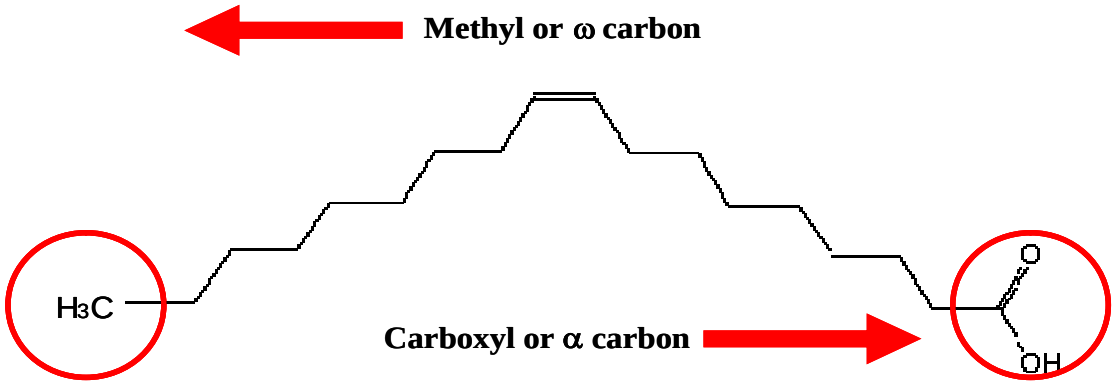
Chapter 1.1

Fatty Acid Synthesis

1.1.1 Introduction

Lipids are broadly defined as "fatty acids, their derivatives, and substances related biosynthetically or functionally to these compounds", which are generally soluble in organic solvents such as chloroform, and are most commonly found in the tissues of plants, animals, and microorganisms (Christie, 2003). The term lipid encompasses a range of bioactive molecules which play critical roles in the biology of all living organisms. These compounds include molecules such as phospholipids which are the major components of cell membranes and key precursors in the production of prostanoids, and cholesterols, which constitute a major component of the cell membrane and which are precursors in the synthesis of steroid hormones (Calder & Burdge, 2004; Calder, 2006; Christie, 2003; Mattos *et al.*, 2000). Additionally, lipids also serve as important energy storage depots (e.g. triacylglycerols) and as signalling molecules.

The general structure of fatty acids of plant, animal or microbial origin is a straight chain of carbons, usually even in number, terminated by a carboxyl group at one end. These molecules may be saturated, containing no double bonds, or unsaturated, containing one or more double bonds in either the *cis* or *trans* conformation (Christie, 2003). Unsaturated fatty acids are further subdivided into monounsaturated fatty acids (one double bond) or polyunsaturated fatty acids (PUFA) (containing two or more double bonds), which are further subdivided based on the position of the first double bond relative to the methyl end of the molecule (Abayasekara & Wathes, 1999; Christie, 2003). Using this system the first double bond is identified as ω-x, where x is the carbon number on which the double bond occurs **(Figure 1.1.1)**. The ω-x nomenclature is also referred to as omega-x or n-x. In addition to their scientific nomenclature, fatty acids are commonly described



***cis* 9- Oleic acid, C18:1, ω-9**

Figure 1.1.1 The structure and nomenclature of fatty acids

Table 1.1.1 The nomenclature of fatty acids

Trivial name	Systematic name	Shorthand
Butyric acid	Butanoic acid	C4:0
Caproic acid	Hexanoic acid	C6:0
Caprylic acid	Octanoic acid	C8:0
Myristic acid	Tetradecanoic acid	C14:0
Palmitic acid	Hexadecanoic acid	C16:0
Stearic acid	Octadecanoic acid	C18:0
Myristoleic acid	9-tetradecenoic acid	C14:1 (ω-5)
Palmitoleic acid	9-hexadecenoic acid	C16:1 (ω-7)
Oleic acid	9-octadecenoic acid	C18:1 (ω-9)
Myristelaidic acid	*trans* 9-tetradecenoic acid	C14:1 (ω-5)
Palmitelaidic acid	*trans* 9-hexadecenoic acid	C16:1 (ω-7)
Elaidic acid	*trans* 9-octadecenoic acid	C18:1 (ω-9)
Linoleic acid	9, 12-octadecadienoic acid	C18:2 (ω-6)
Rumenic acid (*cis* 9, *trans* 11 CLA)	9, *trans* 11-octadecadienoic acid	C18:2 (ω-7)
α-linolenic acid	9, 12, 15-octadecatrienoic acid	C18:3 (ω-3)
γ-linolenic acid	6, 9, 12-octadecatrienoic acid	C18:3 (ω-6)
Arachidonic acid	5, 8, 11, 14-eicosatetraenoic acid	C20:4 (ω-6)
Eicosapentaenoic acid (EPA)	5, 8, 11, 14, 17-eicosapentaenoic acid	C20:5 (ω-3)
Docosapentaenoic acid (DPA)	7, 10, 13, 16, 19-docosapentaenoic acid	C22:5 (ω-3)
Docosahexaenoic acid (DHA)	4, 7, 10, 13, 16, 19-docosahexaenoic acid	C22:6 (ω-3)

