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Rate-Limiting Enzymes in Cardiometabolic Health and Aging in Humans

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Abstract
Introduction: Rate-limiting enzymes (RLEs) are innate slow points in metabolic pathways, and many function in bioprocesses related to nutrient sensing. Many RLEs carry causal mutations relevant to inherited metabolic disorders. Because the activity of RLEs in cardiovascular health is poorly characterized, our objective was to assess their involvement in cardiometabolic health and disease and where altered biophysical and biochemical functions can promote disease. ***Methods:*** A dataset of 380 human RLEs was compared to protein and gene datasets for factors likely to contribute to cardiometabolic disease, including proteins showing significant age-related altered expression in blood and genetic loci with variants that associate with common cardiometabolic phenotypes. The biochemical reactions catalyzed by RLEs were evaluated for metabolites enriched in RLE subsets associating with various cardiometabolic phenotypes. Most significance tests were based on Z-score enrichment converted to *p* values with a normal distribution function. ***Results:*** Of 380 RLEs analyzed, 112 function in mitochondria, and 53 are assigned to inherited metabolic disorders. There was a depletion of RLE proteins known as aging biomarkers. At the gene level, RLEs were assessed for common genetic variants that associated with important cardiometabolic traits of LDL-cholesterol or any of the five outcomes pertinent to metabolic syndrome. This revealed several RLEs with links to cardiometabolic traits, from a minimum of 26 for HDL-cholesterol to a maximum of 45 for plasma glucose. Analysis of these GWAS-linked RLEs for enrichment of the molecular constituents of the catalyzed reactions disclosed a number of significant phenotype-metabolite links. These included blood pressure with acetate ($p = 2.2 \times 10^{-4}$) and NADP+ ($p = 0.0091$), plasma HDL-cholesterol and triglyceride with diacylglycerol

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($p = 2.6 \times 10^{-5}$, 6.4×10^{-5}, respectively) and diolein ($p = 2.2 \times 10^{-6}$, 5.9×10^{-6}), and waist circumference with D-glucosamine-6-phosphate ($p = 1.8 \times 10^{-4}$). ***Conclusion:*** In the context of cardiometabolic health, aging, and disease, these results highlight key diet-derived metabolites that are central to specific rate-limited processes that are linked to cardiometabolic health. These metabolites include acetate and diacylglycerol, pertinent to blood pressure and triglycerides, respectively, as well as diacylglycerol and HDL-cholesterol.

Introduction

Obesity and dyslipidemias develop from metabolic imbalance, and these conditions exacerbate the risk of cardiovascular disease (CVD) incidence. Many factors affect the consumption-expenditure equation, including physical inactivity, which can lower mitochondrial counts per cell [1], thereby altering metabolic capacity. Human aging is associated with reduced mitochondrial function in many tissues [2]. Additionally, metabolic imbalance can promote oxidative stress, which, for example, has been proposed as a unifying factor in hypertension, where activated NADPH oxidases (nicotinamide adenine dinucleotide phosphate oxidases) generate reactive oxygen species, leading to endoplasmic reticulum and mitochondrial stress [3]. Furthermore, the narrowing of phenotypic resilience in older individuals compounds metabolic imbalances, especially those disparities that arise from a sub-optimal diet. The manifestations of this imbalance are many, and one common metric is metabolic syndrome (MetS), a condition based on at-risk levels of plasma triglycerides (TG), HDL-cholesterol (HDL-C), glucose, blood pressure (BP), and waist circumference (WC) [4].

Nutrition, naturally, is a primary influencer of metabolic processes as food is the source of the molecular components, cofactors, coenzymes and prosthetic groups of metabolism and metabolic enzymes [5]. Yet, a sub-optimal diet and regular postprandial metabolic imbalances contribute to cardiometabolic dysfunction and disease, which include the clinical and anthropometrical parameters of MetS, body mass index, plasma LDL-cholesterol (LDL-C), acetylated hemoglobin, insulin resistance, and plasma adiponectin. Characteristics of such sub-optimal diets include excess calories [6, 7], a Western-type dietary pattern [8], low carbohydrate quality [9], and higher saturated to polyunsaturated fat ratios [10]. Similarly, the metabolic capacity and genetic architecture of the individual are important determinants of that individual's nutritional requirements and health span [11]. Regarding dietary carbohydrates, we demonstrated that the consequences on cardiometabolic health stemming from the dietary fat-carbohydrate balance can be traced in part to DNA methylation levels at loci regulating expression of carnitine palmitoyltransferase 1A (CPT1A) [12]. CPT1A, a rate-limiting enzyme (RLE), catalyzes the committed step in mitochondrial oxidation of long-chain fatty acids. Lastly, a nearly linear relationship exists between sodium intake and mean systolic and diastolic BP [13]. These examples and numerous others demonstrate that proper nutrition supports metabolic homeostasis through specific metabolic and transport processes.

Within a biochemical pathway, the forward reaction rate of certain reactions might be limited. Catalysis can be rate-limited for any of several reasons [14]. These include sub-optimal enzyme-substrate binding, small change in free energy of the substrate intermediate, allosteric or non-competitive inhibition of the RLE, or substrate concentration vastly less than K_M, the Michaelis constant. Also, an excess of the reaction's product or byproduct (delta-pH, for example) can temper reaction rates [5]. Importantly, RLEs are key components of metabolic processes that are themselves highly influenced by the diet. Together, these points suggest RLEs are central to metabolic disease. While certain RLEs individually have been linked to metabolic diseases from neonates to the elderly, to our knowledge, no comprehensive assessment of a large set of human RLEs has been performed. Thus, the objective of this study was to identify contributions by RLEs to cardiometabolic health and where compromised biophysical and biochemical functions can promote disease in adults.

Materials and Methods

Data on RLEs

A published set of RLEs for humans and other organisms [15] was not available at the website given in the publication but was acquired via the most recent archived version at www.archive.org, version May 23, 2019. Data extracted from individual gene pages hosted by the (US) National Center for Biotechnology Information for use in this work consists of gene symbol, Entrez gene identifier (ID), and Enzyme Commission (EC) number, for all human RLEs, across all tissues. The analyses draw from 380 unique human RLE proteins and were done to assess enrichment of the RLEs for various other gene sets (see below).

