REVIEW

Metabolic Pathways of Acylcarnitine Synthesis

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Summary

Acylcarnitines are important markers in metabolic studies of many diseases, including metabolic, cardiovascular, and neurological disorders. We reviewed analytical methods for analyzing acylcarnitines with respect to the available molecular structural information, the technical limitations of legacy methods, and the potential of new mass spectrometry-based techniques to provide new information on metabolite structure. We summarized the nomenclature of acylcarnitines based on historical common names and common abbreviations, and we propose the use of systematic abbreviations derived from the shorthand notation for lipid structures. The transition to systematic nomenclature will facilitate acylcarnitine annotation, reporting, and standardization in metabolomics. We have reviewed the metabolic origins of acylcarnitines important for the biological interpretation of human metabolomic profiles. We identified neglected isomers of acylcarnitines and summarized the metabolic pathways involved in the synthesis and degradation of acylcarnitines, including branched-chain lipids and amino acids. We reviewed the primary literature, mapped the metabolic transformations of acyl-CoAs to acylcarnitines, and created a freely available WikiPathway WP5423 to help researchers navigate the acylcarnitine field. The WikiPathway was curated, metabolites and metabolic reactions were annotated, and references were included. We also provide a table for conversion between common names and abbreviations and systematic abbreviations linked to the LIPID MAPS or Human Metabolome Database.

Key words

Acylcarnitines • Mass spectrometry • Beta oxidation • Branched fatty acids • Branched lipids • Nomenclature • Standardization • Shorthand notation for lipid structures • LIPID MAPS

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Introduction

Acylcarnitines are acyl esters of carnitine (L-3-hydroxy-4-aminobutyrobetaine) and are essential for the oxidative catabolism of fatty acids in mitochondria and peroxisomes. They are necessary for healthy and sustainable cellular energy homeostasis, involved in the metabolism of branched-chain amino acids and related metabolic pathways, and serve as a detoxification pathway [1,2]. There are many acylcarnitine molecules described in public databases of metabolites, for instance, 114 records in LIPID MAPS, 2359 records in the Human Metabolome Database, and 308 entries on the ChEBI database, measured in plasma/serum, dried blood spots, urine, bile and amniotic fluid [3], but very little is published about their metabolic origins.

We aimed to help researchers interpret metabolomics data containing acylcarnitines. We reviewed the biologically important acylcarnitines [1] concerning their biochemical origins and generated a pathway map summarizing the reactions. We focused on the isomers and nomenclature and annotated the enzymatic reactions involved in their metabolism. This review should facilitate orientation in the acylcarnitine datasets and link the measured analytes with the correct enzymatic reactions.

Analytical methods using mass spectrometry

Critical evaluation of the limitations of the analytical techniques used to measure acylcarnitine is the basis for correct annotation and biological interpretation.

Sample extraction should consider that the polarity of acylcarnitines can vary by more than 10 orders of magnitude on the log octanol-water partition coefficient (logP) scale. In commonly employed bi-phase extraction methods like methyl *tert*-butyl ether/methanol/water, chloroform/methanol/water, and dichloromethane/methanol/water, it is crucial to analyze both fractions to cover these metabolites [4].

Direct-infusion tandem mass spectrometry (DI-MS/MS) remains a valid approach for identifying patients with inborn errors of metabolism, problems in mitochondrial fatty acid β-oxidation, and certain types of acidemias (reviewed by Miller *et al.* [3]). However, this legacy technique provides only limited information about the analytes, usually the number of carbon atoms and double bonds.

Currently, liquid chromatography-mass spectrometry (LC-MS/MS) is the most common technique for metabolomics profiling, which allows the separation of acylcarnitine isomeric species and improves the sensitivity and selectivity of their detection [1,3]. Although LC-MS/MS provides an insight into the analyte's structure, it does not yield unambiguous molecular identity.

