Celastrol

A century-long journey from the isolation to the biotechnological production and the development of an antiobesity drug

Zhao, Yong; Miettinen, Karel; Kampranis, Sotirios C.

Published in:
Current Opinion in Plant Biology

DOI:
10.1016/j.pbi.2024.102615

Publication date:
2024

Document version
Publisher's PDF, also known as Version of record

Available online at www.sciencedirect.com

ScienceDirect

Current Opinion in Plant Biology

Celastrol: A century-long journey from the isolation to the biotechnological production and the development of an antiobesity drug

Yong Zhao, Karel Miettinen and Sotirios C. Kampranis

Celastrol, a triterpenoid found in the root of the traditional medicinal plant *Tripterygium wilfordii*, is a potent anti-inflammatory and antiobesity agent. However, pharmacological exploitation of celastrol has been hindered by the limited accessibility of plant material, the co-existence of other toxic compounds in the same plant tissue, and the lack of an efficient chemical synthesis method. In this review, we highlight recent progress in elucidating celastrol biosynthesis and discuss how this knowledge can facilitate its scalable bioproduction using cell factories and its further development as an antiobesity and anti-inflammatory drug.

Addresses
Biochemical Engineering Group, Plant Biochemistry Section, Department of Plant and Environment Sciences, Faculty of Science, University of Copenhagen, Thorvaldsensvej 40, 1871 Frederiksberg C, Denmark

Corresponding author: Kampranis, Sotirios C. (soka@plen.ku.dk)

Current Opinion in Plant Biology 2024, **81**:102615

This review comes from a themed issue on **Physiology and metabolism 2024**

Edited by **Vincent Courdavault** and **Anne Osbourn**

For a complete overview see the Issue and the Editorial

Available online xxx

https://doi.org/10.1016/j.pbi.2024.102615

Introduction

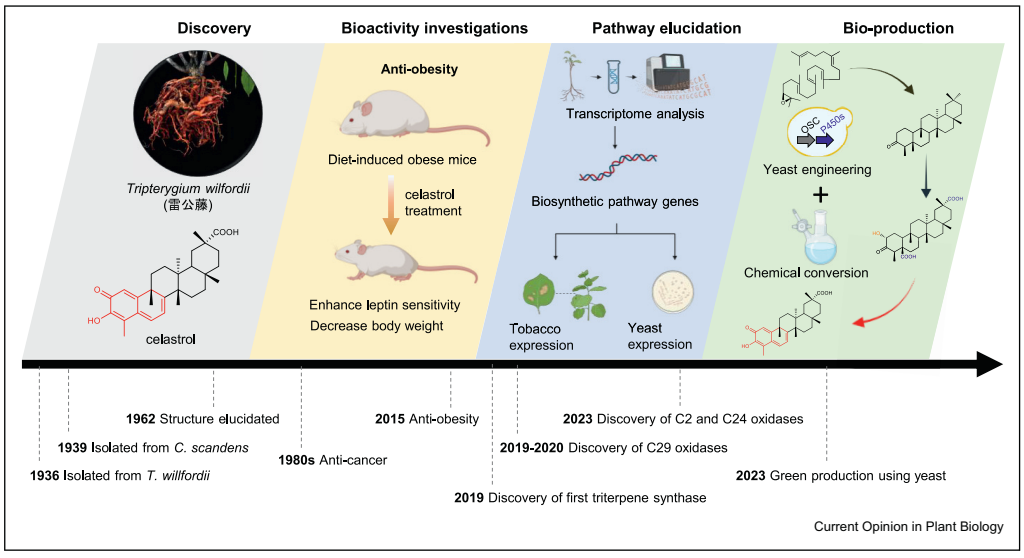
Plants produce a plethora of structurally diverse specialized metabolites that provide essential chemicals for human life, from food to medicine. One such example is celastrol, a potent antiobesity agent isolated from the root of *Tripterygium wilfordii* (thunder god vine) [1,2]. However, when it comes to the development of a natural molecule into a commercial drug, the lack of scalable and cost-effective supply is one of the biggest concerns. Structurally, celastrol is a pentacyclic triterpenoid containing a characteristic quinone methide moiety. The presence of this type of structure, along with the multiple stereocenters typically found in triterpenoid scaffolds, poses a considerable challenge to its chemical synthesis, hindering further studies and pharmaceutical exploitation [3]. Metabolic engineering of industrially amenable microorganisms can provide a sustainable and scalable source of celastrol. The success of such an approach is exemplified by several recent cases in the bioproduction of structurally complex natural products such as artemisinin [4], vinblastine [5], opioids [6], and cannabinoids [7]. Nevertheless, the successful heterologous bio-production of desired molecules relies on a full understanding of their native biosynthesis in plants. In this review, we summarize the recent efforts that led to the complete elucidation of the pathway of celastrol biosynthesis in plants and the *de novo* biosynthesis of celastrol in baker's yeast (*Saccharomyces cerevisiae*), establishing the basis for its pharmaceutical exploitation (Figure 1) [8].

Discovery: a red pigment from the root of a toxic Chinese herb

In China, the root of *T. wilfordii* has been used for centuries in the treatment of rheumatoid arthritis and other autoimmune-related disorders. However, the use of this plant, either alone or in combination with other herbal medicines, has been associated with strong hepatotoxicity and nephrotoxicity, raising significant safety concerns [9,10]. Therefore, the dosage and duration of treatment with extracts of *T. wilfordii* had to be restricted and carefully controlled. Unraveling the individual compounds responsible for the pharmaceutical properties and toxicity of the plant extract could provide improved strategies for its medicinal use. To this end, extensive phytochemical studies have been undertaken, leading to the identification of more than 400 metabolites to date [11]. One of these compounds is celastrol, which was first isolated from the root bark as a blood-red, cubic crystal in 1936 [12]. The same compound was also isolated from *Celastrus scandens* in 1939, from which its name originates [13].

Shortly after the isolation of celastrol, a series of chemical studies was carried out to identify its structure, demonstrating the existence of the main benzoquinone chromophore [14–18]. Nevertheless, it was not until 1962 that the full structure of celastrol and its methyl analog pristimerin were correctly assigned by

Figure 1



Milestones in celastrol discovery, characterization, and biosynthesis. A timeline of milestones in the study and exploitation of celastrol since its initial discovery in 1936. Pivotal moments such as bioactivity investigations, total chemical synthesis endeavors, and recent advancements in comprehending its biosynthetic pathway are highlighted.

both chemical and NMR evidence [19]. The structures of celastrol and pristimerin are characterized by the presence of a quinone methide moiety, which has been found in only very few natural products (e.g. taxodone [20] and kendomycin [21]).