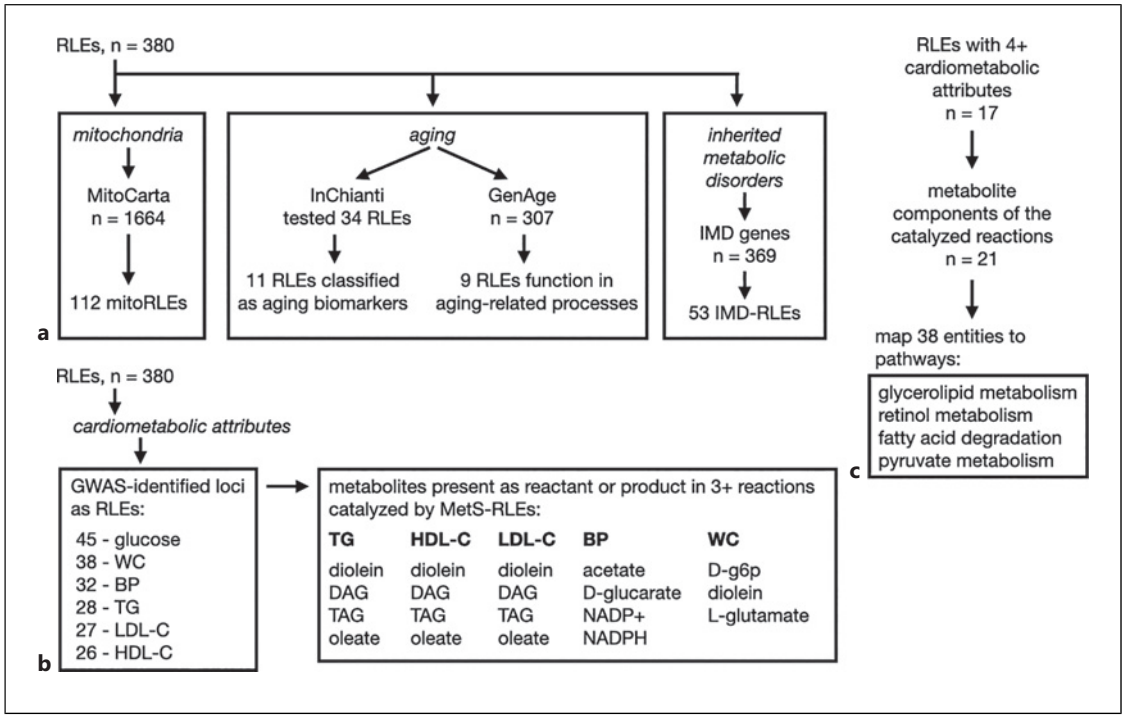

Fig. 1. Schematic of the various analyses performed. Boxed sections indicate principal results. **a** A set of 380 human RLEs was compared to various datasets for the following attributes: mitochondrial protein, aging biomarker or function in aging-related processes, and loci relevant to an inherited metabolic disorder. Full results are presented in online suppl. Table S1, with additional details of the IMD-RLE loci in Table 4. **b** Identification of RLEs relevant to primary cardiometabolic outcomes was attained by comparing the RLE set to loci cataloged from GWAS studies as associating with fasting glucose, WC, BP, TG, LDL-C, and HDL-C. For those loci that are an RLE and associate with one of the six outcomes, individual metabolites involved in three or more of the catalyzed reactions (i.e., different enzymes) are noted for each outcome. For RLE loci associating with fasting glucose, no metabolites were tallied from three or more different catalyzed reactions. These results are presented in online suppl. Table S1 (GWAS loci) and Table 2 (constituents of RLE-catalyzed reactions). D-g6p refers to D-glucosamine-6-phosphate. **c** RLE-centric pathways pertinent to CVD were identified by bundling the RLEs with four or more cardiometabolic attributes (see part **b** of this figure) and the metabolite components of their catalyzed reactions. These RLE proteins and small molecules were mapped to pathways. For example, ten of the combined set of 38 entities function in glycerolipid metabolism. See Table 3 for full results.

Other Protein and Gene Datasets

Mitochondrial proteins originated from predictions in MitoCarta2.0 [16], a set of 1,664 proteins. For a set of human proteins whose expression is significantly altered by age, we used published results from the recent InChianti study and its analysis of the plasma proteome to identify biomarkers of aging [17]. We used the 651 proteins that showed statistically significant changes, per data in Figure 1, source data 1. Of various definitions of MetS [4], we used the criteria of the National Cholesterol Education Program, Adult Treatment Panel (NCEP ATP) III of 2005. The genome-wide association study (GWAS) catalog data [18] were retrieved in September 2020. This resource catalogs genetic associations between common genetic variants and phenotypes pertinent to common diseases and physical characteristics. Genes for which certain variations are known to be the cause of specific inherited metabolic disorders (IMD, or inborn errors of metabolism) were assembled into a single list. This disparate set of genes and their related genetic-based diseases were derived from a multitude of published and online sources.

Outcome Variables

The focus of this work is on the five outcomes that constitute the MetS criteria, any three of which define the syndrome: plasma TG, plasma HDL-C, glucose, BP, and WC. To these five outcomes, we added plasma LDL-C. These six measures relevant to cardiometabolic health and their associated loci were mined from the downloaded GWAS catalog data.

Catalyzed Reactions and Small Molecule Enrichment

For each reaction catalyzed by an RLE (n = 1,899 different biochemical reactions), the reactant and product entities were extracted from the Enzyme database at Expasy (https://enzyme.expasy.org) [19] in September 2020. Using the Enzyme database, which is an extensive resource on enzyme nomenclature and descriptions, including reactants and products, an RLE was linked to each small molecule of the catalyzed reaction. For each small molecule entity, occurrences were tabulated for the entire RLE set and for the subset of RLEs linked to each cardiometabolic outcome in the GWAS data. Small molecules whose counts were between 3

and 30 inclusive [15, 20] were evaluated for enrichment in the phenotype subgroup. Proteins and metabolites were mapped to KEGG biochemical and disease pathways [21]. It is notable that the RLE set gives a large set of small molecules in this regard, unlike most gene or protein datasets and their broad spectrum of functions, many with no assigned EC number and no cataloged interactions with small molecules.

Statistical Tests

Statistical significance of differences between groups was assessed by χ^2 and Z-score measures with standard formulas in R. Enrichment assessment of the RLEs for attributes represented by other datasets was based on a human gene count of 20,000. Specific for the enrichment of small molecules, a Z-score was determined by comparing the ratio of occurrence as reactant or product in the subset to that of the whole [22]. Z-scores were converted to p values with the pnorm function in R: 2 × pnorm(-abs[Z]). A p value below 0.05 was interpreted as significant.

Results

A schematic of the analyses executed in this work is illustrated in Figure 1. To identify key metabolic pathways that influence cardiovascular health, we analyzed expression patterns in the mitochondrion and in the blood with regard to aging of all known RLEs [15], plus their reaction products, for associations with known drivers of CVD. Exactly 380 RLEs extracted from RLEdb [15] were assessed for membership in different protein and gene sets relevant to nutrition, aging, and cardiometabolic health. These datasets included mitochondrial proteins, proteins whose levels in the blood change significantly with age, loci identified by genetic association with MetS phenotypes, and genes implicated in inherited metabolic disorders. Results are summarized in Figure 2, with details in the following sections. All datasets were comprised of human data. Some 162 RLEs were not observed in any of the analyzed datasets. Fully tabulated results are presented in online supplementary Table S1 (for all online suppl. material, see https://doi.org/10.1159/000531350).

Mitochondrion

CPT1A is an RLE [15] that acts in the mitochondria to catalyze the committed step to oxidation of long-chain fatty acids. We theorized that other RLEs also are mitochondrial proteins. Comparing the 380 RLEs to the collection of 1,664 mitochondrial proteins we extracted from MitoCarta2.0 [16] indicated that 112 RLEs function in this organelle. This is a significant 3.5-fold overabundance of mitoRLEs, $p < 1 \times 10^{-6}$ (χ^2).

Aging

Age is a contributing factor to both cardiometabolic disease incidence and the phenotypic resilience that supports health amid certain lifestyle insults like suboptimal diets and physical inactivity. Thus, we sought to determine which RLEs have been identified as having age-associated changes in protein expression. To accomplish this, we examined the InChianti plasma proteomics data [17] for co-occurrence of RLEs. Of 34 RLEs tested in InChianti for being an aging biomarker, 11 were classified as aging biomarkers, which is somewhat less than the expected number of 17 ($p = 0.037$, Z-score), based on 651 aging biomarkers identified from 1,301 proteins assayed [17]. Although the relatively low number of RLEs tested for aging biomarker status could be a consequence of the high number of mitoRLEs and low abundance in plasma, we examined the direction of expression change with aging. We observed no striking difference in the ratio of upregulated to downregulated proteins between the InChianti set and the 11 aging RLEs. Whereas the distribution of downregulated to upregulated expression in the InChianti set showed 145–506 proteins, the aging RLEs were 3–8 (Table 1). These two distributions are not statistically significantly distinct.

Only F2, encoding prothrombin and downregulated with aging, has genetic association data to cardiometabolic outcomes: HDL-C and glucose. Similarly, only renin (REN), a component of the renin-angiotensin-aldosterone system and upregulated with aging, has genetic association evidence to the cardiometabolic outcome of BP.

Because so many RLEs are mitochondrial proteins and the InChianti aging biomarkers were assayed from plasma, which may be enriched for secreted proteins, we compared the RLEs to the GenAge set of genes related to human aging [23]. This is a set of 307 genes whose proteins function in diverse aging-related processes. Results were very similar in that just nine RLEs are found in the GenAge dataset, including F2/thrombin and phosphoenolpyruvate carboxykinase 1 (PCK1) linked to TG. Together, these observations indicate that RLEs in general and specifically those linked to cardiometabolic outcomes are rarely described as age-related. This suggests that expression of RLEs is tightly controlled and not a contributor to age-related decay of metabolic homeostasis.

