighted differences in lipid metabolism between LA and HA women. Analysis of the MS-discriminant metabolites using the Ingenuity Knowledge Base annotations confirmed the significant enrichment of functional categories for "lipid metabolism," "energy production," and "carbohydrate metabolism." In particular, the MS data showed that the levels of four DFAs, suberate, sebacate, azelate, and dodecanedioate, were higher in the sera from LA women compared with HA women (Table 2). This increase in DFA levels is suggestive of an upregulation of the ω-oxidation. In normal conditions, ω-oxidation plays a minor role in overall FA oxidation. However, in certain physiological states in which mitochondrial β-oxidation is blocked or overwhelmed, free FAs are ω-oxidized in the reticulum, by a CYP4A-dependent pathway, to DFAs (16), which can then enter mitochondrial β-oxidation.

To corroborate the proposed interpretation, alongside the increase in DFAs, women with LA also showed lower serum concentrations of several medium- and long-chain FAs (Table 2). This reduction in FA levels is coherent with a more active uptake and oxidation, both via β- and ω-oxidation.

Interestingly, LA women also showed increased levels of 2-hydroxybutyrate, a biomarker for insulin resistance in humans (17) (Table 2). Taken together, these results suggest a pattern of reduced cellular glucose uptake, and a consistent metabolic shift toward lipid exploitation, in LA women. Interestingly, NMR findings suggested a slight increase of glucose levels in LA sera.

In a similar line, Mandel and Breslin (1) recently published an experiment in which they measured the dynamics of postprandial glucose and insulin after controlled starch or glucose meals in healthy LA and HA carriers. Although LA and HA carriers did not show any differences after the glucose-based meal, LA carriers showed significantly higher glucose levels after the starch-based meal, suggesting glucose malabsorption after starch ingestion in people with constitutionally low salivary amylase levels. Mandel and Breslin (1) advocated that this effect might be mediated by delayed preabsorptive insulin response in the LA group after the starch meal.

In summary, our results support the hypothesis that sAA CN might play a role in glucose uptake, offering novel evidence to support further research on its mechanisms of action.

Acknowledgments. The authors thank all participants in this study. The authors would like to express special gratitude to Phillipe Froguel and his team, particularly Dr. Loic Yengo, for their insightful discussions on the whole project and for facilitating access to the samples.

Funding. This study was supported by an intramural grant from the Qatar Biomedical Research Institute, a member of the Qatar Foundation. The D.E.S.I.R. study has been supported by INSERM contracts with CNAMTS, Lilly, Novartis Pharma, and Sanofi and by INSERM (Réseaux en Santé Publique, Interactions entre les déterminants de la santé, Cohortes Santé TGIR 2008), the Association Diabète Risque Vasculaire, the Fédération Française de Cardiologie, La Fondation de France, ALFEDIAM, ONIVINS, Société Francophone du Diabète, Ardix Medical, Bayer Diagnostics, Becton Dickinson, Cardionics, Merck Santé, Novo Nordisk, Pierre Fabre, Roche, and Topcon.

Duality of Interest. No potential conflicts of interest relevant to this article were reported.

Author Contributions. A.A. and M.F. conceived and designed the study, helped to interpret the data, wrote the first draft of the manuscript, and contributed to the final version of the manuscript. M.S. helped to interpret the data, performed the statistical analysis and wrote that respective section of the manuscript, and contributed to the final version of the manuscript. N.C. performed the nuclear magnetic resonance data analysis and wrote that respective section of the manuscript, helped to interpret the data, and contributed to the final version of the manuscript. J.E.-S.M., B.B., and M.M. helped to interpret the data and contributed to the final version of the manuscript. J.T. conceived the D.E.S.I.R. protocol, participated in the conduct of the study over 10 years, and aided in financing the study. T.B. performed the measurement of the amylase levels. The D.E.S.I.R. Study Group provided the serum samples and the demographic data. A.A. and M.F. are the guarantors of this work and, as such, had full access to all the data in the study and take responsibility for the integrity of the data and the accuracy of the data analysis.