using their more common trivial names **(Table 1.1.1)**. The highly reactive nature of the carboxyl group found in fatty acids permits the formation of ester linkages with glycerol and cholesterols forming acylglycerols (e.g. triacylglycerols or triglycerides and phospholipids) (Christie, 2003). The structure of each fatty acid in terms of its carbon chain length, number of double bonds and bond conformation, tend to govern the specific role or function of the molecule.

1.1.2 Polyunsaturated fatty acid (PUFA) synthesis

While most fatty acids have an important role in the mammalian diet, only two are considered essential. These fatty acids are the PUFA, linoleic acid (ω-6) and α-linolenic acid (ω-3) (Calder & Burdge, 2004). The essential nature of these fatty acids in mammals stem from the absence of the desaturase enzymes necessary to convert other dietary fatty acids to both linoleic acid and α-linolenic acid. As a result of this inability, both linoleic acid and α-linolenic acid must be provided in the diet. Indeed, essential fatty acid deficiency in rats has been associated with a number of debilitating conditions outlined in **Table 1.1.2**. More recently it has been suggested that the long chain PUFA, docosahexaenoic acid (DHA), might also be classed as an essential fatty acid as a result of its abundance in most tissues (Gebauer *et al.*, 2006; Spector, 1999).

The majority of the fatty acids present in vertebrates are either absorbed from the diet or are synthesised *de novo*. Saturated fatty acids are built up by the successive addition of two carbon units onto the carbon backbone, with the principal product believed to be palmitic acid (Calder & Burdge, 2004; Gurr *et al.*, 2002). Shorter chained fatty acids can be released from these fatty acids via the action of specific enzymes while in eukaryotes, longer chained fatty acids can be

Table 1.1.2 Symptoms of essential fatty acid deficiency in rats

Organ/system	Deficiency symptoms
Skin	Dermatitis; Increased water permeability; Epithelial hyperplasia
Weight	Decrease
Circulation	Heart enlargement; Decreased capillary resistance; Increased capillary permeability
Kidney	Enlargement; Intertubular haemorrhage
Lung	Cholesterol accumulation
Endocrine glands	Adrenal weight decreased in females and increased in males; Reduced weight of thyroid
Reproduction	Degeneration of seminiferous tubules in males; Irregular oestrus in females and impaired reproduction
Immune system	Spleen and thymic atrophy; Impaired immune function
Metabolism	Changes in the fatty acid composition of most tissues; Decreased efficiency of mitrocondrial energy generation

Adapted from Calder *et al,* (2004)

generated via the action of elongase enzymes (Calder & Burdge, 2004).

Desaturation of fatty acids occurs in the endoplasmic reticulum of the cell, and is common to almost all cells including bacteria, yeasts, algae, higher plants and animals (Calder & Burdge, 2004). This activity is catalysed by a wide range of desaturase enzymes, which are classified according to the location on the carbon chain where they insert a double bond **(Figure 1.1.2)**. For example, the enzyme Δ^9-desaturase is so named, due to its ability to insert a double bond between carbons 9 and 10 of the carbon chain of fatty acids such as stearic acid resulting in the production of the monounsaturated fatty acid, oleic acid.

All eukaryotes and certain bacteria possess the ability to produce PUFA. While higher plants catalyse the addition of a double bond between an existing double bond and the terminal methyl group, animals can only insert new double bonds between existing double bonds and the carboxyl group **(Figure 1.1.2)**. Although mammalian cells cannot synthesise linoleic acid or α-linolenic acid, they can catalyse the conversion of these fatty acids to their longer chained derivatives. This activity is catalysed by the same set of enzymes, namely, Δ^6-desaturase, Δ^5-desaturase and elongases, with β-oxidation playing an important role in the conversion of C24:6 (ω-3) to DHA **(Figure 1.1.2)**. Of these reactions, it is the activity of the Δ^6-desaturase enzyme which is the rate limiting reaction (Foundation, 1992). Furthermore, this enzyme plays an important role in dictating which type of PUFA is produced, displaying a higher preference for ω-3 PUFAs, rather than ω-6 PUFAs such as linoleic acid, or ω-9 fatty acids, such as oleic acid (Foundation, 1992; Simopoulos, 2008). Control of these pathways is maintained hormonally, by nutritional status and by feedback inhibition by the end products (Foundation, 1992).

