Fatty acid acylation of proteins: specific roles for palmitic, myristic and caprylic acids

Vincent Rioux

OCL 2016, 23(3) D304
© V. Rioux, Published by EDP Sciences 2016
DOI: 10.1051/ocl/2015070

OCL Oilseeds & fats Crops and Lipids
Available online at:
www.ocl-journal.org

Research Article – Dossier Open Access

Lipid consumption and functionality: new perspectives
Consommations et fonctionnalités des lipides : Nouveaux horizons

Dossier

Fatty acid acylation of proteins: specific roles for palmitic, myristic and caprylic acids

Vincent Rioux*

Laboratoire de Biochimie-Nutrition Humaine, Agrocampus Ouest, INRA USC 1378, 65 rue de Saint-Brieuc, CS 84251, 35042 Rennes Cedex, France

Received 28 November 2015 – Accepted 14 December 2015

Abstract – Fatty acid acylation of proteins corresponds to the co- or post-translational covalent linkage of an acyl-CoA, derived from a fatty acid, to an amino-acid residue of the substrate protein. The cellular fatty acids which are involved in protein acylation are mainly saturated fatty acids. Palmitoylation (S-acylation) corresponds to the reversible attachment of palmitic acid (C16:0) via a thioester bond to the side chain of a cysteine residue. N-terminal myristoylation refers to the covalent attachment of myristic acid (C14:0) by an amide bond to the N-terminal glycine of many eukaryotic and viral proteins. Octanoylation (O-acylation) typically concerns the formation of an ester bond between octanoic acid (caprylic acid, C8:0) and the side chain of a serine residue of the stomach peptide ghrelin. An increasing number of proteins (enzymes, hormones, receptors, oncogenes, tumor suppressors, proteins involved in signal transduction, eukaryotic and viral structural proteins) have been shown to undergo fatty acid acylation. The addition of the acyl moiety is required for the protein function and usually mediates protein subcellular localization, protein-protein interaction or protein-membrane interaction. Therefore, through the covalent modification of proteins, these saturated fatty acids exhibit emerging specific and important roles in modulating protein functions. This review provides an overview of the recent findings on the various classes of protein acylation leading to the biological ability of saturated fatty acids to regulate many pathways. Finally, the nutritional links between these elucidated biochemical mechanisms and the physiological roles of dietary saturated fatty acids are discussed.

Keywords: Acylated proteins / N-terminal myristoylation / octanoylation / palmitoylation / saturated fatty acids

Résumé – Protéines acylées pas des acides gras : rôles spécifiques des acides palmitique, myristique et caprylique. L'acylation d'une protéine correspond à la formation co- ou post-traductionnelle d'une liaison covalente entre un acide gras activé en acyl-CoA et un résidu d'acide aminé de la protéine substrat. Les acides gras utilisés par la cellule pour acyler des protéines sont très majoritairement saturés. On parle alors, respectivement, de palmitoylation (S-acylation) lorsqu'une liaison thioester se forme entre l'acide palmitique (C16:0) et la chaîne latérale d'une cystéine, de myristoylation N-terminale lorsqu'une liaison amide intervient entre l'acide myristique et la fonction amine d'une glycine N-terminale, et d'octanoylation (O-acylation) lorsqu'une liaison ester apparaît entre l'acide caprylique (ou octanoïque, C8:0) et la chaîne latérale d'une sérine. L'acylation concerne de très nombreuses protéines (enzymes, hormones, récepteurs, oncogènes, suppresseurs de tumeur, protéines impliquées dans la transduction des signaux, protéines de structure eucaryotes et même virales) et exerce donc une grande variété de fonctions dans les régulations cellulaires. La liaison covalente de l'acide gras à la protéine est cruciale pour l'acquisition de la fonction de la protéine, en changeant son hydrophobicité, en régulant l'ancrage de la protéine à la membrane, en modifiant son adressage subcellulaire ou encore en induisant des interactions entre sous-unités protéiques. La découverte progressive de nombreuses protéines acylées, dont la fonction est régulée par l'acylation, donne donc un nouvel intérêt fonctionnel à ces acides gras saturés. L'objectif de cette revue est de synthétiser les découvertes récentes sur les différentes classes d'acylation des protéines et sur les fonctions cellulaires émergentes que cette acylation procure à certains acides gras saturés. Le lien nutritionnel entre ces mécanismes moléculaires et les apports alimentaires en acides gras saturés est finalement discuté.

Mots clés : Acides gras saturés / myristoylation N-terminale / octanoylation / palmitoylation / protéines acylées

* Correspondence: vincent.rioux@agrocampus-ouest.fr

1 Introduction

Fatty acid acylation of proteins (Towler *et al.*, 1988) corresponds to the co- or post-translational covalent linkage of a fatty acid, activated in the form of acyl-CoA, to an amino-acid residue of the substrate protein (Fig. 1). The cellular fatty acids covalently bound to proteins are mainly saturated fatty acids (SFAs). Palmitoylation (S-acylation) corresponds to the reversible attachment of palmitic acid (C16:0) to the side chain of a cysteine residue via a thioester bond (Blaskovic *et al.*, 2014). N-terminal myristoylation refers to the covalent attachment of myristic acid (C14:0) by an amide bond to the N-terminal glycine of many eukaryotic and viral proteins (Johnson, Bhatnagar, *et al.*, 1994). Octanoylation (O-acylation) typically concerns the formation of an ester bond between octanoic acid (caprylic acid, C8:0) (Lemarié, Beauchamp, Legrand, *et al.*, 2015) and the side chain of a serine residue of the stomach ghrelin peptide (Kojima *et al.*, 1999). An increasing number of proteins (enzymes, hormones, receptors, oncogenes, tumor suppressors, proteins involved in signal transduction, eukaryotic and viral structural proteins) have been shown to undergo fatty acid acylation. The acyl moiety can mediate protein subcellular localization, protein-protein interaction or protein-membrane interaction. Therefore, through the covalent modification of proteins, these particular saturated fatty acids exhibit emerging specific and important roles in modulating protein functions (Ezanno *et al.*, 2013).

