The effects of triglycerides and fatty acids on T cells: role in atherosclerosis

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CHAPTER 5

Insights into the role of triglycerides and T cells in cardiovascular risk: T cells of patients with moderate hypertriglyceridemia have a pro-inflammatory transcriptomic profile

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Abstract

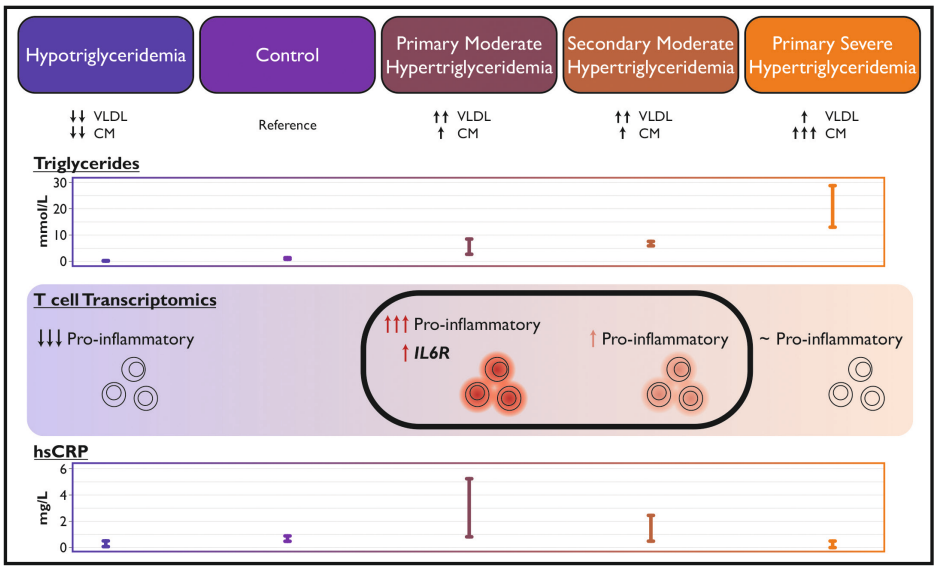
Background and aims: The role of triglycerides and T cells in atherosclerosis, the underlying cause of cardiovascular disease (CVD), is increasingly recognized. Moderately elevated triglycerides have emerged as a causal risk factor of CVD, while T cells are the most prominent immune cell type in the atherosclerotic plaque. Studies in mice and *in vitro* indicate that triglycerides can affect T cell function. Here, we characterize differences in T cells across control and patient groups with distinct triglyceride levels either due to genetic or secondary causes using the transcriptome as a functional read-out.

Methods: We enrolled patients with primary (genetically determined, n=13) and secondary (due to a secondary cause, n=14) moderate hypertriglyceridemia and compared them to controls (n=15). Additionally, we enrolled patients with rare genetic conditions, namely primary severe hypertriglyceridemia (n=3) or hypotriglyceridemia (n=4). From all participants (n=49), we purified $CD4^+$ and $CD8^+$ T cells and extracted RNA for RNA sequencing.

Results: $CD4^+$ T cells of patients with primary moderate hypertriglyceridemia displayed a pro-inflammatory transcriptomic profile ($P_{FDR}<0.05$), including an upregulated interleukin-6 receptor (*IL6R*) expression, a subunit of the IL-6 receptor complex that is causally linked to CVD. The profile was mirrored in $CD8^+$ T cells from these patients and also present in both T cell types from secondary moderate hypertriglyceridemia patients, albeit less pronounced. In accordance, the transcriptomic differences were reversed in patients with hypo- and absent in patients with severe hypertriglyceridemia.

Conclusions: Elevated triglycerides may contribute to CVD by promoting a pro-inflammatory response in T cells.

Graphical abstract



Key Question: What are the differences in T cells across control and patient groups with distinct triglyceride levels either due to genetic or secondary causes using the transcriptome as a functional read-out?

Key Finding: T cells of patients carrying mutations resulting in moderately elevated triglyceride levels show an increased expression of pro-inflammatory genes as compared with controls. Effects in patients with secondary, hypotriglyceridemia and severe hyperglyceridaemia were consistent, reversed and absent, respectively.

Take-home Message: Patients with moderately elevated triglyceride levels have T cells with pro-inflammatory characteristics, a finding that sheds new light on the role of triglycerides in cardiovascular disease.

Introduction

Triglyceride levels in individuals may range from lowered (hypotriglyceridemia, <0.5mmol/L) to normal (0.8–2.0mmol/L) to elevated (hypertriglyceridemia, >2.0mmol/L). Moderate hypertriglyceridemia, between the range of 2–10mmol/L, has been associated, through genome-wide association studies[1-4], with an increased risk of cardiovascular disease (CVD)[5-9], which Mendelian randomization[10-14] studies have indicated to be a causal effect. In accordance, individuals with hypotriglyceridemia were indicated to be at a lower risk for developing CVD[15, 16]. Interestingly, the risk of CVD is no longer increased in individuals with severe hypertriglyceridemia (>10mmol/L), because triglycerides are then stored in chylomicrons which cannot cross the arterial wall[17]. The differences in triglyceride levels can be attributed to primary genetic factors (e.g. pathogenic mutations in *MTTP* or *LPL*) or secondary outcomes of conditions like diabetes[18]. However, the precise pathophysiology of CVD in those with altered triglyceride levels remains poorly understood.

Triglycerides in the circulation are mostly contained within triglyceride-rich lipoproteins (TGRLs), which have also been found as a causal risk factor for CVD[19, 20]. However, CVD, and the main underlying cause thereof, atherosclerosis, develop due to the interplay between lipids, the immune system, and the vascular wall[21]. As such, emerging evidence underscores a significant role not only of triglycerides, but also of the immune system in its pathogenesis[22, 23]. In particular, T cells have been found to make up over half the immune cells in atherosclerotic plaques, of which half are $CD4^+$ and half are $CD8^+$ [24, 25]. Moreover, the biologically functional components of triglycerides, fatty acids, have already been found to substantially impact T cell functionality[26-28]. In patients with hypertriglyceridemia, T cells are exposed to excess triglycerides contained in TGRLs in the circulation. Therefore, the study of patients with primary hyper- or hypotriglyceridemia can shed light on how moderately elevated triglyceride levels can contribute to atherosclerotic disease through interactions with the immune system *in vivo*.

Here, we compare $CD4^+$ and $CD8^+$ T cells derived from patients with varying levels of triglycerides from both primary and secondary causes as compared to a control group using the transcriptome as a functional read-out. We show that patients of moderately elevated triglycerides levels have T cells characterized by a pro-inflammatory transcriptomic profiles shedding light on an unexplored pathway relevant to atherosclerosis and CVD.

Methods

Participant selection and data collection

Participants were enrolled in one of five groups, being control (defined as no known abnormal triglyceride levels and no fibrate use and/or triglyceride levels between 0.8–2.0mmol/L), primary moderate hypertriglyceridemia (a confirmed pathogenic mutation in the *LPL*, *APOC2*, *APOA5*, *LMF1*, *GPIHBP1*, or *APOE2/APOE2* gene and the most recent measured triglyceride level >3.0mmol/L), secondary moderate hypertriglyceridemia (due to a secondary cause, such as diabetes or lifestyle

factors including obesity and excessive alcohol use and the most recent measured triglyceride level >3.0mmol/L), primary severe hypertriglyceridemia (two confirmed pathogenic mutations in the *LPL*, *APOC2*, *APOA5*, *LMF1*, or *GPIHBP1* gene leading to familial hyperchylomicronaemia syndrome (FCS) and the most recent measured triglyceride level >13.0mmol/L), and hypotriglyceridemia group (a pathogenic mutation in the *MTTP* or *APOB* gene resulting in a hypobetalipoproteinaemia or abetalipoproteinaemia phenotype and the most recent measured triglyceride level <0.56mmol/L).

The exclusion criteria were as follows: 1) below the age of 18 years, 2) presence of an infection at the time of blood drawing (e.g. COVID-19), 3) medical history or current treatment for any condition that could impact the immune system at the time of participation (e.g. ongoing treatment for malignancy or immunotherapy), and 4) unwillingness or inability to provide informed consent. For the control group, an additional exclusion criterion was applied in cases where triglyceride levels were unknown, with a threshold set at 2.0mmol/L for the triglyceride levels measured in the sample.

The local ethical board of Erasmus MC in Rotterdam, The Netherlands, approved and assigned a waiver for this study (MEC-2021-0596). Patients were consecutively recruited after informed consent was given at the lipid clinic of the Erasmus MC in Rotterdam, The Netherlands, between March 2022 and December 2022. We included 15, 13, and 14 participants for the control, primary, and secondary moderate hypertriglyceridemia groups respectively (Table 1). However, because FCS and abetalipoproteinemia are very rare conditions, we included 3 and 4 participants in the primary severe hypertriglyceridemia and hypotriglyceridemia group, respectively (Table 1). At the time of inclusion, additional data, including sex, age, BMI, smoking, medication (particularly lipid-lowering therapy), diet, and medical history, were collected. In total, 36mL of fasted whole blood was collected per individual in anticoagulant citrate phosphate dextrose adenine (CPDA) tubes (Greiner Bio-One, 455056) and transported to the Leiden University Medical Centre (LUMC) in Leiden, The Netherlands (Fig. 1).

Plasma and peripheral blood isolation

To obtain plasma and peripheral blood mononuclear cells (PBMCs) from the patients, whole blood samples in the CPDA tubes were transferred into a 50mL tube (Starlab Group, E1450-0200) and spun down at 350g for 10min to isolate the plasma. 1.8mL plasma was collected into a 2mL Sarstedt Microtube (Sarstedt, 72.730.009 and 65.716.002) and stored at -80°C for lipid profiling, see below. Whole blood was mixed and diluted 1:1 in PBMC isolation buffer (buffered natrium chloride (PBS; pH 7.4; Fresen, 15360679), 2% Alburex (CLS Behring GmbH, C1309/490)). To obtain peripheral blood mononuclear cells (PBMCs), freshly collected and diluted whole blood was filtered by Ficoll paque (Pharmacy LUMC, 97902861) gradient centrifugation using Leucosep tubes (Greiner Bio-One, 227290). Cells were washed in PBMC isolation buffer. An additional red blood cell lysis step was carried out using red blood cell lysis buffer (MilliQ water, 0.15M NH_4Cl (Merck Millipore, 101145), 1mM $KHCO_3$ (Merck Millipore, 104854), and 0.1mM Na_2 EDTA (Sigma Aldrich, E5134)). Cell viability was measured by trypan blue (Sigma Aldrich, T8154) exclusion using a 0.0025mm^2 haemocytometer (Bürker-Turk Bright Line, 0640211).