Metabolic Syndrome

MetS can be defined as abnormal values of any three of the following five health parameters: blood levels of TG, HDL-C, and fasting glucose, BP, and WC [4]. To identify

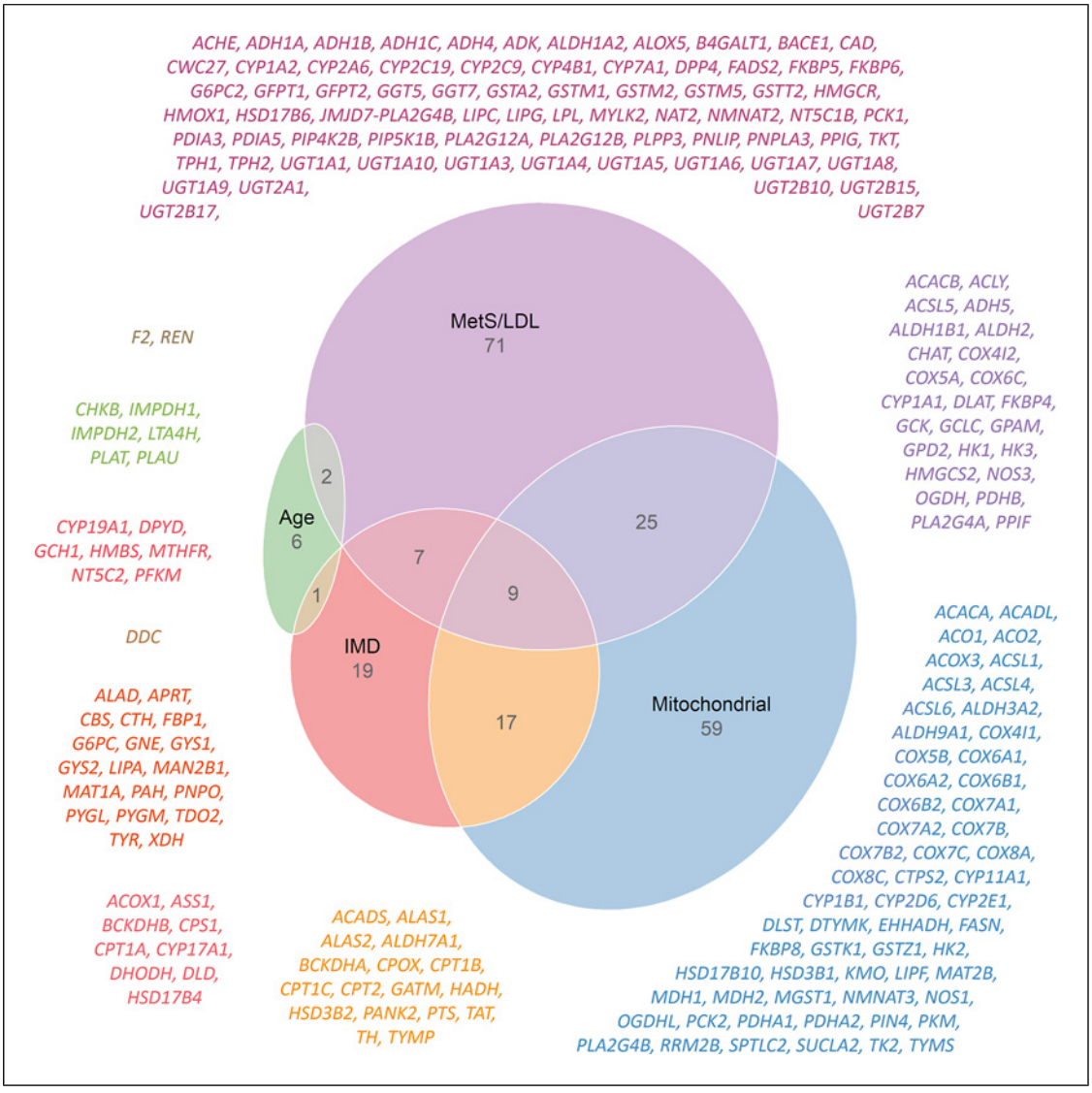

Fig. 2. RLEs are annotated with membership in various gene and protein sets relevant to cardiometabolic health. The numbers given correspond the number of RLEs in the overlap of depicted gene and protein datasets. Note that two RLEs shared by the mitochondrial and age groups are not depicted here; these are GSTP1 and PLA2G2A. IMD, inherited metabolic disorders; MetS/LDL, genes with genetic association data to any component of MetS or LDL-C.

Table 1. Human RLEs with age-associated protein levels in the blood

Age-related expression	RLEs
Downregulated	CHKB, DDC, F2
Upregulated	GSTP1, IMPDH1, IMPDH2, LTA4H, PLA2G2A, PLAT, PLAU, REN

RLEs additionally identified in GenAge include F2, GCLC, GSTA4, GSTP1, MT-CO1, PCK1, PIN1, PLAU, and PTGS2.

those RLEs with some purpose in MetS outcomes, we queried the extensive genetic association study data available in the GWAS catalog [18]. As LDL-C levels trend upward with age in both sexes, LDL-C joined the collection of MetS phenotypes we queried in the GWAS data.

Performing queries of the GWAS catalog data identified a number of RLEs as loci for MetS components plus LDL-C: TG – 28, HDL-C – 26, LDL-C – 27, BP – 32, WC – 38, and glucose – 45 (online suppl. Table S1). There were 114 RLE loci that had GWAS association data with any component of MetS plus LDL-C and 52 RLE loci with GWAS association data for two or more of these outcomes. *ALDH1A2* (aldehyde dehydrogenase 1A2) and *LPL* (lipoprotein lipase) had GWAS data for all six cardiometabolic outcomes. *ADH1B* (alcohol dehydrogenase 1B) and *LIPC* (hepatic lipase) had GWAS data for all but one of these six outcomes, except TG for *ADH1B* and BP for *LIPC*. Notable loci include *LIPG* (endothelial lipase), *LPL*, and *PNPLA3* (patatin-like phospholipase domain containing 3), which all associate with TG and HDL and whose encoded enzymes catalyze reactions involving identical molecules.

Metabolites in RLE-Catalyzed Reactions

Metabolomics profiling has seen extensive development recently, and metabolomics data analysis has become an effective tool to discern mechanisms of genetic and epigenetic associations [24, 25]. Hence, we sought to identify relationships between the metabolites utilized by RLEs and the six tested cardiometabolic attributes from the genetic association data. Of 380 RLEs, 375 have reactant and product information [19]. This makes it feasible to query the biochemical reactions catalyzed by all RLEs with any molecule to assess enrichment in a specific RLE phenotype subset. Thus, for those GWAS-identified RLEs associating with each of the six cardiometabolic outcomes, we determined for any reactant and product molecules present in three or more reactions driven by the GWAS-identified RLEs those molecules that were significantly overrepresented. Results for each phenotype are presented in Table 2.

Strong relationships among TG, HDL-C, and LDL-C are known, and for the small molecules overrepresented in the reactions catalyzed by RLEs associating with these lipid outcomes, our results uphold these relationships. The most significantly overrepresented small molecules were diolein (dioleoylglycerol), diacylglycerol, triacylglycerol, and oleate, used by RLEs LIPC (hepatic lipase), LIPG, LPL, and PNPLA3. These are the gatekeepers of biochemical reactions whose outputs are central to the three lipid phenotypes examined, which are diagnostic of cardiometabolic health. That these three RLEs share associations with TG and other lipids and use the same biochemical components underscores a unique aspect of examining RLEs in the context of cardiometabolic disease and the response to certain dietary interventions.

