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Dietary caprylic acid and ghrelin O-acyltransferase activity to modulate octanoylated ghrelin functions: What is new in this nutritional field?

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ABSTRACT

Caprylic acid (octanoic acid, C8:0) belongs to the class of medium-chain saturated fatty acids (MCFAs). Dairy products and specific oils such as coconut oil are natural sources of dietary caprylic acid. MCFAs display distinct chemico-physical and metabolic properties from those of long-chain saturated fatty acids (LCFAs ≥ 12 carbons) and potential beneficial physiological effects of dietary C8:0 have been studied for many years. More recently, caprylic acid was shown to octanoylate ghrelin, the only known peptide hormone with an orexigenic effect. Through its covalent binding to the ghrelin peptide, caprylic acid exhibits an emerging and specific role in modulating physiological functions themselves regulated by octanoylated ghrelin. Dietary caprylic acid is therefore now suspected to provide the ghrelin O-acyltransferase (GOAT) enzyme with octanoyl-CoA co-substrates necessary for the acyl modification of ghrelin. Recent studies suggest that decreasing the circulating octanoylated ghrelin level through the inhibition of GOAT activity, or simply by modulating the availability of its C8:0 substrate, might constitute a therapeutic strategy against obesity. Both dietary caprylic acid availability and GOAT activity may indeed be important to modulate octanoylated ghrelin concentration and functions. This review highlights recent findings in the field of nutrition.

1. Introduction

Caprylic acid (octanoic acid, C8:0) belongs to the class of medium-chain saturated fatty acids (MCFA), which also includes caproic acid (hexanoic acid, C6:0) and capric acid (decanoic acid, C10:0) [1]. MCFAs are characteristic nutrients present in dairy products [2] and in specific oils such as palm kernel and coconut oils [3]. Caprylic acid is abundant in coconut oil (6–10% of fatty acids (FAs)), and in palm kernel oil (2–5% of FAs), where it occupies the *sn*-1 and -3 positions of triglycerides (TG) [4]. Milk is the only animal source of caprylic acid with strong differences between mammalian species. Caprylic acid represents around 0.5% of FAs in human milk [5], 1–2% in cow milk [2], 3% in goat milk [6], 5–6% in rat milk [7], and up to 15–18% in rabbit milk [8]. MCFAs are primarily esterified at the *sn*-3 position of TGs in cow [2], rat [9], and human [5] milk.

MCFAs display distinct chemico-physical and metabolic properties from those of long-chain saturated fatty acids (LCFAs ≥ 12 carbons), leading to specific physiological effects [1]. First, a part of MCFAs coming from dietary medium chain triglycerides (MCTs) are quickly released after ingestion under the action of preduodenal lipases [10], allowing direct absorption by the stomach mucosa [8,11,12]. Second, small intestinal cells absorb MCFAs (and LCFAs) after the subsequent action of duodenal pancreatic lipases on both dietary remaining MCTs and long chain triglycerides (LCTs). However, unlike LCFAs which are re-esterified with 2-monoglycerides into triglycerides and incorporated into chylomicrons entering the lymphatic system, MCFAs are directly transferred to the portal circulation and transported as free fatty acids (FFAs) with albumin to the liver [13]. Third, hepatic MCFAs are rapidly subjected to mitochondrial β-oxidation [14], since they easily enter the mitochondria independently of the carnitine transport system, as opposed to LCFAs [15].

More recently, caprylic acid was shown to specifically acylate ghrelin [16], the only known peptide hormone with an orexigenic effect. Ghrelin is a 28 amino acid peptide mainly expressed in the

Abbreviations: EI, energy intake; FFA, free fatty acid; GH, growth hormone; GOAT, ghrelin O-acyltransferase; GHS-R1a, growth hormone secretagogue receptor 1a; LCFA, long-chain fatty acid; LCT, long-chain triglyceride; MCFA, medium-chain fatty acid; MCT, medium-chain triglyceride; PORCN, porcupine; PUFA, polyunsaturated fatty acid; TG, triglyceride

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gastrointestinal tract, especially in the stomach [17]. Acylated ghrelin binds the growth hormone secretagogue receptor 1a (GHS-R1a) located in the pituitary gland and the hypothalamus [18,19], and is described to regulate many relevant biological processes including the secretion of the growth hormone (GH), the stimulation of appetite and food intake, the regulation of gastric acid secretion, gastric motility, glucose homeostasis and adiposity [20].

During its maturation in the gastric mucosa and prior to secretion into blood, part of the proghrelin (94 amino acids) is subjected to a unique modification consisting in the addition of an activated octanoyl-CoA onto the N-terminal serine 3 residue through an oxyester linkage (O-acylation) catalyzed by the ghrelin O-acyltransferase (GOAT) enzyme. Ghrelin therefore circulates in the plasma in both its unacylated and acylated forms, but only the latter can bind GHS-R1a. Therefore, the availability of the octanoyl moiety appears crucial for this peptide hormone functions.

Diet is suspected to directly provide the GOAT enzyme with caprylic acid, subsequently activated in octanoyl-CoA co-substrate, necessary for the O-acylation of ghrelin. Indeed, ingestion of MCFAs/MCTs in mice increased the stomach acylated ghrelin concentration [21], without modifying the total ghrelin level. Using GOAT knockout mice, Kirchner et al. [22] demonstrated that GOAT was required to mediate the impact of dietary MCTs on body adiposity. These studies suggest that GOAT might be a therapeutic target against obesity and hyperphagia through inhibition of its activity to decrease the circulating level of acylated ghrelin [23]. Altogether, these data suggest that dietary caprylic acid and GOAT activity regulate octanoylated ghrelin production, circulating concentration and functions. This review highlights the recent findings about ghrelin octanoylation in the nutrition field.