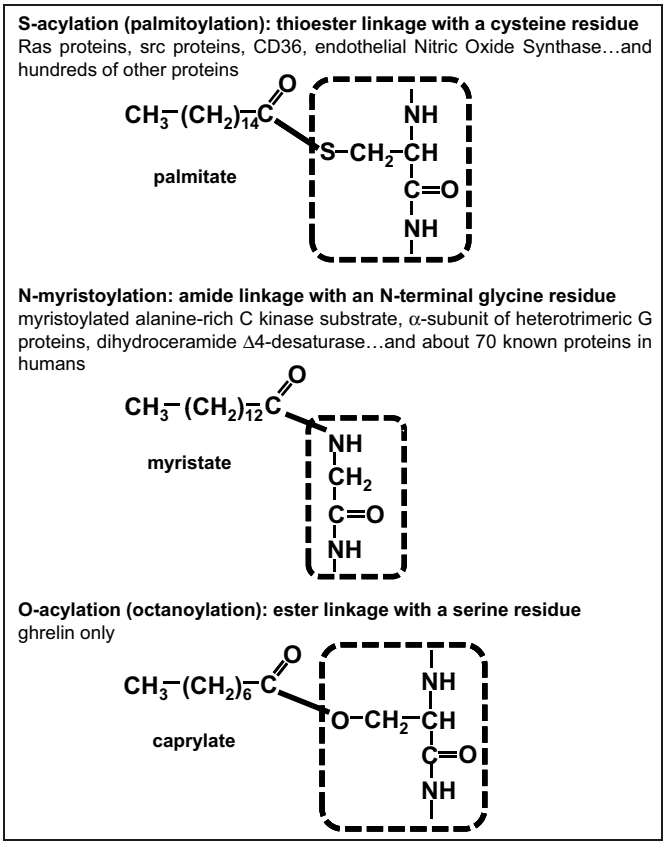

Fig. 1. Classification of the saturated fatty acid protein acylation and examples of acylated proteins. Palmitoylation (S-acylation) corresponds to the reversible attachment of palmitic acid (C16:0) to the side chain of a cysteine residue via a thioester bond. N-terminal myristoylation refers to the covalent attachment of myristic acid (C14:0) by an amide bond to the N-terminal glycine of many eukaryotic and viral proteins. Octanoylation (O-acylation) typically concerns the formation of an ester bond between octanoic acid (caprylic acid, C8:0) and the side chain of a serine residue of the stomach ghrelin peptide.

2 Palmitic acid and protein palmitoylation

Protein S-acylation (Fig. 1) is also called palmitoylation because palmitic acid (C16:0) is the main SFA involved in this posttranslational thioester linkage with the side chain of cysteine residues (Mitchell *et al.*, 2006), but other SFAs like myristic (Rioux *et al.*, 2002) and lauric acids (Rioux *et al.*, 2003) have also been found. Protein palmitoylation is catalyzed by a family of palmitoyltransferases sharing a DHHC motif and including 20 to 24 members in humans (Greaves and Chamberlain, 2011). Palmitoylation is involved in regulatory mechanisms because the association of the protein with the palmitoyl moiety is reversible and facilitates protein-membrane interactions and subcellular trafficking of proteins. Proteins that undergo this modification span almost all cellular functions. Several signal transductions depend for instance on palmitic acid, including proteins that have been shown to undergo successive myristoylation and palmitoylation, like the α subunit of many heterotrimeric G proteins (Chen and Manning, 2001).

3 Myristic acid and protein N-terminal myristoylation

Protein N-myristoylation (Fig. 1) specifically involves myristic acid (C14:0) (Beauchamp, Rioux, *et al.*, 2009; Rioux and Legrand, 2001). Myristoyl-CoA: protein N-myristoyltransferase (NMT), the enzyme catalyzing this stable acylation, has been identified in many organisms. In mammals, two distinct NMT genes referred to as type 1 and 2 have been described (Giang and Cravatt, 1998; Rioux *et al.*, 2006; Rundle *et al.*, 2002). The contribution of each gene transcript to NMT expression and activity in vivo, and the specific role of each NMT isoform in cellular replication, proliferation, and other cellular processes, is however still not clearly described (Ducker *et al.*, 2005; Selvakumar *et al.*, 2006; Yang *et al.*, 2005). Both isoforms seem to have a similar high substrate selectivity for myristic acid (Giang and Cravatt, 1998; Rioux *et al.*, 2006). The myristoyl moiety has been shown to mediate protein subcellular localization, protein-protein interaction or protein-membrane interactions required for the biological activities of the myristoylated proteins (Johnson, Bhatnagar, *et al.*, 1994). Initially described as a co-translational modification, N-myristoylation has more recently been shown to also occur as a post-translational mechanism in apoptotic cells (Martin *et al.*, 2011), after proteolytic cleavage by caspases exposing a previously hidden N-terminal glycine residue.

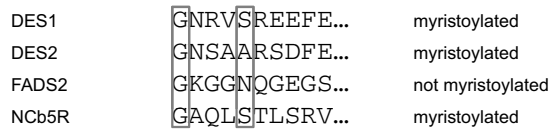

Fig. 2. Alignment and comparison of the N-terminal amino acid sequences of the two isoforms of rat dihydroceramide Δ4-desaturase (DES1 and DES2), rat Δ6-desaturase (Fatty Acid Desaturase 2 or FADS2) and rat NADH-cytochrome b5 reductase (NCb5R). Among these putative myristoylation candidates, only FADS2 was shown to be preclude from myristoylation.

The proteins that are myristoylated all possess an N-terminal glycine residue, but the subsequent so-called "myristoylation consensus sequence" is less well-defined (Fig. 2). Computational prediction suggested that about 0.5% of all proteins in the human genome could be myristoylated (Maurer-Stroh *et al.*, 2004). Indeed, the N-myristoylated proteome was recently studied in human cells, leading to the identification of more than 100 N-myristoylated proteins (Thinon *et al.*, 2014). The myristoylated proteins include key components in intracellular signaling pathways, oncogenes, structural viral proteins but also common constitutive eukaryotic proteins.