Recent advances in analytical techniques push forward our ability to provide a full structural characterization of lipid species in complex biological matrices. These include ion mobility spectrometry (separating stereoisomers, reducing background), ion activation techniques (targeting double bonds by UV photodissociation, providing specific fragment *via* electron capture dissociation), and derivatizations (increasing sensitivity using charged modifiers, separating isomers, localizing double bonds using O_3), [5-9], which allow quantification of isomeric and odd-numbered acylcarnitines, identification of acyl double bond position and configuration, stereochemistry, etc. In the future, these specialized techniques will become a part of routine pipelines, producing hundreds of acylcarnitine species structurally identified at 'Complete structure level'. New tools will be needed to mine these information-rich datasets. Implementation of the Lipidomics Minimal Reporting Checklist, which summarizes all technical details of the analytical technique and defines the level of molecular structural information, would facilitate the interpretation of the acylcarnitine data [10].

Acylcarnitine nomenclature

Common names

According to IUPAC, the common names of acyl groups are typically derived by replacing the **-ic** acid suffix of the corresponding carboxylic acid's common name with **-yl** or the **-oic** acid suffix with **-oyl**, (acetic acid > acetyl- or palmitic acid > palmitoyl-). Systematic names based on hydrocarbon chains are derived similarly (hexadecanoic acid > hexadecanoylcarnitine).

Common abbreviations

Common name acylcarnitine abbreviations in the field of newborn screening are simple and follow these rules: a) a number of all carbon atoms is used to describe the chain length regardless of the branching or functional groups (e.g., glutarylcarnitine as C5-DC); b) unsaturation degree is used only when present (e.g., oleoylcarnitine as C18:1); c) functional groups are used without the positional information (e.g. 3(*S*)-hydroxybutyrylcarnitine as C4-OH) [3,11]. Alternative grammar distinguishes isomeric species, e.g., 3-hydroxybutyrylcarnitine and 3-hydroxyisobutyrylcarnitine as C4-OH and C4-OH-I, respectively [1]. The *iso* prefix can be used for structures where the saturated chain has a branching point on the penultimate (one from the end) carbon atom (e.g., isobutyrylcarnitine). The *anteiso* prefix marks the branching point located on the antepenultimate carbon atom (two from the end). The prefixes cannot be used with unsaturated or modified chains, and the position of the methyl branching group is used instead (e.g., tiglylcarnitine as C5:1-M).

Systematic abbreviations

Common names and abbreviations will soon reach their limit of usability. Abbreviations based on systematic names are needed due to the advent of more powerful analytical techniques. Nowadays, rules designed for unit-resolution mass spectrometers cannot describe structural diversity. Shorthand notation for lipid structures [12-14] allows annotation of the measured analytes based on the level of structural information provided by the analytical technique (Table 1). It combines predefined class (CAR) followed by the acyl chain characteristics, including all available structural

Table 1. Example of structural hierarchy representation of '3-Hydroxyoleoylcarnitine' according to Shorthand notation for lipid structures [12-14].

Level	Name	Description (information gain)
Category	Fatty acyls [FA]	Lipid category
Class	Fatty acyl carnitines [FA0707]	+ lipid class
Species	CAR 18:1;O	+ headgroup, fatty *Acyl* identity, oxidation
Molecular species	CAR 18:1;O1	+ one oxygen
sn-Position	CAR 18:1;O1	(not applicable)
Structure defined	CAR 18:1(9*Z*);OH	+ double bond position, type of oxidation
Full structure	CAR 18:1(9*Z*);3OH	+ position of the oxidation
Complete structure	CAR 18:1(9*Z*);3OH[*S*]	+ stereo configuration

information. For instance, there are at least four different decenoylcarnitines: CAR 10:2(2*E*), CAR 10:1(3*E*), CAR 10:1(3*Z*), and CAR 10:1(4*Z*) in the β-oxidation pathways of common fatty acids (Fig. 1). Two of them, CAR 10:1(3*Z*) and CAR 10:1(2*E*), come from β-oxidation of palmitoleic acid (9*Z*). However, there are at least 15 different isomers of fatty acid 16:1 in human plasma, four containing a double bond at positions 9, 10, and 11 [6]. Therefore, many more CAR 10:1 isomers from the FA 16:1 or other longer FAs could be present in plasma as yet unidentified but describable by shorthand notation. Other examples are quantification of C14:1 acylcarnitine n-5 and n-9 in human and mouse fibroblasts, which levels reflect differences in the expression of long chain acyl-CoA dehydrogenase [15] and quantification of *cis*-3-C12:1 and *cis*-5-C14:1 acylcarnitine in plasma of enoyl-CoA delta isomerase 1-deficient mice [16]. In both examples, the acyl chain identity is crucial for proper interpretation of long chain acyl-CoA dehydrogenase and enoyl-CoA isomerase activities. However, this approach is also limited based on predefined classes. It is impossible to distinguish the L-/D- isomers within the CAR class definition, and a full IUPAC name is the ultimate option.