2. Characterization of the ghrelin O-Acyl-Transferase (GOAT) enzyme

Nineteen years ago, caprylic acid was surprisingly found attached to the ghrelin peptide purified from rat stomach [16]. Although the first members of the membrane-bound O-acyltransferases (MBOAT) family were identified in 2000 [24], eight additional years were necessary to formally identify MBOAT4 (i.e. GOAT) as the enzyme catalyzing the addition of the octanoate moiety onto ghrelin Ser3 residue through an oxyester linkage [25,26]. Out of the 11 MBOAT members identified in humans [27], only a few have been characterized and their catalytic activity identified (acyl-CoA:cholesterol acyltransferase (ACAT) 1 and 2, hedgehog (Hh) acyltransferase (HHAT), porcupine (PORCN) and GOAT) [28]. MBOAT members share highly conserved histidine and asparagine residues and are predicted to contain multiple (8–12) transmembrane domains (TMD) making the study of this protein family challenging.

Following bioinformatic analysis predicting 12 potential TMD in GOAT 3D structure, Taylor et al. confirmed 11 transmembrane-spanning domains and one reentrant loop using selective permeabilization monitored by indirect immunofluorescence and design of multiple GOAT constructs expressing internal epitope tags inserted between predicted TMD [29]. N-terminus and C-terminus are found on opposite sides of the ER membrane (lumen and cytosol, respectively) and therefore required an odd number of TMD for the protein. Interestingly, conserved His-338 and Asn-307 residues are also found on opposite sides suggesting that the latter might not be involved in the catalytic activity of GOAT. This was further confirmed with the GOAT His-338-Ala mutant showing no detectable acylation activity [30]. Additional studies are needed to fully characterize the catalytic site of GOAT, but a recent study using a thiol-modifying reagent (NEM: N-ethylmaleimide) inhibiting the enzyme suggests that a cysteine might be functionally involved [31].

Two stable GOAT isoforms were identified when overexpressed in Sf9 insect cells, and it was confirmed that it was not an artifact due to aggregation. Matrix assisted laser desorption/ionization time of flight (MALDI-TOF) mass spectrometry analysis identified the Met-56 residue as an alternative translation start site, but the physiological relevance of this “short-GOAT” isoform has not been elucidated. Interestingly, Met-56 is predicted to be the last residue of the TMD2 which suggests that the stability of the protein lacking the two first TMD is maintained [29]. GOAT is found in the endoplasmic reticulum and assures the translocation of the octanoyl-CoA from the cytosolic side to the lumen and the addition of the lipid moiety onto ghrelin. However, residual cytoplasmic localization is detected using immunogold electron microscopy [32]. The GOAT enzyme is predicted to be associated with the secretory pathway, but in marrow adipocytes, GOAT is found to be associated with lipid trafficking vesicles suggesting a role in lipid storage regulation [32].

GOAT is mainly expressed in the stomach similarly to ghrelin, but can also be found in the hypothalamus, pituitary gland, serum and intestine [33]. Its abundance in other organs, such as the pancreas might be dependent of the species analyzed [26,34]. Consistent with its major role in the regulation of food intake, GOAT mRNA levels are found 5 times greater in the stomach as compared to the hypothalamus or pituitary [35] but the local activity of GOAT within those organs seems highly regulated [36]. The relative insensitivity of GOAT activity to different pH suggests that the enzyme could be active in different cellular microenvironment [30].

Bioinformatics analysis and peptide specificity studies led to the conclusion that ghrelin is the unique substrate of GOAT within the human proteome [37]. A minimum of four amino acids are required for enzymatic activity [38] and GOAT can acylate both immature des-acyl proghrelin and unacylated mature ghrelin [25]. This confirms that the N-terminal sequence is the major determinant for substrate recognition. However, GOAT shows a 10-fold greater affinity for the mature ghrelin as compared to the decapeptide mimicking N-terminal ghrelin sequence suggesting that the C-terminal peptide, with a net +4 positive charge, contributes also to the binding with the enzyme [30]. Interestingly, like other O-acyltransferases such as PORCN [39], GOAT is able to octanoylate a threonine at lower efficiency compared to the Ser3 [37] but also an alanine through an amide bound [30]. The acylation site position is very specific since serines at position 2 and 6 were not found to be acylated.

A broad range of acyl donor from acetic acid to palmitic acid can be recognized by GOAT but a clear preference for C8:0 has been confirmed in the most recent and sensitive assay (about 50-fold higher affinity as compared to C6:0 or C10:0) [37]. Bioavailability is therefore a major determinant for the nature of the final acyl donor found on the mature ghrelin [21]. From all these information, Darling et al. were able to provide a putative schematic illustration of the active site that will be very important for the future design of GOAT inhibitors.

3. Impact of dietary octanoic acid on ghrelin octanoylation in the stomach and plasma

Although the GOAT enzyme exhibits a clear substrate preference for caprylic acid, the origin of this fatty acid used, in its acyl-CoA activated form, to acylate the ghrelin peptide is still not fully understood. A dietary origin remains the most likely, considering that caprylic acid synthesis has only been described in the lactating mammary glands [40,41]. The impact of dietary MCFAs on ghrelin octanoylation has now been studied in various species (mouse, suckling and adult rat, lactating cow, hatchling chicken, cachexic, anorexic and overweight human) with different experimental conditions (dose, free fatty acid (FFA) or triglyceride (TG)) and methodologies (in-house or commercial immunoassays, HPLC-MS) [12,21,42–46].

When analyzing bibliographic data, one should keep in mind that total and acylated ghrelin levels are regulated by many factors besides diet and hunger, such as stress, sleep, light and circadian rhythm [47–49]. Moreover, ghrelin is quickly cleaved and/or deacylated after blood/tissue collection [50], making its accurate dosage challenging.