Among this last category, we noticed throughout the past years that several members of the mammalian family of membrane-bound desaturases possess a potential site of myristoylation. Both isoforms of dihydroceramide Δ4-desaturase (DES1 and DES2) indeed present a site of myristoylation in rats (Fig. 2), mice and humans (Beauchamp *et al.*, 2007; Mizutani *et al.*, 2004; Ternes *et al.*, 2002). DES1 catalyzes the last step of *de novo* ceramide biosynthesis which consists in the introduction of a *trans* Δ4-double bond in the carbon chain of the dihydroceramide. DES2 possesses a bifunctionnal Δ4-desaturase/C4-hydroxylase activity (Omae *et al.*, 2004). The presence of the *trans* Δ4-double bond seems to be critical for the acquisition of the biological activities of ceramide (Bielawska *et al.*, 1993). Indeed, ceramide is able to induce apoptosis (Garcia-Ruiz *et al.*, 1997; Gudz *et al.*, 1997; Siskind *et al.*, 2002), which is not the case of its precursor dihydroceramide. We showed that both DES1 and DES2 are myristoylated and that this N-terminal modification significantly increased the activity of the recombinant DES1 when expressed in COS-7 cells (Beauchamp *et al.*, 2007). Compared to a recombinant unmyristoylable mutant form of DES1 (N-terminal glycine replaced by an alanine), the desaturase activity of the myristoylable wild-type DES1 was two times higher, in the presence of myristic acid incubated with the cells. The description of this regulatory mechanism highlighted a new potential relationship between myristic acid, the saturated fatty acid capable of binding and activating the enzyme involved in the final *de novo* ceramide biosynthesis step, and lipoapoptosis induced through the ceramide pathway. Indeed, we subsequently showed that the myristoylation of recombinant DES1 can target part of the enzyme to the mitochondria, leading to an increase in ceramide levels (specifically in the mitochondria) which in turn leads to apoptosis in the COS-7 cell model (Beauchamp, Tekpli, *et al.*, 2009). Finally, myristic acid also increased native DES1 activity in cultured rat hepatocytes (Ezanno *et al.*, 2012).

A second example of a membrane-bound desaturase which has been studied for its potential myristoylation is the Δ6-desaturase (Fatty Acid Desaturase 2: FADS2) involved in essential polyunsaturated fatty acid synthesis (Aki *et al.*, 1999; Cho *et al.*, 1999; D'Andrea *et al.*, 2002). Several years ago, myristic acid was shown to trigger a specific and dose-dependent increasing effect on Δ6-desaturase activity in cultured rat hepatocytes (Jan *et al.*, 2004) whatever the substrate used to measure this enzyme activity (oleic acid, linoleic or α-linolenic acid). Because the FADS2 enzyme exhibits an N-terminal glycine residue (Fig. 2), the increase in the activity of Δ6-desaturase by myristic acid was first postulated to be mediated by N-myristoylation. However, bioinformatic predictions indicated and biological experiments confirmed that FADS2 is not myristoylated (Beauchamp *et al.*, 2007). Nevertheless, FADS2 is believed to cooperate with NADH-cytochrome b5 reductase (NCb5R) in the endoplasmic reticulum membrane (Guillou *et al.*, 2004) and this last enzyme is also known (Fig. 2) to be N-terminally myristoylated (Borgese *et al.*, 1996; Colombo *et al.*, 2005; Ozols *et al.*, 1984). The hypothesis according to which the myristoylation of NADH cytochrome b5 reductase could account for the increased Δ6-desaturase activity was therefore proposed (Rioux *et al.*, 2011). Although its linkage with myristic acid is not absolutely required for its association with endoplasmic reticulum membranes (Strittmatter *et al.*, 1993), myristoylation of NCb5R may modify the transfer by lateral diffusion of electrons from NCb5R to the heme of cytochrome b5 and then to the terminal desaturase. It may also change the interaction between NCb5R and the desaturase. Moreover, it may modify the conformation of the whole complex, as the analysis of the relative contribution of the myristoyl moiety in membrane binding in a model of phospholipid vesicles suggests (Strittmatter *et al.*, 1993). In such a hypothesis, not only the Δ6-desaturase but also all the membrane-bound desaturases which are associated with NCb5R would be affected by this regulatory mechanism. This regulation may explain the effect of dietary myristic acid on the overall conversion of α-linolenic acid to longer highly unsaturated fatty acids, like eicosapentaenoic (EPA) and docosahexaenoic (DHA) acids, shown in rat nutritional experiments (Legrand *et al.*, 2010; Rioux *et al.*, 2005, 2008).

These two detailed examples show that through the myristoylation of the dihydroceramide Δ4-desaturase (DES) and of the NADH-cytochrome b5 reductase, myristic acid may therefore be considered as one of the regulators of cellular bioactive lipid concentration such as ceramide and polyunsaturated fatty acids.

4 Caprylic acid and ghrelin octanoylation

Fifteen years ago, caprylic acid (C8:0) was surprisingly found attach to the ghrelin (Fig. 1) purified from rat stomach (Kojima *et al.*, 1999), but only recently the presence of the octanoyl moiety appeared crucial for this peptide hormone (Lemarié, Beauchamp, Legrand, *et al.*, 2015). Ghrelin is a 28 amino acid peptide expressed in the digestive tract and mainly in the stomach. Its octanoylated form binds to the growth hormone secretagogue receptor (GHSR-1a) located in the pituitary gland and hypothalamus (Howard *et al.*, 1996).

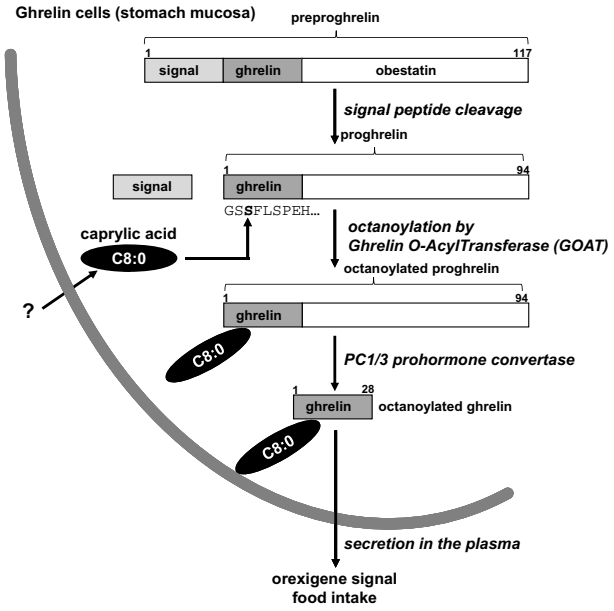

Fig. 3. Ghrelin synthesis, maturation and post-translational octanoylation in stomach cells. Preproghelin is first translated from its corresponding gene and then cleaved to produce the proghrelin. Proghrelin is octanoylated on its 3rd amino-acid residue which corresponds to a serine. The octanoylated proghrelin is finally cleaved a 2nd time and secreted in the plasma. Both octanoylated and non-octanoylated ghrelin may co-exist but only the octanoylated form is able to bind to its hypothalamic receptor.