Appendix

The D.E.S.I.R. Study Group's participating institutions (sites and investigators) are as follows: INSERM-U1018 (Paris: B. Balkau, P. Ducimetière, E. Eschwège); INSERM-U367 (Paris: F. Alhenc-Gelas); CHU d'Angers (A. Girault); Bichat Hospital (Paris: F. Fumeron, M. Marre, R. Roussel); CHU de Rennes (F. Bonnet); CNRS UMR-8199 (Lille: S. Cauchi, P. Froguel); Medical Examination Services (Alençon, Angers, Blois, Caen, Chartres, Chateauroux, Cholet, Le Mans, Orléans, and Tours); Research Institute for General Medicine (J. Cogneau); general practitioners of the region; and the Cross-Regional Institute for Health (C. Born, E. Caces, M. Cailleau, N. Copin, O. Lantieri, J.G. Moreau, F. Rakotozafy, J. Tichet, S. Vol).

References

1. Mandel AL, Breslin PA. High endogenous salivary amylase activity is associated with improved glycemic homeostasis following starch ingestion in adults. J Nutr 2012;142:853–858
2. Lee JG, Park SW, Cho BM, et al. Serum amylase and risk of the metabolic syndrome in Korean adults. Clin Chim Acta 2011;412:1848–1853
3. Nakajima K, Nemoto T, Muneyuki T, Kakei M, Fuchigami H, Munakata H. Low serum amylase in association with metabolic syndrome and diabetes: a community-based study. Cardiovasc Diabetol 2011;10:34
4. Nakajima K, Oshida H, Muneyuki T, et al. Independent association between low serum amylase and non-alcoholic fatty liver disease in asymptomatic adults: a cross-sectional observational study. BMJ Open 2013;3:e002235
5. Perry GH, Dominy NJ, Claw KG, et al. Diet and the evolution of human amylase gene copy number variation. Nat Genet 2007;39:1256–1260
6. Falchi M, El-Sayed Moustafa JS, Takousis P, et al. Low copy number of the salivary amylase gene predisposes to obesity. Nat Genet 2014;46:492–497
7. Mejía-Benítez MA, Bonnefond A, Yengo L, et al. Beneficial effect of a high number of copies of salivary amylase AMY1 gene on obesity risk in Mexican children. Diabetologia 2015;58:290–294
8. Usher CL, Handsaker RE, Esko T, et al. Structural forms of the human amylase locus and their relationships to SNPs, haplotypes and obesity. Nat Genet 2015;47:921–925
9. Balkau B, Eschwege E, Tichet J, Marre M. Proposed criteria for the diagnosis of diabetes: evidence from a French epidemiological study (D.E.S.I.R.). Diabetes Metab 1997;23:428–434
10. Evans AM, DeHaven CD, Barrett T, Mitchell M, Milgram E. Integrated, nontargeted ultrahigh performance liquid chromatography/electrospray ionization tandem mass spectrometry platform for the identification and relative quantification of the small-molecule complement of biological systems. Anal Chem 2009;81:6656–6667
11. Beckonert O, Keun HC, Ebbels TM, et al. Metabolic profiling, metabolomic and metabonomic procedures for NMR spectroscopy of urine, plasma, serum and tissue extracts. Nat Protoc 2007;2:2692–2703
12. Vu TN, Valkenborg D, Smets K, et al. An integrated workflow for robust alignment and simplified quantitative analysis of NMR spectrometry data. BMC Bioinformatics 2011;12:405
13. Chong I-G, Jun C-H. Performance of some variable selection methods when multicollinearity is present. Chemom Intell Lab Syst 2005;78:103–112
14. Wehrens R, Franceschi P, Vrhovsek U, Mattivi F. Stability-based biomarker selection. Anal Chim Acta 2011;705:15–23
15. Carpenter D, Dhar S, Mitchell LM, et al. Obesity, starch digestion and amylase: association between copy number variants at human salivary (AMY1) and pancreatic (AMY2) amylase genes. Hum Mol Genet 2015;24:3472–3480
16. Miura Y. The biological significance of ω-oxidation of fatty acids. Proc Jpn Acad, Ser B, Phys Biol Sci 2013;89:370–382
17. Ferrannini E, Natali A, Camastra S, et al. Early metabolic markers of the development of dysglycemia and type 2 diabetes and their physiological significance. Diabetes 2013;62:1730–1737