Trans-palmitoleic acid (trans-9-C16:1, or trans-C16:1 n-7): Nutritional impacts, metabolism, origin, compositional data, analytical methods and chemical synthesis. A review

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Trans-palmitoleic acid (*trans*-9-C16:1, or *trans*-C16:1 n-7): nutritional impacts, metabolism, origin, compositional data, analytical methods and chemical synthesis. A review.

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trans-palmitoleic acid; *trans*-vaccenic acid; rumenic acid; ruminant meat; ruminant milk; type 2 diabetes.

ABBREVIATIONS
ALA: α-linolenic acid
AOCS: American Oil Chemists' Society
CLA: conjugated linoleic acid
CLnA: conjugated linolenic acid
CPA: *cis*-palmitoleic acid
ELOVL: Elongation of very long chain fatty acid enzyme
FID: flame-ionization detector
GC: gas chromatography
HPLC: high-performance liquid chromatography
LA: linoleic acid
LC: liquid chromatography
MS: mass spectroscopy
MUFA: mono-unsaturated fatty acid
PHFO: partially hydrogenated fish oil
PHO: partially hydrogenated oil
PHVO: partially hydrogenated vegetable oil
RMA: rumenic acid
R-TFA: ruminant (or natural) *trans* fatty acids
SPE: solid-phase extraction
TLC: thin layer chromatography
TPA: *trans*-palmitoleic acid
TVA: *trans*-vaccenic acid

ABSTRACT

Since the early 2010s, dietary *trans*-palmitoleic acid (*trans*-9-hexadecenoic acid, *trans*-9-C16:1 in the Δ-nomenclature, *trans*-C16:1 n-7 in the Ω-nomenclature, TPA) has been epidemiologically associated with a lower risk of type 2 diabetes in humans. Thanks to these findings, TPA has become a nutrient of interest.

However, there is a lot of unresolved crucial questions about this dietary fatty acid. Is TPA a natural *trans* fatty acid? What kind of foods ensures intakes in TPA? What about its metabolism? How does dietary TPA act to prevent type 2 diabetes? What are the biological mechanisms involved in this physiological effect? Clearly, it is high time to answer all these questions with the very first review specifically dedicated to this intriguing fatty acid. Aiming at getting an overview, we shall try to give an answer to all these questions, relying on appropriate and accurate scientific results.

Briefly, this review underlines that TPA is indeed a natural *trans* fatty acid which is metabolically linked to other well-known natural *trans* fatty acids. Knowledge on physiological impacts of dietary TPA is limited so far to epidemiological data, awaiting for supplementation studies. In this multidisciplinary review, we also emphasize on methodological topics related to TPA, particularly when it comes to the quantification of TPA in foods and human plasma. As a conclusion, we highlight promising health benefits of dietary TPA; however, there is a strong lack in well-designed studies in both the nutritional and the analytical area.

1. Introduction

1.1. What is TPA?

Trans-palmitoleic acid (TPA) is a hexadecenoic fatty acid with one *trans* double bond located in the 9th position using the Δ-nomenclature (*trans*-9-C16:1) (**Figure 1A**), or in the n-7 position using the Ω-nomenclature (*trans*-C16:1 n-7) (**Figure 1B**). It is worth stressing the importance of chemical details in this review. Indeed, TPA is usually confused in the literature with its *cis* isomer (*cis*-palmitoleic acid, *cis*-9-C16:1 or *cis*-C16:1 n-7, CPA), or with other *trans*-C16:1 positional isomers. Of note, TPA can also be quoted in the literature as "palmitelaidic acid". According to many reglementary definitions (*e.g.*, Codex Alimentarius, European Food Standard Authority, Food and Drug Administration), TPA belongs to the *trans* fatty acid family [1–3], and more precisely to the *trans*-mono-unsaturated fatty acid (*trans*-MUFA) family.

Figure 1 about here.

1.2. Why focusing on TPA?

Why studying the physiological impacts of dietary TPA? The story began in 2008 when Cao and colleagues described a new lipokine corresponding to the fatty acid CPA [4,5]. More precisely, the CPA coming from adipose tissue, and circulating as free fatty acid, was supposed to protect mice from hepatic *de novo* lipogenesis and insulin resistance [4]. Such a hypothesis is exciting, but hard to verify: the huge part of circulating CPA in humans arises from hepatic *de novo* lipogenesis, and not from adipose tissue [6,7]. Alternatively, one could focus on TPA to better understand the physiological impacts of CPA [8]. Mozaffarian and colleagues, therefore, assumed in 2010 that TPA would exert the same physiological impacts as CPA and would not be endogenously synthesized in humans [8].

Explaining the whole story of TPA helps to understand the "magic" atmosphere that has always prevailed around this fatty acid. Before discovering the CPA as a lipokine, TPA was a barely known fatty acid that had just been reported in dairy products by a couple of studies [9,10]. From this point of view, unravelling benefits TPA is clearly surprising, not to mention its belonging to the *trans* fatty acid family. Therefore, it is high time to make things clearer by gathering all knowledge about TPA.

Because there is often a confusion between TPA and its *cis* isomer in the literature, we shall review and talk about studies focusing only on TPA. The suggested benefits of TPA and potential mechanisms of action shall be discussed, along with its food sources. The very important metabolism of TPA shall be reviewed as well, to make connections with other ruminant (or natural) *trans* fatty acids (R-TFAs) that have been of scientific interest as well. Finally, we shall develop on topics related to TPA, especially about appropriate analytical methods.

2. Dietary TPA and health outcomes in humans

2.1. Benefits of dietary TPA acid arising from epidemiological data

Most knowledge about the physiological impact of dietary TPA comes from epidemiological data (**Table 1**). Prospective epidemiological studies highlighted a neutral role of dietary TPA on type 2 diabetes risk [11–13]. Further, other prospective epidemiological data showed a significant inverse association between high levels of circulating TPA in humans and lower risk of type 2 diabetes [8,14,15]. Such an inverse relationship remained significant after adjustment on C15:0 [15] and C17:0 [8], both fatty acids being also specific of dairy fat and suspected as well to have benefits on human health [16]. Therefore, TPA could certainly have special and unique benefits to human health. Importantly, two meta-analyses confirmed this significant and inverse relationship. Pooling different epidemiological studies, De Souza and colleagues highlighted a significant 42%-reduced risk of developing a type 2 diabetes associated with the highest levels of circulating TPA (mean risk ratio of 0.58, IC-95% = [0.58-0.74], $P < 0.001$) [17]. In agreement with this finding, Imamura and colleagues pooled several prospective analyses and found a significantly lower link between circulating levels of TPA in humans and type 2 diabetes incidence (mean hazard ratio of 0.82, IC-95% = [0.70-0.96], $P < 0.05$) [18].

Table 1 about here.

Regarding associations between circulating levels of TPA and anthropometric/metabolic parameters that might explain the decrease in type 2 diabetes risk, two prospective studies highlighted a better systemic insulin sensitivity (as measured by the HOMA-IR index) associated with high levels of circulating TPA [8,15]. Of note, this higher insulin sensitivity seemed to be explained by a decreased fasting insulinemia rather than a decreased fasting glycemia [8,15,19]. However, better glucose uptake by the liver was pointed out as well to explain the lower systemic insulin resistance [20,21]. These discrepancies underline the need in unravelling potential mechanisms of action of dietary TPA.

Epidemiological data are inconsistent as regards to cardiovascular risk. In the MESA cohort, high levels of circulating TPA had a neutral effect on cardiovascular disease [22]. Consistently, in the NHS and the HPFS cohorts, TPA was neutrally associated with risk of stroke [23]. Three studies highlighted a favourable impact of dietary TPA on systolic blood pressure and hypertension [15,22,24], while two others reported a neutral effect [8]. As regards to the impact on the $\frac{\text{total-cholesterol}}{\text{HDL-cholesterol}}$, there is at least a neutral effect of dietary TPA [15] while a favourable effect was highlighted in the CHS cohort [8]. Likewise, inconsistent outcomes are found concerning concentrations of LDL-cholesterol [8,15,22] and HDL-cholesterol [8,15,22,25]. Overall, there is a need in supplementation studies specifically dedicated to the cardiovascular impact of dietary TPA.

2.2. Scarcity of TPA supplementation studies

The hypothesis of the benefits of dietary TPA on insulin resistance calls for supplementation studies that might unravel mechanisms of action. However, supplementation or nutritional studies involving TPA are quite scarce. The very low availability of pure TPA in enough amounts mainly explains this lack of data (see **Section 6.2**).

At the clinical level, to the best of our knowledge, only Lichtenstein and colleagues are currently carrying out a supplementation study focusing on probable benefits of dietary TPA towards insulin-resistance [26]. The situation is the same as regards to *in vivo* studies performed on rodents. Of note, a TPA-supplementation study carried on $ApoE^{-/-}$ male mice was recently published [27]. Given the chosen rodent model, this study dealt more about atherosclerosis rather than type 2 diabetes and insulin resistance. Indeed, TPA was found to be neutral as regards to the progression of atherosclerosis [27]. Besides, TPA did not impact the ratio between total cholesterol and HDL-cholesterol in plasma, suggesting a neutral effect of TPA on biomarkers of cardiovascular diseases [27].

To our knowledge, there is no supplementation study involving TPA on any rodent model dedicated to type 2 diabetes and insulin resistance. However, it is worth quoting that treatment of insulin-resistance by dietary TPA was patented in 2014 by Mozaffarian and colleagues [28]. Few details are available in this patent, but several results can be found. On the one hand, C2C12 myotubes cultured in the presence of TPA had a higher uptake of extracellular glucose compared with both control and palmitic acid treatment [28]. This result suggests a direct impact of dietary TPA on glycemia, which is in line with the conclusions of Kratz and colleagues [21]. However, this result is contrary to epidemiological studies that pointed out a likely role of dietary TPA on fasting insulinemia rather than on fasting glycemia [8,15,19]. On the other hand, 200 mg/kg body weight TPA supplementation on mice (presumably both *Ob*/*Ob* and healthy C57BL/6 mice fed a normal and a high-fat diet) were carried out and confirmed that TPA could be incorporated in plasma free fatty acids, phospholipids and triglycerides [28]. No further details about other outcomes are provided.