Bioactivities of celastrol

Initially, celastrol was characterized as an antibiotic against various Gram-positive bacteria [22]. Since the 1980s, several studies have revealed that celastrol also possesses anticancer activity, prompting further investigation into its mechanism [23–26]. Celastrol was found to affect tumor cell proliferation and migration, and promote apoptosis, by disrupting different cell signaling pathways (i.e. NF-kB/COX-2) [27,28] or inhibiting oncogenic proteins (i.e. Hsp90) [29]. Furthermore, an increasing number of studies have demonstrated its therapeutic potential for a range of other human disorders, including inflammatory-related, autoimmune, neurodegenerative, and protein-folding diseases [30–32]. The anti-inflammatory activity of celastrol aligns with its ethnopharmacological use in the treatment of rheumatoid arthritis.

Potential in the treatment of obesity

Leptin is a human hormone released from adipose tissue to control overall metabolism and food intake in response to energy store levels. A series of studies have elegantly concluded that obesity is characterized by leptin resistance or insensitivity, rendering the body unable to utilize leptin for controlling energy expenditure and food intake [33–35]. This discovery has highlighted leptin re-sensitization as a promising intervention target, driving efforts to discover small molecule leptin sensitizers. In a groundbreaking 2015 study, Ozcan et al. identified celastrol as a potent leptin re-sensitizer, underscoring its potential in obesity treatment [1]. In this study, celastrol emerged as the most potent leptin sensitizer among over 1000 natural compounds tested. Detailed *in vivo* experiments demonstrated that celastrol suppresses food intake, prevents reduction of energy expenditure, and induces up to 45% weight loss in hyperleptinemic diet-induced obese mice. This action of celastrol was found to be mediated by leptin as celastrol treatment was ineffective in leptin-deficient and leptin receptor-deficient mouse models [1]. In a concurrent study, Zhang et al. corroborated celastrol's effect on weight reduction and revealed its specific binding in a hydrophobic groove of the nuclear receptor Nur77 [2]. The antiobesity effect of celastrol was suppressed in mice deficient for Nur77, highlighting the critical role of Nur77 in this process. Considering that celastrol was also found to inhibit inflammation and induce autophagy, Nur77 may confer the leptin-sensitizing effect of celastrol by regulating high-fat diet-induced hypothalamic inflammation [2]. More recent studies demonstrated that interleukin-1 receptor 1 (IL1R1)-deficient mice are completely resistant to the effects of celastrol in leptin sensitization and treatment of obesity, revealing that IL1R1 is another key player in this process [36]. In addition to its action on leptin, celastrol protects against obesity and metabolic dysfunction by activating the transcription factor HSF1, which regulates PGC1α-dependent metabolic programs in adipose and muscle tissues [37].

Further studies have shown that celastrol protects against nonalcoholic fatty liver disease induced by a high-fat diet, mitigating liver metabolic damage and improving glucose tolerance and insulin sensitivity [38].

Early efforts toward an economical and scalable supply of celastrol

Although the therapeutic potential of celastrol is evident from the studies mentioned above, its medical applications are hindered by its limited availability. Obtaining celastrol for large-scale trials and commercialization from the roots of *T. wilfordii* is not viable because accumulation of the compound requires 2–3 years of plant cultivation and due to the toxic compounds accumulating in the same tissue, the purification process is elaborate and costly [11]. Consequently, various options for obtaining celastrol have been explored. Chemical synthesis is a cost-effective and scalable method for producing active pharmaceutical ingredients. However, despite the total chemical synthesis of celastrol being achieved in 2015 [3], this approach requires at least 30 steps starting from 2,3-dimethylbutadiene, rendering it unsuitable for meeting commercial needs. Alternative options, such as isolating celastrol from plant cell suspensions or hairy root cultures, have also been attempted [39–41]. However, while these efforts have provided valuable insights into celastrol biosynthesis, the low yield and high operating cost of these methods have hindered their industrial application. These challenges prompted the development of biotechnological methods for celastrol production.

Discovery of the enzymatic steps of celastrol biosynthesis

To establish the production of celastrol in microbial cell factories, it is crucial to elucidate its biosynthetic pathway in *T. wilfordii*. The initial step in this endeavor was the discovery of the oxidosqualene cyclase enzyme that catalyzes the first step of the pathway and the cyclization of 2,3-oxidosqualene into the pentacyclic triterpene friedelin. In 2019, Zhou et al. identified two enzymes, *Tw*FRS1 and *Tw*FRS2, capable of synthesizing friedelin when expressed heterologously in yeast cells [42]. Moreover, RNAi-mediated gene silencing of the corresponding genes led to a decrease in celastrol accumulation in *T. wilfordii* suspension cells, indicating friedelin as the precursor of celastrol [42]. A concurrent study confirmed the ability of *Tw*FRS2 to synthesize friedelin using a tobacco heterologous expression system [43]. The structure of *Tw*FRS1 (*Tw*OSC1) has been studied by cryogenic electron microscopy, and the catalytic mechanism of friedelin synthesis was proposed to involve a nine-step structural rearrangement, based on quantum mechanics/molecular mechanics simulations [44].

