# Supplementary Appendix

This appendix has been provided by the authors to give readers additional information about their work.

Supplement to: Petersen KF, Dufour S, Hariri A, et al. Apolipoprotein C3 gene variants in nonalcoholic fatty liver disease. N Engl J Med 2010;362:1082-9.

# ONLINE SUPPLEMENTARY APPENDIX

## Apolipoprotein C3 Gene Variants in Non-Alcoholic Fatty Liver Disease

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**Genotyping**

Genomic DNA was extracted from whole blood using the SDS/Proteinase-K, Phenol-chloroform method. Genotypes at SNP's flanking the transcription start site of Apo C3 (position -455, rs2854116, and position -482, rs2854117) as well as Apo A5 (position -3, rs651821) were determined by amplifying the region encompassing the polymorphic sites followed by direct sequencing. The segment of Apo C3 was amplified by polymerase chain reaction using the following primers: 5'GAAGGTGAACGAGAATCAGTCCTG3' and 5'GCCTCGGGCCCATCTCAGCCTTTCACACTG 3'). The segment of Apo A5 was amplified using the primers: 5'GACCTTTGCGAAGGGGTTAGAGCACCAC3' and 5'CTCGCGAGCCATCTTCTGCTGATGGATC3'. Polymorphic SNPs in the PNPLA3 gene (GCCACTGTAGAAGGGG/CATGAAGCAGGAACAT), encompassing the I148M variant (rs738409) were amplified using primer set PNPLAV1F: 5'-GAGCCAACAA CCCTTGGTCC TGTCTG- and PNPLAV1R: 5'-GCTGCCCGG GTAGCCTGGA AATAG-3'. PCR fragments were sequenced with PNPLAV1F to determine the genotype.

**Oral Fat Tolerance Test**

Plasma triglyceride and retinyl fatty acid absorption were measured in a subgroup of Asian Indian volunteers, which consisted of twenty eight (31±11 years, BMI 25.5±3.7 kg/$m^2$) Apo C3 variant allele carriers and ten (age 28±17 years, BMI 23.3±3.6 kg/$m^2$) Apo C3 wild-type homozygote individuals following an oral fat tolerance test. A liquid meal containing; 20.9 grams of protein, 76.2 grams of fat, 61 grams of carbohydrate and 60,000 IU/ $m^2$ of Vitamin A was administered after collection of fasting blood samples. Blood samples were collected hourly for 8 hours for the determination of glucose, triglyceride, and retinyl fatty acid ester concentrations. Triglyceride concentrations were measured enzymatically (Cobas Mira plus, Roche Diagnostic Corp., Indianapolis, IN). Retinyl fatty acid ester concentrations were measured in order to assess chylomicron and post chylomicron remnant metabolism[14-16] using HPLC-tandem mass spectrometry as previously described.[17]

**Intravenous Fat Tolerance Test**

Plasma triglyceride clearance was assessed during an intravenous fat tolerance test in another subgroup of Asian Indian volunteers, which consisted of fifteen (age 26±9 years, BMI 24.6±4.2

kg/m$^2$) Apo C3 variant allele carriers and four (age 24±7 years, BMI 22.5±3.2 kg/m$^2$) Apo C3 wild-type homozygote individuals. After collection of fasting blood samples a bolus infusion of Liposyn (20%, 0.5 ml/kg, Abbott Laboratories, North Chicago, IL) per kg body weight was administered over 2 minutes and blood samples were collected at 2.5, 5, 7.5, 10, 15, 20, 30, 40, 60, 90, 120, 150, and 180 minutes.

**Online Supplementary Appendix Table 1**

**Individual SNPs and their respective effects on Apo C3 concentrations**

Variant alleles = total # high risk alleles (T482 or C455 alleles)
Variant haplotypes = total # haplotypes carrying at least one risk allele (C482-C455 or T482-C455; the T482-T455 haplotype was not observed in this cohort)
Variant loci = # sites with the high risk allele present