Octanoylated ghrelin is therefore suspected to regulate many relevant biological processes including the secretion of the growth hormone (GH), the stimulation of appetite and food intake, the modulation of gastric acid secretion and motility, the regulation of glucose homeostasis and adiposity (Delporte, 2013).

During its maturation in the gastric mucosa and before secretion in the blood (Fig. 3), the preproghrelin is first cleaved and part of the proghrelin is octanoylated on its N-terminal 3rd serine residue, in the endoplasmic reticulum lumen. The stomach enzyme involved in ghrelin octanoylation is called ghrelin O-acyltransferase (GOAT) and belongs to the family of membrane bound O-acyltransferases (MBOAT), a group of proteins involved in acetyltransferase and acyltransferase activity (Gutierrez *et al.*, 2008). This enzyme is described by some authors as a "fat sensor" informing the brain, *via* the octanoylated ghrelin level, of the nutrient availability in the digestive tract (Taylor *et al.*, 2013). The GOAT is an endoplasmic reticulum membrane protein with 11 transmembrane helices and one reentrant loop (Taylor *et al.*, 2013). The GOAT expression corresponds predominantly to the distribution of the ghrelin (Lim *et al.*, 2011) with a strong co-expression in the mucosa of the digestive tract. Studies showed that GOAT displays a high affinity for caprylic acid but can also link ghrelin to C6:0, C10:0 and C10:1 to a lesser extent (Darling *et al.*, 2015). Little is however still known about the GOAT enzymatic mechanism and the analysis of native stomach GOAT activity still remains a challenge (Taylor *et al.*, 2012).

Whether octanoylated or not, the proghrelin is then subjected to a second cleavage under the action of a prohormone convertase (Fig. 3) which leads to the production of the native ghrelin peptide (octanoylated or not) and to an additional C-terminal peptide which can itself undergo further proteolytic processes generating a smaller peptide called obestatin (Zhang *et al.*, 2005). The mature acylated ghrelin secreted in the plasma is the only form that can bind to its GHSR-1a receptor. However, unacylated ghrelin can also be secreted and seems involved in the regulation of several physiological functions (Delhanty *et al.*, 2013). Ghrelin is for now the only known case of a peptide covalently linked to caprylic acid.

As mentioned above, plasma ghrelin exists in both unacylated and acylated forms, but only the active acylated form can bind its GHSR-1a receptor (Howard *et al.*, 1996). Octanoylated ghrelin is best known for its orexigenic actions in the central nervous system, involved in the regulation of food intake and thereby in weight control (Kamegai *et al.*, 2001). The first studies showed that intracerebral chronic acylated ghrelin administration increased body weight by stimulating food intake and by inhibiting energy expenditure and fat catabolism (Tschöp *et al.*, 2000). However, as demonstrated in recent studies showing that ghrelin-null mice did not exhibit altered food intake nor altered expression of hypothalamic neuropeptides involved in the regulation of appetite, the essentiality of endogenous ghrelin in the regulation of food intake appeared controversial (Albarran-Zeckler *et al.*, 2011).

5 Impact of dietary SFAs on cellular protein acylation

Because of their potential dual origin (diet and endogenous synthesis), the impact of dietary SFAs on the regulation of the protein acylation processes is still questioned. More specifically, human data reporting the balance between intake and *de novo* synthesis of SFAs are not available. In addition, the optimal cellular concentration of saturated fatty acyl-CoAs required for each type of protein acylation is not known and data available suggest that the free cytosolic concentration of fatty acyl-CoA esters is in the low nanomolar range (Faergeman and Knudsen, 1997).

Palmitic acid is universally found in natural fats, representing 15–25% of total fatty acids. Therefore, due to its high dietary level (30–38 g/day in humans) (Katan *et al.*, 1994) and well-described predominant synthesis by the Fatty Acid Synthase (Singh *et al.*, 1984), palmitic acid intracellular concentration may not be considered as the rate-limiting molecule for the palmitoylation mechanism.

Concerning now myristic acid, its endogenous biosynthesis (Rioux *et al.*, 2007) appeared very low in cultured rat hepatocytes. If it is also the case in humans, the diet is therefore the main source (4–8 g/day) for this particular fatty acid (Wolk *et al.*, 2001) which represents about 10% of FAs in ruminant milk fat. Very low dietary intakes of myristic acid may likely lead to insufficient intracellular concentration of myristoyl-CoA to ensure the proper activity of N-myristoyltransferase (NMT), when considering the whole pool of myristoylable proteins. One may therefore wonder about the risk of total eviction of dairy products as the unique source of dietary myristic acid. In yeast, studies analyzing the

activity of NMT have suggested that the enzyme was able to use both exogenous and endogenous myristic acid as substrate (Duronio *et al.*, 1991, 1992; Johnson, Knoll, *et al.*, 1994). The requirement for myristic acid suggests that in certain cases, it could be the rate-limiting molecule in this mechanism or that competition could occur. In addition, the mechanism by which myristic acid initially esterified in the TAG or PL is used for myristoylation is unknown, too.

Concerning finally caprylic acid, no endogenous biosynthesis of this fatty acid has been described in animals except in the lactating mammary gland (Fernando-Warnakulasuriya *et al.*, 1981). On the other hand, natural food sources of caprylic acid are restricted to specific vegetable oils and milk products. Caprylic acid is abundant in coconut oil (6–10% of FAs, with C8:0 mainly in sn-1 and -3 positions on the triglycerides, TG) and in palm kernel oil (2–5% of FAs). Milk is the only natural source of animal caprylic acid with strong differences between mammalian species. C8:0 represents about 0.5% of FAs in human milk (Jensen, 1996), but is higher in cow milk (1–2%) (Jensen *et al.*, 1990), in goat milk (3%) (Alonso *et al.*, 1999), in rat milk (5–6%) (Fernando-Warnakulasuriya *et al.*, 1981) and reaches up to 15–18% in rabbit milk (Perret, 1980). Caprylic acid is primarily esterified in sn-3 position of the TGs in cow (Jensen *et al.*, 1990), rat (Staggers *et al.*, 1981) and human (Jensen, 1996) milks. In western countries, dietary MCFAs represent less than 2% of total dietary energy and caprylic acid is only a minor part of these MCFAs in milk fat (1–2% of cow milk FAs).