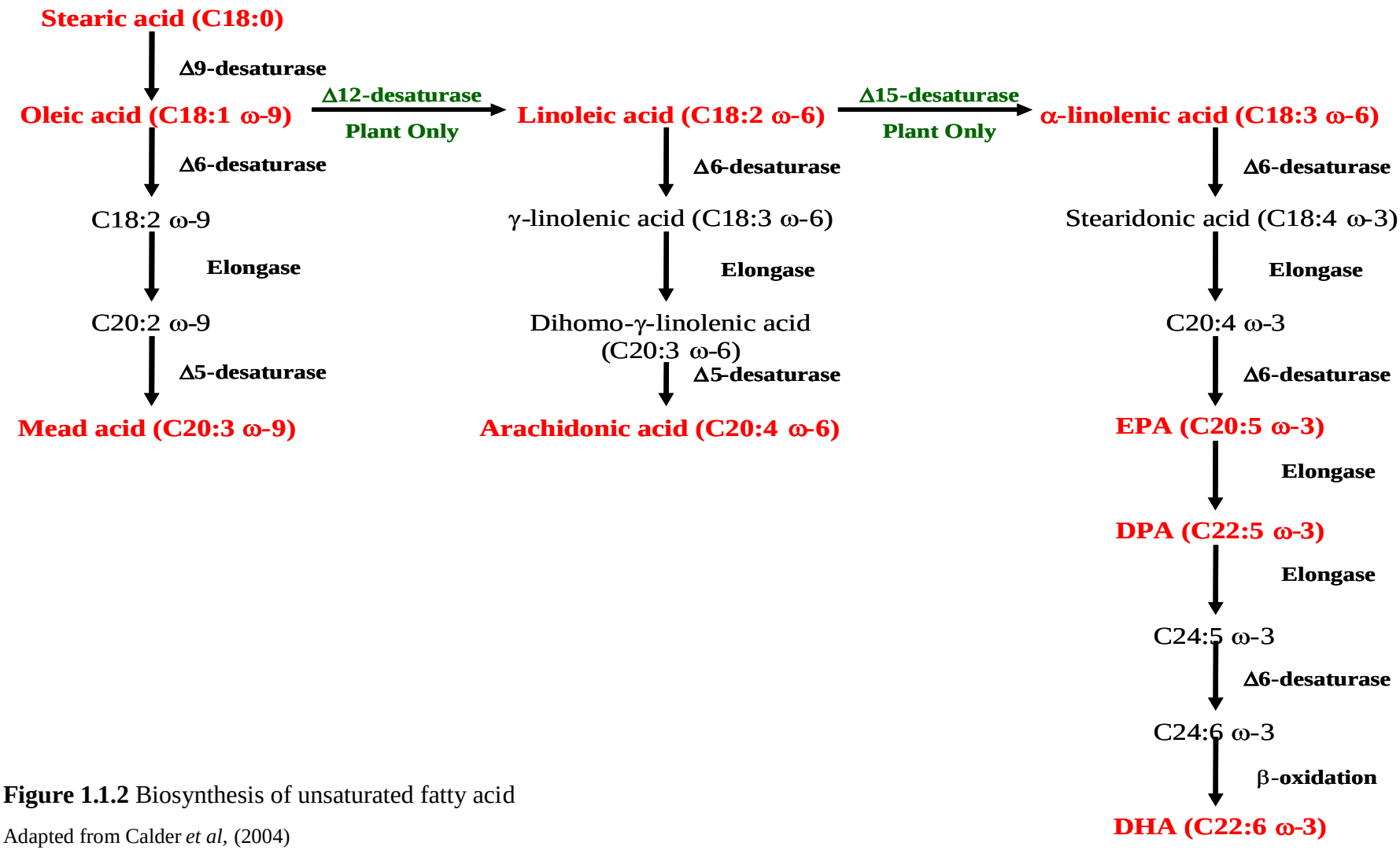


Figure 1.1.2 Biosynthesis of unsaturated fatty acid

Adapted from Calder *et al*, (2004)