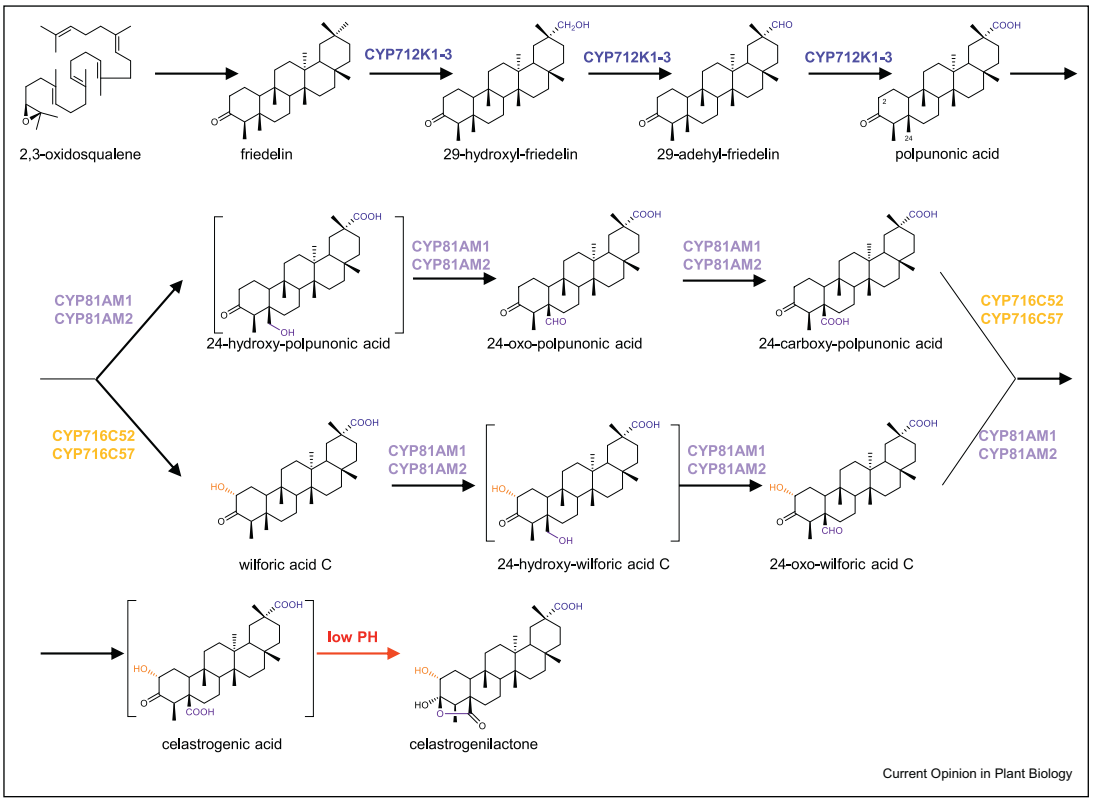
After friedelin synthesis, several successive oxidation events on its A and B rings and the C-29 methyl group are required to produce celastrol. Since cytochrome P450 enzymes (CYPs) account for most of the oxidation reactions in plant-specialized metabolite biosynthesis [45], investigations into the next steps of celastrol biosynthesis focused on identifying CYPs acting on the friedelin skeleton. Because celastrol is found to accumulate predominantly in the roots, Hansen et al. searched for CYPs predominantly expressed in root tissue and identified three enzymes — CYP712K1, CYP712K2, and CYP712K3 — capable of catalyzing the three-step C-29 oxidation reaction from friedelin to polpunonic acid in tobacco and yeast [43,46]. Shortly after, the function of CYP712K1 was corroborated in *T. wilfordii* cell suspension cultures [47]. In addition, a homologous enzyme, CYP712K4, catalyzing the same reaction, was identified in the related plant *Maytenus ilicifolia* [48].

The same differential transcriptomic analysis was utilized to discover CYPs catalyzing the biosynthetic steps downstream of polpunonic acid. Initially, CYP716C52 was identified to hydroxylate the C-2 position of polpunonic acid (A-ring) in yeast and tobacco [8]. The involvement of this enzyme in celastrol biosynthesis was later independently confirmed by RNAi silencing in *T. wilfordii* suspension cells [49]. Subsequently, CYP81AM1 was found to oxidize the C-24 methyl group of polpunonic acid to carboxylic acid [8]. Until now, the order of the oxidation at C-2 and C-24 remains unclear. However, given that both CYP716C52 and CYP81AM1 can catalyze the oxidation of polpunonic acid, it is plausible that a matrix-like biosynthetic route exists (Figure 2). Using the available genomic information for *T. wilfordii* [50], two functional paralogs for CYP716C52 (CYP716C57 and CYP716C52v2) and one paralog for CYP81AM1 (CYP81AM2) were also identified and confirmed to have the same activity. The combination of CYP81AM1 with CYP716C52 led to the production of the key intermediate celastrogenic acid [8], which harbors oxidative modifications at both C-2 and C-24 positions. Celastrogenic acid appears to be unstable in low pH environments, converting to a lactone ring structure involving positions C-2 and C-24. In the acidic environment of yeast cultivation media, this celastrogenic acid-derived lactone (celastogenilactone) exists as a mixture of opened lactone ring isomers due to keto-enol tautomerism on the A ring [8]. Despite extensive investigation, no other *T. wilfordii* CYP has been found to be able to act on celastrogenic acid, prompting further investigation into the events leading from celastrogenic acid to celastrol.

A non-enzymatic oxidation cascade completes celastrol biosynthesis

Up to the synthesis of celastrogenic acid, the biosynthesis of celastrol adheres to the canonical scheme of (tri)terpenoid biosynthesis, initiating with backbone formation and followed by oxidative decoration by CYPs

Figure 2



The biosynthetic pathway leading to the key intermediate celastrogenic acid. These enzymes include a series of triterpene synthases and cytochrome P450s that catalyze celastrol scaffold formation and C-2, C-24, and C-29 oxidations. First, CYP712K1, CYP712K2, or CYP712K3 oxidize C-29 of friedelin to give rise to polpunonic acid. Subsequently, CYP716C52 and its paralogs (CYP716C57 and CYP716C52v2) further oxidize C-2 of polpunonic acid, while CYP81AM1 and its paralogue CYP81AM2 oxidize C-24 of the triterpenoid core structure leading to the key intermediate celastrogenic acid. The black arrows indicate the biosynthetic steps catalyzed by the enzymes; the red arrows indicate steps occurring without enzymes.