Cell sorting and harvesting

To obtain $CD4^+$ and $CD8^+$ T cells, PBMCs were resuspended in FACS buffer (PBS and 0.4% Alburex) and labelled with live dead (Invitrogen, L34964), anti-CD3-PE (BD Biosciences, 555340), anti-CD4-APC (BD Biosciences, 555349), anti-CD8-FITC (BD Biosciences, 555634), anti-CD14-Alexa Fluor® 700 (BD Biosciences, 557923), and anti-CD19-BV786 (BD Biosciences, 563325). PBMCs were sorted into $CD4^+$ T cells, $CD8^+$ T cells, $CD14^+$ monocytes, and $CD19^+$ B cells using the CytoFLEX SRT Benchtop cell sorter (Beckman Coulter, Brea, CA, USA) at the LUMC Flow Cytometry Core Facility (https://www.lumc.nl/research/facilities/fcf/) with the CytExpert SRT v1.0.3.10011 software (Beckman Coulter). Cells were collected in 5% fetal calf serum (FCS) (Bodinco BDC, 16941) DMEM (Dulbecco's Modified Eagle's Serum (Sigma, 05796), 1% Pen-Strep (Lonza, DE17-602E), 1% GlutaMAX-1 (100x) (Gibco, 35050-038)). Post-sorting cell purity was assessed on the CytoFLEX SRT Benchtop cell sorter and shown to be on average 97.2 SE 0.3% for $CD4^+$ T cells and 97.0 SE 0.4% for $CD8^+$ T cells (Supp. Fig. 1a and b).

The cells were subsequently washed in PBS and cell viability and diameter were measured by Via1-Cassette™ (Chemometec, 941-0012) on a NucleoCounter® NC-200™ (Chemometec, 900-0200) and found to be on average 97.4 SE 0.2% and 9.3 SE 0.02µm for $CD4^+$ T cells and 96.8 SE 0.4% and 9.5 SE 0.04µm for $CD8^+$ T cells (Supp. Fig. 1c, d, e and f). A maximum of 2 million cells were harvested by flash freezing in liquid nitrogen and stored at -80°C for RNA isolations. Any additional cells were kept in DMEM supplemented with 30% FCS, 1% Pen-Strep, 1% GlutaMAX-1, and 20% Dimethyl Sulfoxide (DMSO) (WAK-Chemie Medical GmbH, WAK-DMSO-10) medium at a density of ~$25*10^6$ cells/mL, and stored in cryogenic tubes (Greiner Bio-One, 126263) in liquid nitrogen.

Plasma measurements

Plasma samples of the patients were used to determine lipid concentrations, measure high sensitivity C-reactive protein (hsCRP), and profile lipoproteins. Lipid levels, including triglycerides, total cholesterol, LDL-cholesterol, HDL-cholesterol, apolipoprotein (Apo)A-I, and ApoB were measured using standard laboratory techniques. LDL-cholesterol, HDL-cholesterol, total cholesterol and triglycerides were measured with an enzymatic photometric analysis. ApoB and ApoA-I were measured by photometric measurement of antigen-antibody reaction. Lipoprotein (a) [Lp(a)] and hsCRP concentrations were measured using a particle-enhanced immunoturbidimetric assay. All kits were from DiaSys Diagnostic systems GmbH, Holzheim Germany (Diagnostic System #171399910930 and #170459910930 respectively; DiaSys Diagnostic System, GmbH, Holzheim, Germany). This method is largely independent of Apo(a) KIV repeat number. A Mann-Whitney U test was used to determine whether the differences in hsCRP were significantly higher or lower in the patient groups as compared to the control group. Next, chylomicron, VLDL, IDL, LDL, and HDL were isolated using density gradient ultracentrifugation according to the Proudfoot protocol[29, 30]. The cholesterol and triglyceride content of these particles was determined using a Selectra E (DDS Diagnostic system, Istanbul, Turkey), according to the manufacturer's instructions.

RNA isolation

To isolate total RNA for RNA sequencing, RNA was extracted from the cell samples using the Zymo Quick-DNA/RNA Microprep Plus Kit (Zymo Research, D7005) according to manufacturer's instructions. The RNA was quantified using a Qubit® 2.0 Fluorometer (Q32866) with the Qubit® RNA BR Assay Kit (Thermofisher, Q10211) according to manufacturer's instructions. RNA integrity (RIN) was determined using an Agilent 2100 Bioanalyzer Instrument (G2939BA) with the Agilent RNA 6000 Nano Reagents (Agilent, 5067-1511), RIN values were on average 9.7 (SD, 0.6) for $CD4^+$ T cell samples and 9.0 (SD, 0.8) for $CD8^+$ T cell samples. The minimum RNA yield was 160ng and 160ng to 1µg was stored at -80°C for RNA sequencing.

RNA sequencing analysis

RNA sequencing (RNA-seq) was performed to determine the differences in the transcriptome of $CD4^+$ and $CD8^+$ T cells of individuals with primary or secondary moderate hypertriglyceridemia, primary severe hypertriglyceridemia, hypotriglyceridemia, or none (control). The RNA from each of the samples was sent for sequencing (Macrogen, Amsterdam, The Netherlands). RNA-seq libraries were prepared from 200ng RNA using the Illumina Truseq stranded mRNA library prep (Illumina, 20020594) with a poly A selection. Both whole-transcriptome amplification and sequencing library preparations were performed in two 96-well plates with 53 samples in one plate and 45 in another. Quality control steps were included to determine total RNA quality and quantity, the optimal number of PCR preamplification cycles, and fragment size selection. For 48 of the 49 $CD4^+$ and 43 of 49 $CD8^+$ T cell samples, RNA-seq data passed quality control. The samples that did not pass the quality control included 2 control samples, one from $CD4^+$ and one from $CD8^+$ T cells, 1 primary moderate sample from $CD8^+$ T cells and 4 secondary moderate samples from $CD8^+$ T cells. Barcoded libraries were divided across 4 lanes and sequenced separately. Barcoded libraries were sequenced to a read depth of 30 million reads using the Novaseq 6000 (Illumina) to generate 150 base pair paired-end reads.

FastQ files are analysed using the RNAseq pipeline (v5.0.0) from BioWDL (https://zenodo.org/record/5109461), developed by SASC (LUMC). The pipeline performed pre-processing on the FastQ files (including quality control, quality trimming, and adapter clipping), read mapping, and expression quantification. *FastQC* (v0.11.9) is used to check raw reads and *Cutadapt* (v2.10) to perform adapter clipping. Reads are mapped to a reference genome (Ensembl v105) using *STAR aligner* (v2.7.5a), and with *HTSeq Count* (v0.12.4) the number of assigned reads to genes per sample is determined.

Based on count distribution of the sequenced sample it was opted to exclude 1 control and 1 primary severe hypertriglyceridemia sample from the $CD4^+$ T cell analysis as well as 2 control, 1 secondary moderate hypertriglyceridemia, and 1 primary severe hypertriglyceridemia sample in the $CD8^+$ T cell analysis. Based on Ensembl gene biotype annotation, we included only protein coding genes of chromosomes 1-22 for further downstream analysis (19,111 genes in total). We further filtered the background of each group to contain only the counts where at least half of the raw counts per group had a count of 1. Then, for each comparison, only genes were kept that were

expressed in both the control and experimental group, leaving 13,377 expressed genes for primary moderate, 13,386 expressed genes for secondary moderate, 13,421 expressed genes for primary severe, and 13,466 expressed genes for hypotriglyceridemia in the $CD4^+$ T cell analysis, as well as 12,575 expressed genes for primary moderate, 12,671 expressed genes for secondary moderate, 12,396 expressed genes for primary severe, 12,407 expressed genes for hypotriglyceridemia in the $CD8^+$ T cell analysis. We used the Bioconductor package *DESeq2*[31] (v1.42.0) to test whether primary moderate, secondary moderate, primary severe, or hypotriglyceridemia had an effect on gene expression as compared to the control per cell type, $CD4^+$ and $CD8^+$. *DESeq2* fits a generalized linear model (GLM) assuming the negative binomial distribution for the counts. The model expresses the logarithm of the average of the counts in terms of one or more predictors. In this case, we used four models that had one of the groups, sex, and age as predictors each. By including sex and age in the models, we account for the dependence between measurements within the same sex and between different ages of the participants[31]. The Benjamini-Hochberg procedure was manually used to correct for multiple testing and a false discovery rate (FDR) <0.05 was considered statistically significant.

In order to determine whether the similarities in direction of effect sizes of the DEGs in the primary moderate $CD4^+$ T cells expressed in the other $CD4^+$ and $CD8^+$ T cells groups was significant, a binomial test was used. This determines the probability of a particular outcome across a certain number of trials (n=39 DEGs), where there are precisely two possible outcomes (up or down). In order to identify distinct gene expression patterns in the data, the log2 fold change of the DEGs of the $CD4^+$ and $CD8^+$ primary moderate and secondary moderate groups were plotted in heatmaps using *ComplexHeatmap*[32] (v2.18.0). Differentially expressed genes per group were divided into upregulated or downregulated based on the log2 fold change values. 10 human pathway databases (BioPlanet 2019, WikiPathways 2023 Human, KEGG 2021 Human, Elsevier Pathway Collection, BioCarta 2016, Reactome 2022, HumanCyc 2016, NCI-Nature 2016, Panther 2016 and MSigDB Hallmark 2020) were queried using gene symbols. 331 of 406 queried genes for hypotriglyceridemia in $CD8^+$ T cells present in at least 1 database. The identified clusters were then mapped for pathway enrichment using *clusterProfiler*[33] (v4.10.0). The background was set to the 8,799 expressed genes for hypotriglyceridemia in the $CD8^+$ T cells. This was based on at least half of the raw counts per group and control having a count of 1, the gene being expressed in both comparison groups, and the independent filtering of *DESeq2*. Multiple testing using the Benjamini-Hochberg method at 5% FDR was performed over the combined results from the 10 databases. Pathways that included highly similar gene sets were grouped (Jaccard index > 0.7) and only the most significantly enriched pathway per group was retained.

Results

We generated transcriptomic profiles of $CD4^+$ and $CD8^+$ cells for 47 participants (19 (40.4%) women, median age 53 [37-60] years, BMI 26.1 [23.7-29.6] kg/m^2, 10 (21.3%) current smokers) (Table 1). Six patients in the primary moderate hypertriglyceridemia group (n=13) had an *APOE2/*

APOE2 genotype, six an *LPL* mutation, and one patient had a pathogenic *APOA5* mutation. The causes of hypertriglyceridemia in the secondary moderate group (n=14) were diabetes mellitus type 2 (8 (57.1%)) and/or lifestyle related factors (6 (42.9%)). Both patients in the primary severe hypertriglyceridemia group had *LPL/LPL* mutations. In the hypotriglyceridemia group (n=4), 3 patients had a causative *MTTP/MTTP* mutation and 1 patient had an *APOB* variant. The participants included 14 controls. Twenty-five (53.2%) participants used lipid-lowering therapy. None of the participants in the control, primary severe, or hypotriglyceridemia group had a recorded medical history of cardiovascular disease.

Measured triglyceride levels exhibited an increasing trend from the hypotriglyceridemia group (range 0.1–0.3mmol/L), controls (median 1.3 [0.8–1.4] mmol/L), primary moderate group (median 5.2 [2.7–8.5] mmol/L), secondary moderate group (median 6.8 [5.9–7.6] mmol/L), to the primary severe group (range 13.0–28.8 mmol/L; Supp. Fig. 2). A similar trend was observed for chylomicron cholesterol and chylomicron triglycerides (Table 1). The lipoprotein profiles of each participant per group are shown in Supp. Fig. 2.