1.1.3 Production of conjugated fatty acids

In addition to the modification of fatty acids via elongation and desaturation, the functional properties of fatty acids may also be altered via conformational changes in the structure of double bonds. These conformational changes are primarily catalysed by the activity of the microbial isomerase and reductase enzymes found in the rumen or intestine of animals and humans (Griinari & Bauman, 1999). An example is the biohydrogenation of dietary linoleic acid to stearic acid in the rumen involving the enzyme linoleic acid isomerase and ruminal reductase enzymes (Hennessy *et al.*, 2007; Jenkins & McGuire, 2006; Sieber *et al.*, 2004) **(Figure 1.1.3)**. This process results in the production of vaccenic acid (*trans* 11-C18:1) and the *cis* 9, *trans* 11 conjugated linoleic acid (CLA) isomer.

Indeed, the conversion of PUFA to their conjugated isomers by plants, animals, and bacteria has been proven to dramatically impact on their properties. In animals the production of conjugated fatty acids is primarily observed in the ruminant (e.g. bovine, ovine) but occurs by similar mechanisms in almost all mammals including humans (Griinari & Bauman, 1999; Palmquist & Santora, 1999). Ruminant CLA is primarily found in the animal's milk and meat (Dhiman *et al.*, 1999; Griinari & Bauman, 1999). It is estimated that, approximately 75-90% of ruminant CLA is produced endogenously by the animal from ruminally produced vaccenic acid with the remainder produced by the ruminant microbiota as previously described (Hennessy *et al.*, 2007) **(Figure 1.1.3)**. This endogenous CLA production is thought to occur primarily in the mammary gland as a result of the activity of the enzyme Δ^9-desaturase (Griinari & Bauman, 1999). Of the CLA isomers currently identified in mammals, it is the *cis* 9, *trans* 11 CLA isomer which predominates, although a range of others have been detected **(Table 1.1.3)**.