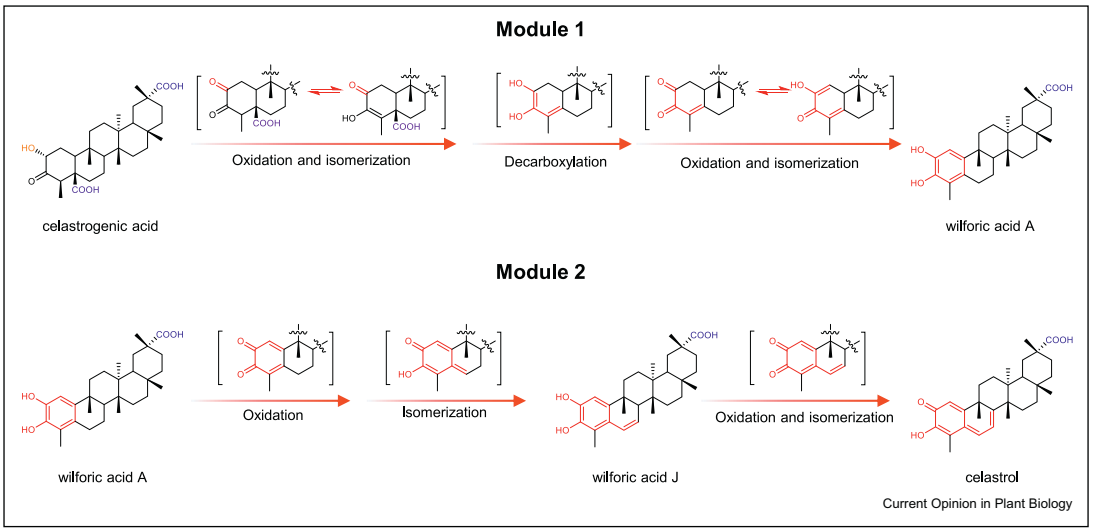
and other enzymes [51]. However, unlike other triterpenoids, such as sapogenins, which are typically inert compounds, celastrogenic acid is relatively unstable due to its A-ring ketol structure, making it susceptible to auto-oxidation. This observation led to the discovery of an unprecedented seven-step auto-oxidation chain reaction that converts celastrogenic acid to celastrol without the need for additional enzymes [8]. This auto-oxidation cascade can be conceptualized as a process involving two modules (Figure 3). The first module commences with a ketol auto-oxidation, followed by a double-bond rearrangement and the decarboxylation of the β,γ-unsaturated COOH group. Decarboxylation triggers further auto-oxidation and aromatization of the A ring, resulting in wilforic acid A. The second module comprises two cycles of tandem catechol oxidation and double-bond rearrangement, culminating in the formation of the quinone methide structure of celastrol [8].

At this stage, the possibility that yet unidentified *T. wilfordii* enzymes could catalyze or accelerate this process cannot be excluded. However, the accumulation of intermediates of the oxidation cascade, including wilforic acid A and wilforic acid J, in adequate amounts in *T. wilfordii* suggests that the conversion of celastrogenic acid to celastrol might happen through a slow, non-enzymatic process rather than a streamlined, enzyme-mediated pathway. It is noteworthy that, despite non-enzymatic oxidation steps being reported in the biosynthesis of other natural products (i.e. strychnine and cannabinoids [52,53]), another elaborate auto-oxidation cascade like the one observed in celastrol biosynthesis has yet to be reported.

Physiological roles of celastrol and its pathway intermediates in *T. wilfordii*

Celastrol is predominantly found in the suberized cell walls of the *T. wilfordii* root periderm and many of the pathway intermediates appear to also accumulate in the roots [8,54]. However, a concise physiological role for celastrol and its precursors in the roots is still lacking. While the antifungal, antibacterial, and antiviral properties of celastrol and its immediate methylation product pristimerin suggest that celastrol could have a role in plant defence [55–58], the findings by Zhao et al. [8] suggest that several pathway intermediates could also

Figure 3



A non-enzymatic oxidation cascade completes celastrol biosynthesis. Module 1 involves the non-enzymatic decarboxylation of C-24, leading to the intermediate wilforic acid A. This process likely initiates with oxidation and isomerization at C-2, resulting in an unstable β,γ-unsaturated acid moiety encompassing C-3, C-4, and C-24, thus initiating the decarboxylation reaction. The resulting intermediate undergoes further oxidation and tautomerization, culminating in the formation of the aromatic structure of wilforic acid A. In **Module 2**, a tandem catechol oxidation cascade completes celastrol biosynthesis. Wilforic acid A undergoes oxidation to ortho-benzoquinone, subsequently tautomerizing to the para-quinone methide form. This transformation facilitates the extension of the double-bond system to the B ring of the triterpenoid backbone. The resulting cyclohexadienone form then converts to wilforic acid J through isomerization. These oxidation and isomerization steps are replicated for wilforic acid J, ultimately yielding the final structure of celastrol.

have biological functions. This is because the late stages of celastrol biosynthesis produce one hydrogen peroxide molecule per oxidation step and involve highly reactive semiquinone radicals and powerful reactive oxygen species such as superoxide. All these molecules have previously been associated with plant defence [59–61] and plant-microbe communication [62]. Furthermore, celastrol, and its pathway intermediates, wilforic acid A and wilforic acid J, have been shown to exhibit remarkable radical scavenging activity that is comparable to known strong antioxidants like ascorbic acid [8]. Therefore, it seems likely that these compounds play roles in the interaction of the plant with microbes, possibly through the modulation of reactive oxygen species content in *T. wilfordii* roots.

Biotechnological production of celastrol

The unveiling of the biosynthetic pathway to celastrol has enabled the *de novo* biosynthesis of this valuable compound from table sugar using yeast fermentation. Zhao et al. achieved this by introducing celastrol biosynthesis genes along with a cytochrome P450 reductase (TwCPR1) into the genome of a platform yeast strain engineered specifically for terpenoid biosynthesis [8]. The resulting strain was capable of producing celastrogenic acid, which was secreted into the culture media and auto-oxidized to celastrol. To enhance the output of this process, isoprenoid precursor production was increased by overexpressing a feedback inhibition-resistant version of a key yeast mevalonate pathway gene *(t)HMG2* and inserting additional copies of bottleneck genes (two copies of *TwFRS1* and seven copies of *CYP81AM*) into the yeast chromosome. Additionally, the approach involved cultivating the yeast in nonacidic buffered media to prevent the formation of side-products and promote celastrol accumulation. Despite these genetic modifications, celastrol production using this method was time-consuming, requiring several weeks. To expedite the process and improve overall yield, Zhao et al. developed a semi-biosynthetic method for celastrol production. This method involved first producing celastrogenic acid through yeast fermentation, isolating the compound from the yeast medium, and then converting it to celastrol using a simple chemical conversion step. The conversion entailed heating celastrogenic acid at 60 °C in MeOH for one week, resulting in the conversion of over 20% of celastrogenic acid to celastrol [8].