Part of caprylic acid coming from dietary medium chain TG (MCTs) can be early released during digestion through the action of preduodenal lipase (Clark *et al.*, 1969), leading to its potential and yet not clearly quantified direct absorption by the stomach mucosa (Lai and Ney, 1998; Perret, 1980). Dietary caprylic acid is therefore suspected to directly provide GOAT enzyme with octanoyl-CoA co-substrates (Fig. 3) necessary for the acyl modification of ghrelin. Indeed, ingestion by mice of either MCFAs or MCTs increased the stomach concentration of acylated ghrelin (Nishi, Hiejima, Hosoda, *et al.*, 2005), without changing the total ghrelin amounts. Nishi *et al.* detected heptanoylghrelin (Nishi, Hiejima, Hosoda, *et al.*, 2005) or decanoylghrelin (Nishi *et al.*, 2013) in the stomachs of mice fed with triheptanoin or tricaprin, confirming that at least part of the ingested MCFAs was directly used for ghrelin acylation. These results are consistent with the hypothesis of gastric absorption of MCTs but part of the caprylic acid present in the stomach may also come from intestinal absorption followed by uncompleted uptake by the liver. In ruminants, the ingestion of MCFAs during 2 weeks by lactating dairy cows increased the plasma acylated ghrelin concentrations (Fukumori *et al.*, 2013). In cachectic patients, a 2-week administration of an enteral nutrition formula containing 3 g/day of caprylic acid enhanced plasma octanoylated ghrelin and also improved the body mass index (Ashitani *et al.*, 2009). A single administration of the formula also increased plasma octanoylated ghrelin 5 h after administration. Conversely, in another study on the role of the gustatory G-protein in the sensing of FAs for octanoylation of ghrelin, ingested MCFAs increased stomach acylated ghrelin but did not change the plasma ghrelin concentration (Janssen *et al.*, 2012).

To further understand the effect of dietary caprylic acid on the concentration of circulating plasma acylated and unacylated ghrelin, we recently designed a nutritional study (Lemarié, Beauchamp, Dayot, *et al.*, 2015) including Sprague-Dawley male rats which were fed during 6 weeks with three dietary C8:0 levels (0, 8 and 21% of FAs). A specific dose-response enrichment of the stomach tissue C8:0 was observed. However, the acylated ghrelin concentration in the plasma was unchanged in spite of the increased C8:0 availability. Conversely, a reproducible decrease in the plasma concentration of unacylated ghrelin was observed, which was consistent with a decrease in the stomach preproghrelin mRNA and stomach ghrelin expression. Additionally, we measured high levels of acylated ghrelin in the plasma of rats receiving no dietary C8:0. Thus, the low stomach C8:0 level observed in these rats could be enough to supply the octanoyl-CoA co-substrate used to acylate the proghrelin (Lemarié, Beauchamp, Dayot, *et al.*, 2015).

In addition, the maturation and secretion of stomach acylated ghrelin are complex processes potentially regulated by dietary LCFAs, MCFAs and GPR120 (Gong *et al.*, 2014). The circulating ghrelin is additionally submitted to clearance and rapid de-acylation or degradation. For instance, a ghrelin deacylation enzyme (acyl-protein thioesterase-1, APT1) has recently been described, that can des-acylate ghrelin in the plasma (Chen and Enriori, 2014). Moreover, acylated ghrelin has been shown to form a complex with larger proteins like immunoglobulins (Ghrelin-reactive IgG) (Takagi *et al.*, 2013) that protects the acylated form from degradation. Some studies assaying total ghrelin in plasma have also reported lower levels in obese subjects, due to lower levels of unacylated ghrelin, whereas acylated ghrelin remained stable, suggesting a specific decreased degradation of acylated ghrelin in obese (Takagi *et al.*, 2013). In humans, it has also been shown that the GOAT enzyme was present in the blood, which could modify the balance between de-acylation and re-acylation (Goebel-Stengel *et al.*, 2013). For all these reasons, the concentration of both the acylated and unacylated plasma ghrelin may not simply reflect the stomach concentration (Nishi, Hiejima, Mifune, *et al.*, 2005b).

6 Conclusion

Focusing on fatty acid acylation of proteins, this review reports new knowledge on cellular and physiological functions of individual SFAs. This review particularly emphasizes that palmitic, myristic and caprylic acids, through their capacity to acylate different proteins, have important and specific roles for which they cannot be a substitute for each other and that cannot be assumed by other fatty acids. For this reason, like for other physiological and pathophysiological aspects (Legrand, 2013; Legrand and Rioux, 2015) not detailed in the present review, SFAs should no longer be considered as a single group in terms of structure, metabolism and functions.

Acknowledgements. The author acknowledges Pr. Philippe Legrand (Head of the laboratory of Biochemistry and Human Nutrition, Agrocampus Ouest-INRA USC 1378, Rennes, France), his former

Ph.D. students Dr. Erwan Beauchamp and Dr. Hélène Ezanno and his current Ph.D. student Fanny Lemarié for their great contributions to researches described in this review.

References

Aki T, Shimada Y, Inagaki K, *et al.* 1999. Molecular cloning and functional characterization of rat delta-6 fatty acid desaturase. *Biochem. Biophys. Res. Commun*. 255: 575–579.

Albarran-Zeckler RG, Sun Y, Smith RG. 2011. Physiological roles revealed by ghrelin and ghrelin receptor deficient mice. *Peptides* 32: 2229–2235.

Alonso L, Fontecha J, Lozada L, Fraga MJ, Juárez M. 1999. Fatty Acid Composition of Caprine Milk: Major, Branched-Chain, and Trans Fatty Acids. *J. Dairy Sci.* 82: 878–884.