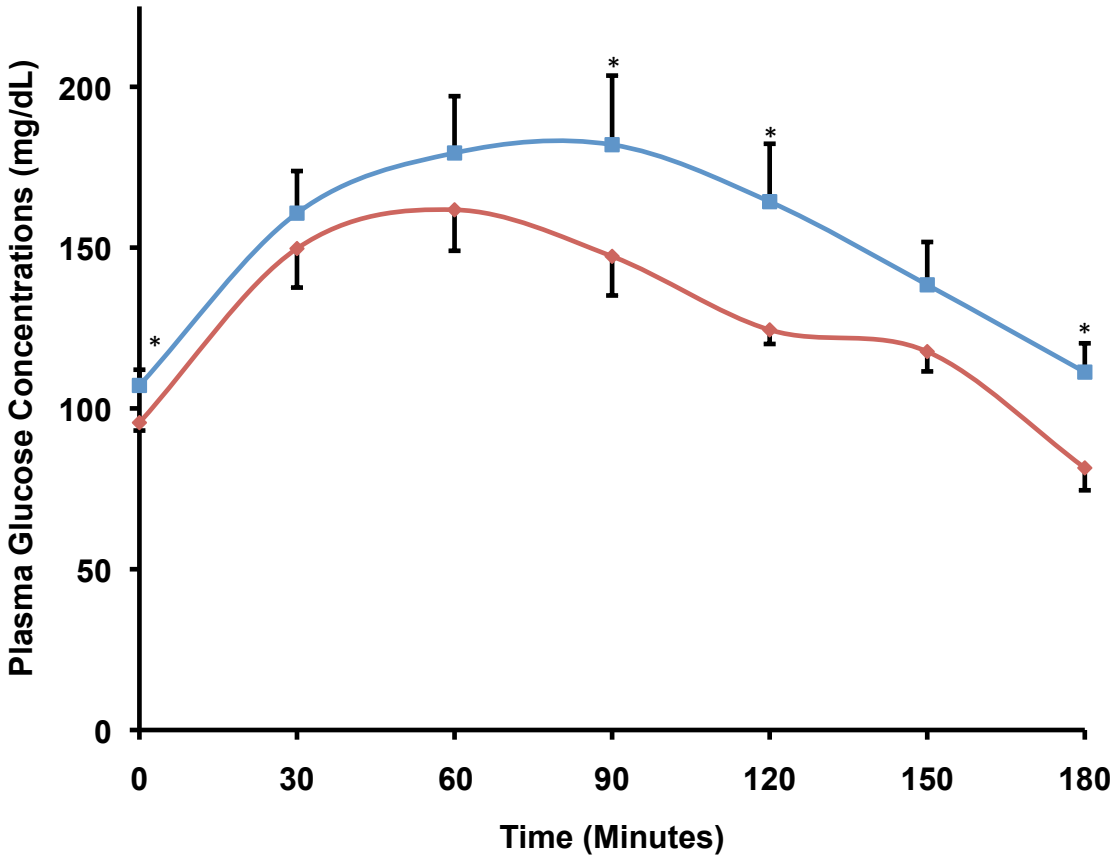
| ApoC3 haplotypes | #Variant alleles | #Variant haplotypes | #Variant loci | N | Mean ApoC3 concentrations (± SEM) (mg/dL) |
|---|---|---|---|---|---|
| C482-T455/C482-T455 | 0 | 0 | 0 | 19 | 8.86±0.61 |
| C482-T455/C482-C455 | 1 | 1 | 1 | 9 | 10.55±1.07 |
| C482-T455/T482-C455 | 2 | 1 | 2 | 45 | 11.55±0.58 |
| C482-C455/C482-C455 | 2 | 2 | 1 | 2 | 12.64±4.93 |
| C482-C455/T482-C455 | 3 | 2 | 2 | 9 | 10.24±1.36 |
| T482-C455/T482-C455 | 4 | 2 | 2 | 11 | 12.43±1.06 |