The systematic abbreviations are backward compatible. Newborn screening acylcarnitine data can be correctly annotated, and metabolomic profiling data, acquired using innovative derivatization or fragmentation techniques, can be annotated with all structural details [6,7]. Broader acceptance of the shorthand notation by major databases would facilitate acylcarnitine annotations, standardization, and further data processing *via* overrepresentation analysis [17].

Lipid databases and metabolite identities

When discussing the biological effect of acylcarnitines, we need to find the most appropriate identifiers in a database considering the analytical limitations and the biological knowledge. This task is challenging and sometimes possible. For instance, an optimized analytical technique can measure the carnitine ester of palmitic acid at the 'Complete structure level' according to shorthand notation for lipid structures [12,13]. We need to find an identifier for (*S*)-3-(palmitoyloxy)-4-(trimethylammonio)butanoate, which is the biologically important molecule commonly known as ‘palmitoylcarnitine.’ First, L-carnitine is essential for the transportation of long-chain fatty acids into mitochondria for β-oxidation, and its optimal isomer, D-carnitine, is a xenobiotic compound [18,19]. Therefore, in most cases, only 'L'-acylcarnitines (or (*R*)- according to Cahn-Ingold-Prelog rules) are biologically relevant for human metabolism. Second, metabolite databases contain records covering different levels of knowledge of metabolite structure (Table 2). Some records lack stereochemistry, and some databases contain the 'D-' forms. Pre-calculated and non-curated databases might also contain wrong records, e.g., LIPID MAPS record LMFA07070079 was up to recently called L-palmitoylcarnitine, which was an incorrect common name for (*S*)-3-(palmitoyloxy)-4-(trimethylammonio) butanoate aka D-palmitoylcarnitine. The involvement of the community is needed to report and curate database records. Another example would be the identity of ‘hydroxybutyrylcarnitine’ – deficiency of a short-chain-3-hydroxyacyl-coenzyme A dehydrogenase leads to the accumulation of CAR 4:0;3OH[S] [20] while ketosis leads to the accumulation of CAR 4:0;3OH[R] [19].