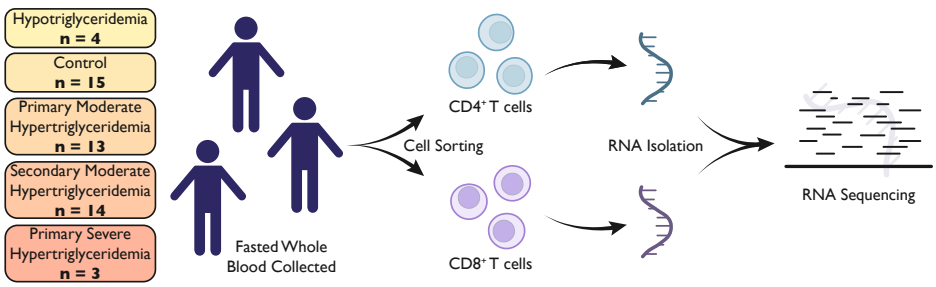


Fig. 1 | Experimental setup. Experimental setup for RNA sequencing of isolated $CD4^+$ and $CD8^+$ T cells derived from participants with primary or secondary moderate hypertriglyceridemia, primary severe hypertriglyceridemia, hypotriglyceridemia, or control.

Moderate hypertriglyceridemia induced transcriptomic effects on $CD4^+$ and $CD8^+$ T cells

In order to discover whether moderately elevated triglycerides have an effect on T cell gene expression, RNA sequencing was performed on $CD4^+$ and $CD8^+$ T cells isolated from individuals with primary moderate and secondary moderate hypertriglyceridemia. Gene expression differences in each cell type, $CD4^+$ and $CD8^+$, from both groups, primary and secondary moderate, were compared to $CD4^+$ and $CD8^+$ T cells isolated from the control group, respectively. This allowed us to discern between the effects of moderately elevated triglyceride exposure on $CD4^+$ and $CD8^+$ T cells separately.

We first focused on the transcriptomic profile in T cells from patients with primary moderate hypertriglyceridemia as compared with that in controls. The most prominent difference in transcriptomic profile was observed in $CD4^+$ T cells, in which 39 differentially expressed genes (DEGs) were found (P_{FDR}<0.05; Supp. Table. 1a), 19 (48.7%) of which were upregulated.

Table 1 | Participant characteristics. Participant characteristics in total and per group, including lipid levels and high sensitivity CRP at the time of inclusion. Participant characteristics in total and per group, including lipid levels and high sensitivity CRP at the time of inclusion. Continuous data shown as median [IQR] or in case of n < 5 as minimum-maximum. Categorical data are provided in count (%). BMI = body mass index, PCSK9 = proprotein convertase subtilisin/kexin type 9, TIA = transient ischemic attack. Remnants = total cholesterol - LDL cholesterol - HDL cholesterol.

	Total	Control	Primary Moderate Hypertriglyceridemia	Secondary Moderate Hypertriglyceridemia	Primary Severe Hypertriglyceridemia	Primary Hypotriglyceridemia
N	47	14	13	14	2	4
General Characteristics						
Age	53 [37-60]	48 [36-59]	56 [47-60]	56 [53-64]	34 [34-34]	26 [25-33]
Sex, female	19 (40.4%)	8 (57.1%)	3 (23.1%)	5 (35.7%)	0	3 (75.0%)
BMI, kg/m2	26.1 [23.7-29.6]	24.3 [22.4-25.8]	27.5 [26.0-30.1]	28.5 [27.6-31.5]	25.6 [23.3-28.0]	21.2 [20.0-23.0]
Smoking (ever)	19 (40.4%)	1 (7.1%)	8 (61.5%)	7 (50%)	2 (100%)	1 (25.0%)
Smoking (current)	10 (21.3%)	0	5 (38.5%)	3 (21.4%)	1 (50.0%)	1 (25.0%)
Diabetes mellitus type 2	14 (29.8%)	0	6 (46.2%)	8 (57.1%)	0	0
Lipid-Lowering Therapy						
Statin	18 (38.3%)	1 (7.1%)	8 (61.5%)	9 (64.3%)	0	0
High intensity statin	8 (17.0%)	0	3 (23.1%)	5 (35.7%)	0	0
Ezetimibe	7 (14.9%)	0	4 (30.8%)	3 (21.4%)	0	0
Fibrate	8 (17.0%)	0	4 (30.8%)	4 (28.6%)	0	0
PCSK9 Inhibitor	4 (8.5%)	0	1 (7.7%)	3 (21.4%)	0	0

[continued on next page]

Table 1 | Participant characteristics. *[continued]*

	Total	Control	Primary Moderate Hypertriglyceridemia	Secondary Moderate Hypertriglyceridemia	Primary Severe Hypertriglyceridemia	Primary Hypotriglyceridemia
Medical History						
Pancreatitis	7 (14.9%)	0	2 (15.4%)	3 (21.4%)	2 (100%)	0
Cardiovascular Disease	9 (19.1%)	0	2 (15.4%)	7 (50.0%)	0	0
Myocardial infarction	3 (6.4%)	0	1 (7.7%)	2 (14.3%)	0	0
Ischemic Stroke or TIA	2 (4.3%)	0	0	2 (14.3%)	0	0
Laboratory Levels in mmol/L						
Triglycerides	3.2 [1.4-6.9]	1.3 [0.8-1.4]	5.2 [2.7-8.5]	6.8 [5.9-7.6]	13.0-28.8	0.1-0.3
Total cholesterol	4.9 [4.0-5.4]	5.0 [4.5-5.1]	4.8 [4.1-5.8]	4.9 [4.2-5.6]	9.0-11.5	2.0-2.8
LDL-cholesterol	1.9 [1.0-3.0]	3.1 [2.7-3.9]	1.7 [1.1-2.4]	1.6 [1.1-2.9]	0.0-0.0	0.1-0.9
HDL-cholesterol	1.06 [0.85-1.31]	1.37 [1.18-1.58]	0.84 [0.81-1.06]	0.97 [0.89-1.08]	0.44-1.23	0.64-1.14
Apolipoprotein A1, g/L	0.95 [0.84-1.09]	1.09 [1.00-1.14]	0.93 [0.83-0.97]	1.00 [0.88-1.09]	0.36-0.54	0.37-0.89
Apolipoprotein B, g/L	0.78 [0.59-0.94]	0.86 [0.68-1.02]	0.74 [0.54-0.91]	0.84 [0.74-1.08]	0.01-0.71	0.01-0.24
Lipoprotein(a), g/L	0.05 [0.02-0.23]	0.07 [0.03-0.12]	0.06 [0.01-0.13]	0.16 [0.03-0.44]	0.00-0.02	0.01-0.04
High sensitivity CRP, mg/L	0.82 [0.43-1.48]	0.75 [0.48-0.89]	1.55 [0.82-5.24]	1.09 [0.49-2.45]	0.00-0.50	0.15-0.51
Chylomicrons cholesterol	0.029 [0.007-0.387]	0.007 [0.002-0.011]	0.165 [0.021-0.682]	0.263 [0.166-0.388]	4.119-8.812	0.000-0.001
Chylomicrons triglyceride	0.143 [0.030-1.199]	0.028 [0.010-0.034]	0.503 [0.113-1.905]	1.028 [0.616-1.412]	11.300-13.615	0.001-0.022
Remnants	1.40 [0.34-2.25]	0.10 [0.00-0.31]	1.55 [1.46-2.31]	2.16 [1.94-2.42]	7.72-11.10	0.77-1.21

Most notably, this included *ILR6* which encodes a subunit of the interleukin 6 (IL-6) receptor complex and plays an important role in the immune response[34] (Fig. 2a). The effects of IL-6 on $CD4^+$ T cells include stimulating T cell survival and proliferation as well as promoting T_H2 and T_H17 differentiation[35]. Furthermore, a Mendelian randomization study of *IL6R* constituted the first evidence for a causal role of inflammation in coronary heart disease[36]. A pro-inflammatory expression profile of $CD4^+$ T cells of primary moderate patients was further indicated by the upregulation of the genes *NCOR1, DOCK8,* and *LPAR6*. *NCOR1* has been shown to aid the function of T_H1 and T_H17 effector cells[37], *DOCK8* is necessary for T cell migration, survival, and immune responses[38], and *LPAR6* has been shown to contribute to T cell activation and effector cell function[39]. On the other hand, genes that were found to be downregulated in $CD4^+$ T cells included *MLF2* and *MMP14*. *MLF2* is a negative regulator of p53, a transcription factor required for T cell homeostasis[40, 41] and *MMP14* expression has been related to surface MHC shedding and immune evasion[42]. These findings contribute to the suggested pro-inflammatory transcriptional profile by downregulating genes involved in T cell regulation. Many of the DEGs had a function in inflammation, however we also identified DEGs involved in other processes. Particularly, genes involved in fatty acid metabolism (*SLC25A34* and *IDH3B*), the cytoskeleton (*KRT10, B4GALT4, SPTAN1,* and *MICAL3*), as well as translation and posttranslational modifications (*PARP4, EIF4B,* and *PABPN1*) were also found. Finally, *APOE* was downregulated, a finding which is likely a direct effect of the mutation carried by a subgroup of patients rather than a downstream effect of elevated triglycerides.

In $CD8^+$ T cells, only 2 DEGs were found in the primary moderate group, which were both upregulated (Supp. Table 1b). These two genes were *NFE2L3* and *MAST1*. *NFE2L3* encodes a transcription factor involved in binding antioxidant response elements and increased expression of this gene has been correlated to effector $CD8^+$ T cells[43]. *MAST1* encodes a protein that associates with microtubules. In the secondary moderate group, no differential expression was observed as compared with controls except for one gene in $CD8^+$ cells, namely *COLQ*, which encodes a subunit of a collagen-like molecule (Supp. Table 1c).

Although the individual DEGs observed for the two T cells and patient groups were different, the patterns of differential expression when combining all DEGs observed across analysis were remarkably similar (Fig. 2b; n=42). Additionally, we found that the effect sizes observed in $CD4^+$ cells of patients with primary moderate hypertriglyceridemia (n=39) matched in direction for 87% of the genes in primary moderate $CD8^+$ cells ($P_{binomial}<0.001$), and 98% of the genes in the secondary moderate $CD4^+$ cells ($P_{binomial}<0.001$), of which 19% and 24%, respectively, were also nominally significant.

To corroborate the pro-inflammatory transcription profiles of T cells, we measured hsCRP levels. As compared to controls (median, 0.75 [0.48-0.89] mg/L), hsCRP levels were particularly higher among patients with primary moderate hypertriglyceridemia (median 1.55 [0.82–5.24] mg/L; P=0.029) and to a lesser extent among patient with secondary hypertriglyceridemia (median 1.09 [0.49–2.45] mg/L; P=0.265; Table 1).