To unravel potential mechanisms of action of dietary TPA, one should rely on a couple of *in vitro* studies. First, compared with dietary oleic acid, TPA did not impact insulin secretion on perfused rat pancreas. However, TPA stimulated insulin secretion to a higher extent than palmitic acid [29]. In a straight relationship with these results, TPA rose PPAR-γ activity and pancreatic and duodenal homeobox 1 (PDX-1) in pancreatic β INS-1 cells [30]. Taken together, these *in vitro* outcomes are in line with epidemiological studies which highlighted a lower insulin secretion in subjects having high circulating levels of TPA. Pancreas might, therefore, be a target for dietary TPA to exert its benefits. Second, on endothelial HUVEC cells, TPA led to a significant decrease in the expression of tumor necrosis factor-α (TNF-α), vascular cell adhesion molecule 1 (VCAM-1) and superoxide-dismutase-2 (SOD-2) [31]. Likewise, TNF-α was significantly downshifted in hepatic HepG2 cells incubated with TPA [31]. In both HUVEC and HepG2 cells, these outcomes were independent of PPAR-γ activation. Thus, the anti-inflammatory effect of dietary TPA is suggested, in agreement with epidemiological data. However, such outcomes were found neither on healthy human endothelial cells nor on diabetic human ones [32].

3. ***Explaining the circulating levels of TPA in humans: dietary TPA as a direct origin***

Circulating levels of TPA in humans can be explained by its dietary origin, as TPA is found in several foods. Because TPA arises from ruminal biohydrogenation, ruminant-derived foods such as ruminant milk and ruminant meat do contain detectable amounts of TPA. Since TPA is a *trans* fatty acid, there is also evidence of its presence in partially hydrogenated oils.

3.1. Ruminant-derived foods as the only forthcoming source of TPA

Epidemiological data pointed out that ruminant-derived foods are among the sources of dietary TPA (**Table 2**). First, cross-sectional studies highlighted a positive correlation between intakes of dairy products and circulating TPA in humans [24,33,34]. Second, studies carried out at the prospective level also confirmed the positive association between ruminant-derived foods and circulating levels of TPA in humans. In the NHS cohort, levels of TPA in both plasma and RBC were significantly and positively correlated to dairy fat consumption [35]. Micha and colleagues reported a significant contribution of both dairy products and red meat in explaining circulating levels of TPA in the CHS cohort [36]. In strong agreement with these findings, circulating levels of TPA were significantly explained by both red meat and dairy product consumption in the MESA cohort [22]. Positive and significant correlations between ruminant-derived foods and circulating levels of TPA were also found in the HPFS cohort [14,23]. Finally, a recent meta-analysis confirmed that dietary TPA stems from consumption of dairy products [37].

Table 2 about here.

As TPA is a naturally occurring *trans* fatty acid, one should look at ruminal biohydrogenation to explain its presence in ruminant-derived foods. The workflow of the ruminal biohydrogenation mainly starts with the dietary linoleic (C18:2 n-6, LA) and α-linolenic (C18:3 n-3, ALA) acids, and ends with the stearic acid (C18:0). R-TFAs with 18 carbons arise as intermediates of this ruminal biohydrogenation. Because ruminal biohydrogenation involves a wide range of bacteria species and metabolic pathways, the diversity of R-TFAs is high. As such, R-TFAs include isomers of *trans*-C18:1 acids, conjugated linoleic acids (CLA), conjugated linolenic acids (CLnA) and C18:2 fatty acids with one *trans* double bond, as previously reviewed [38–40]. Importantly, very accurate metabolic pathways are naturally favoured in the rumen. Thus, *trans*-vaccenic acid (*trans*-11 C18:1, or *trans*-C18:1 n-7, TVA) is the main fatty acid among the *trans*-C18:1 positional isomers naturally generated [41]. Likewise, rumenic acid (*cis*-9, *trans*-11 C18:2, RMA) is the main isomer among the CLA isomers naturally generated by ruminal biohydrogenation [42].

TPA being a *trans* C16:1 fatty acid, where does it stem from? So far, two hypotheses may explain the presence of TPA in ruminant-derived foods (**Figure 2**). First, chain-shortening of TVA might occur. Such a pathway has been proposed for a long time to explain the presence of TPA in ruminant milk [43,44]. Second, TPA might also arise from the ruminant biohydrogenation of dietary C16:3 n-3 fatty acid (C16:3 7-*cis*,10-*cis*,13-*cis*) [10] which occurs in "C16:3 plants" [45,46]. Assuming a similar workflow to that of ALA biohydrogenation, TPA can indeed be obtained from C16:3 n-3 (**Figure 3**) [10]. In fact, both hypotheses might be true, so that TPA in ruminant milk would be explained by two metabolic pathways. Of note, the involvement of dietary C16:2 n-6 fatty acid in TPA synthesis in ruminants was also assumed similarly to biohydrogenation of dietary LA [47], but this hypothesis has remained unexplored so far.

Figure 2 about here.

Figure 3 about here.

When it comes to human nutrition, ruminant meat and milk are of high interest. TPA generated through ruminal biohydrogenation may enter the general circulation of the ruminant and be incorporated in muscle and mammary gland. Because fatty acids including *trans* ones can be metabolised, another question arises about chain-shortening of TVA: does peroxisomal β-oxidation occur in the rumen, in ruminant tissues, or in both? So far, this question remains unresolved but peroxisomal β-oxidation is very likely to occur in ruminant tissues. On the contrary, there are uncertainties about chain-shortening of TVA in the rumen (**Figure 2**).

Reliable analytical data confirm the presence of TPA in ruminant-derived foods available at retail. Regarding ruminant milk, only Destaillats and colleagues reported very reliable TPA contents in ruminant milk available at retail in 2000, based on silver-ion thin-layer chromatography (Ag^+-TLC) and appropriate gas-chromatography (GC) analysis [10]. Regardless of the ruminant species, TPA was the major *trans*-C16:1 positional isomer and accounts for 31.7, 26.6 and 31.1% of total *trans*-C16:1 in cow, goat and ewe milk available at retail, respectively [10]. Our research group also found similar relative levels of TPA (as % of total *trans*-C16:1) in ruminant milk available in France in 2018 [48] (**Figure 4A**). It is worth reminding that having several positional *trans*-C16:1 isomers is compatible with having multiple ruminal biohydrogenation pathways, including one being naturally favoured and leading to TPA. As regards to absolute concentrations, TPA accounted for 54, 41 and 80 mg/100 g of total fatty acids for cow, goat and ewe milk, respectively [10], while total *trans*-C16:1 fatty acids accounted for 0.13% (± 0.05, standard deviation) of total fatty acids in cow milk available at retail [9].

As for ruminant meat available at retail, to the best of our knowledge, our research group was the very first to report accurate content in TPA, based on reliable analytical methods [48]. Like ruminant milk available at retail, TPA was the major *trans*-C16:1 isomer accounting for approx. 40% of total *trans*-C16:1 (**Figure 4B**) and weighted 0.03% of total fatty acids [48].

It is interesting to note a similar pattern between TPA, TVA and RMA in ruminant products. More precisely, TPA, TVA and RMA are the major positional isomers among *trans*-C16:1, *trans*-C18:1 and CLA, respectively. Such a result may be interpreted in two ways. First, it may reflect the metabolic links connecting *trans*-C16:1, *trans*-C18:1 and CLA between each other, once these fatty acids are incorporated in tissues of ruminant species. As a result, one can easily understand the closeness between the *trans*-C16:1, *trans*-C18:1 and CLA profiles in ruminant-derived products. Second, it may also highlight that accurate metabolic pathways are favoured when it comes to ruminal biohydrogenation of dietary C16:3 n-3, yielding TPA as the major *trans*-C16:1 isomer. In this case, these metabolic pathways are the same as those yielding TVA and RMA among the *trans*-C18:1 and CLA, respectively.

Figure 4 about here.

3.2. Partially hydrogenated oils as a former source of TPA

Given that TPA is a *trans*-MUFA, the link with industrial processes that generate such fatty acids seems logical. Several industrial processes have a well-known ability to generate such *trans* fatty acids: partial (or catalytic) hydrogenation, deodorization and deep-frying. Again, most knowledge about *trans*-MUFAs of industrial origin is related to *trans*-C18:1 positional isomers. On the contrary, barely anything is known about *trans*-C16:1 positional isomers, including TPA.

Deodorization of crude oils generate *trans*-PUFAs, but not any *trans*-MUFA (*i.e.*, neither *trans*-C18:1 nor *trans*-C16:1 positional isomers) [49,50]. It is now a process in which the formation of *trans*-PUFAs is highly controlled [49]. As for deep-frying, studies carried out in the 1990s did underline the formation of *trans*-MUFAs (*trans*-C18:1 and presumably *trans*-C16:1) [51], but at the current time, no *trans*-MUFAs (neither *trans*-C18:1 nor *trans*-C16:1 positional isomers) are reported following this step [52–54].

Partial (or catalytic) hydrogenation has been applied for several decades to crude oils, yielding the famous partially hydrogenated oils (PHOs). During this process, the *cis* ethylenic double bond of fatty acid (*cis*-MUFAs or *cis*-PUFAs) can be isomerised into a *trans* double bond and can also change of position across the carbon chain [55]. Here, two cases should be distinguished: partially hydrogenated vegetable oils (PHVOs), and partially hydrogenated fish oils (PHFOs).