For BP, the most significantly overrepresented small molecules were acetate (p = 0.00023), D-glucarate (p = 0.011), and coenzymes NADP+ and NADPH (nicotinamide adenine dinucleotide phosphate; p = 0.0091). Regarding acetate, six enzyme reactions within the entire RLE set involve acetate, and three of these are associated with BP: ACHE (acetylcholinesterase), ALDH2 (aldehyde dehydrogenase 2), and CYP17A1 (cytochrome P450 17A1). These enzymes operate in pathways that exert effects on BP: cholinergic synapse, glycerophospholipid metabolism, glycolysis/gluconeogenesis, fatty acid degradation, and cortisol synthesis and secretion. For WC, significantly overrepresented small molecules included D-glucosamine-6-phosphate (p = 0.00018), diolein (p = 0.00018), and L-glutamate (p = 0.0022). Enzymes GFPT1 (glutamine-fructose-6-phosphate transaminase 1), GFPT2, and HK3 (hexokinase 3) use D-glucosamine-6-phosphate. Diolein is used by the lipases LIPC, LPL, and PNLIP (pancreatic lipase). In contrast, L-glutamate is used by GCLC (glutamate-cysteine ligase), the two glutamine-fructose-6-phosphate transaminases GFPT1 and GFPT2, and two gamma-glutamyltransferases GGT5 and GGT7. The enzymes catalyzing reactions that involve D-glucosamine-6-phosphate, diolein, and L-glutamate and whose genes harbor alleles that associate with WC operate in several different pathways (Table 2). These pathways include fat digestion and absorption, insulin resistance, and glutathione metabolism.

For the outcome of blood glucose, the top overrepresented small molecule present in reactions catalyzed by at least three RLEs was D-glucose, an enrichment that was not significant (p = 0.072). Reported GWAS signals for blood glucose include RLEs B4GALT1 (beta-1,4-galactosyltransferase 1), G6PC2 (glucose-6-phosphatase catalytic subunit 2), and GCK (glucokinase), all of which catalyze reactions utilizing D-glucose.

A set of pathways relevant to CVD was identified by combining those RLEs with four or more cardiometabolic attributes (n = 17) and the metabolites of the reactions catalyzed by those enzyme EC numbers (n = 21), and then mapping the combined set to biochemical and disease pathways. For example, ten entities consisting of RLEs

Table 2. Small molecule components of RLE-catalyzed reactions overrepresented in MetS genetic associations

Phenotype	Small molecule	RLE counts (in phenotype group/in all RLEs)	Z-score	*p* value	RLE symbols	Key RLE pathways*
TG	Diolein	3/5	4.53	5.94E-06	LIPC, LPL, PNPLA3	PG1: cholesterol metabolism, glycerolipid metabolism
TG	Diacylglycerol	4/10	4.00	6.40E-05	LIPC, LIPG, LPL, PNPLA3	PG2: cholesterol metabolism, glycerolipid metabolism
TG	Triacylglycerol	4/10	4.00	6.40E-05	LIPC, LIPG, LPL, PNPLA3	*Refer to PG2 at TG-diacylglycerol*
TG	A carboxylate	7/30	3.48	4.96E-04	ALDH2, LIPC, LIPG, LPL, PLA2G12A, PLA2G12B, PNPLA3	Cholesterol metabolism, fatty acid degradation, glycerolipid metabolism, glycerophospholipid metabolism
TG	Oleate	3/9	3.01	2.58E-03	LIPC, LPL, PNPLA3	*Refer to PG1 at TG-diolein*
HDL	Diolein	3/5	4.73	2.21E-06	LIPC, LPL, PNPLA3	*Refer to PG1 at TG-diolein*
HDL	Diacylglycerol	4/10	4.30	2.63E-05	LIPC, LIPG, LPL, PNPLA3	*Refer to PG2 at TG-diacylglycerol*
HDL	Triacylglycerol	4/10	4.20	2.63E-05	LIPC, LIPG, LPL, PNPLA3	*Refer to PG2 at TG-diacylglycerol*
HDL	A ketone	3/7	3.80	1.42E-04	ADH1B, ADH1C, ADH5	PG3: fatty acid degradation, retinol metabolism
HDL	A secondary alcohol	3/7	3.80	1.42E-04	ADH1B, ADH1C, ADH5	*Refer to PG3 at HDL-ketone*
HDL	An alcohol	3/7	3.80	1.42E-04	ADH1B, ADH1C, ADH5	*Refer to PG3 at HDL-ketone*
HDL	Ethanol	3/8	3.47	5.27E-04	ADH1B, ADH1C, ADH5	*Refer to PG3 at HDL-ketone*
HDL	Oleate	3/9	3.18	1.46E-03	LIPC, LPL, PNPLA3	*Refer to PG1 at TG-diolein*
HDL	A carboxylate	6/30	2.97	2.97E-03	LIPC, LIPG, LPL, PLA2G12A, PLA2G12B, PNPLA3	Cholesterol metabolism, fat digestion and absorption, glycerolipid metabolism, glycerophospholipid metabolism
HDL	R-S-glutathione	4/20	2.39	1.68E-02	GSTA2, GSTM1, GSTM2, GSTM5	PG4: glutathione metabolism
HDL	An aldehyde	3/14	2.20	2.78E-02	ADH1B, ADH1C, ADH5	*Refer to PG3 at HDL-ketone*
HDL	Glutathione	4/25	1.87	6.09E-02	GSTA2, GSTM1, GSTM2, GSTM5	*Refer to PG4 at R-S-glutathione*
LDL	Diacylglycerol	3/10	2.85	4.34E-03	LIPC, LIPG, LPL	PG5: cholesterol metabolism, glycerolipid metabolism
LDL	Triacylglycerol	3/10	2.85	4.34E-03	LIPC, LIPG, LPL	*Refer to PG5 at LDL*
BP	Acetate	3/6	3.69	2.23E-04	ACHE, ALDH2, CYP17A1	Cholinergic synapse, cortisol synthesis and secretion
BP	NADP+	5/21	2.61	9.08E-03	CYP2C19#, CYP2C9, MTHFR, NOS3	PG6: nitric oxide synthesis, one carbon pool by folate, retinol metabolism, serotonergic synapse
BP	NADPH	5/21	2.61	9.08E-03	CYP2C19#, CYP2C9, MTHFR, NOS3	*Refer to PG6 at BP-NADP+*

Table 2 (continued)

Phenotype	Small molecule	RLE counts (in phenotype group/in all RLEs)	Z-score	*p* value	RLE symbols	Key RLE pathways*
BP	An aldehyde	3/14	1.78	7.45E-02	ADH1B, ALDH1B1, ALDH2	Retinol metabolism
BP	[Oxidized NADPH--hemoprotein reductase]	5/29	1.78	7.55E-02	CYP17A1, CYP1A1, CYP1A2, CYP2C19, CYP2C9	PG7: caffeine metabolism, cortisol synthesis and secretion, retinol metabolism, serotonergic synapse
BP	[Reduced NADPH--hemoprotein reductase]	5/29	1.78	7.55E-02	CYP17A1, CYP1A1, CYP1A2, CYP2C19, CYP2C9	*Refer to PG7 at BP-[oxidized NADPH--hemoprotein reductase]*
BP	A carboxylate	5/30	1.69	9.05E-02	ALDH1B1, ALDH2, LIPG, LPL, PLA2G12B	Vascular smooth muscle contraction
WC	D-glucosamine-6-phosphate	3/5	3.75	1.79E-04	GFPT1, GFPT2, HK3	PG8: fructose and mannose metabolism, glycolysis/gluconeogenesis, insulin resistance, insulin signaling pathway
WC	Diolein	3/5	3.75	1.79E-04	LIPC, LPL, PNLIP	PG9: cholesterol metabolism, fat digestion and absorption, glycerolipid metabolism, PPAR signaling pathway
WC	L-glutamate	5/15	3.07	2.14E-03	GCLC, GFPT1, GFPT2, GGT5, GGT7	Arachidonic acid metabolism, glutathione metabolism, insulin resistance
WC	Oleate	3/9	2.36	1.84E-02	LIPC, LPL, PNLIP	*Refer to PG9 at waist-diolein*
WC	D-fructose-6-phosphate	3/10	2.13	3.29E-02	GFPT1, GFPT2, HK3	*Refer to PG8 at waist-D-glucosamine-6-phosphate*
WC	Diacylglycerol	3/10	2.13	3.29E-02	LIPC, LPL, PNLIP	*Refer to PG9 at waist-diolein*
WC	Triacylglycerol	3/10	2.13	3.29E-02	LIPC, LPL, PNLIP	*Refer to PG9 at waist-diolein*
Glucose	D-glucose	3/10	1.80	7.21E-02	B4GALT1, G6PC2, GCK	Glucagon signaling pathway, glycolysis/gluconeogenesis, insulin signaling pathway

*Pathway groups (PG) are numbered for better legibility of the table for identical sets of RLEs. #RLE participates in reactions defined by two different EC numbers.