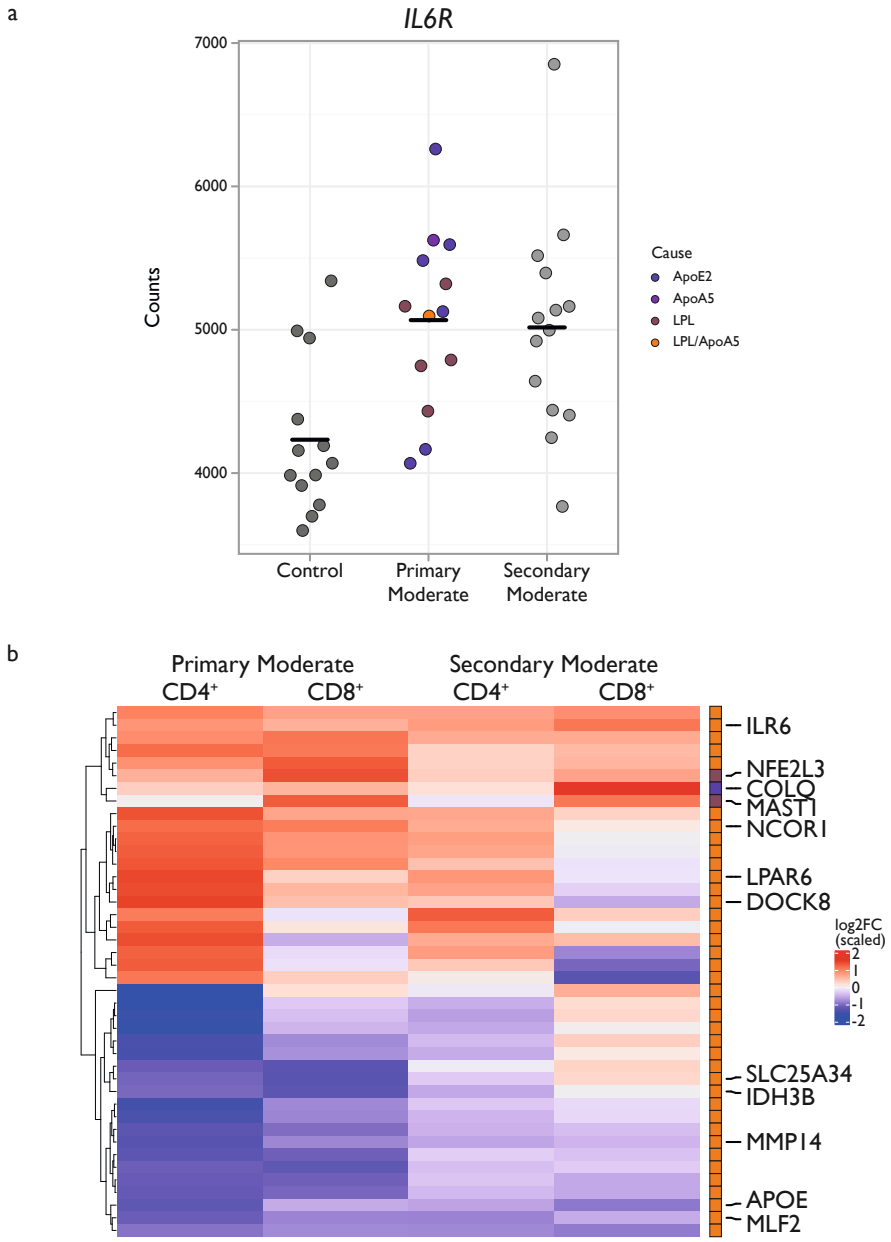


Fig. 2 | (a) Dot plot of the raw count data for the gene *IL6R* in $CD4^+$ T cells for the control, primary moderate, and secondary moderate groups. Dots are coloured by cause of hypertriglyceridemia. **(b)** Differentially expressed genes (DEGs) in $CD4^+$ and $CD8^+$ T cells from primary and secondary moderate groups as compared to the control group. Heatmap obtained from the *DESeq2* analysis resulting in 42 DEGs ($P_{FDR} < 0.05$). DEGs are clustered based on effect sizes. Bar on the right indicates the group in which the gene is differentially expressed (blue = $CD4^+$ primary moderate, orange = $CD8^+$ primary moderate, and green = $CD8^+$ secondary moderate. Genes of interest are labelled, $CD4^+$ T cells primary moderate n = 13, $CD8^+$ T cells primary moderate n = 12, $CD4^+$ T cells secondary moderate n = 14, and $CD8^+$ T cells secondary moderate n = 9.

Primary severe hypertriglyceridemia and hypotriglyceridemia induced transcriptomic effects on CD4⁺ and CD8⁺ T cells

We also enrolled a small number of participants with primary hypotriglyceridemia (n=4) and primary severe hypertriglyceridemia (n=2). In the hypotriglyceridemia group, we would expect opposite effects on the transcription profile in T cells. Indeed, for the 39 DEGs of the primary moderate CD4⁺ T cell group, 30 genes in CD4⁺ and 32 genes in CD8⁺ T cells showed the opposite effect size, 77% and 82%, respectively ($P_{binomial}$<0.001). Although triglycerides levels are very high in the primary severe group, this patient population is not considered to be at increased risk of CVD because the extreme triglyceride levels are mostly transported in chylomicrons[17]. This was reflected in that little to no difference in effect sizes was observed in the 39 DEGs of the primary moderate CD4⁺ T cell group. Specifically, 24 genes in the primary severe CD4⁺ group and 15 genes in primary severe CD8⁺ group showed the same effect size, 62% and 39%, respectively ($P_{binomial}$>0.05). In fact, the primary severe CD8⁺ group was more similar to the hypotriglyceridemia CD8⁺ group in direction of effect sizes with 27 genes or 69% going in the same direction ($P_{binomial}$<0.05). This result was visualized by plotting the effect sizes of the DEGs of the primary moderate and secondary moderate groups (n=42) in each of the groups and cell types tested (Fig. 3).

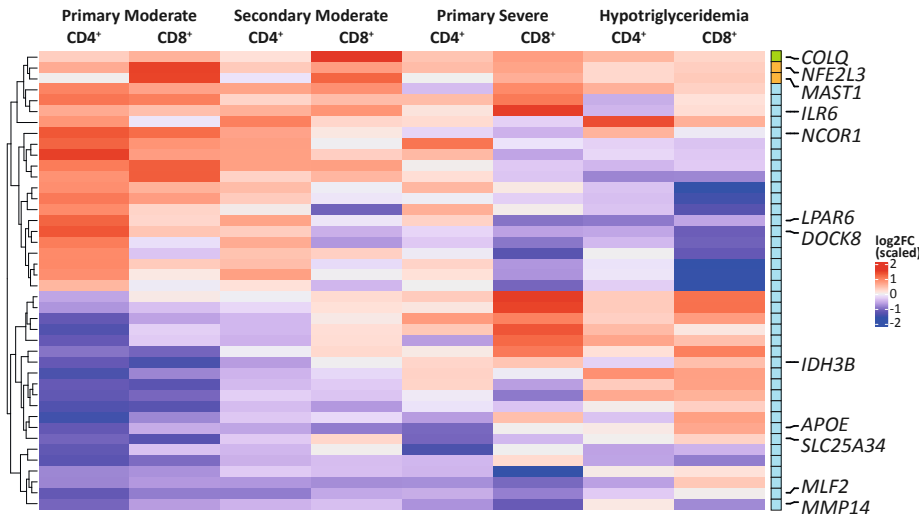


Fig. 3 | Differentially expressed genes (DEGs) in CD4⁺ and CD8⁺ T cells from primary and secondary moderate groups as compared to the control group. Heatmap shows the expression of the aforementioned DEGs in the CD4⁺ and CD8⁺ T cells from primary severe hypertriglyceridemia and hypotriglyceridemia groups as well. Heatmap obtained from the *DESeq2* analysis resulting in 42 DEGs (P_{FDR}<0.05). DEGs are clustered based on effect sizes. Genes of interest are labelled, CD4⁺ T cells primary moderate n=13, CD8⁺ T cells primary moderate n=12, CD4⁺ T cells secondary moderate n=14, CD8⁺ T cells secondary moderate n=9, CD4⁺ T cells primary severe n=2, CD8⁺ T cells primary severe n=2, CD4⁺ T cells hypotriglyceridemia n=4, and CD8⁺ T cells hypotriglyceridemia n=4.

Next, we further explored the associations of severely elevated and reduced triglycerides with CD4⁺ and CD8⁺ T cell gene expression as compared to the control. The primary severe group showed no DEGs in CD4⁺ T cells and 4 DEGs in CD8⁺ T cells that were unique to this group and

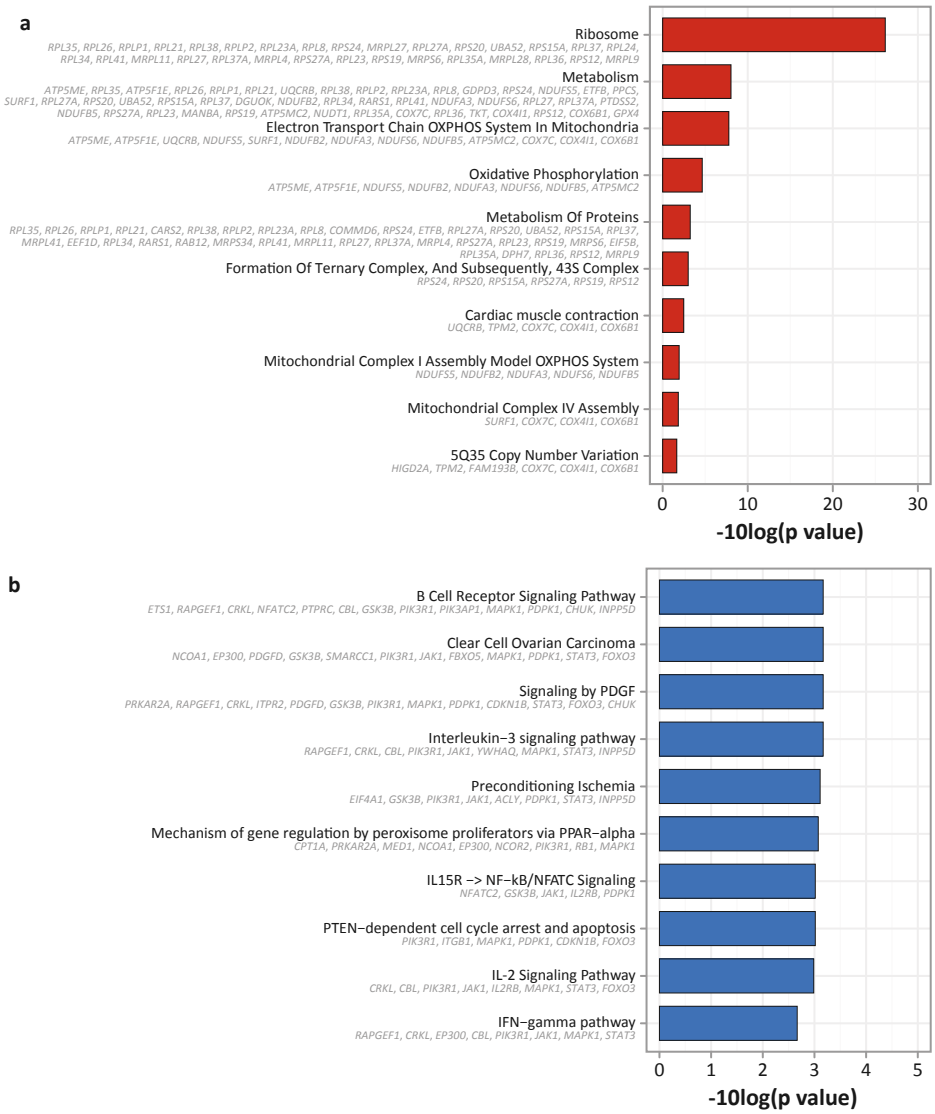


Fig. 4 | Pathway enrichment analysis of DEGs in the CD8⁺ T cells hypotriglyceridemia group. (a) Pathway enrichment analysis of all upregulated CD8⁺ T cell hypotriglyceridemia group DEGs generated using *clusterProfiler* using 10 human pathway databases. Top 10 enrichments are shown. **(b)** Pathway enrichment analysis of all downregulated CD8⁺ T cell hypotriglyceridemia group DEGs generated using *clusterProfiler* using 10 human pathway databases. Top 10 enrichments are shown.

downregulated (Supp. Table 1d). Strikingly, the hypotriglyceridemia group showed 66 DEGs in CD4⁺ T cells and 406 DEGs in CD8⁺ T cells (Supp. Table 1e and f). DEGs of CD4⁺ T cells showed upregulation of fatty acid and cholesterol biosynthesis and transport genes (*SCD*, *DHCR24*, and *LDLR*) as well as downregulation of pro-inflammatory T cell differentiation and immune response genes (*ETS1* and *CD84*). In CD8⁺ T cells, a formal analysis of enriched biological processes

showed 15 upregulated pathways (Fig. 4a; Supp. Table 1g) and 203 downregulated pathways (Fig. 4b; Supp. Table 1h). Upregulated pathways contained the ribosome (including *RPL35, RPL26, RPLP1,* and *RPL21*) and oxidative phosphorylation (including *ATP5ME, NDUFA3,* and *NDUFS6*). Downregulated pathways included B cell signalling (including *ETS1, NFATC2, GSK3B,* and *PIK3AP1*), IL-2 signalling (including *CRKL, JAK1, IL2RB,* and *STAT3*) and IFNγ pathway (including *RAPGEF1, EP300, PIK3R1,* and *MAPK1*).