3.2.1. Partially hydrogenated vegetable oils: a lack of reliable data and the question of their current use

Several epidemiological studies suggest the presence of TPA in PHVOs (**Table 2**). In 2002, Baylin and colleagues highlighted a significant and positive correlation between TPA content in human subcutaneous adipose tissue and vegetable fat consumption in Costa Rica [56]. Consistently in the MESA cohort, consumption of margarine and salted biscuits significantly contributed to circulating levels of TPA [22]. Finally, a significant and positive link was found between PHVO consumption and circulating levels of TPA in the IRAS cohort [13]. These results underline that ruminant-derived foods may not be the only contributors to TPA intakes in humans and underline the need for reliable data about *trans*-C16:1 positional isomers in PHVOs.

CPA is commonly found in crude vegetable oils, accounting for a couple of % of total fatty acids depending on the vegetable oil [6]. From a theoretical point of view, getting TPA after partial hydrogenation of such oils does make sense. Qualitatively speaking, the situation might be the same as for the *trans*-C18:1 profile in PHVOs, which is characterized by a wide range of positional isomers in almost equal amounts [57]. As a result, the theoretical *trans*-C16:1 profile should contain TPA plus other *trans*-C16:1 positional isomers in similar amounts. The amount of *trans*-C16:1 should, however, be way lower than that of *trans*-C18:1 fatty acids. However, to the best of our knowledge, no reliable data exist so far about *trans*-C16:1 positional isomers in PHVOs. Dietz Precht and Joachim Molkentin previously attempted to do so, but the content in *trans*-C16:1 in such oils was too low for allowing an accurate resolution of *trans*-C16:1 positional isomers and assessing TPA content [9,58]. Nonetheless, total *trans*-C16:1 were accurately and reliably quantified in PHVOs, based on exactly the same analytical methods as that of Destaillats and colleagues [10]. Commercially available German PHVOs in

1997 had a mean content of *trans*-C16:1 equal to 0.01% of total fatty acids (± 0.03, standard deviation) [9]. In the early 2000s, still in Germany, total *trans*-C16:1 accounted for 0.01% of total fatty acids (± 0.01, standard deviation) in sunflower margarines and 0.01% (± 0.02, standard deviation) in cooking fats/shortenings available at retail [58]. Taken together, these data confirm that very low amounts of *trans*-C16:1 are found in PHVOs. Based on this quantitative data, plus on the *trans*-C16:1 content in German maternal milk, it was concluded that PHVOs could not be deemed as a reliable contributor to TPA intakes, contrary to dairy products [59].

Despite accurate analytical data, the hypothesis according to which PHVOs contribute to TPA intakes seems plausible. Indeed, most epidemiological data rely on blood sampling carried out in the 1990s (**Table 2**). At that time, PHVOs were still used in a wide range of foods. Therefore, such epidemiological data cannot be used to claim with certainty that TPA is a biomarker of ruminant-derived food consumption. But right now, the main question is whether PHVOs are still in use nowadays at least in Western countries. At the regulation level, countries such as the USA [3], Canada [60] and Denmark [61] were trailblazers in the early 2000s, taking the very first steps to progressively decrease the use of PHVOs in foods. In 2018, both the USA and Canada settled a ban on PHVOs [62,63]. The European Commission has recently adopted a legal 2%-limit of industrial *trans* fatty acids in all foods which should enter into force by April 2021 [64]. Such steps clearly shed light on the PHVO issue which received high media coverage and encouraged industries to look for alternative solutions (*i.e.*, the voluntary reduction approach). Both the regulation steps and the voluntary reduction approach led to decreased levels of industrial *trans* fatty acids, which are now barely detectable in foods [53,65–69]. This trend is confirmed by epidemiological data highlighting a decrease in *trans*-C18:1 content in human plasma and red blood cells, as reviewed by Craig-Schmidt and colleagues [70]. However, recent studies underlined a boundary between Western European countries and Eastern ones, the latter still having elevated amounts of industrial *trans* fatty acids compared to the former [71–74]. Thus, further research is therefore needed to conclude about the current use of PHVOs and its contribution to TPA intakes by analysing bakery foods and biscuits.

3.2.2. Partially hydrogenated fish oils: a former and geographically restricted source of TPA

In addition to the application on vegetable oils, partial hydrogenation can also be applied to crude fish oils to yield PHFOs. Crude fish oils do contain CPA which accounts for 7% of total fatty acids in cod liver oil [6]. As a result, partial hydrogenation of crude fish oils should theoretically lead to TPA plus plenty of other positional *trans*-C16:1 isomers.

To the best of our knowledge, there is no epidemiological data linking PHFO consumption to circulating levels of TPA in humans. Instead, very accurate data exist concerning the *trans*-C16:1 positional isomers in PHFOs. TPA was the major *trans*-C16:1 positional isomer in German cooking fats containing PHFOs and available at retail at the end of the 1990s, and weighted 24% of total *trans*-C16:1 in these oils [9]. Other *trans*-C16:1 positional isomers, especially *trans*-6-8 C16:1 and *trans*-10-C16:1, weighed almost as much as TPA [9]. Similar patterns in PHFOs were obtained in two other studies [75,76] and by our research group [77]. It is worth underlining that all these studies relied on appropriate analytical methods, thus

generating reliable data. Regarding absolute concentrations, *trans*-C16:1 in PHFOs accounted for 1.89% of total fatty acids (± 0.63, standard deviation) in Germany in 1997 [9].

Given the complexity of the fatty acid composition of crude fish oils, one may also assume that TPA can naturally be found in crude fish oils. To our knowledge, only Fardin-Kia and colleagues analysed with high accuracy *trans*-C16:1 positional isomers in crude menhaden oil and highlighted the presence of only *trans*-6-C16:1 but not TPA [78]. Thus, only partial hydrogenation can explain the presence of TPA and other *trans*-C16:1 positional isomers (excepted the *trans*-6-C16:1) in PHFOs.

Because of these high amounts of TPA in PHFOs, such oils presumably contributed to TPA intakes in combination with dairy products in Germany in the late 1990s [59]. This conclusion suggests that PHFOs were still used in Germany at that time. In fact, little information exists about the use of these oils. Importantly, PHFOs are described as excellent alternatives to PHVOs when it comes to the production of shortenings, cooking fats and margarines [79–81]. Still, the use of PHFOs was likely to be restricted to European countries [82], and more precisely Northern European countries like Germany, Netherlands, the United Kingdom or Norway [57,83,84]. In addition, there is evidence about an end of use and consumption of PHFOs in these countries in the late 1990s (Bente Kirkhus, NOFIMA, personal communication). Thus, PHFOs were a strong but former contributor of TPA dietary intakes in Northern European countries and did not impact on epidemiological studies that were conducted in the USA.

4. Explaining the circulating levels of TPA in humans: endogenous retro-conversion of dietary TVA as an indirect origin

4.1. Evidence of endogenous retro-conversion of dietary TVA to TPA

Generally speaking, *trans*-C16:1 fatty acids in non-ruminants have always been hypothesized to arise from their dietary *trans*-C18:1 counterparts. Rats fed a PHVO containing *trans*-C18:1 but deprived of *trans*-C16:1 isomers had detectable levels of *trans*-C16:1 isomers in lipid classes of the liver [85–87]. Recently, Jaudzsus and colleagues reported an increase in TPA content in human red blood cells following a TVA supplementation [88]. It is interesting to note that such a hypothesis corresponds to one assumption that is made to explain the origin of TPA in ruminant milk and meat. Consistently, adding TVA to the culture medium of RINm5F cells led to the detection of TPA in these cells [89].

Our research group accurately demonstrated that TVA could indeed undergo one cycle of peroxisomal β-oxidation, yielding TPA [90]. We first demonstrated that the growing concentrations of TVA in the culture medium of cultured rat hepatocytes led to growing amounts of intracellular TPA, but also to growing amounts of TPA in secreted triglycerides of these hepatocytes [90]. The conversion rate was estimated at 10%, in accordance with the assumptions of Jaudszus and colleagues [88]. It is worth underlining that we formally characterized TPA by a combination of Ag^+-TLC, appropriate GC analysis and DMOX derivatives. Second, we highlighted that specific blockade of peroxisomal β-oxidation in these hepatocytes significantly decreased the amount of intracellular TPA, but not specific blockade of mitochondrial β-oxidation [90]. Third, pregnant rats fed either a low-TVA (0.1% of total energy) or a high-TVA diet (4% of total energy) had detectable levels of TPA in the liver,

accounting for 0.02% and 0.11% of total fatty acids, respectively [90]. Taken together, our findings demonstrated that dietary TVA can undergo peroxisomal β-oxidation yielding TPA. The liver is very likely to ensure a great part of this retro-conversion, and the subsequent TPA can be exported to other tissues through secreted triglycerides.

4.2. Dietary sources of TVA: the same as dietary sources of TPA

In addition to dietary sources of TPA, one should therefore also look at dietary sources of TVA to explain circulating levels of TPA in humans. At the epidemiological level and of course considering solely TVA and not total *trans*-C18:1 fatty acids, there is to our knowledge only the Lifelines Biobank and Cohort Study which concluded that dietary TVA was supplied by dairy products in the Netherlands in the late 2000s [34,91]. This result is consistent with what was said above. Indeed, TVA typically arises as an intermediate of ruminal biohydrogenation, and more precisely as the major *trans*-C18:1 isomer. Accordingly, our research group found that TVA accounted for the major *trans*-C18:1 isomer in a range of commercially available French dairy products, weighing approx. 50% of total *trans*-C18:1 fatty acids (**Figure 5A**) [48]. As regards to absolute concentrations, TVA accounted for approx. 1.8% of total fatty acids [48]. Both our qualitative and quantitative data are in good agreement with previous accurate results obtained from dairy products commercially available in France, Germany, Canada and Japan [92–97]. As regards to ruminant meat available at retail (beef and lamb), the presence of TVA is now well-reported (**Figure 5B**) [48,93]. Of note, TVA is sometimes not the major *trans*-C18:1 isomer in commercial ruminant meat [97–100], ranging from 10% to 60% of total *trans*-C18:1 in Canadian stores [98]. Consequently, absolute concentrations vary as well, ranging from 0.5% to 3.1% of total fatty acids in Canada [98,99].

Figure 5 about here.