GPAM (glycerol-3-phosphate acyltransferase, mitochondrial), LIPC, LIPG, and LPL, plus metabolites acetate, D-glucose, diacylglycerol, diolein, ethanol, and NADP+, all function in glycerolipid metabolism. Within the retinol metabolism pathway, RLEs ADH1B and ALDH1A2, plus metabolites ethanol, NADP+, and oxidized NADPH-hemoprotein reductase, can be found. A list of the top pathways is presented in Table 3. These pathways, populated by RLEs and metabolites involved in those EC-catalyzed reactions, highlight biochemical processes where bottlenecks in pathway flux are relevant to CVD and related outcomes.

Inherited Metabolic Disorders

An inherited metabolic disorder is typically caused by a deleterious variation in a gene encoding a metabolic enzyme, resulting in accumulation of compounds that are toxic at elevated levels or compromised biosynthesis of vital compounds. Most newborns in the USA are tested for 30–35 core IMDs [26] or 25 in Norway [27]. Many of these potentially fatal cases are treated by modifying the diet coupled with close monitoring. Thus, at least some IMDs represent a certain class of gene-diet interaction, where diet customization overcomes, to a degree, a severe deficiency

Table 3. Pathways relevant to cardiometabolic diseases identified from RLEs having several cardiometabolic attributes and the metabolites involved in the catalyzed reactions

Pathway	RLEs	Metabolites
Glycerolipid metabolism	GPAM, LIPC, LIPG, LPL	Acetate, D-glucose, diacylglycerol, dioxin, ethanol, NADP+
Glycolysis/gluconeogenesis	ADH1B, GCK	Acetate, D-fructose-6-phosphate, D-glucosamine-6-phosphate, D-glucose, ethanol, NADP+
Fatty acid degradation	ACOX1, ADH1B, CPT1A	Acetate, ethanol, NADP+, [oxidized NADPH--hemoprotein reductase]
Steroid hormone biosynthesis	CPS1, CYP17A1, CYP19A1	Acetate, NADP+, [oxidized NADPH--hemoprotein reductase]
Beta-alanine metabolism	ACOX1	Acetate, ethanol, L-glutamate, NADP+
Drug metabolism – cytochrome P450	ADH1B	Glutathione, NADP+*, [oxidized NADPH--hemoprotein reductase]
Galactose metabolism	GCK	D-fructose-6-phosphate, D-glucosamine-6-phosphate, D-glucose, NADP+
Metabolism of xenobiotics by cytochrome P450	ADH1B	Ethanol, glutathione, NADP+, [oxidized NADPH--hemoprotein reductase]
Pyruvate metabolism	ADH1B	Acetate, ethanol*, NADP+
Retinol metabolism	ADH1B, ALDH1A2	Ethanol, NADP+, [oxidized NADPH--hemoprotein reductase]

*Metabolite participates in reactions defined by two different EC numbers.

in a specific enzyme. With this in mind, we wished to see if IMDs also are RLEs, as this would offer some indication of how tolerant RLE genes are to severe variation. We compared our collection of 369 IMD genes gathered from various sources with the 380 RLE genes. We observed 53 genes in common, and these fall into 13 different IMD-based disease classes (Table 4). The classes with the greatest representation by RLEs were disorders of amino acid metabolism (11 RLEs, with 3 involved in phenylketonuria [phenylalanine, GCH1, PAH, and PTS, respectively, GTP cyclohydrolase 1, phenylalanine hydroxylase, and 6-pyruvoyltetrahydropterin synthase] and 2 related to methionine [CTH and MAT1A, respectively, cystathionine gamma-lyase and methionine adenosyltransferase 1A]), disorders of fatty acid oxidation and mitochondrial metabolism (9, with 4 related to carnitine [CPT1A, CPT1B, CPT1C, and CPT2, respectively, carnitine palmitoyltransferases 1A, 1B, 1C, and 2]), and disorders of carbohydrate metabolism (7, with 6 involved in glycogen storage diseases). Of the 4 RLEs involved in metabolic disorders of vitamins, coenzymes, and cofactors, 3 relate to tetrahydrobiopterin deficiency: GCH1, MTHFR (methylenetetrahydrofolate reductase), and PTS.

Summary

Comparisons to various datasets showed that of 380 RLEs, 112 function in the mitochondria, 11 are classified as aging biomarkers in the InChianti dataset, and 53 are loci of interest in inherited metabolic disorders. Using loci identified by GWAS as associating with any of six cardiometabolic outcomes, we identified a number of RLEs related to these clinically relevant outcomes: 45 for glucose, 38 for WC, 32 for BP, 28 for TG, 27 for LDL-C, and 26 for HDL-C. All these results are presented in online suppl. Table S1. Combining RLEs associated with greater than three cardiometabolic attributes with the components of those RLE-catalyzed reactions call attention to ten biochemical processes where restricted pathway flux is relevant to CVD, including glycerolipid metabolism, retinol metabolism, and fatty acid degradation (Table 3).

Discussion

Overview

From published reports and online databases, we collected sets of all known human RLEs and genes and proteins associated with cardiometabolic phenotypes to

Table 4. Genes ascribed to specific inherited metabolic disorders and identified as RLEs

Gene ID	RLE	Protein name	Disease
Disorders of amino acid metabolism			
1373	CPS1	Carbamoyl-phosphate synthase 1	Carbamoyl-phosphate synthetase I deficiency
875	CBS	Cystathionine beta-synthase	Homocystinuria
1738	DLD	Dihydrolipoamide dehydrogenase	Maple syrup urine disease
1491	CTH	Cystathionine gamma-lyase	Methionine
4143	MAT1A	Methionine adenosyltransferase 1A	Methionine
2643	GCH1	GTP cyclohydrolase 1	Phenylketonuria
5805	PTS	6-Pyruvoyltetrahydropterin synthase	Phenylketonuria
5053	PAH	Phenylalanine hydroxylase	Phenylketonuria
6999	TDO2	Tryptophan 2,3-dioxygenase	Tryptophan
7299	TYR	Tyrosinase	Tyrosine-melanin
6898	TAT	Tyrosine aminotransferase	Tyrosinemia
Disorders of carbohydrate metabolism			
2538	G6PC1	Glucose-6-phosphatase catalytic subunit 1	Glycogen storage disease
2997	GYS1	Glycogen synthase 1	Glycogen storage disease
2998	GYS2	Glycogen synthase 2	Glycogen storage disease
5213	PFKM	Phosphofructokinase, muscle	Glycogen storage disease
5836	PYGL	Glycogen phosphorylase L	Glycogen storage disease
5837	PYGM	Glycogen phosphorylase, muscle associated	Glycogen storage disease
2203	FBP1	Fructose-bisphosphatase 1	Hepatic fructose 1,6-biphosphate(FBPase) deficiency
Disorders of fatty acid oxidation and mitochondrial metabolism			
1374	CPT1A	Carnitine palmitoyltransferase 1A	Carnitine related
1375	CPT1B	Carnitine palmitoyltransferase 1B	Carnitine related
126129	CPT1C	Carnitine palmitoyltransferase 1C	Carnitine related
1376	CPT2	Carnitine palmitoyltransferase 2	Carnitine related
35	ACADS	Acyl-CoA dehydrogenase short chain	Coenzyme A dehydrogenase deficiencies
3033	HADH	Hydroxyacyl-CoA dehydrogenase	Coenzyme A dehydrogenase deficiencies
22978	NT5C2	5′-Nucleotidase, cytosolic II	Hereditary spastic paraplegia SPG45
126129	CPT1C	Carnitine palmitoyltransferase 1C	Hereditary spastic paraplegia SPG73
3988	LIPA	Lipase A, lysosomal acid type	Lipid storage
Disorders of organic acid metabolism (organic acidurias)			
593	BCKDHA	Branched chain keto acid dehydrogenase E1 subunit alpha	Maple syrup urine disease
594	BCKDHB	Branched chain keto acid dehydrogenase E1 subunit beta	Maple syrup urine disease
Disorders of peroxisomal function			
51	ACOX1	Acyl-CoA oxidase 1	Acyl-CoA oxidase deficiency
3295	HSD17B4	Hydroxysteroid 17-beta dehydrogenase 4	D-bifunctional protein deficiency