As compared to controls (median, 0.75 [0.48-0.89] mg/L), hsCRP levels were not elevated among patients with primary severe hypertriglyceridemia (min-max 0-0.50mg/L; P=0.15) and decreased in hypotriglyceridemia (min-max 0.15-0.51mg/L; P=0.025; Table 1).

Discussion

Triglycerides and T cells have re-emerged as risk factors for atherosclerosis and CVD[22, 23]. However, whether triglycerides can affect T cell responses, which may contribute to atherosclerosis and CVD development, remains unknown. We studied patients with hypertriglyceridemia as a natural experiment of *in vivo* exposure to elevated triglyceride levels to determine the interaction between triglycerides and T cells. We found that transcriptomic landscapes of T cells markedly depended on the levels of circulating triglycerides. Specifically, we found that moderately elevated triglyceride levels, between 2.7–8.5mmol/L associated with a pro-inflammatory transcriptomic profile, while decreased triglyceride levels, between 0.1–0.3mmol/L associated with an anti-inflammatory transcriptomic profile, in both $CD4^+$ and $CD8^+$ T cells. Furthermore, we observed stronger effects among patients with primary moderate than with secondary moderate hypertriglyceridemia, possibly reflecting the persistent exposure to elevated triglycerides in patients with a genetic cause of the disorder. These results were reflected in high-sensitivity CRP levels, which were elevated more strongly in primary than secondary moderate patients. Our findings imply that moderately elevated triglycerides may induce a pro-inflammatory response in circulating T cells, which is attenuated in severely elevated and reversed in depleted triglyceride environments.

Moderately elevated triglyceride levels between 2–10mmol/L are associated with a higher risk of CVD[6]. We found that these levels of triglycerides also associated with a pro-inflammatory transcriptomic profile in T cells, specifically in $CD4^+$ T cells. $CD4^+$ T cells are generally thought to be pro-atherogenic, where depletion of $CD4^+$ T cells is atheroprotective[44], while addition of these cells aggravated atherosclerosis in an *Apoe*$^{-/-}$ mouse model[45, 46]. We found increased expression of multiple pro-inflammatory and pro-atherogenic genes such as *IL6R, NCOR1, DOCK8,* and *LPAR6,* in purified $CD4^+$ T cells from the primary moderate patients. The profile was also detectable in $CD8^+$ T cells, although not significantly. While the role of $CD8^+$ T cells in atherosclerosis has not yet been established[47, 48], the proportion of this cell type is enriched in atherosclerotic plaques as compared with the circulation[25]. These results suggest that $CD4^+$ T cells, and to a lesser extent $CD8^+$ T cells, are influenced by triglycerides to establish a pro-inflammatory phenotype, that may play role in T cell-mediated effects of triglycerides on atherosclerosis and CVD.

The pro-inflammatory transcriptomics profile discovered in the T cells of the primary moderate group was largely reproduced in both the transcriptomic profiles of the $CD4^+$ and $CD8^+$ T cells of secondary moderate patients. This was further reflected in the levels of high sensitivity CRP measured in both the primary and secondary moderate groups. Both groups showed increased levels of high sensitivity CRP above normal, but the levels were higher in the primary moderate group than in the secondary moderate group. Thus, our findings point to the consistent upregulation of pro-inflammatory transcriptomic profile in $CD4^+$ and $CD8^+$ T cells associated with moderately elevated triglyceride levels.

We also compared the results to T cells derived from patients with primary severe hypertriglyceridemia (>13mmol/L) and hypotriglyceridemia (<0.3mmol/L). However, because the mutations that lead to these disorders are very rare, only a small number of participants could be included. Nevertheless, we observed that in the primary severe group, the pro-inflammatory transcriptomic profile dissipates. The gene expression patterns here less closely resembled that of the moderately elevated groups, and very few genes were differentially expressed in both $CD4^+$ and $CD8^+$ T cells. In individuals with severe hypertriglyceridemia, excess triglycerides are stored in chylomicrons. Unlike smaller lipoproteins such as VLDL, chylomicrons have a limited ability to penetrate the arterial wall and typically have a relatively short lifespan in circulation, with a half-life of approximately 5min[17, 49]. Additionally, chylomicrons follow a route through the lymphatic system before entering circulation, while VLDL and other TGRLs are directly synthesized and released into circulation by the liver[50, 51]. Therefore, one possible explanation could be that chylomicrons may have fewer interactions with endothelial cells and the arterial wall, potentially resulting in reduced inflammatory responses in T cells.

On the contrary, T cells derived from the hypotriglyceridemia group expressed an anti-inflammatory transcriptional landscape, with pathways such as B cell signalling, IL-2 signalling, and IFNγ pathway being downregulated. Furthermore, the gene expression patterns of the hypotriglyceridemia group were opposite that of the primary and secondary moderate groups. Hypotriglyceridemia has been suggested to be atheroprotective[15, 16], which is supported by the anti-inflammatory transcriptional profile induced in T cells derived from these patients.

Conclusions

We report that circulating triglycerides are associated with T cell transcriptional landscapes that match the hypothesized pro-inflammatory effects of triglyceride levels and T cells in atherosclerosis and CVD[5-9, 52, 53]. Where patients with elevated triglycerides within the range of 2–10mmol/L, known to be atherogenic, associated with pro-inflammatory effects in T cells, but the effect dissipated above this range, known to be less atherogenic, and reversed in diminished triglyceride levels, thought to be atheroprotective. In conclusion, our findings stipulate that triglycerides may influence circulating T cells to promote the expression of a pro- or anti-inflammatory transcriptional landscape. The implication of this finding for atherosclerosis and CVD pathogenesis warrants further investigation.

Limitations of the study

To our knowledge, this is the first study investigating T cell transcriptomics over the full spectrum of triglyceride levels. Although the results of this study are based on a relatively small and heterogenous sample size, they do provide a meaningful comparison and insight into how different circulating levels of triglycerides might influence the immune system around it, particularly T cells. Furthermore, our use of individuals with hyper- and hypotriglyceridemia due to defined genetic mutations provides a natural *in vivo* exposure, which allowed us to study the effects in human T cells.

Translational perspectives

We report that patients with moderately elevated triglycerides have T cells with pro-inflammatory characteristics, including a higher interleukin-6 receptor expression, and increased levels of the inflammatory marker high sensitivity CRP. This observation is important to better understand why elevated triglycerides are a risk factor for CVD and helps the interpretation of the unexpectedly high benefit of triglyceride-lowering treatment using icosapent ethyl in the REDUCE-IT trial. Also, it suggests that this patient group at risk of CVD may benefit from anti-inflammatory treatments like canakinumab (CANTOS trial) and colchicine (COLCOT trial), or in the future, novel treatment like the interleukin-6 inhibitor ziltivekimab.

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Author contributions
B.T.H, J.W.J, and J.R.v.L. conceived the project. N.A.R. and J.M. designed and conducted the experiments, analysed the results, and drafted the manuscript. K.F.D. designed the analysis model and analysed the RNA sequencing data. T.B.K. aligned the RNA sequencing data. L.C.V-Z. and M.T.M. performed the plasma measurements including the analysis of the lipoprotein profiling. All authors contributed to the writing of the manuscript.

Competing interests
The authors declare no competing interests.

Data availability statement
Upon reasonable request, it can be expected that specific anonymous data will be shared to a qualified researcher.