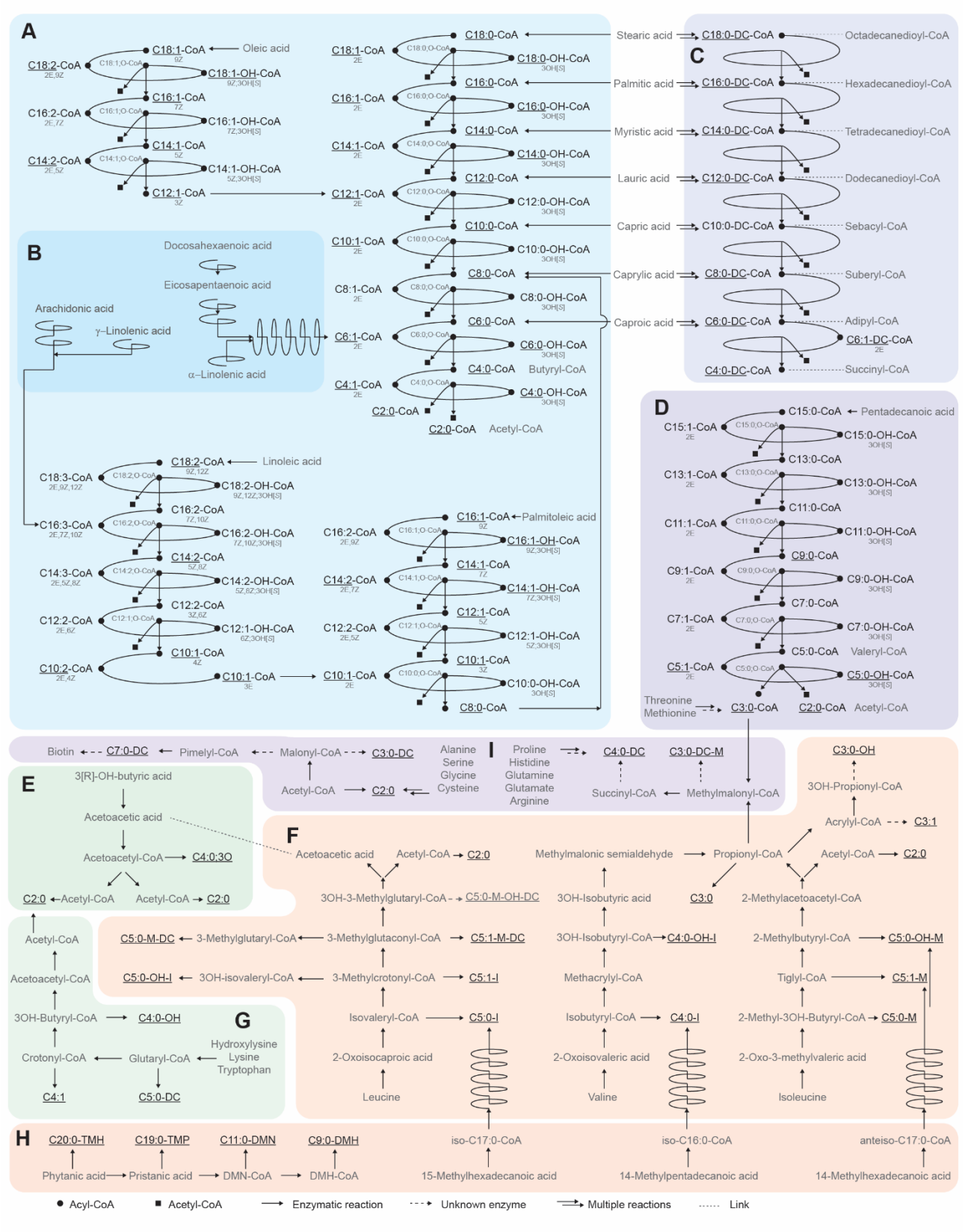

Fig. 1. Acylcarnitine pathways. (**A**) β-oxidation of straight fatty acids; (**B**) β-oxidation of straight polyunsaturated fatty acids; (**C**) β-oxidation of dicarboxylic fatty acids; (**D**) β-oxidation of odd-chain fatty acids; (**E**) Ketogenic pathway; (**F**) Metabolism of branched-chain amino acids; (**G**) Degradation of amino acids; (**H**) β-oxidation of branched-chain fatty acids; (**I**) Metabolism of amino acids and short-chain acylcarnitines. See Table 1 for annotations and WikiPathway WP5423 for details.

Table 1. Biologically important acylcarnitines.