Because PHOs in general (*i.e.*, PHVOs and PHFOs) are characterised by a wide range of *trans*-C18:1 positional isomers, these oils do contain TVA. In the late 1990s, Wolff and colleagues reported that TVA accounted for 13.7% of total *trans*-C18:1 fatty acids in PHVO-containing foods available in France [57]. Lower amounts were reported by Nestlé, who found a mean TVA content of 5.6% of total *trans*-C18:1 in commercial PHVOs [55]. As for PHFOs, analytical chromatograms showed the presence of TVA but without providing any relative amount [75,76].

Therefore, independently of the hypothetic presence of TPA in PHVOs, the TVA present in such oils may in part explain the epidemiological correlation between circulating levels of TPA and intakes of PHVOs [13,15,22,56] through peroxisomal β-oxidation. Whatever the origin, TVA may indeed undergo peroxisomal β-oxidation and yield TPA. Therefore, both ruminant-derived foods and PHOs do contribute to indirect TPA intakes *via* TVA (**Figure 6**). Of note, the role of foods which composition is based both on ruminant fat and vegetable fat should be underlined, because they will contribute to dietary TVA intakes. Thus, it is interesting to focus on the relative contribution of ruminant-derived foods and PHVOs to TVA intakes in the general population. To the best of our knowledge, only Wolff and Precht estimated and updated until 2003 these proportions in Europe and the USA based on accurate data on *trans*-C18:1

profiles in foods [57,93,101]. For instance, in France and Germany in the late 1990s, ruminant-derived foods supplied approx. 80% of the total dietary TVA intakes, the latter being estimated at 900 mg/person/day. Conversely, the relative contribution of ruminant-derived foods in dietary TVA intakes in the USA at the same time was lower than 50%, such intakes being assessed at 1400 mg/person/day.

As said above, it is very likely that PHOs are not used anymore in Western countries, including Western European countries. Should this hypothesis be verified, ruminant-derived foods would, therefore, be the only contributors to TPA intakes in the concerned countries (**Figure 7**).

Figure 6 about here.

Figure 7 about here.

5. *Metabolism of TPA in rodents and humans: bridging the gap with other natural trans fatty acids of nutritional interest*

Despite the inability for humans to synthesise *de novo trans* fatty acids, humans are able to metabolise dietary *trans* fatty acids like any usual fatty acid. Therefore, 2 carbons can be added/removed through the elongation/β-oxidation step, or a *cis* ethylenic bond can be added in the Δ5, Δ6 or Δ9 position of the chain backbone.

The elongation step of dietary TPA is described in several cell models. In bovine pre-adipocytes and adipocytes, the addition of TPA in the culture medium led to an increase in intracellular TPA, but also in intracellular TVA [102]. This elongation step is also reported in endothelial HUVEC cells and hepatic HepG2 cells [31]. Our research group confirmed the existence of this elongation step as well in cultured rat hepatocytes: the increase in TPA content in the culture medium was followed by a similar increase in TVA content in hepatocytes (Guillocheau et al, unpublished results). The use of [1-^{13}C]-TPA in the culture medium allowed the detection of ^{13}C-labelled TVA, demonstrating that the synthesised TVA did stem from incubated TPA (Guillocheau et al, unpublished results). So far, it remains unknown whether Elongation of very-long chain fatty acids enzyme (ELOVL)-5 or ELOVL-6 is involved in this elongation step.

Conversely, dietary TPA can be chain-shortened. Using cultured rat hepatocytes, we pointed out a 14:1 fatty acid that arose when TPA was added to the culture medium. Relying on [1-^{13}C]-TPA, the 14:1 fatty acid was not labelled, consistent with a 2 carbon-removal step from TPA (Guillocheau et al, unpublished results). Like TVA being retro-converted to TPA by one cycle of peroxisomal β-oxidation, TPA is also likely to be shortened as well through the same pathway [90].

Apart from elongation and peroxisomal β-oxidation, dietary TPA can theoretically be desaturated. Given that TPA has already a double bond in the Δ9 position, its Δ9-desaturation is not possible. The few studies carried out on cell models did not report any *trans*-C16:2 fatty acids following the addition of TPA in the culture medium [31,102]. Using cultured rat hepatocytes and increasing amounts of TPA in the culture medium, our research group also failed at highlighting *trans*-C16:2 fatty acids (Guillocheau et al, unpublished results). Thus, TPA is not likely a suitable substrate for any of the desaturases present in Mammals.

To sum up, dietary TPA undergoes only elongation and peroxisomal β-oxidation reactions in rodents and humans (**Figure 8**). The elongation step is quite interesting since there is greater knowledge about the metabolism of TVA and its physiological effects. Therefore, it is the opportunity to review the reactions as well that TVA (both dietary and obtained from TPA) undergoes.

Figure 8 about here.

TVA can first be desaturated. It is now well-demonstrated that TVA can be Δ9-desaturated to yield RMA: such a step was pointed out in both rodents [103–105] and humans [106–108] and would occur with a 20%-conversion efficiency. However, to the best of our knowledge, there is no proof of neither Δ5 nor Δ6-desaturation of TVA even if the reaction could theoretically take place. Of note, however, our research group has recently demonstrated the ability for TVA to undergo a Δ13-desaturation step yielding a conjugated *trans*-11, *cis*-13 C18:2 fatty acid. We formally demonstrated the occurrence of such a step in cultured rat hepatocytes [109], bovine mammary MAC-T and BME-UV endothelial cells [110], and in the mammary gland of pregnant rats fed a high-TVA diet [111]. Apart from desaturation, TVA can be chain-shortened yielding TPA as previously described. Conversely, TVA can be converted to the *trans*-13 C20:1 fatty acid thanks to an elongation step [109].

Because RMA has a conjugated double bond system, all the fatty acids that metabolically arise from it will have such a system as well. The main characterized pathway in rodents starting from RMA consists first in a Δ6-desaturation step (*cis*-6, *cis*-9, *trans*-11 C18:3 conjugated fatty acid), second in an elongation step (*cis*-8, *cis*-11, *trans*-13 C20:3 conjugated fatty acid), and third in a probably Δ5-desaturation step (*cis*-5, *cis*-8, *cis*-11, *trans*-13 C20:4 conjugated fatty acid) [112–116]. Apart from this pathway, RMA is likely to be elongated and converted to the *cis*-11, *trans*-13 C20:2 conjugated fatty acid. Of note, several studies pointed out C16:2 and C16:3 conjugated fatty acids probably arising from peroxisomal β-oxidation of RMA and its derivatives [112–114,117,118].

6. Methodological topics related to TPA

6.1. Analysing and quantifying TPA in foods and human tissues

TPA is commercially available as a pure fatty acid methyl ester standard. Thus, relying on gas-chromatography (GC, most of the time coupled with a flame ionization detector, FID) and by comparison of the retention time of a suspected peak, one is, at first sight, able to identify and quantify TPA. For that reason, quantification of TPA is frequently reported in many studies, including epidemiological and food composition ones.

Theoretical considerations about fatty acids analysis by GC are beyond the scope of this review. Still, this review is the occasion to remind how much attention the *trans* fatty acid analysis requires. Most knowledge is about *trans*-C18:1 positional isomers, but such knowledge holds true for *trans*-C16:1 positional isomers as well. Very often, it is believed that TPA is the only *trans*-C16:1 fatty acid that can be encountered. Instead, when analysing foods [9], human plasma [88] or human milk [59], a wide range of *trans*-C16:1 positional isomers can be encountered (**Figure 4**). Resolving all *trans*-C16:1 positional isomers is therefore mandatory

for proper quantification of TPA. For that purpose, both AOCS Ce 1h-05 [119] and AOCS Ce 1j-07 [120] official methods advocate the use of long and highly polar GC columns: these methods are efficient as regards to the resolution of *trans*-C18:1 isomers, but they do work as well for *trans*-C16:1 fatty acids. More precisely, 100 m CP-Sil 88 (Agilent Technologies) [9,10], 100 m SP-2560/Rt-2560 (Supelco/Restek) [47,76], 100 m BPX-90 (SGE Analytical Science) (**Figure 4**) [48,90], 100 m ZB-88 (Phenomenex) [77] and 100 m SLB-IL 111 (Supelco) [78,121,122] GC columns are a strict minimum to resolve *trans*-C16:1 positional isomers. The 200 m CP-Select FAME (Agilent Technologies) also offers a decent resolution of *trans*-C16:1 positional isomers [88], allowing proper quantification of TPA in foods and plasma.

Furthermore, great attention should be paid to the oven temperature. It is now well demonstrated that isothermal temperature programs are more efficient than gradient temperature programs at resolving *trans*-C18:1 and *trans*-C16:1 positional isomers. More precisely, low-temperature isothermal programs should be applied [123]. Both AOCS official methods recommend a 175 °C isothermal-program [119,120]. However, this temperature is more suitable for the resolution of *trans*-C18:1 positional isomers and will not allow a decent resolution of *trans*-C16:1 positional isomers. In fact, way lower isothermal temperature-programs (*i.e.*, approx. 125 °C) were successfully used for very nice resolution of *trans*-C16:1 positional isomers [9,59,76,90,124]. Of course, the main drawbacks of using very low-temperature isothermal programs are the higher retention times of *trans*-C16:1 fatty acids, which are comprised between 55 and 110 min depending on the carrier gas (H_2 and He, respectively).

Above all, dairy fat contains *iso*-C17:0 fatty acid which overlaps with TPA, even when using highly polar GC columns. The case of *iso*-C17:0 was pointed out several times as a serious issue when quantifying the so-called "TPA" [10,59,125], not only in ruminant-derived foods but also plasma or red blood cells for epidemiological purposes. Furthermore, the widely used flame-ionization detector (FID) cannot distinguish TPA from *iso*-C17:0, contrary to the mass spectrometer (MS), so that no co-elution can be suspected. To tackle this issue and get reliable quantification of TPA, saturated/*cis*/*trans* fractionation based on silver-ion chromatography is mandatory. One can get a fraction containing only *trans*-MUFA, and perform adequate GC method for analysis. Such a step can be carried out by Ag^+-TLC [90,126], Ag^+-high-performance liquid chromatography (Ag^+-HPLC) [121] or Ag^+-solid-phase extraction (Ag^+-SPE) [43,47,76].