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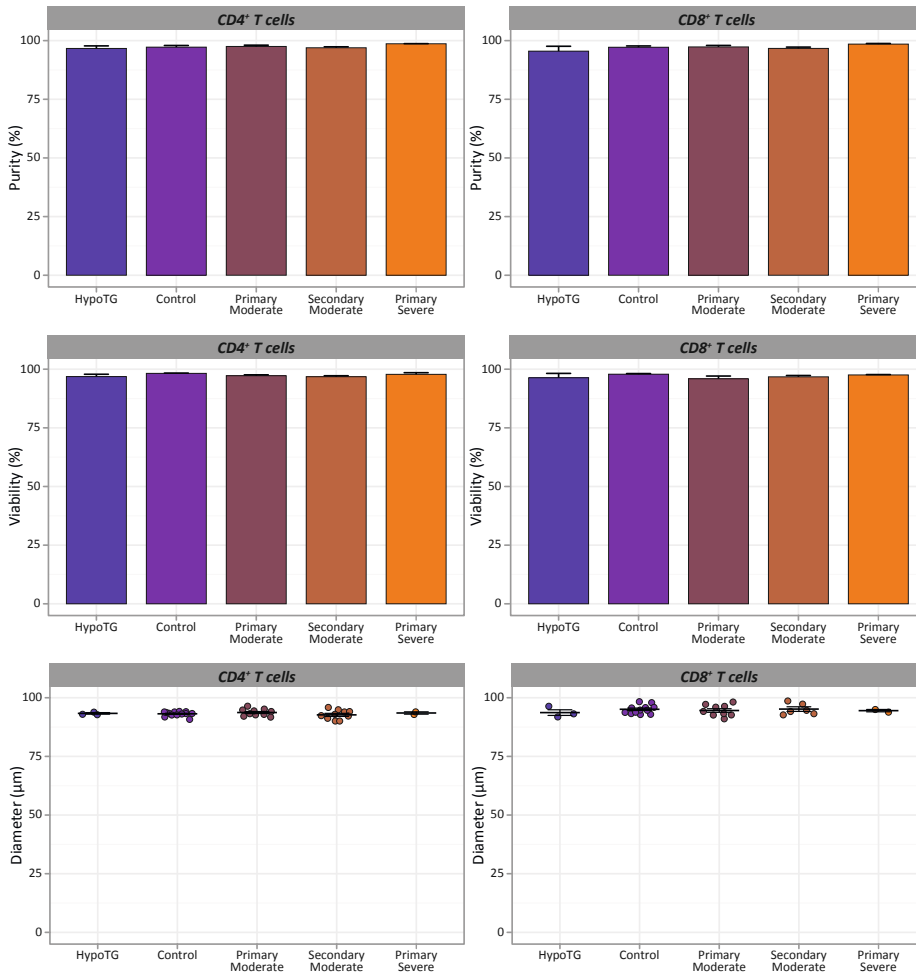
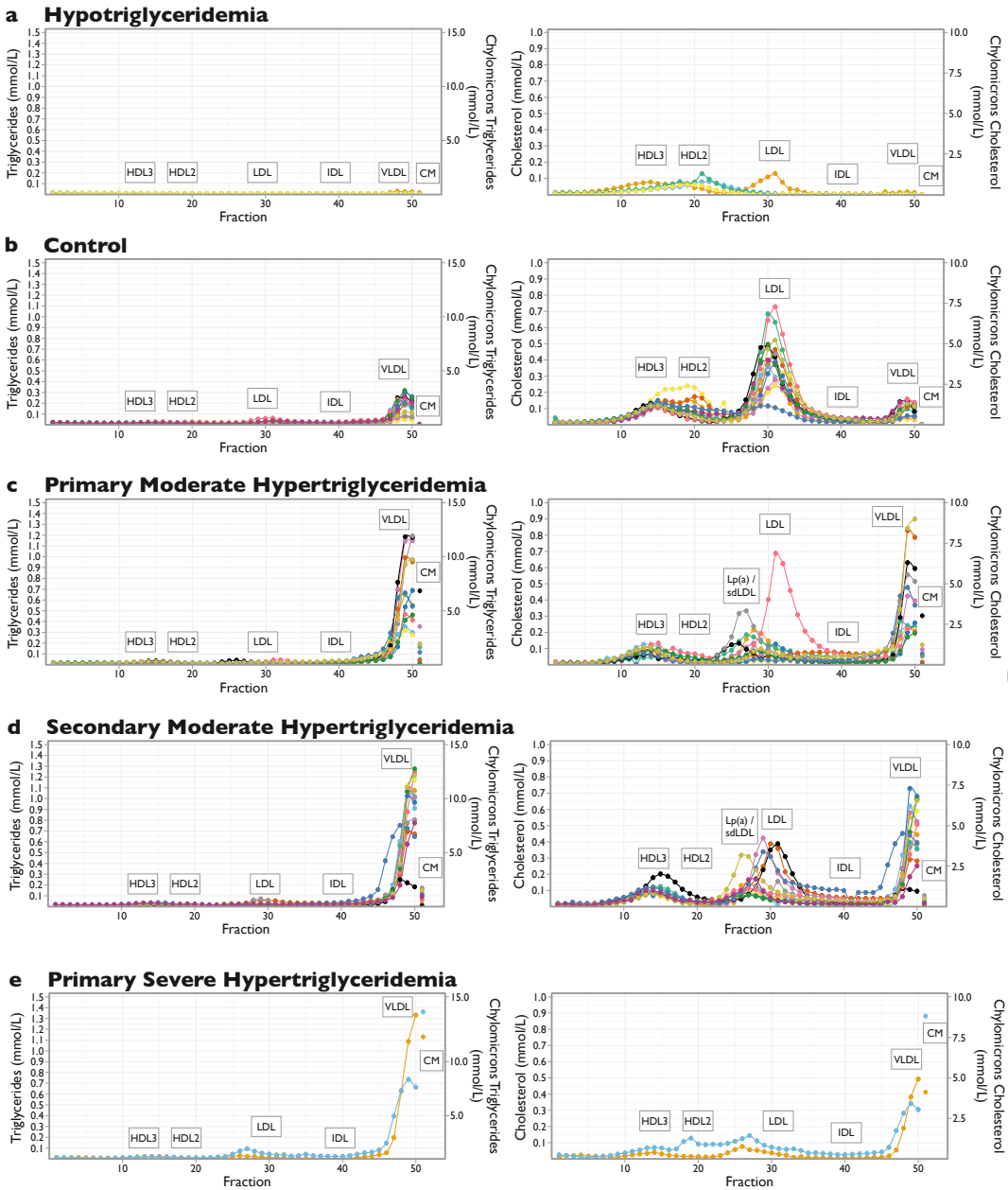
Supplemental information



Supplemental Fig. 1 | Cell purity, viability, and diameter post-sorting. (a) Bar plot showing the average cell purity and standard error in percent of $CD4^+$ T cells isolated from each group as determined by the CytoFLEX SRT Benchtop cell sorter. On average the cell purity of $CD4^+$ T cells was 97.2 SE 0.7% for the control group, 97.5 SE 0.6% for the primary moderate group, 97.0 SE 0.4 for the secondary moderate group, 98.7 SE 0.1% for the primary severe, and 96.7 SE 1.1% for the hypotriglyceridemia group. **(b)** Bar plot showing the average cell purity and standard error in percent of $CD8^+$ T cells isolated from each group as determined by the CytoFLEX SRT Benchtop cell sorter. On average the cell purity of $CD8^+$ T cells was 97.2 SE 0.6% for the control group, 97.3 SE 0.6% for the primary moderate group, 96.7 SE 0.6 for the secondary moderate group, 98.5 SE 0.3% for the primary severe, and 95.5 SE 2.1% for the hypotriglyceridemia group. **(c)** Bar plot showing the average viability and standard error in percent, as determined by Via1-Cassette™ on a NucleoCounter® NC-200™ of $CD4^+$ T cells post-sorting per group. On average the cell viability of $CD4^+$ T cells was 98.2 SE 0.1% for the control group, 97.2 SE 0.4% for the primary moderate group, 96.8 SE 0.4% for the secondary moderate group, 97.8 SE 0.7% for the primary severe, and 96.9 SE 1.0% for the hypotriglyceridemia group. **(d)** Bar plot showing the average viability and standard error in percent, as determined by Via1-Cassette™ on a NucleoCounter® NC-200™ of $CD8^+$ T cells post-sorting per group. On average the cell viability of $CD8^+$ T

cells was 97.9 SE 0.3% for the control group, 95.9 SE 1.2% for the primary moderate group, 96.7 SE 0.6% for the secondary moderate group, 97.6 SE 0.2% for the primary severe, and 96.4 SE 1.9% for the hypotriglyceridemia group. **(e)** Dot plot showing the average cell diameter and standard error in µm, as determined by Via1-Cassette™ on a NucleoCounter® NC-200™ of $CD4^+$ T cells post-sorting per group. On average the cell diameter of $CD4^+$ T cells was 9.3 SE 0.03µm for the control group, 9.4 SE 0.04µm for the primary moderate group, 9.3 SE 0.07µm for the secondary moderate group, 9.4 SE 0.05µm for the primary severe, and 9.3 SE 0.03µm for the hypotriglyceridemia group. **(f)** Dot plot showing the average cell diameter and standard error in µm, as determined by Via1-Cassette™ on a NucleoCounter® NC-200™ of $CD8^+$ T cells post-sorting per group. On average the cell diameter of $CD8^+$ T cells was 9.5 SE 0.05µm for the control group, 9.5 SE 0.08µm for the primary moderate group, 9.5 SE 0.10µm for the secondary moderate group, 9.5 SE 0.05µm for the primary severe, and 9.4 SE 0.12µm for the hypotriglyceridemia group.



Supplemental Fig. 2 | Lipoprotein profiles per individual. Triglycerides are in the lefthand figures and cholesterol in the righthand figures of the **(a)** hypotriglyceridemia, **(b)** control, **(c)** primary moderate hypertriglyceridemia, **(d)** secondary moderate hypertriglyceridemia, and **(e)** primary severe hypertriglyceridemia group. Chylomicrons are depicted as separate values.

Supplemental Table 1 | Changes in gene expression in $CD4^+$ and $CD8^+$ T cells derived from patients with hypertriglyceridemia or hypotriglyceridemia.

Supplemental Table 1 | (a) DEGs for $CD4^+$ T cells from primary moderate hypertriglyceridemia group in order of significance along with their Ensembl ID, gene symbol, UniProt ID, base mean, log2 fold change, log fold change standard error, p value, and adjusted p value (FDR).

Order	Ensembl ID	Gene Symbol	UniProt	baseMean	Log2FC	Log2FC SE	P value	P_{FDR}
1	ENSG00000069329	*VPS35*	Q96QK1	1310.900	0.348	0.067	2.17E-07	0.002
2	ENSG00000141027	*NCOR1*	O75376	2275.859	0.334	0.071	2.30E-06	0.011
3	ENSG00000154165	*GPR15*	P49685	656.740	2.143	0.471	5.36E-06	0.011
4	ENSG00000089693	*MLF2*	Q15773	2214.182	-0.251	0.054	3.10E-06	0.011
5	ENSG00000169021	*UQCRFS1*	P47985	1370.786	-0.345	0.076	6.05E-06	0.011
6	ENSG00000175390	*EIF3F*	O00303	9104.610	-0.217	0.048	5.11E-06	0.011
7	ENSG00000107099	*DOCK8*	Q8NF50	4372.962	0.464	0.107	1.44E-05	0.016
8	ENSG00000123104	*ITPR2*	Q14571	1040.976	0.361	0.084	1.63E-05	0.016
9	ENSG00000131558	*EXOC4*	Q96A65	821.362	0.266	0.062	1.73E-05	0.016
10	ENSG00000125245	*GPR18*	Q14330	238.422	-0.966	0.224	1.65E-05	0.016
11	ENSG00000162910	*MRPL55*	Q7Z7F7	826.591	-0.290	0.066	1.22E-05	0.016
12	ENSG00000186395	*KRT10*	P13645	616.223	-0.324	0.074	1.12E-05	0.016
13	ENSG00000175536	*LIPT2*	A6NK58	161.772	-0.643	0.151	1.93E-05	0.017
14	ENSG00000121578	*B4GALT4*	O60513	891.611	-0.404	0.095	2.22E-05	0.018
15	ENSG00000110200	*ANAPC15*	P60006	765.260	-0.206	0.049	2.45E-05	0.018
16	ENSG00000185591	*SP1*	P08047	2182.606	0.304	0.073	3.07E-05	0.022
17	ENSG00000205413	*SAMD9*	Q5K651	1599.738	0.438	0.107	4.41E-05	0.028
18	ENSG00000126005	*MMP24OS*	A0A0U1RRL7	1002.648	-0.306	0.075	4.18E-05	0.028
19	ENSG00000159377	*PSMB4*	P28070	4855.277	-0.226	0.056	5.22E-05	0.031
20	ENSG00000144840	*RABL3*	Q5HYI8	676.009	0.287	0.072	6.11E-05	0.032
21	ENSG00000160712	*IL6R*	P08887	4629.771	0.291	0.073	6.16E-05	0.032
22	ENSG00000111786	*SRSF9*	Q13242	2738.199	-0.137	0.034	5.85E-05	0.032
23	ENSG00000198064	*NPIPB13*	A6NJU9	62.888	0.865	0.217	6.79E-05	0.033

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Supplemental Table 1 | (a) *[continued]*

Order	Ensembl ID	Gene Symbol	UniProt	baseMean	Log2FC	Log2FC SE	P value	P_{FDR}
24	ENSG00000139679	*LPAR6*	P43657	2493.899	0.672	0.169	7.29E-05	0.034
25	ENSG00000125835	*SNRPB*	P14678	2318.209	-0.251	0.063	7.45E-05	0.034
26	ENSG00000162461	*SLC25A34*	Q6PIV7	123.128	-0.499	0.126	7.72E-05	0.034
27	ENSG00000157227	*MMP14*	P50281	38.752	-0.736	0.187	8.24E-05	0.035
28	ENSG00000130203	*APOE*	P02649	31.750	-0.839	0.214	9.03E-05	0.036
29	ENSG00000102699	*PARP4*	Q9UKK3	1744.975	0.352	0.091	0.00011	0.041
30	ENSG00000063046	*EIF4B*	P23588	29254.798	-0.285	0.074	0.00011	0.041
31	ENSG00000100836	*PABPN1*	Q86U42	1088.730	-0.271	0.070	0.00011	0.041
32	ENSG00000177311	*ZBTB38*	Q8NAP3	1667.448	0.405	0.106	0.00014	0.045
33	ENSG00000178440	*TIMM23B-AGAP6*		18.591	1.063	0.279	0.00014	0.045
34	ENSG00000197694	*SPTAN1*	Q13813	7831.595	0.293	0.077	0.00014	0.045
35	ENSG00000101365	*IDH3B*	O43837	2118.595	-0.197	0.052	0.00014	0.045
36	ENSG00000177570	*SAMD12*	Q8N8I0	306.832	0.736	0.195	0.00016	0.049
37	ENSG00000182134	*TDRKH*	Q9Y2W6	300.104	0.368	0.098	0.00017	0.049
38	ENSG00000092068	*SLC7A8*	Q9UHI5	139.980	-2.390	0.633	0.00016	0.049
39	ENSG00000243156	*MICAL3*	Q7RTP6	296.014	0.698	0.186	0.00017	0.049

Supplemental Table 1 | (b) DEGs for $CD8^+$ T cells from primary moderate hypertriglyceridemia group in order of significance along with their Ensembl ID, gene symbol, UniProt ID, base mean, log2 fold change, log fold change standard error, p value, and adjusted p value (FDR).