Group	Panel	Common abbreviation	Common name	Systematic abbreviation	LIPID MAPS ID
Saturated straight	NBS	C2:0	Acetylcarnitine	CAR 2:0	LMFA07070050
	NBS	C3:0	Propionylcarnitine	CAR 3:0	LMFA07070005
	NBS	C4:0	Butyrylcarnitine	CAR 4:0	LMFA07070003
		C5:0	Valerylcarnitine	CAR 5:0	LMFA07070111
	NBS	C6:0	Caproylcarnitine	CAR 6:0	LMFA07070001
	NBS	C7:0	Heptanoylcarnitine	CAR 7:0	LMFA07070068
	NBS	C8:0	Octanoylcarnitine	CAR 8:0	LMFA07070002
		C9:0	Nonanoylcarnitine	CAR 9:0	LMFA07070082
	NBS	C10:0	Decanoylcarnitine	CAR 10:0	LMFA07070006
		C11:0	Undecanoylcarnitine	CAR 11:0	LMFA07070110
	NBS	C12:0	Lauroylcarnitine	CAR 12:0	LMFA07070062
		C13:0	Tridecanoylcarnitine	CAR 13:0	
	NBS	C14:0	Myristoylcarnitine	CAR 14:0	LMFA07070107
		C15:0	Pentadecanoylcarnitine	CAR 15:0	
	NBS	C16:0	Palmitoylcarnitine	CAR 16:0	LMFA07070004
	NBS	C18:0	Stearoylcarnitine	CAR 18:0	LMFA07070008
		C22:0	Behenoylcarnitine	CAR 22:0	LMFA07070089
		C24:0	Lignoceroylcarnitine	CAR 24:0	
		C26:0	Cerotoylcarnitine	CAR 26:0	LMFA07070069
Unsaturated straight	NBS	C3:1	Propenoylcarnitine	CAR 3:1(2*E*)	LMFA07070104
		C4:1	Butenylcarnitine	CAR 4:1(2*E*)	LMFA07070053
		C5:1	2-Pentenoylcarnitine	CAR 5:1(2*E*)	
		C6:1	Hexenoylcarnitine	CAR 6:1(2*E*)	LMFA07070031
	NBS	C8:1	Octenoylcarnitine	CAR 8:1(2*E*)	LMFA07070035
	NBS	C10:1	Decenoylcarnitine	CAR 10:1(2*E*)	
				CAR 10:1(3*E*)	
				CAR 10:1(3*Z*)	
				CAR 10:1(4*Z*)	LMFA07070017
	NBS	C10:2	Decadienoylcarnitine	CAR 10:2(2*E*,4*Z*)	LMFA07070015
	NBS	C12:1	Dodecenoylcarnitine	CAR 12:1(2*E*)	
				CAR 12:1(3*Z*)	
				CAR 12:1(5*Z*)	
		C12:2	Dodecadienoylcarnitine	CAR 12:2(2*E*,5*Z*)	
				CAR 12:2(2*E*,6*Z*)	
				CAR 12:2(3*Z*,6*Z*)	

	NBS	C14:1	Tetradecenoylcarnitine	CAR 14:1(2*E*)	
				CAR 14:1(5*Z*)	LMFA07070057
				CAR 14:1(7*Z*)	
	NBS	C14:2	Tetradecedienoylcarnitine	CAR 14:2(2*E*,5*Z*)	
				CAR 14:2(2*E*,7*Z*)	
				CAR 14:2(5*Z*,8*Z*)	LMFA07070020
	NBS	C16:1	Hexadecenoylcarnitine	CAR 16:1(2*E*)	LMFA07070109
				CAR 16:1(7*Z*)	
				CAR 16:1(9*Z*)	LMFA07070097
		C16:2	Hexadecadienoylcarnitine	CAR 16:2(2*E*,7*Z*)	
				CAR 16:2(2*E*,9*Z*)	
				CAR 16:2(7*Z*,10*Z*)	LMFA07070021
	NBS	C18:1	Octadecenoylcarnitine	CAR 18:1(2*E*)	
				CAR 18:1(9*Z*)	LMFA07070096
	NBS	C18:2	Octadecadienylcarnitine	CAR 18:2(2*E*,9*Z*)	
				CAR 18:2(9*Z*,12*Z*)	LMFA07070092
Branched	NBS	C4:0-I	Isobutyrylcarnitine	CAR 3:0;2Me	LMFA07070075
	NBS	C5:0-I	Isovalerylcarnitine	CAR 4:0;3Me	LMFA07070077
	NBS	C5:0-M	2-Methylbutyrylcarnitine	CAR 4:0;2Me	LMFA07070034
		C5:1-I	3-Methylcrotonylcarnitine	CAR 4:1(2*E*);3Me	
	NBS	C5:1-M	Tiglylcarnitine	CAR 4:1(2*E*);2Me	LMFA07070108
		C20:0-TMH	Phytanoylcarnitine*	CAR 16:0;3Me;7Me; 11Me;15Me	
		C19:0-TMP	Pristanoylcarnitine*	CAR 15:0;2Me;6Me; 10Me;14Me	
		C11:0-DMN	4,8-dimethylnonanoyl carnitine*	CAR 9:0;4Me;8Me	
		C9:0-DMH	2,6-dimethylheptanoyl carnitine*	CAR 7:0;2Me;6Me	
Oxidized		C3:0-OH	3-Hydroxypropionyl carnitine	CAR 3:0;3OH[*S*]	LMFA07070074
	NBS	C3:0-DC	Malonylcarnitine	CAR 2:0;2COOH	LMFA07070093
	NBS	C3:0-DC-M	Methylmalonylcarnitine	CAR 2:0;2Me;2COOH	LMFA07070094
	NBS	C4:0-OH	3-Hydroxybutyrylcarnitine	CAR 4:0;3OH[*S*]	LMFA07070037
		C4:0-OH-I	3-Hydroxyisobutyryl carnitine	CAR 3:0;2Me;3OH[*S*]	
		C4:0;O	Acetoacetylcarnitine	CAR 4:0;3oxo	
		C4:0-DC	Succinylcarnitine	CAR 3:0;3COOH	LMFA07070101
		C5:0-OH	3-Hydroxyvaleryl	CAR 5:0;3OH[*S*]	