Given that TPA may co-elute with other *trans*-C16:1 positional isomers and/or *iso*-C17:0, overestimation of TPA content is at risk. Yet, methods to get reliable amounts of TPA are still expensive and burdensome, making them hardly feasible in studies involving many samples or volunteers. Therefore, several compositional studies overestimated the content in TPA in various matrixes. For instance, the so-called "TPA" accounted for 0.8% of total fatty acids in dairy fat according to the TRANSFAIR Study [127]. This corresponds to a 2,000% overestimation compared with the more reliable data of Destaillats and colleagues who used Ag^+-TLC combined with appropriate GC analysis [10]. As regards to epidemiological studies (**Table 1**), overestimation is hard to assess given the few available details concerning the methods used. In addition, there is no consensus about what a "normal" plasmatic concentration of TPA in humans is. To our knowledge, only Boué and colleagues applied proper analytical

methods on subcutaneous fat of French women to properly assess the content in TPA [126]. In that study, TPA accounted for 0.06% of total fatty acids; but this value cannot really be compared to epidemiological studies that were focused on plasma.

6.2. Chemical synthesis and isolation of pure TPA for nutritional studies

The exciting outcomes of epidemiological studies about dietary TPA call for *in vivo* supplementation/nutritional studies (carried out either on rodent or better humans) to unravel hypothetic physiological effects of this fatty acid. Two requests should be met to settle such nutritional studies. On the one hand, one goes beyond actual intakes of TPA and uses high levels of this fatty acid to maximizes chances to highlight probable benefits (*i.e.*, the amount criteria). On the other hand, TPA should be as pure as possible to avoid any bias at drawing conclusions (*i.e.*, the purity criteria).

There is a real challenge which explains why only one *in vivo* nutritional study focusing on TPA is published so far [27]. On the one hand, usual chemical suppliers (*e.g.*, Sigma-Aldrich) do offer a > 98%-purity TPA but only several hundreds of mg are available. Besides, food sources of TPA meet neither the purity criteria (*e.g.*, presence of other fatty acids that require to be removed) nor the amount criteria. Of note, however, the Palmitoleic Isomer Study relies on PHVOs for the clinical supplementation in TPA, suggesting an ability to remove the other *trans*-C16:1 positional isomers that are contained in PHVOs [26].

Chemical synthesis is maybe the best strategy to get high amounts of pure TPA. Despite no published method to synthesize TPA so far, one can rely on the couple of studies that investigated the physiological effects of TVA and relied on chemical synthesis. Because TVA is a *trans*-MUFA, any methodology used for chemical synthesis of TVA could be translated to the case of TPA. The typical workflow consists of a Wittig reaction for the synthesis of the corresponding *cis*-MUFA, then followed by an isomerisation and afterwards by a large-scale *cis*/*trans* fractionation [90,111,128–136]. As regards to the purity criteria, the Wittig reaction is strategic: the position of the double bond is certain, thus avoiding any other positional isomer of the *cis*-MUFA [129,135]. The subsequent isomerisation reaction is nowadays well described and yields a mix of *trans*/*cis* MUFA (approx. 80/20) [129,135]. Large scale *cis*/*trans* purification to remove the *cis*-MUFA is usually achieved by means of low-temperature solvent crystallization [128,129,133,135,136]: acetone and ether are widely used, but methanol is also a suitable solvent for that purpose. Alternatively, flash liquid-chromatography (LC) was used by our research group for successful large-scale *cis*/*trans* fractionation of TVA. In all case, purities greater than 95% were achieved for TVA [90,111].

Otherwise, and specifically to the case of TPA, one can also use food sources of *CPA* instead of relying on Wittig reaction. CPA is contained in elevated amounts in macadamia nut oil (approx. 15% of total fatty acids) or better in the dietary complement Provinal® (> 50% of total fatty acids). Importantly, these sources have very low amounts of other C16:1 positional isomers. Flash-LC can be used for purification of CPA, and the previously described steps for getting pure TPA can be subsequently applied [137].

7. Conclusion and perspectives

To our knowledge, this is the very first review specifically dedicated to TPA. We discussed here a very wide range of aspects related to TPA: suggested nutritional benefits, origin, occurrence in foods and metabolism. We also developed on methodological topics related to this fatty acid, which are quite important for future epidemiological and supplementation studies. Importantly, we reviewed articles dealing specifically with TPA, to avoid any confusion with its *cis* counterpart.

We began our review reminding the biochemical definition of TPA, that is the location and the configuration of its double bond. Both nomenclatures (Ω and Δ) were used to makes things quite clear. Based on the wide range of denominations that can be found in the literature, it is very easy for non-specialists to be confused: "palmitoleic acid", "palmitelaidic acid", "C16:1", "*trans*-C16:1", "C16:1 n7", "tC16:1 n-9"… We believe it is mandatory for future research dealing with TPA to accurately state the biochemical formula of the given fatty acid and the nomenclature used, to avoid any misunderstanding.

Because TPA is a *trans* fatty acid, methodological aspects are of great importance. This review debunked the too widespread idea according to which quantifying TPA in foods or human plasma can be easily performed. Our research group is now experienced with quantification of TPA in foods, and this review is the opportunity to say that quantifying TPA has nothing to do with the measurement of other well-known fatty acids. For instance, proper assessment of circulating levels of EPA and DHA can be easily performed by direct GC, without any burdensome steps. Instead, specific and crucial steps are mandatory for proper quantification of TPA. Thus, future research focusing on TPA should be very accurate on this point as well, detailing the methods performed and the operating conditions if GC is used. Furthermore, we reviewed the methods allowing chemical synthesis and purification of TPA, making supplementation studies on rodents or even humans feasible.

To sum up, TPA is an R-TFA which intakes are exclusively ensured by ruminant-derived foods (*i.e.*, ruminant milk and ruminant meat). Several epidemiological studies (cross-sectional and prospective) suggested positive physiological impacts of dietary TPA with respect to the risk of type 2 diabetes. Importantly, they indicated that dietary TPA could prevent insulin-resistance by targeting insulinemia. *In vitro* studies provided complementary results and showed that TPA could favourably target pancreas and modulate β-cell function. Therefore, there is a call for further research and especially TPA-supplementation studies focused on type 2 diabetes, to unravel mechanisms of action. Furthermore, one cannot exclude that dietary TPA targets other tissues (*e.g.*, liver, adipose tissue).

The metabolism of TPA is a crucial feature. Indeed, it reveals that the whole topic of TPA is strongly tied to that of TVA and RMA. Of note, TVA and RMA have been extensively explored for their health benefits. Thus, it is striking that epidemiological studies focusing on TPA did not make a hint to the metabolism of TPA. Indeed, supplementation studies on rodents dealing with TVA and RMA also pointed out the ability to prevent from insulin-resistance and type 2 diabetes: such results strongly concur with epidemiological outcomes. Therefore, epidemiological studies are totally complementary to supplementation studies carried out on rodents, giving for the first time evidence of benefits of R-TFAs in humans. Beyond, one can wonder if the shared *trans* n-7 double bond confers special properties to these fatty acids. It is worth underlining that TVA and RMA have been proposed as powerful ligands of PPAR-α and PPAR-γ to explain their benefits in rodents [131,138]. Can dietary TPA act the same way?

Awaiting for well-designed studies, we can reasonably assume that TPA, TVA and RMA are ligands of PPAR-α and PPAR-γ and can act independently of each other.

Finally, the very first question on TPA was about the closeness with CPA, with respect to physiological impacts [8]. Can a *cis*-MUFA and a *trans*-MUFA exert the same physiological effects? Once again, the relationship between fatty acid structure (double bond configuration) and function is crucial. To our knowledge, similar functions between a *cis*-MUFA and a *trans*-MUFA have never been proven, making such assumption very intriguing. Thus, supplementation studies on rodents or humans are needed.

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Conflicts of interest

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Author contributions

All authors have equally contributed to the review.

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TABLE 1

Epidemiological associations between circulating *trans*-palmitoleic acid in humans and risk of type 2 diabetes, risk of cardiovascular diseases, risk of stroke, anthropometric parameters and metabolic disorders.

Reference	Type of study	Country	Year at blood sampling	Participants *n*	Circulating TPA			Outcomes
					Human tissue analysed	Assessment of TPA[1]	TPA content[2]	
[139]	Cross-sectional	Costa Rica	1994-1998	1785	Subcutaneous adipose tissue	GC-FID SP-2560 100 m × 0.25 mm × 0.20 µm 90-240 °C, gradient, H_2	Not reported	↑ TPA ⇒ ↑ BMI ↑ TPA ⇒ ↓ Skinfold thickness
[24]	Cross-sectional	Canada	> 2009	233	Plasma (PL)	GC-FID CP-Select 100 m × 0.25 mm × 0.20 µm 185-245 °C, gradient	0.16%[3]	↑ TPA ⇒ ↓ Systolic blood pressure ↑ TPA ⇒ ↓ BMI
[20]	Cross-sectional	USA	Not reported	106	Serum (PL)	GC-FID SP-2560 100 m × 0.25 mm × 0.20 µm 160-240 °C, gradient	Not reported	↑ TPA ⇒ NAFLD score
[140]	Cross-sectional	Spain	> 2014	282	RBC (PL)	GC-FID Suprawax-280 30 m	0.23%[3]	↑ TPA ⇒ ↓ IFN-γ ↑ TPA ⇒ ↓ IL-6 ↑ TPA ⇒ ↓ IL-8 ↑ TPA ⇒ ↓ IL-10 ↑ TPA ⇒ ↓ IL-1β
[91]	Cross-sectional	Netherlands	2006-2013	864	Plasma (PL)	GC-FID CP-Select 200 m × 0.25 mm × 0.20 µm 190-240 °C, gradient	0.020%[4]	↑ TPA ⇒ ✕ Waist circumference ↑ TPA ⇒ ✕ BMI ↑ TPA ⇒ ↓ Fasting plasma TAG ↑ TPA ⇒ ✕ Fasting glycemia ↑ TPA ⇒ ↓ CRP ↑ TPA ⇒ ↓ Total-cholesterol ↑ TPA ⇒ ↓ HDL-cholesterol ↑ TPA ⇒ ✕ LDL-cholesterol ↑ TPA ⇒ ✕ Diastolic blood pressure ↑ TPA ⇒ ✕ Systolic blood pressure
[21]	Case-control (healthy/NAFLD)	USA	Not reported	32	Plasma (PL)	GC-FID SP-2560 100 m × 0.25 mm × 0.20 µm 160-240 °C, gradient	Healthy PL: 0.20%[3] NAFLD PL: 0.15%[3]	↑ TPA ⇒ ↓ Fasting glycemia ↑ TPA ⇒ ↓ Hepatic lipids ↑ TPA ⇒ ↓ Hepatic insulin-resistance ↑ TPA ⇒ ↓ Systemic insulin-resistance ↑ TPA ⇒ ✕ Pancreatic β-cell function

TABLE 1

Continued.