While semi-synthetic bioproduction of celastrol shows promise as a scalable and potentially cost-effective method, further efforts are necessary to improve production from the mg/L scale to commercial viability. Considering that triterpenoids similar to celastrogenic acid have been produced at high titers (up to 15 g/L for protopanaxadiol and 5.7 g/L for ginsenoside compound K) [63,64], it is reasonable to expect that high titers of

celastrogenic acid could be achieved through metabolic engineering approaches. These approaches may include: 1) Enhancing triterpenoid precursor production through increasing the expression of genes in the mevalonate pathway, including the bottleneck enzyme 3-hydroxy-3-methylglutaryl-coenzyme A reductase, along with early sterol pathway genes to increase the metabolic flux toward 2,3-oxidosqualene. 2) Suppressing genes in pathways that compete for the same triterpenoid precursors in the sterol biosynthetic pathway, such as ERG7. 3) Optimization of fermentation processes via fine-tuning fermentation conditions, including pH, temperature, and carbon source. Additionally, other proven strategies for enhancing triterpenoid production, such as pathway compartmentalization and selecting enzyme homologs with optimal expression in yeast, could be employed [5,65,66]. For the non-enzymatic late part of celastrol synthesis, coordinated transition metal catalysts [67–69] could be utilized to enhance critical oxidation steps. Moreover, various types of promiscuous enzymes, such as polyphenol oxidases or laccases, could be employed to accelerate catechol moiety oxidation [70,71].

Developing celastrol into a drug

Celastrol holds great promise as a drug lead in obesity treatment. Recent advances in obesity therapies have identified semaglutide and other GLP-1 receptor ligands as effective weight loss drugs [72]. Celastrol-based or inspired therapeutics could complement weight control strategies, particularly in the maintenance phase following semaglutide treatment. Semaglutide-based therapies have a strong appetite-reducing effect, which can impart the quality of life for individuals undergoing treatment and reduce long-term adherence to the protocol. Thus, celastrol can be used to attain weight control while allowing individuals to enjoy the taste and social aspects of food intake after achieving sufficient weight loss [72]. However, several challenges remain in establishing celastrol or its derivatives as antiobesity drugs. First, the beneficial effects of celastrol for humans need validation in non-human primate models, while its safety for human administration must be confirmed. Other challenges hindering introduction of celastrol to the market include its poor bioavailability due to hydrophobicity and the possible occurrence of side-effects [73,74]. To address these issues, various interventions have been proposed, such as developing celastrol analogs with hydrophilic sidechains [75] or incorporating it into macromolecular complexes [76]. For instance, studies have demonstrated that loading celastrol into albumin nanoparticles significantly improves its bioavailability and reduces systemic toxicity, particularly in the treatment of inflammatory metabolic syndrome and kidney inflammation [77,78]. Additionally, celastrol macromolecular assemblies containing tissue-specific targeting molecules, such as cancer surface marker protein recognizing aptamers or neutrophil membrane proteins, have been shown to effectively deliver celastrol to colorectal cancer cells and inflamed pancreatic tissue, respectively [78,79]. In light of these advancements, it is clear that celastrol is getting closer to fulfilling its promise as a potent and versatile anti-inflammatory drug.

Declaration of competing interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: Sotirios C. Kampranis, Yong Zhao, and Karel Miettinen are coinventors in patent application #PCT/DK2023/050292 pending to University of Copenhagen.

Data availability

No data was used for the research described in the article.

Acknowledgements

This work was financially supported by the Novo Nordisk Foundation grants NNF17OC0027646, NNF16OC0021760, and NNF19OC0055204 (to S.C.K).

References

Papers of particular interest, published within the period of review, have been highlighted as:

* of special interest
** of outstanding interest

1. ** Liu J, Lee J, Salazar Hernandez MA, Mazitschek R, Ozcan U: **Treatment of obesity with celastrol**. *Cell* 2015, **161**:999–1011.
This work identifies celastrol as a powerful leptin sensitizer and a promising agent for obesity treatment.

2. Hu M, Luo Q, Alitongbieke G, Chong S, Xu C, Xie L, Chen X, Zhang D, Zhou Y, Wang Z, *et al.*: **Celastrol-induced Nur77 interaction with TRAF2 alleviates inflammation by promoting mitochondrial ubiquitination and autophagy**. *Mol Cell* 2017, **66**:141–153.

3. Camelio AM, Johnson TC, Siegel D: **Total synthesis of celastrol, development of a platform to access celastroid natural products**. *J Am Chem Soc* 2015, **137**:11864–11867.

4. Paddon CJ, Westfall PJ, Pitera DJ, Benjamin K, Fisher K, McPhee D, Leavell MD, Tai A, Main A, Eng D, *et al.*: **High-level semi-synthetic production of the potent antimalarial artemisinin**. *Nature* 2013, **496**:528–532.

5. Zhang J, Hansen LG, Gudich O, Viehrig K, Lassen LMM, Schrübbers L, Adhikari KB, Rubaszka P, Carrasquer-Alvarez E, Chen L, *et al.*: **A microbial supply chain for production of the anti-cancer drug vinblastine**. *Nature* 2022, **609**:341–347.

6. Galanie S, Thodey K, Trenchard IJ, Interrante MF, Smolke CD: **Complete biosynthesis of opioids in yeast**. *Science* 2015, **349**:1095–1100.

7. Luo X, Reiter MA, d'Espaux L, Wong J, Denby CM, Lechner A, Zhang Y, Grzybowski AT, Harth S, Lin W, *et al.*: **Complete biosynthesis of cannabinoids and their unnatural analogues in yeast**. *Nature* 2019, **567**:123–126.

8. ** Zhao Y, Hansen NL, Duan Y-T, Prasad M, Motawia MS, Møller BL, Pateraki I, Staerk D, Bak S, Miettinen K: **Biosynthesis and biotechnological production of the anti-obesity agent celastrol**. *Nat Chem* 2023, **15**:1236–1246.
The authors elucidate the complete biosynthetic pathway of celastrol and combine metabolic engineering with chemical conversion to enable its scalable production using baker's yeast.

9. Song L, Wang J, Nie J, Zhang Y, Han R, Liu H, Ma N, Yang Z, Li Y: **Study on toxicity/efficacy related substances and metabolic mechanism of *Tripterygium wilfordii* Hook. f based on O2LPS correlation analysis**. *J Ethnopharmacol* 2024, **318**, 116949.

10. Luo Y, Hou X, Xi A, Luo M, Wang K, Xu Z: **Tripterygium wilfordii Hook F combination therapy with methotrexate for rheumatoid arthritis: an updated meta-analysis**. *J Ethnopharmacol* 2023, **307**, 116211.