			carnitine		
	NBS	C5:0-OH-I	3-Hydroxyisovaleryl carnitine	CAR 4:0;3Me;3OH[*S*]	LMFA07070041
	NBS	C5:0-OH-M	2-Methyl-3-hydroxybutyrylcarnitine	CAR 4:0;2Me;3OH[*S*]	
	NBS	C5:0-DC	Glutarylcarnitine	CAR 4:0;4COOH	LMFA07070091
		C5:0-M-DC	3-Methylglutarylcarnitine	CAR 4:0;3Me[*R*];4COOH	
		C5:1-M-DC	3-Methylglutaconyl carnitine	CAR 4:1(2*E*);3Me;4COOH	
		C5:0-M-OH-DC	3-Hydroxy-3-methylglutarylcarnitine	CAR 4:0;3OH[*S*];3Me;4COOH	
	NBS	C6:0-OH	3-Hydroxy-hexanoylcarnitine	CAR 6:0;3OH[*S*]	LMFA07070072
		C6:0-DC	Adipylcarnitine	CAR 5:0;5COOH	LMFA07070087
		C6:1-DC	Dehydroadipylcarnitine	CAR 5:1(2*E*);5COOH	LMFA07070013
		C7:0-DC	Pimelylcarnitine	CAR 6:0;6COOH	
		C8:0-DC	Suberylcarnitine	CAR 7:0;7COOH	
	NBS	C10:0-OH	3-Hydroxydecanoyl carnitine	CAR 10:0;3OH[*S*]	
	NBS	C10:1-OH	3-Hydroxydecenoyl carnitine	CAR 10:1;OH	
	NBS	C12:0-OH	3-Hydroxydodecanoyl carnitine	CAR 12:0;3OH[*S*]	LMFA07070039
		C12:0-DC	Dodecanedioylcarnitine	CAR 11:0;11COOH	LMFA07070083
	NBS	C12:1-OH	3-Hydroxydodecenoyl carnitine	CAR 12:1(5*Z*);3OH[*S*]	
	NBS	C14:0-OH	3-Hydroxytetradecanoyl carnitine	CAR 14:0;3OH[*S*]	LMFA07070045
		C14:0-DC	Tetradecanedioylcarnitine	CAR 13:0;13COOH	LMFA07070084
	NBS	C14:1-OH	3-Hydroxytetradecenoyl carnitine	CAR 14:1(5*Z*);3OH[*S*] CAR 14:1(7*Z*);3OH[*S*]	
	NBS	C16:0-OH	3-Hydroxyhexadecanoyl carnitine	CAR 16:0;3OH[*S*]	
	NBS	C16:1-OH	3-Hydroxyhexadecenoyl carnitine	CAR 16:1(7*Z*);3OH[*S*] CAR 16:1(9*Z*);3OH[*S*]	LMFA07070044
		C16:0-DC	Hexadecanedioylcarnitine	CAR 15:0;15COOH	LMFA07070007
	NBS	C18:0-OH	3-Hydroxystearoyl carnitine	CAR 18:0;3OH[*S*]	LMFA07070043
	NBS	C18:1-OH	3-Hydroxyoleoylcarnitine	CAR 18:1(9*Z*);3OH[*S*]	LMFA07070025
		C18:0-DC	Octadecanedioylcarnitine	CAR 17:0;17COOH	LMFA07070085
	NBS	C18:2-OH	3-Hydroxyoctadecadienoyl carnitine	CAR 18:2(9*Z*,12*Z*);3OH[*S*]	LMFA07070042

Note: Zero (':0') was included in newborn screening (NBS) names to unify various dialects. * denotes mixture of unknown isomers.