Reference	Type of study	Country	Year at blood sampling	Participants *n*	Circulating TPA			Outcomes
					Human tissue analysed	Assessment of TPA[1]	TPA content[2]	
[19]	Case-control (healthy/obese)	Canada	2004-2007	200	Plasma (PL)	GC-FID HP-88 100 m × 0.25 mm × 0.20 µm He	Not reported	↑ TPA ⇒ ↓ BMI ↑ TPA ⇒ ↓ Insulinemia
[11,25]	Prospective EPIC-Potsdam Study	Germany	1994-1998	2724	RBC (PL)	GC-FID CP-Select 100 m × 0.25 mm × 0.20 µm 185-245 °C, gradient, N_2	Q1: 0.12%[3] Q4: 0.27%[3]	✗ *RR Type 2 diabetes*
[8]	Prospective CHS Study	USA	1989-1993	3736	Plasma (PL)	GC-FID SP-2560 100 m × 0.25 mm × 0.20 µm 160-240 °C, gradient	Q1: 0.13%[3] Q4: 0.25%[3]	↓ *HR Type 2 diabetes* ✗ BMI ↓ Waist girth ↓ Fasting plasma TAG ✗ Fasting glycemia ↓ Fasting insulinemia ↓ HOMA-IR ↓ CRP ✗ LDL-cholesterol ↑ HDL-cholesterol ↓ Total cholesterol / HDL-cholesterol ✗ Diastolic blood pressure ✗ Systolic blood pressure
[12]	Prospective EPIC-Norfolk Study	UK	1993-1997	383	Plasma (PL) RBC (PL)	GC-FID	Plasma 0.06%[3] RBC 0.18%[3]	✗ *OR Type 2 diabetes*

TABLE 1

Continued.

Reference	Type of study	Country	Year at blood sampling	Participants *n*	Circulating TPA			Outcomes
					Human tissue analysed	Assessment of TPA[1]	TPA content[2]	
[15,22]	Prospective MESA Study	USA	2000-2002	2617 [15] 2837 [22]	Plasma (PL)	GC-FID CP-Select 100 m × 0.25 mm × 0.20 µm 190-240 °C, gradient	Q1: 0.03%[3] Q5: 0.10%[3]	↓ *HR Type 2 diabetes* ✗ *HR Cardiovascular disease* ↓ Fasting plasma TAG ✗ Fasting glycemia ↓ Fasting insulinemia ↓ HOMA-IR ✗ CRP ↑ LDL-cholesterol ✗ HDL-cholesterol ✗ Total cholesterol / HDL-cholesterol ↓ Diastolic blood pressure ↓ Systolic blood pressure
[13,141]	Prospective IRAS Study	USA	1992-1994	659	Serum (total lipids)	GC-FID and GC-MS HP-88 30 m × 0.25 mm × 0.20 µm	0.30%[3]	↑ TPA ⇒ ✗ Insulin sensitivity ↑ TPA ⇒ ✗ Pancreatic β-cell function
[14,23]	Prospective HPFS Study NHS Study	USA	HPFS Study 1993-1994 NHS Study 1989-1990	3333 [14] 1188 [23]	Plasma (total lipids)	GC-FID SP-2560 100 m × 0.25 mm × 0.20 µm 90-250 °C, gradient, H_2	HPFS Study Q1: 0.10%[3] Q4: 0.22%[3] NHS Study Q1: 0.13%[3] Q4: 0.28%[3]	Pooled cohorts ↓ *HR Type 2 diabetes* ↓ *HR Stroke*

[1]Details about the method for assessment of TPA content are given as follows (if reported in the corresponding study): type of chromatography-type of detector/type of GC column/length of the GC column × inner diameter of the GC column × film thickness of the GC column/starting-ending temperature of the oven, type of temperature program (gradient or isothermal), carrier gas.

[2]Mean TPA content (if reported in the corresponding study) of either the total subject sample or of the quartiles/quintiles of the corresponding study.

[3]Mean TPA content expressed as % of total fatty acids.

[4]Mean TPA content expressed as % mol of total fatty acids.

Abbreviations. BMI, body mass index; CHS, Cardiovascular Health Study; CRP, C-reactive protein; EPIC, European Prospective Investigation into Cancer and Nutrition; GC-FID, gas chromatography coupled with flame-ionization detector; GC-MS, gas-chromatography coupled with mass spectrometry; HOMA-IR, Homeostasis Model Assessment of Insulin Resistance; HPFS, Health Professionals Follow-up Study; HR, hazard ratio; IFN-γ, Interferon-γ; IL, Interleukin; IRAS, Insulin Resistance Atherosclerosis Study; MESA, Multi-Ethnic Study of Atherosclerosis; NAFLD, non-alcoholic fatty liver disease; NHS, Nurses' Health Study; OR, odds ratio; PL, phospholipids; Q, quartile/quintile (depending on studies); RBC, red blood cells; RR, relative risk; TAG, triacylglycerol.

Symbols. ×, non-significant impact; ↑ significant increase; ↓ significant decrease.

TABLE 2

Epidemiological associations between dietary intakes and circulating levels of *trans*-palmitoleic acid in humans.

Reference	Type of study	Country	Year at blood sampling	Participants *n*	Dietary TPA		Circulating TPA			Conclusions Dietary sources of TPA
					Assessment of dietary intake	Food composition data	Human tissue analysed	Assessment of TPA[1]	TPA content[2]	
[56]	Cross-sectional	Costa-Rica	1994-1998	503	FFQ	USDA database	Subcutaneous adipose tissue	GC-FID SP-2560 100 m × 0.25 mm × 0.20 µm 90-240 °C, gradient, H_2	0.08%[3]	Vegetable fat
[33]	Cross-sectional	Australia	Not reported	86	4-day record	Australian Food Composition tables	Plasma (PL)	GC-MS BPX-70	Not reported	Dairy products
[24]	Cross-sectional	Canada	> 2009	233	FFQ	NDSR Software	Plasma (PL)	GC-FID CP-Select 100 m × 0.25 mm × 0.20 µm 185-245 °C, gradient	0.16%[3]	Dairy products
[34,91]	Cross-sectional	Netherlands	2006-2013	769 [34] 864 [91]	FFQ	Dutch food database	Plasma (PL)	GC-FID CP-Select 200 m × 0.25 mm × 0.20 µm 190-240 °C, gradient	0.020%[4]	Dairy products
[35]	Prospective NHS Study	USA	1989-1990	313	FFQ	USDA database	Plasma (total lipids) RBC (total lipids)	GC-FID SP-2560 100 m × 0.25 mm × 0.20 µm 90-250 °C, gradient, H_2	Plasma 0.15%[3] RBC 0.14%[3]	Dairy products
[8,36]	Prospective CHS Study	USA	1989-1993	3330	FFQ	Harvard University nutrition database	Plasma (PL)	GC-FID SP-2560 100 m × 0.25 mm × 0.20 µm 160-240 °C, gradient	Not reported	Dairy products Red meat
[15,22]	Prospective MESA Study	USA	2000-2002	2617 [15] 2837 [22]	FFQ	Not reported	Plasma (PL)	GC-FID CP-Select 100 m × 0.25 mm × 0.20 µm 190-240 °C, gradient	0.05%[3]	Dairy products Red meat PHVOs
[13,141]	Prospective IRAS Study	USA	1992-1994	659	FFQ	HHHQ-DIETSYS Software	Serum (total lipids)	GC-FID and GC-MS HP-88 30 m × 0.25 mm × 0.20 µm	0.30%[3]	PHVOs

TABLE 2

Continued.

Reference	Type of study	Country	Year at blood sampling	Participants *n*	Dietary TPA		Circulating TPA			Conclusions Dietary sources of TPA
					Assessment of dietary intake	Food composition data	Human tissue analysed	Assessment of TPA[1]	TPA content[2]	
[14,23]	Prospective HPFS Study NHS Study	USA	HPFS 1993-1994 NHS 1989-1990	3333 [14] 1188 [23]	FFQ	USDA database	Plasma (total lipids)	GC-FID SP-2560 100 m × 0.25 mm × 0.20 µm 90-250 °C, gradient, H_2	HPFS Study Q1: 0.10%[3] Q4: 0.22%[3] NHS Study Q1: 0.13%[3] Q4: 0.28%[3]	Dairy products Meat
[142]	Randomized control trial 3 groups of dairy intake	Canada	2014 3 blood samplings per group	94	24h-recall	Canadian Nutrient File 2010b	Plasma (RBC)	GC-FID HP-88 60 m × 0.25 mm 60-220 °C, gradient, H_2	At the end of the trial G.1: 0.06%[3] G.2: 0.09%[3] G.3: 0.08%[3]	Dairy products

[1]Details about the method for assessment of TPA content are given as follows (if reported in the corresponding study): type of chromatography-type of detector/type of GC column/length of the GC column × inner diameter of the GC column × film thickness of the GC column/starting-ending temperature of the oven, type of temperature program (gradient or isothermal), carrier gas.