11. Shan Y, Zhao J, Wei K, Jiang P, Xu L, Chang C, Xu L, Shi Y, Zheng Y, Bian Y, *et al.*: **A comprehensive review of *Tripterygium wilfordii* hook. f. in the treatment of rheumatic and autoimmune diseases: bioactive compounds, mechanisms of action, and future directions**. *Front Pharmacol* 2023, **14**, 1282610.

12. Chou TQ, Mei PF: **The principle of the Chinese drug Lei-Kung-Teng, Tripterygium wilfordii, Hook. I. The coloring substance and the sugars**. *Zhongguo Shenglixue Zazhi* 1936, **10**:529.

13. Gisvold O: **The pigments contained in the bark of the root of Celastrus scandens**. *J Am Pharmaceut Assoc (1912-1977)* 1939, **28**:440.

14. Gisvold O: **The constitution of celastrol. II**. *J Am Pharmaceut Assoc (1912-1977)* 1940, **29**:12–14.

15. Gisvold O: **Constitution of celastrol. III**. *J Am Pharmaceut Assoc (1912-1977)* 1940, **29**:432.

16. Gisvold O: **The constitution of celastrol. IV**. *J Am Pharmaceut Assoc (1912-1977)* 1942, **31**:529.

17. Nakanishi K, Kakisawa H, Hirata Y: **Structure of pristimerin and celastrol**. *J Am Chem Soc* 1955, **77**:3169.

18. Grant PK, Johnson AW, Pristimerin I: **The nature of the chromophore**. *J Chem Soc* 1957, **4079**.

19. Harada R, Kakisawa H, Kobayashi S, Musya M, Nakanishi K, Takahashi Y: **Structure of pristimerin, a quinonoid triterpene**. *Tetrahedron Lett* 1962, **603**.

20. Kupchan SM, Karim A, Marcks C: **Tumor inhibitors. XXXIV. Taxodione and taxodone, two novel diterpenoid quinone methide tumor inhibitors from *Taxodium distichum***. *J Am Chem Soc* 1968, **90**:5923–5924.

21. Bode HB, Zeeck A: **Structure and biosynthesis of kendomycin, a carbocyclic ansa-compound from Streptomyces**. *J Chem Soc Perkin Trans* 2000, **1**:323–328.

22. Goncalves de Lima O, Leoncio d'Albuquerque I, Maciel GM: **Antimicrobial substances in higher plants. XXIX. Antibiotic activity of celastrol**. *Rev Inst Antibiot, Univ Fed Pernambuco, Recife* 1969, **9**:75.

23. Pang X, Yi Z, Zhang J, Lu B, Sung B, Qu W, Aggarwal BB, Liu M: **Celastrol suppresses angiogenesis-mediated tumor growth through inhibition of AKT/mammalian target of rapamycin pathway**. *Cancer Res* 2010, **70**:1951–1959.

24. Yang H, Chen D, Cui QC, Yuan X, Dou QP: **Celastrol, a triterpene extracted from the Chinese "Thunder of God Vine," is a potent proteasome inhibitor and suppresses human prostate cancer growth in nude mice**. *Cancer Res* 2006, **66**:4758–4765.

25. Uttarkar S, Dassé E, Coulibaly A, Steinmann S, Jakobs A, Schomburg C, Trentmann A, Jose J, Schlenke P, Berdel WE, *et al.*: **Targeting acute myeloid leukemia with a small molecule inhibitor of the Myb/p300 interaction**. *Blood* 2016, **127**:1173–1182.

26. Hassane DC, Guzman ML, Corbett C, Li X, Abboud R, Young F, Liesveld JL, Carroll M, Jordan CT: **Discovery of agents that eradicate leukemia stem cells using an in silico screen of public gene expression data**. *Blood* 2008, **111**:5654–5662.

27. Sethi G, Ahn KS, Pandey MK, Aggarwal BB: **Celastrol, a novel triterpene, potentiates TNF-induced apoptosis and suppresses invasion of tumor cells by inhibiting NF-kappaB-regulated gene products and TAK1-mediated NF-kappaB activation**. *Blood* 2007, **109**:2727–2735.

28. Freudlsperger C, Bian Y, Contag Wise S, Burnett J, Coupar J, Yang X, Chen Z, Van Waes C: **TGF-β and NF-κB signal pathway cross-talk is mediated through TAK1 and SMAD7 in a subset of head and neck cancers**. *Oncogene* 2013, **32**:1549–1559.

29. * Hieronymus H, Lamb J, Ross KN, Peng XP, Clement C, Rodina A, Nieto M, Du J, Stegmaier K, Raj SM, *et al.*: **Gene expression signature-based chemical genomic prediction identifies a novel class of HSP90 pathway modulators**. *Cancer Cell* 2006, **10**:321–330.
Pioneering study revealing the potential of celastrol in cancer treatment.

30. Nanjundaiah SM, Venkatesha SH, Yu H, Tong L, Stains JP, Moudgil KD: **Celastrus and its bioactive celastrol protect against bone damage in autoimmune arthritis by modulating osteoimmune cross-talk**. *J Biol Chem* 2012, **287**:22216–22226.

31. * Mu TW, Ong DS, Wang YJ, Balch WE, Yates 3rd JR, Segatori L, Kelly JW: **Chemical and biological approaches synergize to ameliorate protein-folding diseases**. *Cell* 2008, **134**:769–781.
Highlights the potential of celastrol in the treatment of protein-folding diseases.

32. Yang C, Swallows CL, Zhang C, Lu J, Xiao H, Brady RO, Zhuang Z: **Celastrol increases glucocerebrosidase activity in Gaucher disease by modulating molecular chaperones**. *Proc Natl Acad Sci U S A* 2014, **111**:249–254.

33. Considine RV, Sinha MK, Heiman ML, Kriauciunas A, Stephens TW, Nyce MR, Ohannesian JP, Marco CC, McKee LJ, Bauer TL, *et al.*: **Serum immunoreactive-leptin concentrations in normal-weight and obese humans**. *N Engl J Med* 1996, **334**:292–295.

34. Frederich RC, Hamann A, Anderson S, Löllmann B, Lowell BB, Flier JS: **Leptin levels reflect body lipid content in mice: evidence for diet-induced resistance to leptin action**. *Nat Med* 1995, **1**:1311–1314.