Order	Ensembl ID	Gene Symbol	UniProt	baseMean	Log2FC	Log2FC SE	P value	P_{FDR}
1	ENSG00000050344	*NFE2L3*	Q9Y4A8	131.314	1.551	0.300	2.28E-07	0.003
2	ENSG00000105613	*MAST1*	Q9Y2H9	14.915	4.616	1.001	4.00E-06	0.025

Supplemental Table 1 | (c) DEGs for $CD8^+$ T cells from secondary moderate hypertriglyceridemia group in order of significance along with their Ensembl ID, gene symbol, UniProt ID, base mean, log2 fold change, log fold change standard error, p value, and adjusted p value (FDR).

Order	Ensembl ID	Gene Symbol	UniProt	baseMean	Log2FC	Log2FC SE	P value	P_{FDR}
1	ENSG00000206561	*COLQ*	Q9Y215	566.855	1.374	0.294	2.96E-06	0.037

Supplemental Table 1 | (d) DEGs for $CD8^+$ T cells from primary severe group in order of significance along with their Ensembl ID, gene symbol, UniProt ID, base mean, log2 fold change, log fold change standard error, p value, and adjusted p value (FDR).

Order	Ensembl ID	Gene Symbol	UniProt	baseMean	Log2FC	Log2FC SE	P value	P_{FDR}
1	ENSG00000080493	*SLC4A4*	Q9Y6R1	246.560	-1.657	0.342	1.26E-06	0.016
2	ENSG00000109756	*RAPGEF2*	Q9Y4G8	722.977	-1.454	0.316	4.08E-06	0.025
3	ENSG00000169554	*ZEB2*	O60315	3472.877	-1.738	0.398	1.28E-05	0.042
4	ENSG00000189190	*ZNF600*	Q6ZNG1	2194.249	-1.463	0.336	1.34E-05	0.042

Supplemental Table 1 | (e) (partial) DEGs for $CD4^+$ T cells from hypotriglyceridemia group in order of significance along with their Ensembl ID, gene symbol, UniProt ID, base mean, log2 fold change, log fold change standard error, p value, and adjusted p value (FDR). Top 30 most significantly expressed genes are shown here.

Order	Ensembl ID	Gene Symbol	UniProt	baseMean	Log2FC	Log2FC SE	P value	P_{FDR}
1	ENSG00000099194	*SCD*	O00767	167.515	2.360	0.398	2.90E-09	3.60E-05
2	ENSG00000136541	*ERMN*	Q8TAM6	354.683	1.060	0.185	9.65E-09	5.99E-05
3	ENSG00000116133	*DHCR24*	Q15392	220.084	1.602	0.286	2.11E-08	8.75E-05
4	ENSG00000144152	*FBLN7*	Q53RD9	277.620	-0.650	0.124	1.44E-07	0.00045
5	ENSG00000198064	*NPIPB13*	A6NJU9	64.361	1.456	0.289	4.67E-07	0.00116
6	ENSG00000186166	*CENATAC*	Q86UT8	1448.013	0.615	0.132	3.16E-06	0.00654
7	ENSG00000075292	*ZNF638*	Q14966	3579.615	0.495	0.110	7.34E-06	0.00757
8	ENSG00000104133	*SPG11*	Q96JI7	2772.839	0.378	0.085	8.53E-06	0.00757

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Supplemental Table 1 | (e) (partial) *[continued]*

Order	Ensembl ID	Gene Symbol	UniProt	baseMean	Log2FC	Log2FC SE	P value	P_{FDR}
9	ENSG00000115239	*ASB3*	Q9Y575	484.747	0.547	0.122	7.75E-06	0.00757
10	ENSG00000150401	*DCUN1D2*	Q6PH85	394.946	0.494	0.109	6.39E-06	0.00757
11	ENSG00000160447	*PKN3*	Q6P5Z2	83.384	1.032	0.227	5.76E-06	0.00757
12	ENSG00000196757	*ZNF700*	Q9H0M5	666.493	0.795	0.179	8.37E-06	0.00757
13	ENSG00000221914	*PPP2R2A*	P63151	1330.896	0.406	0.089	5.28E-06	0.00757
14	ENSG00000117298	*ECE1*	P42892	3252.266	-0.377	0.083	6.16E-06	0.00757
15	ENSG00000100055	*CYTH4*	Q9UIA0	4291.201	0.381	0.088	1.47E-05	0.01139
16	ENSG00000116350	*SRSF4*	Q08170	2338.237	0.499	0.115	1.42E-05	0.01139
17	ENSG00000185946	*RNPC3*	Q96LT9	1394.700	-0.620	0.143	1.56E-05	0.01139
18	ENSG00000108474	*PIGL*	Q9Y2B2	1201.827	-0.340	0.079	1.67E-05	0.01152
19	ENSG00000204178	*MACO1*	Q8N5G2	889.374	0.334	0.079	2.08E-05	0.01292
20	ENSG00000111276	*CDKN1B*	P46527	7866.116	-0.559	0.131	2.00E-05	0.01292
21	ENSG00000103222	*ABCC1*	P33527	2898.805	-0.272	0.065	2.57E-05	0.01443
22	ENSG00000115705	*TPO*	P07202	18.123	-3.135	0.746	2.62E-05	0.01443
23	ENSG00000258728	*ENSG 00000258728*		701.586	-0.627	0.149	2.67E-05	0.01443
24	ENSG00000106077	*ABHD11*	Q8NFV4	275.336	0.538	0.129	3.07E-05	0.01464
25	ENSG00000110628	*SLC22A18*	Q96BI1	299.581	1.153	0.277	3.06E-05	0.01464
26	ENSG00000139624	*CERS5*	Q8N5B7	959.383	0.499	0.120	3.18E-05	0.01464
27	ENSG00000206418	*RAB12*	Q6IQ22	450.507	0.567	0.136	3.18E-05	0.01464
28	ENSG00000121542	*SEC22A*	Q96IW7	252.048	0.448	0.108	3.58E-05	0.01588
29	ENSG00000170260	*ZNF212*	Q9UDV6	324.595	0.739	0.181	4.33E-05	0.01791
30	ENSG00000240053	*LY6G5B*	Q8NDX9	197.842	-0.464	0.113	4.30E-05	0.01791

Supplemental Table 1 | (f) (partial) DEGs for $CD8^+$ T cells from hypotriglyceridemia group in order of significance along with their Ensembl ID, gene symbol, UniProt ID, base mean, log2 fold change, log fold change standard error, p value, and adjusted p value (FDR). Top 30 most significantly expressed genes are shown here.

Order	Ensembl ID	Gene Symbol	UniProt	baseMean	Log2FC	Log2FC SE	P value	P_{FDR}
1	ENSG00000084112	*SSH1*	Q8WYL5	1657.780	-0.803	0.146	3.54E-08	0.00031
2	ENSG00000110090	*CPT1A*	P50416	1258.597	-1.728	0.326	1.12E-07	0.00049
3	ENSG00000139514	*SLC7A1*	P30825	702.125	-0.709	0.136	1.74E-07	0.00051
4	ENSG00000172493	*AFF1*	P51825	2084.541	-0.726	0.144	4.93E-07	0.00108
5	ENSG00000204472	*AIF1*	P55008	988.474	1.423	0.289	8.56E-07	0.00116
6	ENSG00000166446	*CDYL2*	Q8N8U2	292.277	-1.258	0.257	9.86E-07	0.00116
7	ENSG00000177311	*ZBTB38*	Q8NAP3	1843.038	-1.181	0.242	1.05E-06	0.00116
8	ENSG00000178502	*KLHL11*	Q9NVR0	423.669	-1.461	0.298	9.68E-07	0.00116
9	ENSG00000160218	*TRAPPC10*	P48553	2748.232	-0.805	0.167	1.34E-06	0.00131
10	ENSG00000172428	*COPS9*	Q8WXC6	659.556	0.642	0.134	1.59E-06	0.00140
11	ENSG00000114302	*FAAH*	P13861	1122.716	-0.785	0.167	2.73E-06	0.00210
12	ENSG00000135272	*MDFIC*	Q9P1T7	1620.508	-0.767	0.164	2.87E-06	0.00210
13	ENSG00000060237	*WNK1*	Q9H4A3	5648.666	-0.605	0.130	3.41E-06	0.00231
14	ENSG00000040199	*PHLPP2*	Q6ZVD8	406.036	-0.903	0.199	5.53E-06	0.00257
15	ENSG00000109452	*INPP4B*	O15327	1429.090	-0.974	0.213	4.88E-06	0.00257
16	ENSG00000111252	*SH2B3*	Q9UQQ2	1498.342	-0.561	0.124	5.84E-06	0.00257
17	ENSG00000125686	*MED1*	Q15648	1683.447	-0.759	0.167	5.66E-06	0.00257
18	ENSG00000133657	*ATP13A3*	Q9H7F0	1044.678	-0.958	0.211	5.40E-06	0.00257
19	ENSG00000134954	*ETS1*	P14921	26654.754	-0.683	0.149	4.57E-06	0.00257
20	ENSG00000164307	*ERAP1*	Q9NZ08	2230.601	-1.023	0.224	4.88E-06	0.00257
21	ENSG00000048707	*VPS13D*	Q5THJ4	2677.249	-0.558	0.124	6.92E-06	0.00257
22	ENSG00000084676	*NCOA1*	Q15788	2543.318	-0.730	0.162	6.96E-06	0.00257
23	ENSG00000104419	*NDRG1*	Q92597	1323.154	-0.587	0.130	6.61E-06	0.00257
24	ENSG00000173706	*HEG1*	Q9ULI3	1004.858	-1.213	0.270	7.23E-06	0.00257

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Supplemental Table 1 | (f) (partial) *[continued]*

Order	Ensembl ID	Gene Symbol	UniProt	baseMean	Log2FC	Log2FC SE	P value	P_{FDR}
25	ENSG00000224470	*ATXN1L*	P0C7T5	2024.319	-0.828	0.185	7.31E-06	0.00257
26	ENSG00000024862	*CCDC28A*	Q8IWP9	363.350	0.769	0.172	7.66E-06	0.00259
27	ENSG00000066294	*CD84*	Q9UIB8	2499.072	-0.823	0.184	8.09E-06	0.00264
28	ENSG00000186469	*GNG2*	P59768	3931.442	-0.851	0.192	9.67E-06	0.00304
29	ENSG00000169020	*ATP5ME*	P56385	877.665	0.634	0.144	1.06E-05	0.00310
30	ENSG00000145734	*BDP1*	A6H8Y1	1555.640	-0.910	0.206	1.03E-05	0.00310

Supplemental Table 1 | (g) (partial) Pathway enrichment analysis of all upregulated CD8⁺ T cell hypotriglyceridemia DEGs generated using *clusterProfiler* using 10 human pathway databases. Enriched pathways are shown with information on which group that term clusters into based on the Jaccard index > 0.70, pathway term, number of DEGs that overlap with that databases gene set for that pathway, p value, adjusted p value, number of DEGs in that pathway that were upregulated, number of DEGs in that pathway that were downregulated, and gene names of all DEGs included in that pathway. Only the top term of the 15 groups are shown here.