[2]Mean TPA content (if reported in the corresponding study) of either the total subject sample or of the quartiles of the corresponding study.

[3]Mean TPA content expressed as % of total fatty acids.

[4]Mean TPA content expressed as % mol of total fatty acids.

Abbreviations. CHS, Cardiovascular Health Study; GC-FID, gas chromatography coupled with flame-ionization detector; GC-MS, gas-chromatography coupled with mass spectrometry; HPFS, Health Professionals Follow-up Study; IRAS, Insulin Resistance Atherosclerosis Study; MESA, Multi-Ethnic Study of Atherosclerosis; NHS, Nurses' Health Study; PL, phospholipids; Q, quartile; RBC, red blood cells; USDA, United States Department of Agriculture.

FIGURE LEGENDS

FIGURE 1

Structure of *trans*-palmitoleic acid: the position of the *trans* double bond is indicated using either (A) the Δ-nomenclature or (B) the Ω-nomenclature.

FIGURE 2

Origin of *trans*-palmitoleic acid in ruminant-derived foods: from ruminal biohydrogenation to uptake and metabolism in key ruminant tissues. Hypotheses are illustrated by dotted arrows.
Abbreviations. ALA, α-linolenic acid; LA, linoleic acid; RMA, rumenic acid; TPA, *trans*-palmitoleic acid; TVA, *trans*-vaccenic acid.
Symbols. β-ox, peroxisomal β-oxidation; Δ, desaturation ε, elongation.

FIGURE 3

Generation of *trans*-palmitoleic acid from ruminal biohydrogenation of dietary C16:3 n-3, similarly to ruminal biohydrogenation of dietary α-linolenic acid. Red-labelled double bonds undergo subsequent isomerization or saturation reaction.
Abbreviations. ALA, α-linolenic acid; TPA, *trans*-palmitoleic acid; TVA, *trans*-vaccenic acid.

FIGURE 4

Typical chromatogram of *trans*-C16:1 isomers in ruminant-derived foods available at retail in France in 2018, to accurately quantify *trans*-palmitoleic acid. A: ruminant milk. B: ruminant meat. Fatty acids were analysed by Ag^+-TLC followed by GC-MS analysis carried out with the BPX-90 column. Data from [48].
Abbreviations. TPA, *trans*-palmitoleic acid.

FIGURE 5

Typical chromatogram of *trans*-C18:1 isomers in ruminant-derived foods available at retail in France in 2018, to accurately quantify *trans*-vaccenic acid. A: ruminant milk. B: ruminant meat. Fatty acids were analysed by Ag^+-TLC followed by GC-MS analysis carried out with the BPX-90 column. Data from [48].
Abbreviations. TVA, *trans*-vaccenic acid.

FIGURE 6

Dietary sources of *trans*-palmitoleic acid at the time of the use of partially hydrogenated oils.
Abbreviations. TPA, *trans*-palmitoleic acid; TVA, *trans*-vaccenic acid.

FIGURE 7

Dietary sources of *trans*-palmitoleic acid after regulation measures on partially hydrogenated oils.
Abbreviations. PHFO, partially hydrogenated fish oil; PHO, partially hydrogenated oil; PHVO, partially hydrogenated vegetable oil; TPA, *trans*-palmitoleic acid; TVA, *trans*-vaccenic acid.

FIGURE 8

1226 Metabolism of dietary *trans*-palmitoleic acid in humans and rodents.
1227 *Abbreviations.* TPA, *trans*-palmitoleic acid; TVA, *trans*-vaccenic acid.
1228 *Symbols.* β-ox, peroxisomal β-oxidation; Δ, desaturation ε, elongation.

FIGURE 1

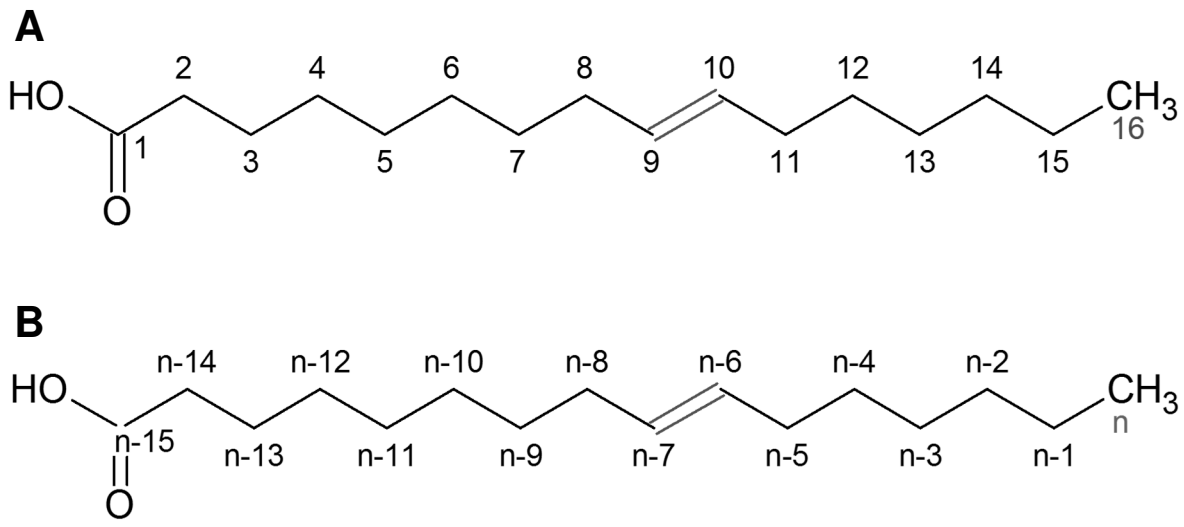

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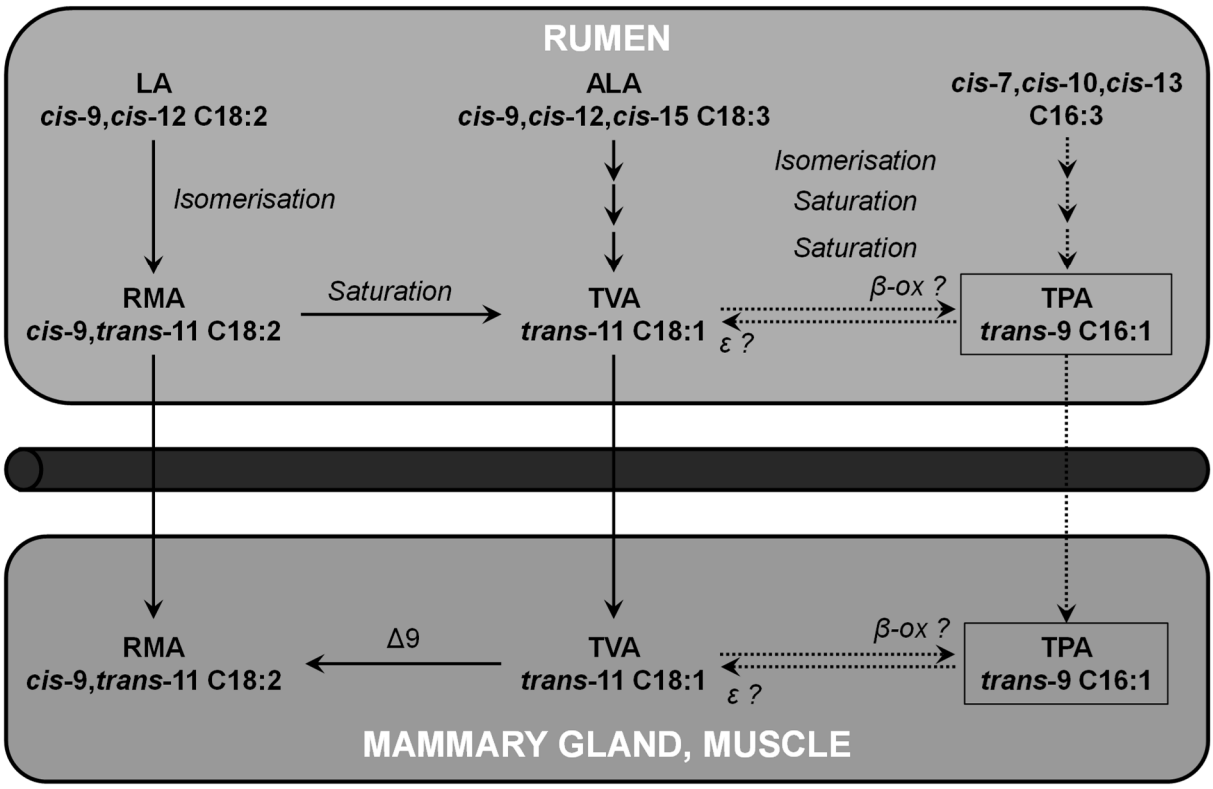
FIGURE 2



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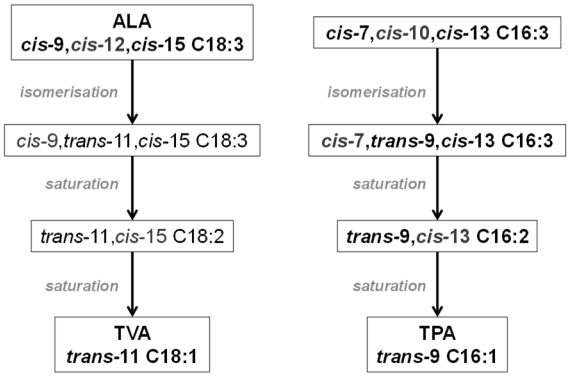