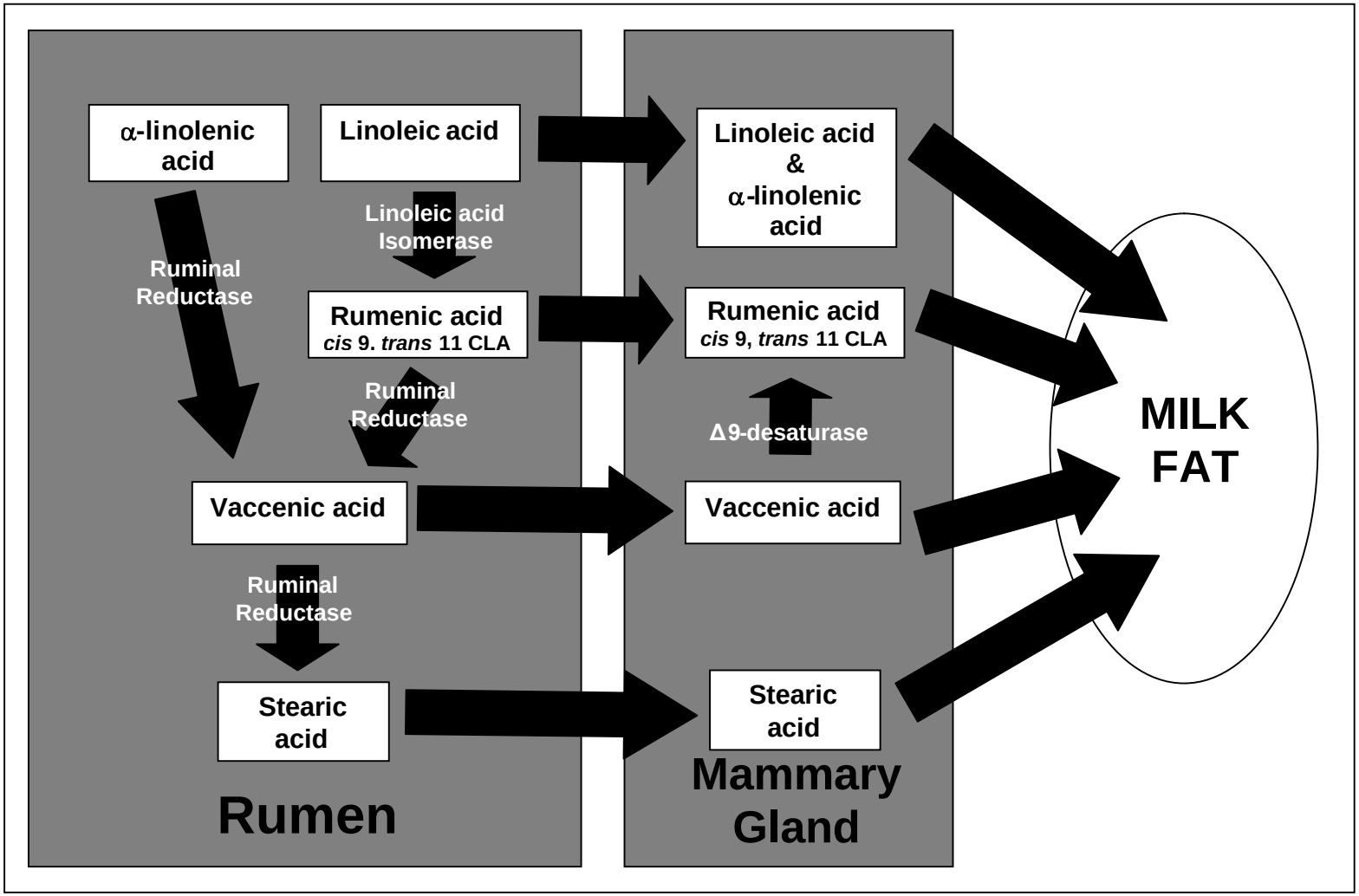


Figure 1.1.3 Ruminal biohydrogenation of dietary fatty acids

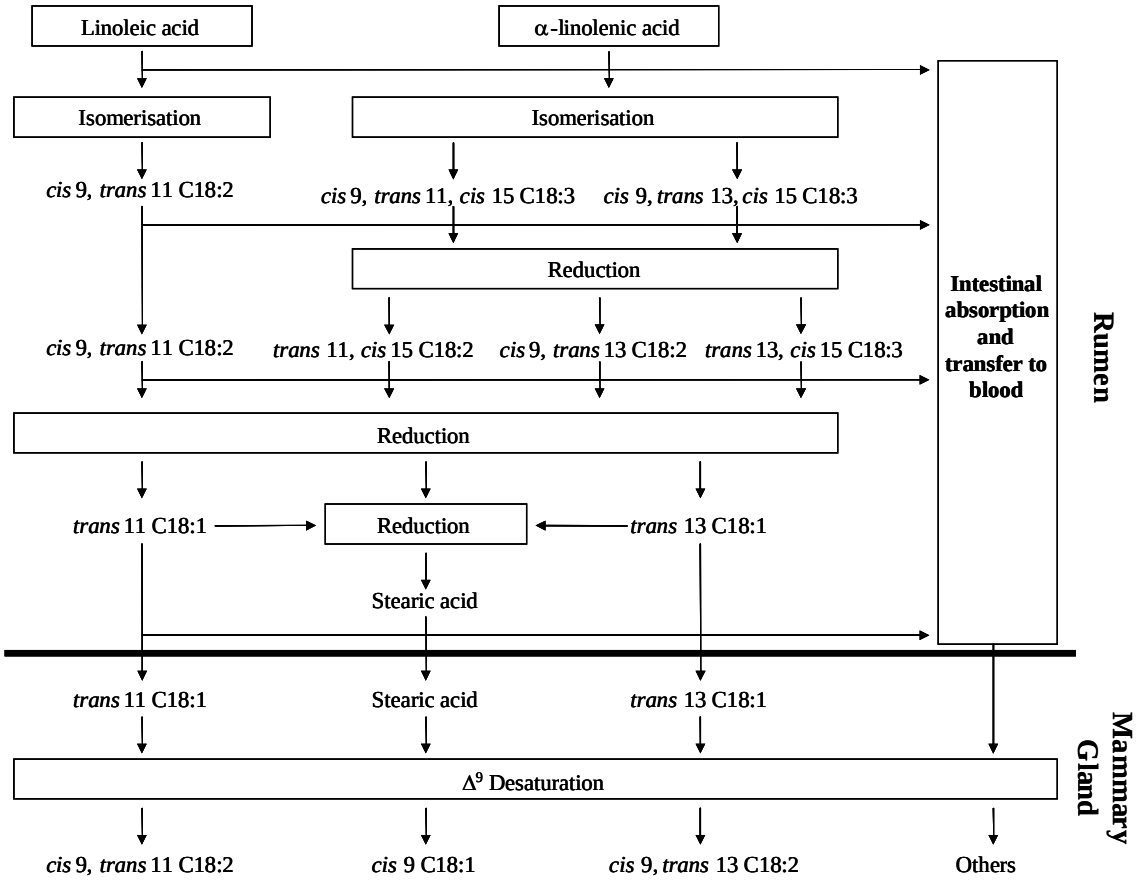
In addition to CLA, other conjugated isomers are often detected in animals and in particular, ruminants. The most predominant of these are the *cis* 9, *trans* 11, *cis* 15-C18:3 and *cis* 9, *trans* 13, *cis* 15-C18:3 conjugated α-linolenic acid (CALA) isomers, which constitute 0.03% of the milk fat derived from Canadian cattle (Destaillats *et al.*, 2005). Like the CLA isomers, the CALA isomers are present as a result of ruminal reductase and isomerase activity **(Figure 1.1.4)**.