Ashitani J-I, Matsumoto N, Nakazato M. 2009. Effect of octanoic acid-rich formula on plasma ghrelin levels in cachectic patients with chronic respiratory disease. *Nutr. J.* 8: 25.

Beauchamp E, Goenaga D, Le Bloc'h J, Catheline D, Legrand P, Rioux V. 2007. Myristic acid increases the activity of dihydroceramide Delta4-desaturase 1 through its N-terminal myristoylation. *Biochimie*. 89: 1553–1561.

Beauchamp E, Rioux V, Legrand P. 2009. Acide myristique: nouvelles fonctions de régulation et de signalisation. *Med. Sci. Paris* 25: 57–63.

Beauchamp E, Tekpli X, Marteil G, Lagadic-Gossmann D, Legrand P, Rioux V. 2009. N-Myristoylation targets dihydroceramide Delta4-desaturase 1 to mitochondria: partial involvement in the apoptotic effect of myristic acid. *Biochimie* 91: 1411–1419.

Bielawska A, Crane HM, Liotta D, Obeid LM, Hannun YA. 1993. Selectivity of ceramide-mediated biology. Lack of activity of erythro-dihydroceramide. *J. Biol. Chem*. 268: 26226–26232.

Blaskovic S, Adibekian A, Blanc M, van der Goot GF. 2014. Mechanistic effects of protein palmitoylation and the cellular consequences thereof. *Chem. Phys. Lipids* 180: 44–52.

Borgese N, Aggujaro D, Carrera P, Pietrini G, Bassetti M. 1996. A role for N-myristoylation in protein targeting: NADH-cytochrome b5 reductase requires myristic acid for association with outer mitochondrial but not ER membranes. *J. Cell. Biol*. 135: 1501–1513.

Chen CA, Manning DR. 2001. Regulation of G proteins by covalent modification. *Oncogene*. 20: 1643–1652.

Chen W, Enriori PJ. 2014. Ghrelin: a journey from GH secretagogue to regulator of metabolism. *Transl. Gastrointest. Cancer* 4: 14–27.

Cho HP, Nakamura MT, Clarke SD. 1999. Cloning, expression, and nutritional regulation of the mammalian Δ-6 desaturase. *J. Biol. Chem*. 274: 471–477.

Clark SB, Brause B, Holt PR. 1969. Lipolysis and absorption of fat in the rat stomach. *Gastroenterology* 56: 214–222.

Colombo S, Longhi R, Alcaro S, *et al.* 2005. N-myristoylation determines dual targeting of mammalian NADH-cytochrome b5 reductase to ER and mitochondrial outer membranes by a mechanism of kinetic partitioning. *J. Cell. Biol*. 168: 735–745.

D'Andrea S, Guillou H, Jan S,*et al.* 2002. The same rat Δ6-desaturase not only acts on 18- but also on 24-carbon fatty acids in very-long-chain polyunsaturated fatty acid biosynthesis. *Biochem. J*. 364: 49–55.

Darling JE, Zhao F, Loftus RJ, Patton LM, Gibbs RA, Hougland JL. 2015. Structure–activity analysis of human ghrelin O-acyltransferase reveals chemical determinants of ghrelin selectivity and acyl group recognition. *Biochemistry (Mosc.)*. 54: 1100–1110.

Delhanty PJ, Neggers SJ, AJ van der Lely. 2013. Des-acyl ghrelin: a metabolically active peptide. *Endocr. Dev.* 25: 112–121.

Delporte C. 2013. Structure and physiological actions of ghrelin. *Scientifica* 2013: 518909.

Ducker CE, Upson JJ, French KJ, Smith CD. 2005. Two N-myristoyltransferase isozymes play unique roles in protein myristoylation, proliferation, and apoptosis. *Mol. Cancer Res*. 3: 463–476.

Duronio RJ, Rudnick DA, Johnson RL, Johnson DR, Gordon JI. 1991. Myristic acid auxotrophy caused by mutation of *S. cerevisiae* myristoyl-CoA:protein N-myristoyltransferase. *J. Cell. Biol*. 113: 1313–1330.

Duronio RJ, Reed SI, Gordon JI. 1992. Mutations of human myristoyl-CoA:protein N-myristoyltransferase cause temperature-sensitive myristic acid auxotrophy in *Saccharomyces cerevisiae*. *Proc. Natl. Acad. Sci. USA* 89: 4129–4133.

Ezanno H, le Bloc'h J, Beauchamp E, Lagadic-Gossmann D, Legrand P, Rioux V. 2012. Myristic acid increases dihydroceramide Delta4-desaturase 1 (DES1) activity in cultured rat hepatocytes. *Lipids* 47: 117–128.

Ezanno H, Beauchamp E, Lemarié F, Legrand P, Rioux V. 2013. L'acylation des protéines: une fonction cellulaire importante des acides gras saturés. *Nutr. Clin. Metab*. 27: 10–19.

Faergeman NJ, Knudsen J. 1997. Role of long-chain fatty acyl-CoA esters in the regulation of metabolism and in cell signalling. *Biochem. J*. 323: 1–12.

Fernando-Warnakulasuriya GJ, Staggers JE, Frost SC, Wells MA. 1981. Studies on fat digestion, absorption, and transport in the suckling rat. I. Fatty acid composition and concentrations of major lipid components. *J. Lipid Res.* 22: 668–674.

Fukumori R, Sugino T, Shingu H, *et al.* 2013. Ingestion of medium chain fatty acids by lactating dairy cows increases concentrations of plasma ghrelin. *Domest. Anim. Endocrinol.* 45: 216–223.

Garcia-Ruiz C, Colell A, Mari M, Morales A, Fernandez-Checa JC. 1997. Direct effect of ceramide on the mitochondrial electron transport chain leads to generation of reactive oxygen species. Role of mitochondrial glutathione. *J. Biol. Chem*. 272: 11369–11377.

Giang DK, Cravatt BF. 1998. A second mammalian N-myristoyltransferase. *J. Biol. Chem*. 273: 6595–6598.

Goebel-Stengel M, Hofmann T, Elbelt U, *et al.* 2013. The ghrelin activating enzyme ghrelin-O-acyltransferase (GOAT) is present in human plasma and expressed dependent on body mass index. *Peptides* 43: 13–19.