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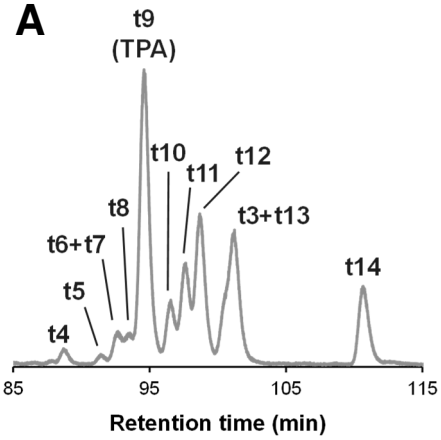

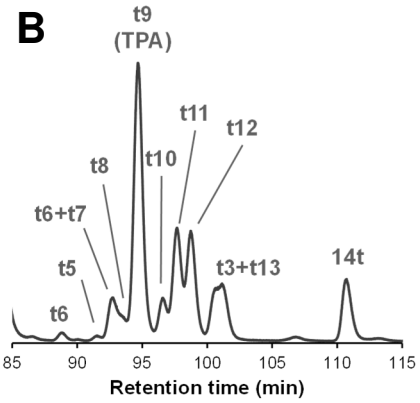

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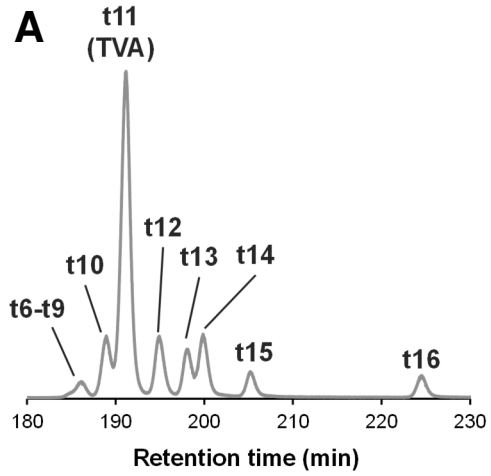
FIGURE 5



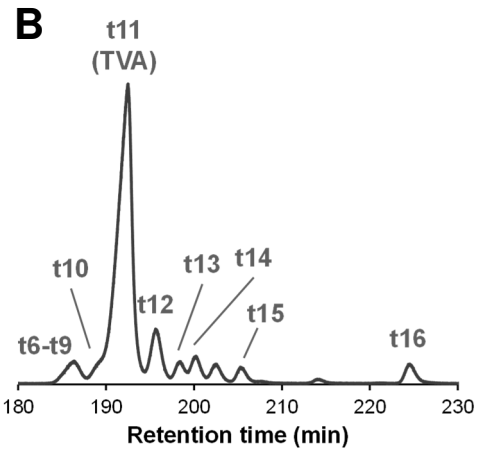


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FIGURE 6

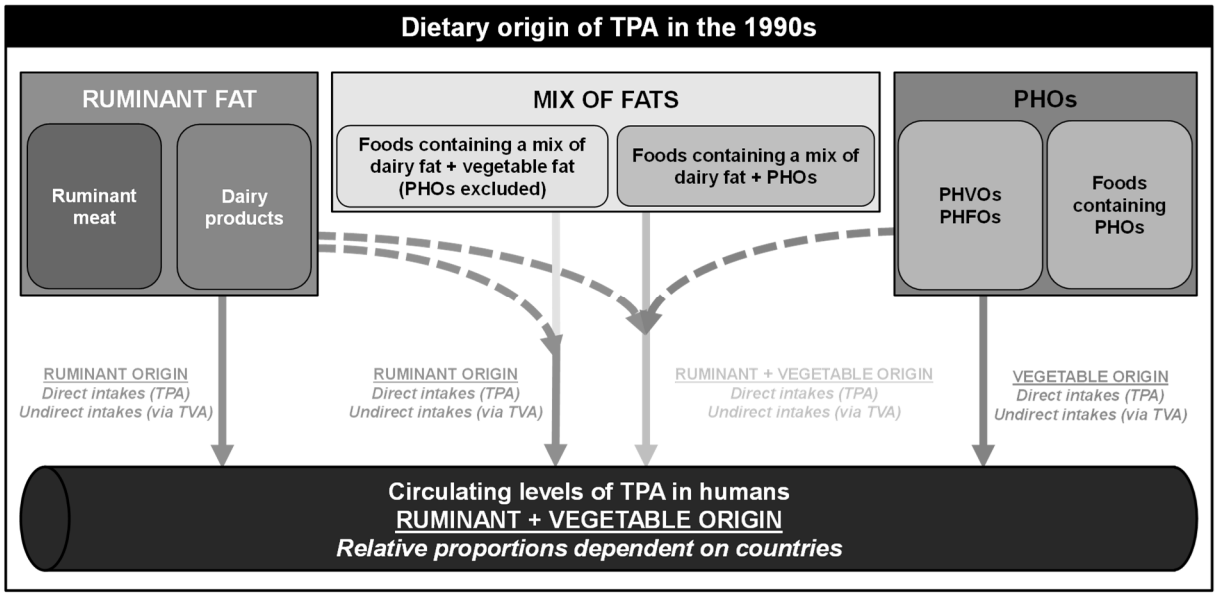

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FIGURE 7

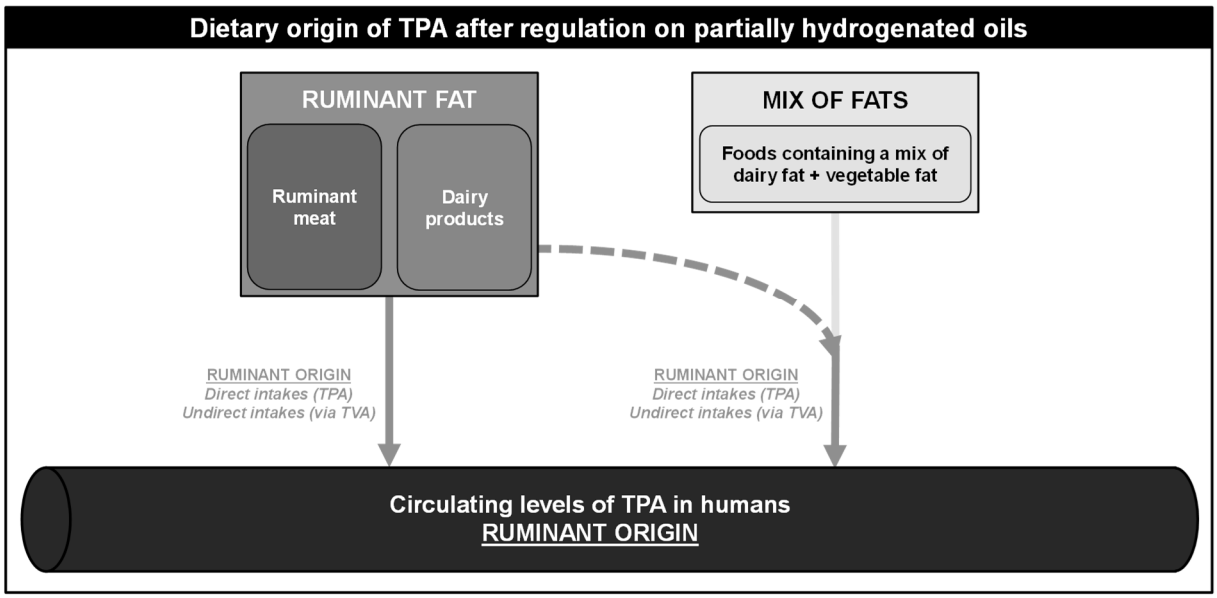

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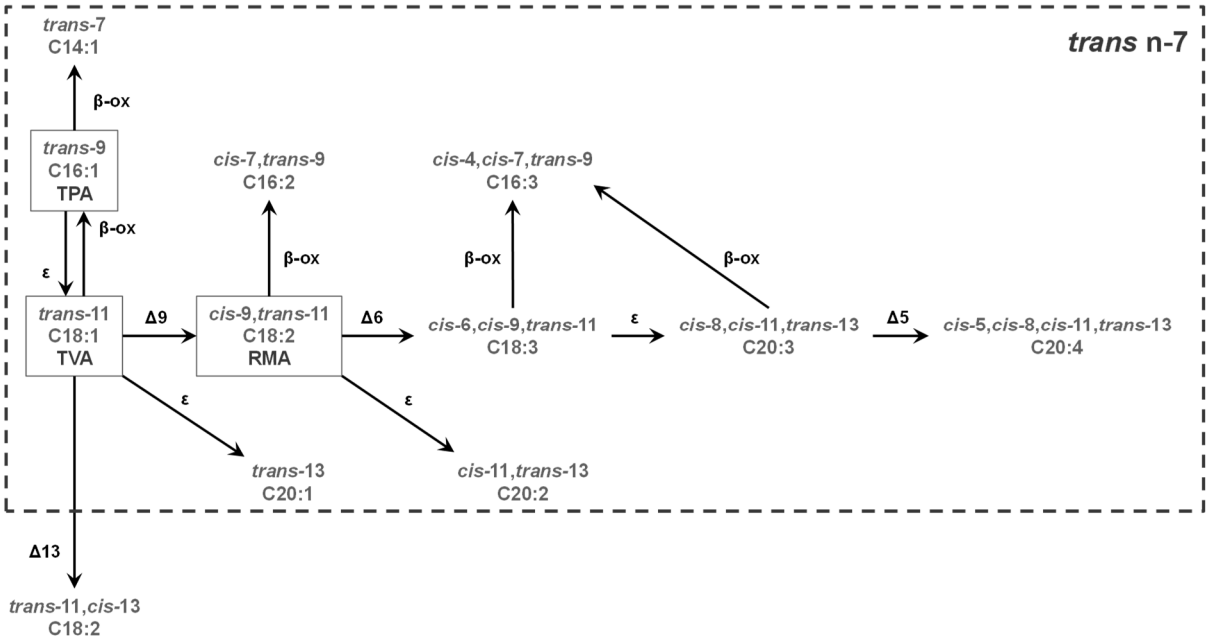
FIGURE 8



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GRAPHICAL ABSTRACT