**Online Supplementary Appendix Table 2**

**Clinical characteristics of study participants by genotype classification**

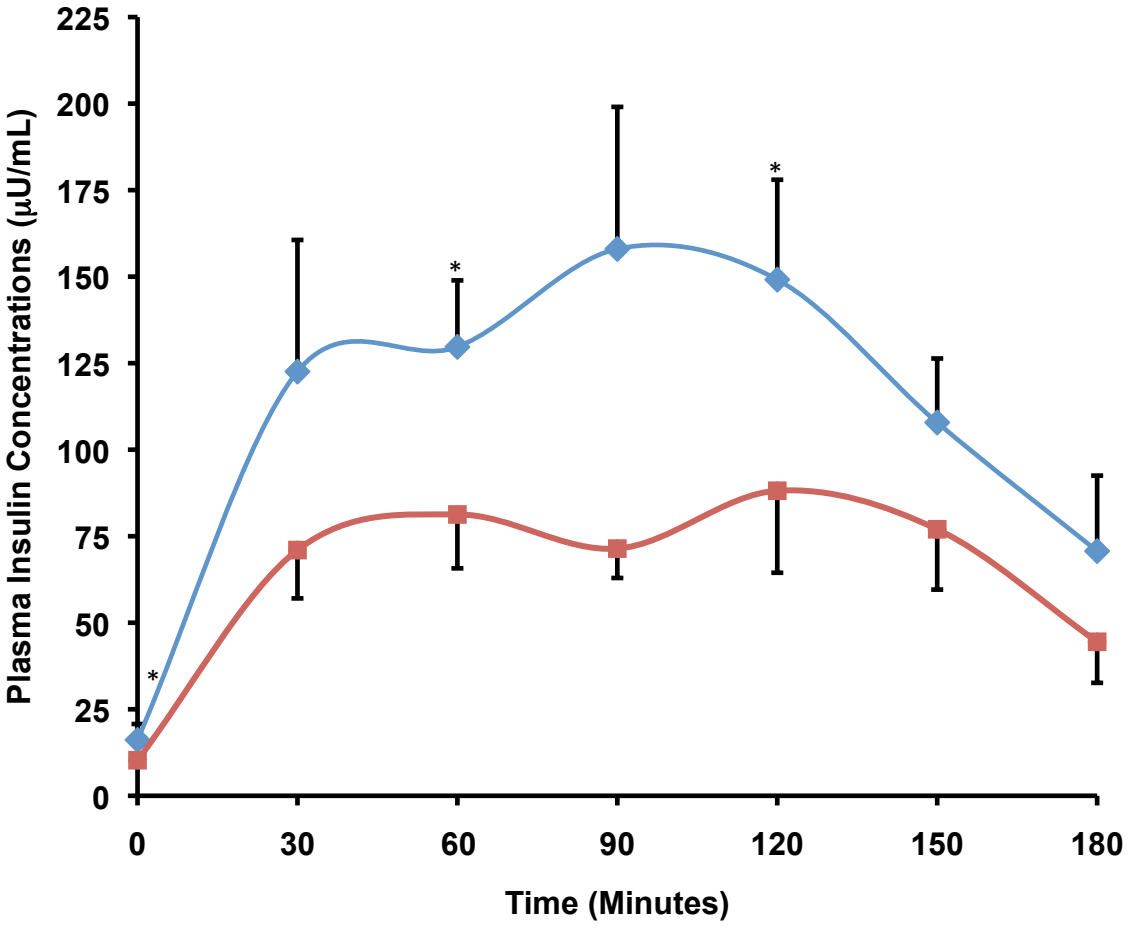
| | Asian Indian Men | | Confirmation Cohort | |
|---|---|---|---|---|
| | **Variant Allele Carriers (N=76)** | **Wild-Type Homozygotes (N=19)** | **Variant Allele Carriers (N=124)** | **Wild-Type Homozygotes (N=39)** |
| **Age (years)** | 33±13 | 30±16 | 29±14 | 24±8 |
| **Height (m)** | 1.72±0.09 | 1.76±0.10 | 1.76±0.07 | 1.79±0.08 |
| **Weight (kg)** | 74±11 | 72±13 | 75±10 | 77±10 |
| **Body Mass Index (kg/m$^2$)** | 25.0±3.6 | 23.1±3.4 | 24.2±3.0 | 24.0±2.5 |
| **Fat Intake (%)** | 27±12 | 28±12 | 32±8 | 31±9 |
| **Carbohydrate Intake (%)** | 57±14 | 57±9 | 49±11 | 50±10 |
| **Protein Intake** | 15±6 | 15±4 | 18±5 | 18±6 |
| **Alcohol Intake (gram/day)** | 2±3 | 3±8 | 5±8 | 4±7 |
| **Pedometer Activity (km/day)** | 5.8±3.0 | 3.7±1.9 | 6.6±2.9 | 6.3±2.9 |
| **Exercise Index** | 2.4±1.1 | 2.6±0.8 | 2.5±0.9 | 2.5±1.0 |
| **Fasting Glucose (mg/dL)** | 101±15 | 97±12 | 95±9 | 94±6 |
| **Fasting Insulin (µU/mL)** | 13±6 | 11±4 | 9±5 | 9±4 |
| **Fasting Triglycerides (mg/dL)** | 118±87 | 74±31 | 80±49 | 87±54 |

Supplemental Figure 1A




Plasma glucose concentrations during the oral glucose tolerance test before (blue) and after (red) weight loss. Mean±SEM, *P<0.05

Supplemental Figure 1B




Plasma insulin concentrations during the oral glucose tolerance test before (blue) and after (red) weight loss.
Mean ±SEM, *P≤0.02