Gong Z, Yoshimura M, Aizawa S, *et al.* 2014. G protein-coupled receptor 120 signaling regulates ghrelin secretion *in vivo* and *in vitro*. *Am. J. Physiol. - Endocrinol. Metab.* 306: E28–E35.

Greaves J, Chamberlain LH. 2011. DHHC palmitoyl transferases: substrate interactions and (patho)physiology. *Trends Biochem. Sci*. 36: 245–253.

Gudz TI, Tserng KY, Hoppel CL. 1997. Direct inhibition of mitochondrial respiratory chain complex III by cell-permeable ceramide. *J. Biol. Chem*. 272: 24154–24158.

Guillou H, D'Andrea S, Rioux V, *et al.* 2004. Distinct roles of endoplasmic reticulum cytochrome b5 and fused cytochrome b5-like domain for rat Delta6-desaturase activity. *J. Lipid Res*. 45: 32–40.

Gutierrez JA, Solenberg PJ, Perkins DR, *et al.* 2008. Ghrelin octanoylation mediated by an orphan lipid transferase. *Proc. Natl. Acad. Sci. USA* 105: 6320–6325.

Howard AD, Feighner SD, Cully DF, *et al.* 1996. A receptor in pituitary and hypothalamus that functions in growth hormone release. *Science* 273: 974–977.

Jan S, Guillou H, D'Andrea S, Daval S, Bouriel M, Rioux V, Legrand P. 2004. Myristic acid increases delta6-desaturase activity in cultured rat hepatocytes. *Reprod. Nutr. Dev*. 44: 131–140.

Janssen S, Laermans J, Iwakura H, Tack J, Depoortere I. 2012. Sensing of Fatty Acids for Octanoylation of Ghrelin Involves a Gustatory G-Protein. *PLoS One*. 7: e40168.

Jensen RG. 1996. The lipids in human milk. *Prog. Lipid Res.* 35: 53–92.

Jensen RG, Ferris AM, Lammi-Keefe CJ, Henderson RA. 1990. Lipids of bovine and human milks: a comparison. *J. Dairy Sci*. 73: 223–240.

Johnson DR, Bhatnagar RS, Knoll LJ, Gordon JI. 1994a. Genetic and biochemical studies of protein N-myristoylation. *Annu. Rev. Biochem*. 63: 869–914.

Johnson DR, Knoll LJ, Levin DE, Gordon JI. 1994b. Saccharomyces cerevisiae contains four fatty acid activation (FAA) genes: an assessment of their role in regulating protein N-myristoylation and cellular lipid metabolism. *J. Cell Biol*. 127: 751–62.

Kamegai J, Tamura H, Shimizu T, Ishii S, Sugihara H, Wakabayashi I. 2001. Chronic central infusion of ghrelin increases hypothalamic neuropeptide Y and Agouti-related protein mRNA levels and body weight in rats. *Diabetes*. 50: 2438–2443.

Katan MB, Zock PL, Mensink RP. 1994. Effects of fats and fatty acids on blood lipids in humans: an overview. *Am. J. Clin. Nutr*. 60: 1017S–1022S.

Kojima M, Hosoda H, Date Y, Nakazato M, Matsuo H, Kangawa K. 1999. Ghrelin is a growth-hormone-releasing acylated peptide from stomach. *Nature* 402: 656–660.

Lai HC, Ney DM. 1998. Gastric digestion modifies absorption of butterfat into lymph chylomicrons in rats. *J. Nutr.* 128: 2403–2410.

Legrand P. 2013. Nouvelle approche pour les recommandations nutritionnelles en lipides. *OCL* 20: 75–78.

Legrand P, Rioux V. 2015. Specific roles of saturated fatty acids: Beyond epidemiological data. *Eur. J. Lipid Sci. Technol.* 117: 1489–1499.

Legrand P, Beauchamp E, Catheline D, Pedrono F, Rioux V. 2010. Short chain saturated fatty acids decrease circulating cholesterol and increase tissue PUFA content in the rat. *Lipids* 45: 975–86.

Lemarié F, Beauchamp E, Dayot S, Duby C, Legrand P, Rioux V. 2015. Dietary Caprylic Acid (C8:0) Does Not Increase Plasma Acylated Ghrelin but Decreases Plasma Unacylated Ghrelin in the Rat. *PloS One* 10: e0133600.

Lemarié F, Beauchamp E, Legrand P, Rioux V. 2015. Revisiting the metabolism and physiological functions of caprylic acid (C8:0) with special focus on ghrelin octanoylation. *Biochimie*

Lim CT, Kola B, Grossman A, Korbonits M. 2011. The expression of ghrelin O-acyltransferase (GOAT) in human tissues. *Endocr. J.* 58: 707–710.

Martin DD, Beauchamp E, Berthiaume LG. 2011. Post-translational myristoylation: Fat matters in cellular life and death. *Biochimie* 93: 18–31.

Maurer-Stroh S, Gouda M, Novatchkova M, *et al.* 2004. MYRbase: analysis of genome-wide glycine myristoylation enlarges the functional spectrum of eukaryotic myristoylated proteins. *Genome Biol*. 5: R21.

Mitchell DA, Vasudevan A, Linder ME, Deschenes RJ. 2006. Protein palmitoylation by a family of DHHC protein S-acyltransferases. *J. Lipid Res*. 47: 1118–1127.

Mizutani Y, Kihara A, Igarashi Y. 2004. Identification of the human sphingolipid C4-hydroxylase, hDES2, and its up-regulation during keratinocyte differentiation. *FEBS Lett*. 563: 93–97.

Nishi Y, Hiejima H, Hosoda H, *et al.* 2005a. Ingested medium-chain fatty acids are directly utilized for the acyl modification of ghrelin. *Endocrinology* 146: 2255–2264.

Nishi Y, Hiejima H, Mifune H, Sato T, Kangawa K, Kojima M. 2005b. Developmental changes in the pattern of ghrelin's acyl modification and the levels of acyl-modified ghrelins in murine stomach. *Endocrinology* 146: 2709–2715.

Nishi Y, Mifune H, Yabuki A, *et al.* 2013. Changes in subcellular distribution of n-octanoyl or n-decanoyl ghrelin in ghrelin-producing cells. *Front. Endocrinol.* 4: 84.