Group	Term	Overlap	P value	Adjusted P value	Up-regulated	Down-regulated	Genes
1	Ribosome	31/116	4.12E-30	6.78E-27	31	0	*RPL35, RPL26, RPLP1, RPL21, RPL38, RPLP2, RPL23A, RPL8, RPS24, MRPL27, RPL27A, RPS20, UBA52, RPS15A, RPL37, RPL24, RPL34, RPL41, MRPL11, RPL27, RPL37A, MRPL4, RPS27A, RPL23, RPS19, MRPS6, RPL35A, MRPL28, RPL36, RPS12, MRPL9*
2	Metabolism	47/1130	2.04E-10	9.34E-09	47	0	*ATP5ME, RPL35, ATP5F1E, RPL26, RPLP1, RPL21, UQCRB, RPL38, RPLP2, RPL23A, RPL8, GDPD3, RPS24, NDUFS5, ETFB, PPCS, SURF1, RPL27A, RPS20, UBA52, RPS15A, RPL37, DGUOK, NDUFB2, RPL34, RARS1, RPL41, NDUFA3, NDUFS6, RPL27, RPL37A, PTDSS2, NDUFB5, RPS27A, RPL23, MANBA, RPS19, ATP5MC2, NUDT1, RPL35A, COX7C, RPL36, TKT, COX4I1, RPS12, COX6B1, GPX4*
3	Electron transport chain OXPHOS system in mitochondria	13/79	3.76E-10	1.67E-08	13	0	*ATP5ME, ATP5F1E, UQCRB, NDUFS5, SURF1, NDUFB2, NDUFA3, NDUFS6, NDUFB5, ATP5MC2, COX7C, COX4I1, COX6B1*
4	Oxidative phosphorylation	8/46	6.86E-07	2.21E-05	8	0	*ATP5ME, ATP5F1E, NDUFS5, NDUFB2, NDUFA3, NDUFS6, NDUFB5, ATP5MC2*
5	Metabolism of proteins	38/1186	2.02E-05	0.00057	38	0	*RPL35, RPL26, RPLP1, RPL21, CARS2, RPL38, RPLP2, RPL23A, RPL8, COMMD6, RPS24, ETFB, RPL27A, RPS20, UBA52, RPS15A, RPL37, MRPL41, EEF1D, RPL34, RARS1, RAB12, MRPS34, RPL41, MRPL11, RPL27, RPL37A, MRPL4, RPS27A, RPL23, RPS19, MRPS6, EIF5B, RPL35A, DPH7, RPL36, RPS12, MRPL9*

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Supplemental Table 1 | (g) (partial) *[continued]*

Group	Term	Overlap	P value	Adjusted P value	Up-regulated	Down-regulated	Genes
6	SARS-CoV-2 modulates host translation machinery	6/39	3.79E-05	0.00099	6	0	*RPS24, RPS20, RPS15A, RPS27A, RPS19, RPS12*
7	Cardiac muscle contraction	5/31	0.00014	0.00333	5	0	*UQCRB, TPM2, COX7C, COX4I1, COX6B1*
8	Mitochondrial complex I assembly model OXPHOS system	5/41	0.00053	0.01153	5	0	*NDUFS5, NDUFB2, NDUFA3, NDUFS6, NDUFB5*
9	Mitochondrial complex IV assembly	4/25	0.00069	0.01440	4	0	*SURF1, COX7C, COX4I1, COX6B1*
10	5Q35 copy number variation	6/71	0.00108	0.02198	6	0	*HIGD2A, TPM2, FAM193B, COX7C, COX4I1, COX6B1*
11	Mitochondrial translation elongation	6/73	0.00125	0.02512	6	0	*MRPL41, MRPS34, MRPL11, MRPL4, MRPS6, MRPL9*
12	Signaling by constitutively active EGFR	3/16	0.00212	0.03964	3	0	*HRAS, UBA52, RPS27A*
13	Formation pf ATP by chemiosmotic coupling	3/16	0.00212	0.03964	3	0	*ATP5ME, ATP5F1E, ATP5MC2*
14	Cellular response to chemical stress	8/142	0.00236	0.04364	8	0	*SURF1, UBA52, NUDT2, RPS27A, COX7C, TKT, COX4I1, COX6B1*
15	Modulation by Mtb of host immune system	2/5	0.00263	0.04763	2	0	*UBA52, RPS27A*

Supplemental Table 1 | (h) (partial) Pathway enrichment analysis of all downregulated CD8⁺ T cell hypotriglyceridemia DEGs generated using *clusterProfiler* using 10 human pathway databases. Enriched pathways are shown with information on which group that term clusters into based on the Jaccard index > 0.70, pathway term, number of DEGs that overlap with that databases gene set for that pathway, p value, adjusted p value, number of DEGs in that pathway that were upregulated, number of DEGs in that pathway that were downregulated, and gene names of all DEGs included in that pathway. Only the top term of the first 20 groups are shown here.

Group	Term	Overlap	P value	Adjusted P value	Up-regulated	Down-regulated	Genes
1	B cell receptor signaling pathway	13/74	3.49E-07	0.00068	0	13	*ETS1, RAPGEF1, CRKL, NFATC2, PTPRC, CBL, GSK3B, PIK3R1, PIK3AP1, MAPK1, PDPK1, CHUK, INPP5D*
2	Clear cell ovarian carcinoma	12/65	5.71E-07	0.00068	0	12	*NCOA1, EP300, PDGFD, GSK3B, SMARCC1, PIK3R1, JAK1, FBXO5, MAPK1, PDPK1, STAT3, FOXO3*
3	Signaling by PDGF	13/80	8.91E-07	0.00068	0	13	*PRKAR2A, RAPGEF1, CRKL, ITPR2, PDGFD, GSK3B, PIK3R1, MAPK1, PDPK1, CDKN1B, STAT3, FOXO3, CHUK*
4	Interleukin-3 signaling pathway	9/36	1.05E-06	0.00068	0	9	*RAPGEF1, CRKL, CBL, PIK3R1, JAK1, YWHAQ, MAPK1, STAT3, INPP5D*
5	Preconditioning ischemia	8/28	1.40E-06	0.00078	0	8	*EIF4A1, GSK3B, PIK3R1, JAK1, ACLY, PDPK1, STAT3, INPP5D*
6	Mechanism of gene regulation by peroxisome proliferators via PPARα	9/39	2.18E-06	0.00084	0	9	*CPT1A, PRKAR2A, MED1, NCOA1, EP300, NCOR2, PIK3R1, RB1, MAPK1*
7	IL15R -> NF-κB/NFATC signaling	5/9	3.17E-06	0.00096	0	5	*NFATC2, GSK3B, JAK1, IL2RB, PDPK1*
8	PTEN-dependent cell cycle arrest and apoptosis	6/15	3.35E-06	0.00096	0	6	*PIK3R1, ITGB1, MAPK1, PDPK1, CDKN1B, FOXO3*
9	IL 2 signaling pathway	8/32	4.26E-06	0.00103	0	8	*CRKL, CBL, PIK3R1, JAK1, IL2RB, MAPK1, STAT3, FOXO3*
10	IFNγ pathway	8/36	1.10E-05	0.00216	0	8	*RAPGEF1, CRKL, EP300, CBL, PIK3R1, JAK1, MAPK1, STAT3*
11	Prostate cancer	11/72	1.17E-05	0.00216	0	11	*ETS1, EP300, GSK3B, JAK1, RB1, ABCC1, RUNX3, MAPK1, PDPK1, CDKN1B, STAT3*

[continued on next page]

Supplemental Table 1 | (h) (partial) *[continued]*

Group	Term	Overlap	P value	Adjusted P value	Up-regulated	Down-regulated	Genes
12	Interleukin-7 signaling pathway	7/27	1.34E-05	0.00221	0	7	*EP300, GSK3B, PIK3R1, JAK1, MAPK1, CDKN1B, STAT3*
13	Signaling events mediated by stem cell factor receptor (c-Kit)	8/37	1.37E-05	0.00221	0	8	*SH2B3, CRKL, CBL, GSK3B, PIK3R1, PDPK1, STAT3, FOXO3*
14	ALK associated neuroblastoma	6/19	1.64E-05	0.00244	0	6	*RAPGEF1, CRKL, MAPK1, PDPK1, STAT3, FOXO3*
15	Signal transduction	63/1228	1.73E-05	0.00248	0	63	*CPT1A, PRKAR2A, PHLPP2, SH2B3, NCOA1, GNG2, RAPGEF1, WIPF1, IKZF1, REST, CRKL, ARAP2, CLIP1, LATS2, EP300, STRN, KMT2D, BMPR2, ITPR2, CBL, ARHGAP35, PDGFD, NCOR2, GSK3B, TFDP2, LLGL1, LRIG1, F2R, DOCK2, PIK3R1, ITGB1, TAB2, SLK, ACTR2, JAK1, B4GALT1, PRKCH, NOTCH2, IL2RB, ERBIN, RAPGEF2, SEPTIN7, AKAP13, YWHAQ, PIK3AP1, RUNX3, INCENP, GFOD1, MAPK1, PDPK1, CDKN1B, WASF2, KTN1, STAT3, FOXO3, DOCK9, ARHGEF3, CHUK, TNKS2, CAB39, MYH9, FASN, PIP4K2A*
16	PI3K-Akt signaling pathway	16/153	1.94E-05	0.00259	0	16	*PHLPP2, GNG2, PDGFD, GSK3B, F2R, PIK3R1, ITGB1, JAK1, IL2RB, YWHAQ, PIK3AP1, MAPK1, PDPK1, CDKN1B, FOXO3, CHUK*
17	Kit receptor signaling pathway	8/39	2.07E-05	0.00267	0	8	*CRKL, EP300, CBL, PIK3R1, MAPK1, STAT3, FOXO3, INPP5D*
18	Interleukin-3, interleukin-5 and GM-CSF signaling	7/29	2.23E-05	0.00273	0	7	*RAPGEF1, CRKL, CBL, PIK3R1, JAK1, IL2RB, INPP5D*
19	Signaling by interleukins	11/77	2.25E-05	0.00273	0	11	*RAPGEF1, CRKL, CBL, PIK3R1, TAB2, JAK1, IL2RB, MAPK1, STAT3, CHUK, INPP5D*
20	IL-2 receptor beta chain in T cell activation	8/40	2.51E-05	0.00292	0	8	*CRKL, CBL, PIK3R1, JAK1, IL2RB, RB1, MAPK1, PDPK1*