The incorrect mapping of database identifiers can affect further data processing, like over-representation or pathway analysis. For instance, the Rhea database contains only 'L'-acylcarnitine reactions (https://www.rhea-db.org/rhea/12663), and 'D'-identifiers would produce false negative results.

In parallel, acylcarnitine data measured using direct infusion unit-resolution mass spectrometry yield annotations at 'Species level' [12,13], and careful identifier mapping should be performed to prevent over-interpretation. For instance, 3-hydroxyisovalerylcarnitine (C5-OH-I), LIPID MAPS identification LMFA07070041, is abbreviated as CAR 5:0;O and the same systematic abbreviation is used for 3-hydroxyvalerylcarnitine. However, these two metabolites come from entirely different pathways and precursors (leucine degradation and odd-chain fatty acid degradation, Fig. 1).

Acylcarnitines important for biological interpretation of human metabolomic profiles

Recently, Dambrova *et al.* published an excellent review on acylcarnitines [1]. We decided to update the older acylcarnitine nomenclature (based on newborn screening rules) into systematic nomenclature and map the metabolites associated with a disease or necessary for the biological interpretation of datasets into identifiers and pathways (Table 2, Fig. 1). We primarily focused on the identity and source of acylcarnitines and successfully mapped most of the metabolites. However, some acylcarnitines defined by newborn screening methods cannot be unambiguously identified [11].

Although the 3-hydroxydecenoylcarnitine (C10:1-OH) has been officially part of the newborn screening panel [3,11], we could not find its molecular identity. It is not a product of the major metabolic pathways, and the position of the double bond is unknown. Violante *et al.* performed a detailed analysis of mitochondrial carnitine acyltransferase substrate preference and showed that the acyltransferase enzyme for some short-chain-CoAs is still unknown. For instance, an enzyme(s) responsible for the synthesis of succinylcarnitine, malonylcarnitine, or 3-hydroxy-3-methylglutarylcarnitine is still unknown [21-23]. 3-Hydroxy-3-methylglutarylcarnitine (C5:1-M-OH-DC) was observed only using low-resolution MS, and its molecular identity was not confirmed by other techniques [24].

Limitations of analytical techniques often prevent accurate identification of the compounds. For instance, isovalerylcarnitine (C5:0-I), which is a diagnostic indicator of isovaleric acidemia, is isobaric with pivaloylcarnitine, 2-methylbutyrylcarnitine, and *n*-valerylcarnitine [25,26]. Pivaloylcarnitine derived from antibiotics and pimeloylcarnitine synthesized by microbiota are typical members of exposome [25,27]. Mixtures of straight and branched saturated acylcarnitines (e.g., CAR 20:0 as arachidylcarnitine or phytanoyl-carnitine) should be considered when annotating unknown features [28,29].

Acylcarnitine pathways

We designed a map of acylcarnitine metabolism in WikiPathways format (WP5423) to facilitate data interpretation and better orientation in various acylcarnitines' metabolic origins. It is important to interpret the data and pathways carefully based on the type of the sample (isolated mitochondria, cells, tissue biopsies, serum/plasma, urine). Not all pathways are active in all cell types, inborn errors of metabolism have specific signs, and the circulating acylcarnitines reflect tissue acylcarnitine metabolism only transiently and inadequately [2,30,31]. The map is divided into three sections: straight-chain fatty acids, branched-chain lipids, and odd-chain fatty acids.