35. Halaas JL, Boozer C, Blair-West J, Fidahusein N, Denton DA, Friedman JM: **Physiological response to long-term peripheral and central leptin infusion in lean and obese mice**. *Proc Natl Acad Sci U S A* 1997, **94**:8878–8883.

36. * Feng X, Guan D, Auen T, Choi JW, Salazar Hernández MA, Lee J, Chun H, Faruk F, Kaplun E, Herbert Z, *et al.*: **IL1R1 is required for celastrol's leptin-sensitization and antiobesity effects**. *Nat Med* 2019, **25**:575–582.
Key study revealing the involvement of the interleukin-1 receptor in the anti-obesity effect of celastrol.

37. * Ma X, Xu L, Alberobello AT, Gavrilova O, Bagattin A, Skarulis M, Liu J, Finkel T, Mueller E: **Celastrol protects against obesity and metabolic dysfunction through activation of a HSF1-PGC1α transcriptional Axis**. *Cell Metabol* 2015, **22**:695–708.
One of the key early observations of the positive effect of celastrol on obesity.

38. Zhang Y, Geng C, Liu X, Li M, Gao M, Liu X, Fang F, Chang Y: **Celastrol ameliorates liver metabolic damage caused by a high-fat diet through Sirt1**. *Mol Metabol* 2017, **6**:138–147.

39. Zhao Y, Zhang Y, Su P, Yang J, Huang L, Gao W: **Genetic transformation system for woody plant *Tripterygium wilfordii* and its application to product natural celastrol**. *Front Plant Sci* 2018, **8**, 300122.

40. Liu Y-J, Zhao Y-J, Su P, Zhang M, Tong Y-R, Hu T-Y, Huang L-Q, Gao W: **The MVA pathway genes expressions and accumulation of celastrol in *Tripterygium wilfordii* suspension cells in response to methyl jasmonate treatment**. *J Asian Nat Prod Res* 2016, **18**:619–628.

41. Zhang B, Chen L, Huo Y, Feng J, Ma Z, Zhang X, Zhu C: **Enhanced production of celastrol in *Tripterygium wilfordii* hairy root cultures by overexpression of TwSQS2**. *Biochem Eng J* 2020, **161**, 107681.

42. * Zhou J, Hu T, Gao L, Su P, Zhang Y, Zhao Y, Chen S, Tu L, Song Y, Wang X, *et al.*: **Friedelane-type triterpene cyclase in celastrol biosynthesis from *Tripterygium wilfordii* and its application for triterpenes biosynthesis in yeast**. *New Phytol* 2019, **223**:722–735.
This work identifies two enzymes, TwFRS1 and TwFRS2, that synthesize friedelin in *T. wilfordii*.

43. * Hansen NL, Miettinen K, Zhao Y, Ignea C, Andreadelli A, Raadam MH, Makris AM, Møller BL, Stærk D, Bak S, *et al.*:

Integrating pathway elucidation with yeast engineering to produce polpunonic acid the precursor of the anti-obesity agent celastrol. *Microb Cell Factories* 2020, **19**:15.
This work identifies TwFRS2 as a friedelin synthase and three cytochrome P450 enzymes, CYP712K1, CYP712K2, and CYP712K3, that oxidize C29 of friedelin to synthesize polpunonic acid.

44. Luo Y, Ma X, Qiu Y, Lu Y, Shen S, Li Y, Gao H, Chen K, Zhou J, Hu T, *et al.*: **Structural and catalytic insight into the unique pentacyclic triterpene synthase TwOSC**. *Angew Chem Int Ed* 2023, **62**, e202313429.

45. Pateraki I, Heskes AM, Hamberger B: **Cytochromes P450 for terpene functionalisation and metabolic engineering**. *Adv Biochem Eng Biotechnol* 2015, **148**:107–139.

46. Hansen NL: *Terpenoid pathway discovery in Tripterygium wilfordii : a Chinese medicinal plant*. Doctoral thesis. University of Copenhagen, Faculty of Science, Department of Plant and Environmental Sciences, Section of Plant Biochemistry; 2017.

47. Zhou J, Hu T, Liu Y, Tu L, Song Y, Lu Y, Zhang Y, Tong Y, Zhao Y, Su P, Wu X, Huang L, Gao W: **Cytochrome P450 catalyses the 29-carboxyl group formation of celastrol**. *Phytochemistry* 2021, **190**, 112868.

48. Bicalho KU, Arendt P, Goossens A, Pollier J, Bicalho KU, Arendt P, Goossens A, Pollier J, Bicalho KU, Santoni MM, *et al.*: **CYP712K4 catalyzes the C-29 oxidation of friedelin in the Maytenus ilicifolia quinone methide triterpenoid biosynthesis pathway**. *Plant Cell Physiol* 2019, **60**:2510–2522.

49. Lu Y, Liu Y, Zhang Y, Gao H, Chen X, Tu L, Luo Y, Jiang Z, Yin Y, Zhou J: **Characterization of the cytochrome P450 CYP716C52 in celastrol biosynthesis and its applications in engineered *Saccharomyces cerevisiae***. *J Nat Prod* 2024, **87**:176–185.

50. Tu L, Su P, Zhang Z, Gao L, Wang J, Hu T, Zhou J, Zhang Y, Zhao Y, Liu Y, *et al.*: **Genome of *Tripterygium wilfordii* and identification of cytochrome P450 involved in triptolide biosynthesis**. *Nat Commun* 2020, **11**:971.

51. Thimmappa R, Geisler K, Louveau T, O'Maille P, Osbourn A: **Triterpene biosynthesis in plants**. *Annu Rev Plant Biol* 2014, **65**:225–257.

52. Hong B, Grzech D, Caputi L, Sonawane P, López CER, Kamileen MO, Hernández Lozada NJ, Grabe V, O'Connor SE: **Biosynthesis of strychnine**. *Nature* 2022, **607**:617–622.

53. Tahir MN, Shahbazi F, Rondeau-Gagné S, Trant JF: **The biosynthesis of the cannabinoids**. *J Cannabis Res* 2021, **3**:7.

54. Lange BM, Fischedick JT, Lange MF, Srividya N, Šamec D, Poirier BC: **Integrative approaches for the identification and localization of specialized metabolites in *Tripterygium* Roots**. *Plant Physiol* 2016, **173**:456–469.