In microbes the production of conjugated fatty acids is catalysed primarily by two different isomerase enzymes. The more common of these is the enzyme linoleic acid isomerase which catalyses the conversion of free linoleic acid and α-linolenic acid to the *cis* 9, *trans* 11 and *trans* 9, *trans* 11 CLA isomers, and to the *cis* 9, *trans* 11 *cis* 15 and *trans* 9, *trans* 11, *cis* 15 CALA isomers, respectively (Coakley *et al.*, 2009; Hennessy *et al.*, 2007; Ogawa *et al.*, 2005). This enzyme is found in both the ruminal and intestinal microbiota, and has been particularly associated with strains of *Lactobacillus*, *Bifidobacterium*, and *Butyrivibrio fibrisolvens*, although the enzyme has also been found in strains of dairy starter such as *Propionibacterium*, *Lactococcus*, and *Enterococcus* (Hennessy *et al.*, 2007; Sieber *et al.*, 2004). Studies to characterise the activity of this enzyme have been performed by Ogawa *et al.*, (2001) using the strain *Lactobacillus acidophilus* AKU 1137, and these researchers found the activity of the enzyme is characterised by two distinct steps consisting of the hydration of linoleic acid to 10-hydroxy-C18:1 and the subsequent conversion of these hydroxy fatty acids to the *cis* 9, *trans* 11 CLA isomer and *trans* 9, *trans* 11 CLA isomer **(Figure 1.1.5)**. The other fatty acid isomerase, termed *Propionibacterium acnes* isomerase, is most commonly isolated from strains of *Propionibacterium acnes* (Liavonchanka *et al.*, 2006; Verhulst *et al.*, 1987). This isomerase has been attributed primarily with the bioconversion

Table 1.1.3 Commonly identified conjugated linoleic acid isomers

CLA isomer	Primary source
cis 9, *trans* 11-C18:2	Milk and meat of mammals; Microbially produced
trans 10, *cis* 12-C18:2	Milk and meat of mammals; Microbially produced
trans 7, *cis* 9-C18:2	Milk of ruminants
cis 9, *trans* 13-C18:2	Milk of ruminants
trans 9, *trans* 11-C18:2	Microbially produced