The straight-chain fatty acid part describes the intermediates of β-oxidation from stearic acid to acetyl-CoA, intermediates of oleic acid, linoleic acid, α- and γ-linolenic acids, palmitoleic acid, docosahexaenoic and eicosapentaenoic acid complete degradation leading to acetyl-CoA, ω- and β-oxidation of dicarboxylic fatty acids, and partial β-oxidation of eicosanoids and docosanoids illustrated by prostaglandin E_2 path [16,32-37].

The branched-chain lipids part includes α- and β-oxidation of phytanic and pristanic acids and degradation of branched-chain fatty acids of the iso and *anteiso* series leading to intermediates of branched-chain amino acid degradation [29,38,39]. Amino acid-related metabolism, including ketogenesis and biotin synthesis [19,27], is included to cover all metabolic origins of short-chain acylcarnitines [1] and branched acyl side chains of cholestanoic acids are β-oxidized in the pathway of bile acid synthesis [40]. Degradation of branched-acyls leads to the production of both acetyl-CoA and propionyl-CoA and their respective acylcarnitines.

The odd-chain fatty acids part illustrates the metabolic fate of pentadecanoic acid *via* β-oxidation,

leading to six molecules of acetyl-CoA and one propionyl-CoA and their acylcarnitine counterparts.

All metabolites are annotated using shorthand notation for lipid structures [12,13], common names, and the most appropriate database identifiers from LIPID MAPS or the Human metabolome database. When the database contained records with (un)defined stereochemistry, the closest structure was selected. Acylcarnitines discussed in the review by Dambrova *et al.* [1] and acylcarnitines important for the pathway flow are highlighted in bold font and provided with literature citations. Metabolic conversions are depicted by arrows and enzymatic reactions yielding acylcarnitines are highlighted in red. Five acylcarnitine transferases: carnitine acetyltransferase (CRAT), carnitine O-octanoyl-transferase (CROT), carnitine palmitoyltransferase 1A (CPT1A), carnitine palmitoyltransferase 1B (CPT1B), and carnitine palmitoyltransferase 2 (CPT2) convert acyl-CoAs into acylcarnitines according to their substrate specificity, which is documented by references linked to the red arrows (and reviewed in Dambrova *et al.* [1]). Most of the pathways contain the β-oxidation catabolic spiral, which is composed of acyl-CoA dehydrogenase and the mitochondrial trifunctional protein (TFP) [41,42]. The fatty acyl-CoA dehydrogenase produces 2,3-enoyl-CoA, the TFP 2,3-enoyl-CoA hydratase adds water and yields 3-hydroxyacyl-CoA, the TFP 3-hydroxyacyl-CoA dehydrogenase oxidizes the intermediate to 3-ketoacyl-CoA, and the TFP 3-ketoacyl-CoA thiolase releases a two-carbon unit in the form of acetyl-CoA [41]. Carnitine-dependent peroxisomal oxidation reactions, starting with acyl-CoA oxidase, have been reviewed by Houten *et al.* [38,40].

Carnitine acyltransferases ensure the reversible shuttling of acyl groups between free CoA and carnitine, and even the acylcarnitine forms of the intermediates can be detected using very specific and targeted techniques [43]. However, the stability and enzymatic accessibility of the 3-ketoacyl intermediates is much lower compared to the final acyl-CoAs and acylcarnitines, and the 3-ketoacylcarnitines are not depicted in the map for clarity. To further reduce the complexity of the pathways, enzymes involved in α-, β-, and ω-oxidations are omitted but can be explored in the linked WikiPathways or Reactome maps [36,37]. Reactions populated in the Rhea knowledgebase are annotated with a Rhea identifier [44].

We created a simplified poster version of the WP5423, which can be printed on A3 format paper (Fig. S1). The poster uses newborn screening-based acylcarnitine nomenclature, and the alternative systematic metabolite names can be found in Table 1.

Conclusions

We reviewed the metabolic origins of acylcarnitines, mapped their metabolic conversions, and created a freely available WikiPathway and a poster scheme to help researchers orient themselves in the acylcarnitine realm. We propose the use of shorthand notation for lipid structures [12,13] as a way to standardize the acylcarnitine reporting.

Availability of data and materials

WikiPathway WP5423 is freely available online at https://classic.wikipathways.org/index.php/Pathway:WP5423

Conflict of Interest

There is no conflict of interest.

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