Table 4 (continued)

Gene ID	RLE	Protein name	Disease
Disorders of porphyrin metabolism			
1371	CPOX	Coproporphyrinogen oxidase	Coproporphyria, hereditary
210	ALAD	Aminolevulinate dehydratase	Porphyria, acute hepatic
3145	HMBS	Hydroxymethylbilane synthase	Porphyria, acute intermittent
212	ALAS2	5′-Aminolevulinate synthase 2	Pyridoxine-responsive sideroblastic anemia, X-linked
211	ALAS1	5′-Aminolevulinate synthase 1	Sideroblastic anemia, hereditary
Disorders of purine metabolism			
353	APRT	Adenine phosphoribosyltransferase	Adenine phosphoribosyltransferase deficiency
1890	TYMP	Thymidine phosphorylase	Mitochondrial neurogastrointestinal encephalopathy syndrome
7498	XDH	Xanthine dehydrogenase	Xanthinuria
Disorders of pyrimidine metabolism			
1806	DPYD	Dihydropyrimidine dehydrogenase	Dihydropyrimidine dehydrogenase deficiency
1723	DHODH	Dihydroorotate dehydrogenase	Miller syndrome
Disorders of steroid metabolism			
1586	CYP17A1	Cytochrome P450 family 17 subfamily A member 1	Adrenal hyperplasia, congenital
3284	HSD3B2	Hydroxy-delta-5-steroid dehydrogenase, 3 beta- and steroid delta-isomerase 2	Adrenal hyperplasia, congenital
1588	CYP19A1	Cytochrome P450 family 19 subfamily A member 1	Aromatase deficiency
1588	CYP19A1	Cytochrome P450 family 19 subfamily A member 1	Aromatase excess
1586	CYP17A1	Cytochrome P450 family 17 subfamily A member 1	Combined 17alpha-hydroxylase/17,20-lyase deficiency
Lysosomal storage diseases			
10020	GNE	Glucosamine (UDP-N-acetyl)-2-epimerase/ N-acetylmannosamine kinase	French type sialuria
4125	MAN2B1	Mannosidase alpha class 2B member 1	α-Mannosidosis (type I and II)
Metabolic disorders of vitamins, coenzymes, and cofactors			
80025	PANK2	Pantothenate kinase 2	Pantothenate kinase-associated neurodegeneration
2643	GCH1	GTP cyclohydrolase 1	Tetrahydrobiopterin deficiency
4524	MTHFR	Methylenetetrahydrofolate reductase	Tetrahydrobiopterin deficiency
5805	PTS	6-Pyruvoyltetrahydropterin synthase	Tetrahydrobiopterin deficiency
Neurometabolic defects			
501	ALDH7A1	Aldehyde dehydrogenase 7 family member A1	AASA dehydrogenase deficiency (antiquitin)
2628	GATM	Glycine amidinotransferase	Arginine:glycine amidinotransferase deficiency
1644	DDC	Dopa decarboxylase	Aromatic L-amino acid decarboxylase deficiency (AADC)

Table 4 (continued)

Gene ID	RLE	Protein name	Disease
55163	PNPO	Pyridoxamine 5′-phosphate oxidase	Pyridoxamine 5′-phosphate oxidase deficiency (PNPO)
7054	TH	Tyrosine hydroxylase	Tyrosine hydroxylase deficiency
Urea cycle disorder or urea cycle defects			
445	ASS1	Argininosuccinate synthase 1	Citrullinemia
1373	CPS1	Carbamoyl-phosphate synthase 1	Urea cycle/hyperammonemia

assess the relevance of RLEs to aging and CVD. We observed that there is extensive, moderate, and minimal concurrence, respectively, with mitochondrial proteins, inherited metabolic disorders, and expression profiles indicative of aging. For 114 of the 380 RLE loci, there is cataloged evidence of genetic association with at least one outcome that constitutes MetS or LDL-C, indicating considerable relevance to CVD. Using data from the reactions catalyzed by RLEs linked to MetS, we identified several small molecules relevant to specific MetS outcomes. This work adds the dimension of reaction kinetics to the processes by which certain genes and their encoded proteins contribute to cardiometabolic health and the age-related diseases of the cardiovascular system.

Mitochondria, IMDs, and Aging

That there is such overrepresentation of mitochondrial proteins among RLEs is not surprising. The source RLE dataset [15] was identified from important, well-documented metabolic processes, many of which are mitochondrial. Similarly, it is logical that many IMDs trace to enzymes that are inherent limiters of a particular metabolic flux, as variation in these can be expected to have large phenotypic effects. This is precisely what we observed: 53 of 369 IMD loci encode an RLE. Yet, evaluating the full extent of the overlap between RLEs and IMD genes is difficult because a complete IMD set has not been established [28]. In parallel, we tabulated a moderately significant reduction in RLEs exhibiting age-related altered expression in blood versus the expected (11 observed, 17 expected). This implies tight control of the availability and expression of these gatekeeper enzymes into old age. Furthermore, this observation suggests that survival to very old age requires consistent metabolic homeostasis, while age-related decline in metabolic homeostasis that eventually manifests as cardiometabolic disease occurs at genes and proteins more accepting of variation in expression. This is supported by the observation that only two InChianti aging proteins are found in the GWAS data for MetS outcomes.

GWAS RLEs

GWAS are a ubiquitous research vehicle to identify common genetic variants linked to any within a wide spectrum of phenotypes [28]. We believe our observations that genes encoding 114 different RLEs also carry variants that associate with any of five MetS-defining outcomes plus LDL-C contribute to characterizing the mechanism of the observed association in two ways. One, as the associating locus encodes an RLE, any explanation of how the candidate gene and its variants affect the phenotype must consider reaction kinetics and bottlenecks in pathway flux. Two, biochemical entities that are constituents of the catalyzed reactions accentuate key metabolites that could affect the genotype-phenotype relationship, with potential exploratory tests of gene-by-environment interactions.

Noteworthy Genes

Of many genes and metabolites presented here that warrant emphasis, focus is drawn on a select few. Carnitine palmitoyltransferase CPT1A catalysis in mitochondria supports a homeostatic response in the liver to nutritional modulators [12, 29, 30]. Our survey of RLEs cataloged connections between CPT1A and carnitine-related disorders of fatty acid oxidation, an inherited metabolic disorder, and genetic associations with TG and HDL-C. Variation in DNA methylation at *CPT1A* has been associated with *CPT1A* mRNA expression, plasma TG and glucose levels, and body mass index [12].

Of nine aldehyde dehydrogenases in the RLE dataset, ALDH1A2, which synthesizes retinoic acid (RA) from

retinaldehyde, has at least twice as many cardiometabolic attributes as any of the other eight. GWAS data for *ALDH1A2* show associations with all six cardiometabolic outcomes assessed here. Dose-dependent RA effects on triacylglycerol levels in 3T3-L1 preadipocytes have been observed [31]. Replacing dietary saturated fatty acids with polyunsaturated fatty acids showed a similar association between RA and HDL-C, LDL-C, and other cholesterol moieties [32]. Thus, fine-tuning of RA levels could be one important mechanism by which to manage blood lipids and adipogenesis in dyslipidemic individuals.