Figure 1.1.4 Ruminal biohydrogenation of linoleic acid and α-linolenic acid



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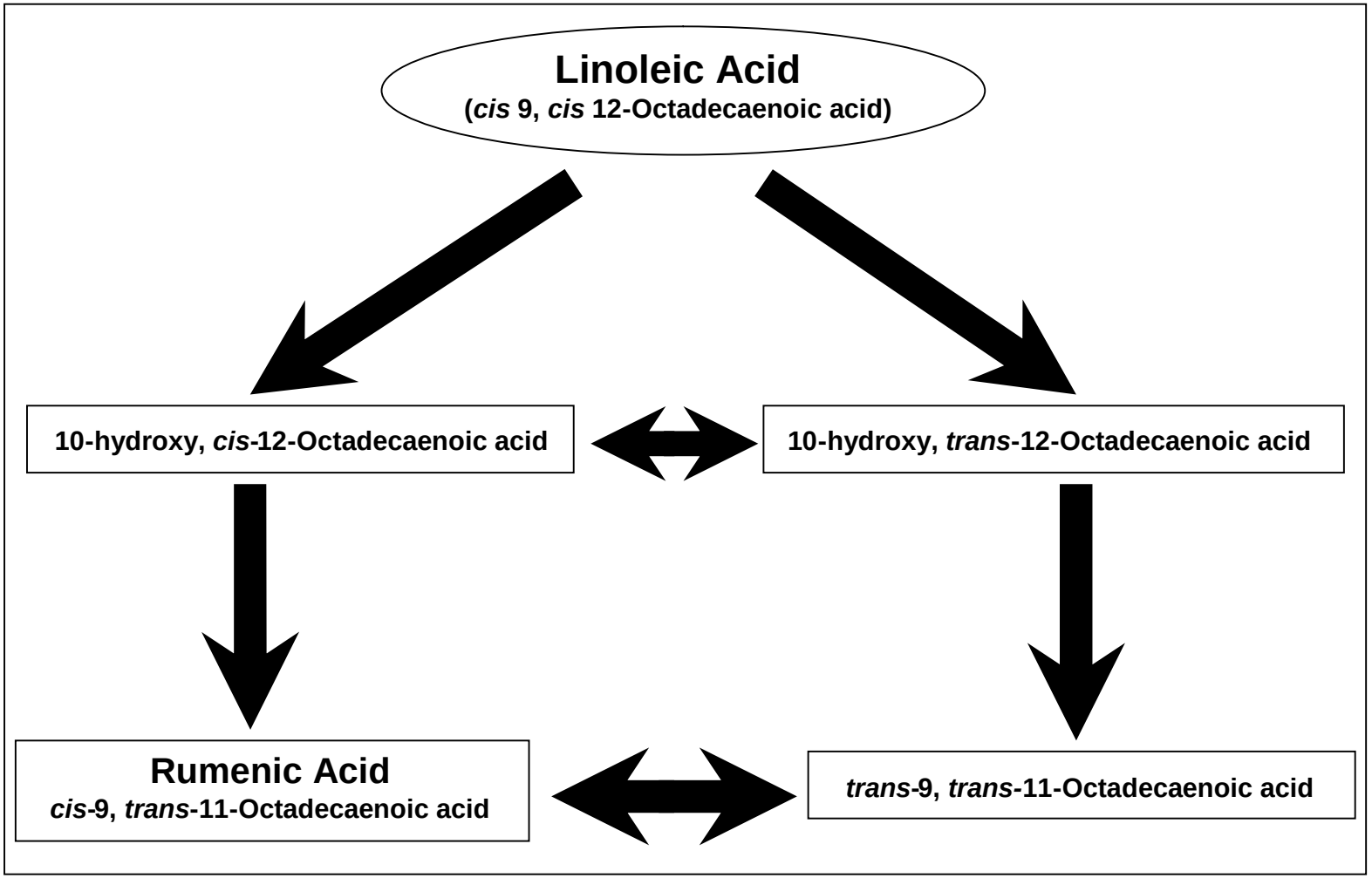

Figure 1.1.5 Formation of *cis* 9, *trans* 11 CLA and *trans* 9, *trans* 11 CLA by *Lactobacillus acidophilus* AKU 1137

of linoleic acid to the *trans* 10, *cis* 12 CLA isomer, but has also been observed to conjugate a range of other fatty acid substrates **(Table 1.1.4)**.

Conjugated fatty acids are found in the seed oils of a number of plants and trees **(Table 1.1.5)**. The most common of these conjugates include punicic acid (*cis* 9, *trans* 11, *cis* 13-C18:3), α-eleostearic acid (*cis* 9, *trans* 11, *trans* 13-C18:3), catalpic acid (*trans* 9, *trans* 11, *cis* 13-C18:3), and calendic acid (*trans* 8, *trans* 10, *cis* 12-C18:3). The presence of these fatty acids in these seed oils is primarily attributed to the activity of a number of divergent forms of "conjugase" enzymes which display high homology to the Δ^{12}-oleate desaturase (FAD2) family of enzymes (Cahoon *et al.*, 1999; Cahoon *et al.*, 2001; Dyer *et al.*, 2002). Recent attempts have been made to identify the pathways responsible for the production of two of these conjugated fatty acids, namely, α-eleostearic acid from *Aleurites fordii* Hemsl. (Tung) and calendic acid from *Calendula officinalis*. In the biosynthetic pathway of α-eleostearic acid, researchers identified the involvement of two closely related forms of Δ^{12}-oleate desaturase (FAD2) (Dyer *et al.*, 2002). The first of these, termed FAD2, catalyses the conversion of oleic acid to linoleic acid, and the second, FADX, catalyses the conversion of linoleic acid to α-eleostearic acid **(Figure 1.1.6)**. In addition to their ability to catalyse the conversion of linoleic acid to α-eleostearic acid, the FADX enzyme was found to facilitate the conversion of a wide range of fatty acid substrates to their conjugated derivatives (Dyer *et al.*, 2002). The second pathway investigated targeted the production of calendic acid in *Calendula officinalis* seeds, and resulted in the identification of the role of two FAD2 related enzymes, namely, CoFADX-1 and CoFADX-2 which convert the *cis* 9 double bond of linoleic acid to the *trans* 8, *trans* 10 double bond found in calendic acid (Cahoon *et al.*, 2001) **(Figure 1.1.6)**.