55. Luo DQ, Wang H, Tian X, Shao H-J, Liu J-K: **Antifungal properties of pristimerin and celastrol isolated from Celastrus hypoleucus**. *Pest Manag Sci* 2005, **61**:85–90.

56. Abdel-Halim MS, Askoura M, Mansour B, Yahya G, El-Ganiny AM: ***In vitro* activity of celastrol in combination with thymol against carbapenem-resistant *Klebsiella pneumoniae* isolates**. *J Antibiot* 2022, **75**:679–690.

57. Yu JS, Tseng CK, Lin CK, Hsu YC, Wu YH, Hsieh CL, Lee JC: **Celastrol inhibits dengue virus replication via up-regulating type I interferon and downstream interferon-stimulated responses**. *Antivir Res* 2017, **137**:49–57.

58. Padilla-Montaño N, de León Guerra L, Moujir L: **Antimicrobial activity and mode of action of celastrol, a nortriterpen quinone isolated from natural sources**. *Foods* 2021, **10**.

59. Barbehenn RV, Peter Constabel C: **Tannins in plant-herbivore interactions**. *Phytochemistry* 2011, **72**:1551–1565.

60. Uchimiya M: **Proton-coupled electron transfers of defense phytochemicals in sorghum (*sorghum bicolor* (L.) moench)**. *J Agric Food Chem* 2020, **68**:12978–12983.

61. Eilenberg H, Pnini-Cohen S, Rahamim Y, Sionov E, Segal E, Carmeli S, Zilberstein A: **Induced production of antifungal naphthoquinones in the pitchers of the carnivorous plant *Nepenthes khasiana***. *J Exp Bot* 2010, **61**:911–922.

62. Nordzieke DE, Fernandes TR, El Ghalid M, Turrà D, Di Pietro A: **NADPH oxidase regulates chemotropic growth of the fungal pathogen *Fusarium oxysporum* towards the host plant**. *New Phytol* 2019, **224**:1600–1612.

63. Zhu Y, Li J, Peng L, Meng L, Diao M, Jiang S, Li J, Xie N: **High-yield production of protopanaxadiol from sugarcane molasses by metabolically engineered *Saccharomyces cerevisiae***. *Microb Cell Factories* 2022, **21**:230.

64. Wang P, Wang J, Zhao G, Yan X, Zhou Z: **Systematic optimization of the yeast cell factory for sustainable and high efficiency production of bioactive ginsenoside compound K**. *Synth Syst Biotechnol* 2021, **6**:69–76.

65. Dale MP, Moses T, Johnston EJ, Rosser SJ: **A systematic comparison of triterpenoid biosynthetic enzymes for the production of oleanolic acid in *Saccharomyces cerevisiae***. *PLoS One* 2020, **15**, e0231980.

66. Dinday S, Ghosh S: **Recent advances in triterpenoid pathway elucidation and engineering**. *Biotechnol. Adv.s* 2023, **68**, 108214.

67. Mukherjee D, Nag P, Shteinman AA, Vennapusa SR, Mandal U, Mitra M: **Catechol oxidation promoted by bridging phenoxo moieties in a bis(μ-phenoxo)-bridged dicopper(ii) complex**. *RSC Adv* 2021, **11**:22951–22959.

68. Titi A, Dahmani M, Abbaoui Z, El Kodadi M, Et-Touhami A, Yahyi A, Yousfi EB, Touzani R, Siaj M: **New catalysts based on carboxylate Sn(IV) complexes used in the oxidation reaction of 3,5-di-tert-butylcatechol to 3,5-di-tert-butyl-o-benzoquinone**. *React Kinet Mech Catal* 2024, **137**:133–148.

69. Titi A, Zaidi K, Alzahrani AYA, El Kodadi M, Yousfi EB, Moliterni A, Hammouti B, Touzani R, Abboud M: **New in situ catalysts based on nitro functional pyrazole derivatives and copper (II) salts for promoting oxidation of catechol to O-quinone**. *Catalysts* 2023, **13**:162.

70. Mayer AM: **Polyphenol oxidases in plants and fungi: going places? A review**. *Phytochemistry* 2006, **67**:2318–2331.

71. Aktaş N, Tanyolaç A: **Kinetics of laccase-catalyzed oxidative polymerization of catechol**. *J Mol Catal B Enzym* 2003, **22**:61–69.

72. Sorli C, Harashima S-i, Tsoukas GM, Unger J, Karsbøl JD, Hansen T, Bain SC: **Efficacy and safety of once-weekly semaglutide monotherapy versus placebo in patients with type 2 diabetes (SUSTAIN 1): a double-blind, randomised, placebo-controlled, parallel-group, multinational, multicentre phase 3a trial**. *Lancet Diabetes Endocrinol* 2017, **5**:251–260.

73. Zha J, Zhang Q, Li M, Wang JR, Mei X: **Improving dissolution properties by polymers and surfactants: a case study of celastrol**. *J Pharmaceut Sci* 2018, **107**:2860–2868.

74. Wang C, Dai S, Zhao X, Zhang Y, Gong L, Fu K, Ma C, Peng C, Li Y: **Celastrol as an emerging anticancer agent: current status, challenges and therapeutic strategies**. *Biomed Pharmacother* 2023, **163**, 114882.

75. Hou W, Liu B, Xu H: **Celastrol: progresses in structure-modifications, structure-activity relationships, pharmacology and toxicology**. *Eur J Med Chem* 2020, **189**, 112081.

76. Sun Y, Wang C, Li X, Lu J, Wang M: **Recent advances in drug delivery of celastrol for enhancing efficiency and reducing the toxicity**. *Front Pharmacol* 2024, **15**, 1137289.

77. Guo L, Luo S, Du Z, Zhou M, Li P, Fu Y, Sun X, Huang Y, Zhang Z: **Targeted delivery of celastrol to mesangial cells is effective against mesangioproliferative glomerulonephritis**. *Nat Commun* 2017, **8**:878.

78. Ge P, Niu B, Wu Y, Xu W, Li M, Sun H, Zhou H, Zhang X, Xie J: **Enhanced cancer therapy of celastrol in vitro and in vivo by smart dendrimers delivery with specificity and biosafety**. *Chem Eng J* 2020, **383**, 123228.

79. Zhou X, Cao X, Tu H, Zhang Z-R, Deng L: **Inflammation-targeted delivery of celastrol via neutrophil membrane-coated nanoparticles in the management of acute pancreatitis**. *Mol Pharm* 2019, **16**:1397–1405.