Like ALDH1A2, the gene encoding LPL, the rate-limiting lipase in catabolism of plasma TGs, shows associations with all six cardiometabolic outcomes. *LPL* variants that are associated with lower TG are also associated with reduced risk of coronary heart disease [33]. LPL also appears to have effects on weight gain in response to high-fat or hypercaloric diets via insulin signaling and glucose homeostasis [34]. Taken together, these vignettes exemplify that RLEs with genetic associations to cardiometabolic outcomes have important, central functions in metabolic homeostasis, CVD, and response to diet.

Noteworthy Metabolites

Acetate, the shortest of short-chain fatty acids, is derived from gut bacteria, foods such as cheese and processed meats, and alcohol, or via de novo synthesis from pyruvate [35]. A sizable body of literature links BP and acetate, for example [36]. However, the acetate-BP link as one that involves RLEs is rarely invoked. We observed that RLE genes genetically associated with BP showed an enrichment of catalyzed reactions yielding acetate. This suggests that acetate, as an energy source and precursor to acetyl-CoA, and the affiliated reactions might participate in BP control with certain sensitivity pertinent to enzyme kinetics.

Our data imply that NADP+ and NADPH are linked to BP via RLEs CYP2C9, CYP2C19 (cytochrome P450 family 2 subfamily C member 9, and member 19), MTHFR, and NOS3 (nitric oxide synthase 3), involved in nitric oxide synthesis, the one carbon-folate pool, retinol metabolism, and the serotonergic synapse. Much is known about these processes and BP, such as links between vitamin A deficiency and the renin-angiotensin system [37]. Several natural phenolic compounds have been shown to inhibit reactive oxygen species-derived NADPH oxidase and modulate BP [38]. In addition, much has been written on the bioactivity and genetic associations that link MTHFR and NOS3 to BP.

Several RLE lipase genes showed associations with both plasma TG and HDL-C. These lipases catalyze reactions that use di- and triacylglycerols, important elements of TG-rich lipoprotein and cholesterol particles. Substitution of dietary triacylglycerol for diacylglycerol was shown to improve blood lipid profiles in humans [39]. That these metabolites are components of the RLE-catalyzed reaction suggests the need to account for reaction kinetics and the potential impact of even slight changes in gene expression and enzyme function.

Merging the RLEs linked to each cardiometabolic outcome and the metabolites present in any reaction with that RLE's EC number and then mapping the combined set of proteins and metabolites onto pathways identified prime CVD-relevant processes. This demonstrated the importance of glycerolipid metabolism. The combined set of RLEs and metabolites that highlighted glycerolipid metabolism contained lipases and diacylglycerol, and the associations of these entities to blood lipid outcomes make this an expected finding. However, the presence of acetate and NADP+ in this pathway and their connections to BP (Table 2) suggest new considerations that link lipases LIPG and LPL to risk of hypertension. Similarly, the other identified pathways should be explored in ways that take into account the enzymes and metabolites that participate in rate-limited reactions.

As these gatekeeper enzymes regulate flux through their pathways, it is of keen interest to know if that regulation is allele-specific, especially as data mining revealed numerous associations with determinants of MetS. Such tests for allele-specific regulatory activity of the RLE will be guided by the strong links between nutrition and metabolism, followed by assessing if RLE-gene variation supports gene-environment interactions. An inspection of CardioGxE, a catalog of genetic associations for cardiometabolic outcomes that are modified by various lifestyle factors [40], shows that over 25 RLEs have documented allele-specific interactions with diet, physical activity, and other lifestyle factors on cardiometabolic phenotypes.

Limitations

The results and interpretation of the work presented herein are limited in a number of ways. First, because ongoing and future research may add to the protein, gene, and catalyzed reaction datasets we analyzed, some of our reported outcomes will be affected. Second, 162 RLEs have no relationship with the datasets examined in this work; these proteins may have roles in other diseases. Third, we tested for overrepresentation of small molecule reactants and products of the RLEs that associate with various MetS outcomes, but we did not thoroughly examine cofactors such as metal cations and heme. Fourth, we did not evaluate RLE-gene expression and protein degradation rates in specific tissues.

Exploratory analysis suggests that RLEs with any association with MetS plus LDL-C outcomes, and with BP in particular, are significantly enriched for elevated protein turnover rates, which may expose cells to damaging oxidized molecules and a higher energy demand [41].

Conclusion

Our survey of RLEs and their involvement in cardiometabolic health and disease has shown that, for certain genes and enzymes, aspects of rate-limiting kinetics are an important attribute. Specific attributes of human RLEs that are relevant to cardiometabolic phenotypes include that many function in the mitochondria, some are relevant to inherited metabolic disorders, but very few can be described as aging biomarkers. We then proceeded to establish links between RLE genes, the molecular components of their catalyzed reactions, and pathways relevant to CVD. We first mined GWAS data to identify that several loci encoding RLEs are relevant to principal cardiometabolic outcomes, including *ALDH1A2*, *CPT1A*, *CYP2C9*, *CYP2C19*, *LPL*, *MTHFR*, and *NOS3* plus others. We next examined the molecular components of the reactions catalyzed by those RLEs, which identified acetate, di- and triacylglycerols, D-glucosamine-6-phosphate, L-glutamate, NADP+, and NADPH, among others as important metabolites. Lastly, we combined genes and metabolites to identify CVD-relevant pathways. Regarding those pathways, RLEs involved in glycerolipid metabolism are linked to blood lipids, with interesting connections made between acetate, NADP+, LIPG, LPL and risk of hypertension. Altogether, RLE is a useful label to interpret genetic association data encompassing cardiometabolic outcomes, especially when components of the catalyzed reactions are derived from the diet.

Statement of Ethics

This study did not use primary data from individual human study participants or any primary data from animal studies. The results of this study are derived from the reuse of published datasets that are available in an unrestricted manner. Specific datasets include published RLEs [15], predicted mitochondrial proteins [16], published sets of protein biomarkers of aging [17, 23], and genome-wide association study data in the GWAS catalog [18]. Furthermore, no human study participants were recruited for this project. As such, this work does not require ethics approval.

Conflict of Interest Statement

J.J.C. has received research grants and/or personal fees from Mills DA and Amgen, unrelated to the content of this manuscript. All other authors have declared no conflict of interest.

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Author Contributions

L.D.P. conceived the study, established the analyses, and wrote the manuscript. A.W.B., J.J.C., and C.D.W. contributed to the design of analyses. L.D.P., K.S.M., and A.W.B. collected and analyzed the data. L.D.P., K.S.M., A.W.B., C.E.S., C.-Q.L., J.J.C., C.D.W., and J.M.O. interpreted the results and critically reviewed the manuscript.

Data Availability Statement

All results generated from this study are available in online suppl. Table S1. This includes the RLEs that share attributes with any of the datasets described in the Materials and Methods and Results sections. Further inquiries can be directed to the corresponding author.

References

1 Manfredi TG, Fielding RA, O'Reilly KP, Meredith CN, Lee HY, Evans WJ. Plasma creatine kinase activity and exercise-induced muscle damage in older men. Med Sci Sports Exerc. 1991;23(9):1028–34.

2 Kauppila TES, Kauppila JHK, Larsson NG. Mammalian mitochondria and aging: an update. Cell Metab. 2017;25(1):57–71.

3 Touyz RM, Rios FJ, Alves-Lopes R, Neves KB, Camargo LL, Montezano AC. Oxidative stress: a unifying paradigm in hypertension. Can J Cardiol. 2020;36(5):659–70.