Table 1.1.4 Conjugated fatty acid production by strains of *Propionibacterium acnes*

Substrate fatty acid	Conjugate produced
Linoleic acid, *cis* 9, *cis* 12-C18:2	*trans* 10, *cis* 12-CLA
α-linolenic acid, *cis* 9, *cis* 12, *cis* 15-C18:3	Negative
γ-linolenic acid, *cis* 6, *cis* 9, *cis* 12-C18:3	*cis* 6, *trans* 10, *cis* 12-octadecatrienoic acid
Homo-γ-linolenic acid, *cis* 8, *cis* 11, *cis* 14-C20:3	*cis* 8, *trans* 12, *cis* 14-eicosatrienoic acid
Arachidonic acid, *cis* 5, *cis* 8, *cis* 11, *cis* 14-C20:4	5, 8, 12, 14-eicosatetraenoic acid
Nonadecadienoic acid *cis* 10, *cis* 13-C19:2	*trans* 11, *cis* 13-nonadecadienoic acid

Adapted from Verhulst *et al.,* (1987)

Table 1.1.5 The principal conjugated plant fatty acids and their sources

Conjugate	Source	Conc.	Reference
C18:3 CALA (ω-3)			
cis 9, *trans* 11, *cis* 13-C18:3	Pomegranate seed	83%	(Takagi & Itabashi, 1981)
cis 9, *trans* 11, *trans* 13-C18:3	Tung seed	67.7%	(Takagi & Itabashi, 1981)
	Bitter gourd seed	56.2%	(Takagi & Itabashi, 1981)
	Snake gourd seed	30-50%	(Dhar & Bhattacharyya, 1998)
	Parwal seed	30-50%	(Dhar & Bhattacharyya, 1998)
trans 9, *trans* 11, *cis* 13-C18:3	Catalpa seed	42.3%	(Takagi & Itabashi, 1981)
trans 8, *trans* 10, *cis* 12-C18:3	Pot marigold seed	62.2%	(Takagi & Itabashi, 1981)
trans 8, *trans* 10, *trans* 12-C18:3	Pot marigold seed	4.7%	(Nagao & Yanagita, 2005)

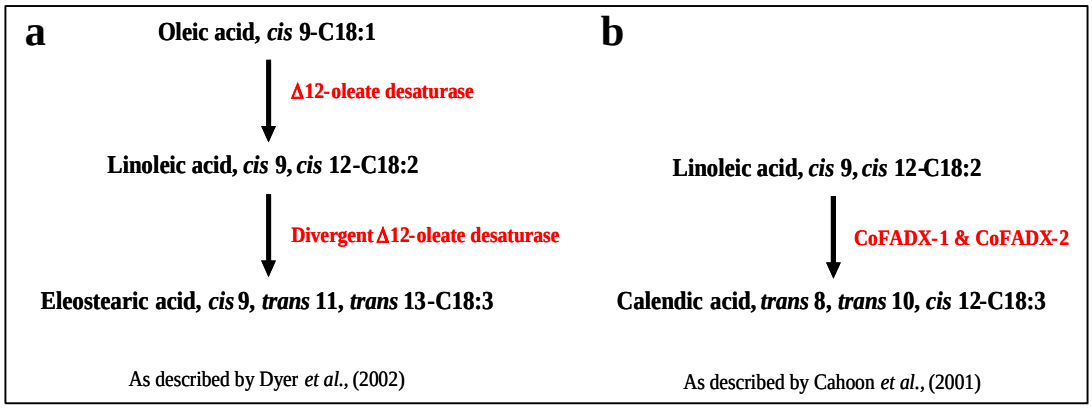

Figure 1.1.6 Biosynthesis of conjugated fatty acids by **a)** *Calendula officinalis* and **b)** *Aleurites fordii* Hemsl (Tung tree).

1.1.4 Conclusions

At their most basic, all fatty acids share a common origin. This basic structure can undergo a wide range of structural changes of which elongation, desaturation, reduction, conjugation and various forms of oxidation are the most widely recognised. Furthermore, these fatty acids may also form ester linkages with other molecules via their highly reactive carboxyl group. As a whole, these reactions give rise to a range of fatty acids with a wide variety of physical and chemical properties which play a vital role in every facet of life.

1.1.5 References

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