Omae F, Miyazaki M, Enomoto A, Suzuki M, Suzuki Y, Suzuki A. 2004. DES2 protein is responsible for phytoceramide biosynthesis in the mouse small intestine. *Biochem. J*. 379: 687–695.

Ozols J, Carr SA, Strittmatter P. 1984. Identification of the NH2-terminal blocking group of NADH-cytochrome b5 reductase as myristic acid and the complete amino acid sequence of the membrane-binding domain. *J. Biol. Chem*. 259: 13349–13354.

Perret JP. 1980. Gastric lipolysis of maternal milk triglycerides, gastric absorption of medium chain fatty acids in the young rabbit (author's transl). *J. Physiol. (Paris)* 76: 159–166.

Rioux V, Legrand P. 2001. Métabolisme et fonctions de l'acide myristique. *OCL* 8: 161–166.

Rioux V, Galat A, Jan G, Vinci F, D'Andrea S, Legrand P. 2002. Exogenous myristic acid acylates proteins in cultured rat hepatocytes. *J. Nutr. Biochem*. 13: 66–74.

Rioux V, Daval S, Guillou H, Jan S, Legrand P. 2003. Although it is rapidly metabolized in cultured rat hepatocytes, lauric acid is used for protein acylation. *Reprod Nutr Dev*. 43: 419–430.

Rioux V, Catheline D, Bouriel M, Legrand P. 2005. Dietary myristic acid at physiologically relevant levels increases the tissue content of C20:5 n-3 and C20:3 n-6 in the rat. *Reprod. Nutr. Dev*. 45: 599–612.

Rioux V, Beauchamp E, Pedrono F, Daval S, Molle D, Catheline D, Legrand P. 2006. Identification and characterization of recombinant and native rat myristoyl-CoA: protein N-myristoyltransferases. *Mol. Cell. Biochem*. 286: 161–170.

Rioux V, Catheline D, Legrand P. 2007. In rat hepatocytes, myristic acid occurs through lipogenesis, palmitic shortening and lauric acid elongation. *Animal* 1: 820–826.

Rioux V, Catheline D, Beauchamp E, Le Bloc'h J, Pédrono F, Legrand P. 2008. Substitution of dietary oleic for myristic acid increases the tissue storage of a-linolenic acid and the concentration of docosahexaenoic acid in brain, red blood cells and plasma in the rat. *Animal*. 2: 636–644.

Rioux V, Pedrono F, Legrand P. 2011. Regulation of mammalian desaturases by myristic acid: N-terminal myristoylation and other modulations. *Biochim. Biophys. Acta*. 1811: 1–8.

Rundle DR, Rajala RV, Anderson RE. 2002. Characterization of Type I and Type II myristoyl-CoA:protein N-myristoyltransferases with the Acyl-CoAs found on heterogeneously acylated retinal proteins. *Exp. Eye. Res*. 75: 87–97.

Selvakumar P, Smith-Windsor E, Bonham K, Sharma RK. 2006. N-myristoyltransferase 2 expression in human colon cancer: cross-talk between the calpain and caspase system. *FEBS Lett*. 580: 2021–2026.

Singh N, Wakil SJ, Stoops JK. 1984. On the question of half- or full-site reactivity of animal fatty acid synthetase. *J. Biol. Chem*. 259: 3605–3611.

Siskind LJ, Kolesnick RN, Colombini M. 2002. Ceramide channels increase the permeability of the mitochondrial outer membrane to small proteins. *J. Biol. Chem*. 277: 26796–26803.

Staggers JE, Fernando-Warnakulasuriya GJ, Wells MA. 1981. Studies on fat digestion, absorption, and transport in the suckling rat. II. Triacylglycerols: molecular species, stereospecific analysis, and specificity of hydrolysis by lingual lipase. *J. Lipid Res.* 22: 675–679.

Strittmatter P, Kittler JM, Coghill JE, Ozols J. 1993. Interaction of non-myristoylated NADH-cytochrome b5 reductase with cytochrome b5-dimyristoylphosphatidylcholine vesicles. *J. Biol. Chem*. 268: 23168–23171.

Takagi K, Legrand R, Asakawa A, *et al.* 2013. Anti-ghrelin immunoglobulins modulate ghrelin stability and its orexigenic effect in obese mice and humans. *Nat. Commun.* 4.

Taylor MS, Hwang Y, Hsiao P-Y, Boeke JD, Cole PA. 2012. Ghrelin O-acyltransferase assays and inhibition. *Methods Enzymol.* 514: 205–228.

Taylor MS, Ruch TR, Hsiao P-Y, *et al.* 2013. Architectural organization of the metabolic regulatory enzyme ghrelin O-acyltransferase. *J. Biol. Chem.* 288: 32211–32228.

Ternes P, Franke S, Zahringer U, Sperling P, Heinz E. 2002. Identification and characterization of a sphingolipid delta 4-desaturase family. *J. Biol. Chem*. 277: 25512–25518.

Thinon E, Serwa RA, Broncel M, *et al.* 2014. Global profiling of co- and post-translationally N-myristoylated proteomes in human cells. *Nat. Commun.* 5.

Towler DA, Gordon JI, Adams SP, Glaser L. 1988. The biology and enzymology of eukaryotic protein acylation. *Annu. Rev. Biochem*. 57: 69–99.

Tschöp M, Smiley DL, Heiman ML. 2000. Ghrelin induces adiposity in rodents. *Nature* 407: 908–913.

Wolk A, Furuheim M, Vessby B. 2001. Fatty acid composition of adipose tissue and serum lipids are valid biological markers of dairy fat intake in men. *J. Nutr*. 131: 828–833.

Yang SH, Shrivastav A, Kosinski C, *et al.* 2005. N-myristoyltransferase 1 is essential in early mouse development. *J. Biol. Chem*. 280: 18990–18995.

Zhang JV, Ren P-G, Avsian-Kretchmer O, *et al.* 2005. Obestatin, a peptide encoded by the ghrelin gene, opposes ghrelin's effects on food intake. *Science* 310: 996–999.

Cite this article as: Vincent Rioux. Fatty acid acylation of proteins: specific roles for palmitic, myristic and caprylic acids. OCL 2016, 23(3) D304.