4 Huang PL. A comprehensive definition for metabolic syndrome. Dis Model Mech. 2009;2(5–6):231–7.

5 Robinson PK. Enzymes: principles and biotechnological applications. Essays Biochem. 2015;59:1–41.
6 Schrauwen P, Westerterp KR. The role of high-fat diets and physical activity in the regulation of body weight. Br J Nutr. 2000;84(4):417–27.
7 Saklayen MG. The global epidemic of the metabolic syndrome. Curr Hypertens Rep. 2018;20(2):12.
8 van Dam RM, Rimm EB, Willett WC, Stampfer MJ, Hu FB. Dietary patterns and risk for type 2 diabetes mellitus in U.S. men. Ann Intern Med. 2002;136(3):201–9.
9 Zamanillo-Campos R, Chaplin A, Romaguera D, Abete I, Salas-Salvadó J, Martín V, et al. Longitudinal association of dietary carbohydrate quality with visceral fat deposition and other adiposity indicators. Clin Nutr. 2022;41(10):2264–74.
10 Larsen SV, Holven KB, Christensen JJ, Flatberg A, Rundblad A, Leder L, et al. Replacing saturated fat with polyunsaturated fat modulates peripheral blood mononuclear cell gene expression and pathways related to cardiovascular disease risk using a whole transcriptome approach. Mol Nutr Food Res. 2021;65(24):e2100633.
11 Moat SJ, George RS, Carling RS. Use of dried blood spot specimens to monitor patients with inherited metabolic disorders. Int J Neonatal Screen. 2020;6(2):26.
12 Lai CQ, Parnell LD, Smith CE, Guo T, Sayols-Baixeras S, Aslibekyan S, et al. Carbohydrate and fat intake associated with risk of metabolic diseases through epigenetics of CPT1A. Am J Clin Nutr. 2020;112(5):1200–11.
13 Filippini T, Malavolti M, Whelton PK, Naska A, Orsini N, Vinceti M. Blood pressure effects of sodium reduction: dose-response meta-analysis of experimental studies. Circulation. 2021;143(16):1542–67.
14 Stryer L. Biochemistry. New York (NY): WH Freeman & Co; 1988.
15 Zhao M, Chen X, Gao G, Tao L, Wei L. RLEdb: a database of rate-limiting enzymes and their regulation in human, rat, mouse, yeast and E. coli. Cell Res. 2009;19(6):793–5.
16 Calvo SE, Clauser KR, Mootha VK. MitoCarta2.0: an updated inventory of mammalian mitochondrial proteins. Nucleic Acids Res. 2016;44(D1):D1251–7.
17 Tanaka T, Basisty N, Fantoni G, Candia J, Moore AZ, Biancotto A, et al. Plasma proteomic biomarker signature of age predicts health and life span. Elife. 2020;9:e61073.
18 Buniello A, MacArthur JAL, Cerezo M, Harris LW, Hayhurst J, Malangone C, et al. The NHGRI-EBI GWAS catalog of published genome-wide association studies, targeted arrays and summary statistics 2019. Nucleic Acids Res. 2019;47(D1):D1005–12.
19 Bairoch A. The ENZYME database in 2000. Nucleic Acids Res. 2000;28(1):304–5.
20 Zhao M, Qu H. Human liver rate-limiting enzymes influence metabolic flux via branch points and inhibitors. BMC Genomics. 2009;10(Suppl 3):S31.
21 Kanehisa M, Goto S, Sato Y, Kawashima M, Furumichi M, Tanabe M. Data, information, knowledge and principle: back to metabolism in KEGG. Nucleic Acids Res. 2014;42(Database issue):D199–205.
22 Mangano KM, Noel SE, Lai CQ, Christensen JJ, Ordovas JM, Dawson-Hughes B, et al. Diet-derived fruit and vegetable metabolites show sex-specific inverse relationships to osteoporosis status. Bone. 2021;144:115780.
23 Tacutu R, Thornton D, Johnson E, Budovsky A, Barardo D, Craig T, et al. Human ageing genomic resources: new and updated databases. Nucleic Acids Res. 2018;46(D1):D1083–90.
24 Tabassum R, Rämö JT, Ripatti P, Koskela JT, Kurki M, Karjalainen J, et al. Genetic architecture of human plasma lipidome and its link to cardiovascular disease. Nat Commun. 2019;10(1):4329.
25 Nguyen TTL, Liu D, Ho MF, Athreya AP, Weinshilboum R. Selective serotonin reuptake inhibitor pharmaco-omics: mechanisms and prediction. Front Pharmacol. 2021;11:614048.
26 NewSTEPs. Newborn screening status for all disorders. 2021. Available at: https://www.newsteps.org/resources/data-visualizations/newborn-screening-status-all-disorders (accessed March 23, 2021).
27 Oslo Universitetssykehus. Nyfødtscreening. 2020. Available at: https://oslo-universitetssykehus.no/avdelinger/barne-og-ungdomsklinikken/nyfodtscreeningen/nyfodtscreening (accessed April 12, 2021).
28 Lekka DE, Brucknerova J, Salingova A, Sebova C, Ostrozlikova M, Ziburova J, et al. Congenital disorders of glycosylation: an umbrella term for rapidly expanding group of rare genetic metabolic disorders–importance of physical investigation. Bratisl Lek Listy. 2021;122(3):190–5.
29 Schlaepfer IR, Joshi M. CPT1A-mediated fat oxidation, mechanisms, and therapeutic potential. Endocrinology. 2020;161(2):bqz046.
30 Lee YC, Christensen JJ, Parnell LD, Smith CE, Shao J, McKeown NM, et al. Using machine learning to predict obesity based on genome-wide and epigenome-wide gene-gene and gene-diet interactions. Front Genet. 2022;12:783845.
31 Kim DH, Lee JW, Lee K. Supplementation of all-trans-retinoic acid below cytotoxic levels promotes adipogenesis in 3T3-L1 cells. Lipids. 2019;54(1):99–107.
32 Ulven SM, Christensen JJ, Nygård O, Svardal A, Leder L, Ottestad I, et al. Using metabolic profiling and gene expression analyses to explore molecular effects of replacing saturated fat with polyunsaturated fat-a randomized controlled dietary intervention study. Am J Clin Nutr. 2019;109(5):1239–50.
33 Ference BA, Kastelein JJP, Ray KK, Ginsberg HN, Chapman MJ, Packard CJ, et al. Association of triglyceride-lowering LPL variants and LDL-C-lowering LDLR variants with risk of coronary heart disease. JAMA. 2019;321(4):364–73.
34 Nakamura T, Tanimoto H, Okamoto M, Takeuchi M, Tsubamoto Y, Noda H. GIP receptor antagonist, SKL-14959 indicated alteration of the lipids metabolism to catabolism by the inhibition of plasma LPL activity, resulting in the suppression of weight gain on diets-induced obesity mice. Diabetes Metab Syndr Obes. 2021;14:1095–105.
35 Bose S, Ramesh V, Locasale JW. Acetate metabolism in physiology, cancer, and beyond. Trends Cell Biol. 2019;29(9):695–703.
36 Poll BG, Xu J, Jun S, Sanchez J, Zaidman NA, He X, et al. Acetate, a short-chain fatty acid, acutely lowers heart rate and cardiac contractility along with blood pressure. J Pharmacol Exp Ther. 2021;377(1):39–50.
37 Liang X, Chen M, Wang D, Wen J, Chen J. Vitamin A deficiency indicating as low expression of LRAT may be a novel biomarker of primary hypertension. Clin Exp Hypertens. 2021;43(2):151–63.
38 Yousefian M, Shakour N, Hosseinzadeh H, Hayes AW, Hadizadeh F, Karimi G. The natural phenolic compounds as modulators of NADPH oxidases in hypertension. Phytomedicine. 2019;55:200–13.
39 Matsuyama T, Shoji K, Takase H, Kamimaki I, Tanaka Y, Otsuka A, et al. Effects of phytosterols in diacylglycerol as part of diet therapy on hyperlipidemia in children. Asia Pac J Clin Nutr. 2007;16(1):40–8.
40 Parnell LD, Blokker BA, Dashti HS, Nesbeth PD, Cooper BE, Ma Y, et al. CardioGxE, a catalog of gene-environment interactions for cardiometabolic traits. BioData Min. 2014;7:21.
41 Swovick K, Firsanov D, Welle KA, Hryhorenko JR, Wise JP Sr, George C, et al. Interspecies differences in proteome turnover kinetics are correlated with life spans and energetic demands. Mol Cell Proteomics. 2021;20:100041.
42 Visscher PM, Wray NR, Zhang Q, Sklar P, McCarthy MI, Brown MA, et al. Ten years of GWAS discovery: biology, function, and translation. Am J Hum Genet. 2017;101(1):5–22.