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Review

Crosstalk among dietary polyunsaturated fatty acids, urolithiasis, chronic inflammation, and urinary tract tumor risk

Aldo R. Eynard M.D., M.Sc., Ph.D. [a,*], Alicia Navarro M.Sc., Ph.D. [b]

[a] Instituto de Biología Celular, INICSA, Córdoba, Argentina
[b] Escuela de Nutrición, FCM-Universidad Nacional de Córdoba, Córdoba, Argentina

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ABSTRACT

Based on a consistent bulk of experimental and epidemiologic works, we proposed that abnormal metabolism and/or dietary deprivation of essential polyunsaturated fatty acids by inducing a chronic and subclinical essential fatty acid deficiency (EFAD) in urothelial cell membranes may enhance the risk for urinary tract tumor (UTT) development. This threat may be enhanced by the unusual fact that the fatty-acid profile of the normal urothelium is similar to that reported in EFAD. The risk for UTT may be worsened when coexisting with a low-grade chronic inflammation (LGCI) state induced by urolithiasis or disbalance management of peroxides, free radical molecules, and their quenchers. There is cumulative evidence linking the LGCI of the urinary tract mucosa, calculi, and UTT, due to the long-standing release of promitotic, promutagen, and pro-inflammatory antiapoptotic cytokines in these conditions. The dual role played by pro- and anti-inflammatory eicosanoids and bioactive lipids, cytokines, and the disbalance of lipid peroxidation is discussed, concluding that the moderate, long-standing consumption or dietary supplementation of ω-3 PUFAs may improve the chances of avoiding UTT development.

Introduction

Urinary tract tumor (UTT) in humans continues to be a major concern as death rates due to bladder neoplasms have not diminished appreciably over recent decades (revised in Andreatta [1,2]). It is possible that the tumorigenic effect of urothelial mutagens, whether ingested in the diet and/or flushed by urine, may be avoided by the normal integrity of the "passive barrier" to water-soluble molecules located at the luminal surface of the mammalian urothelium in normal conditions [3,4]. UTT are the 10th cause of cancer worldwide, with annual age standardized incidence rates being 100 per 100,000 for men and 36 per 100,000 for women, respectively, according to worldwide data from 2002 [1,5,6]. Even if this mortality is not remarkably high, the morbidity and recurrence of these tumors provides a serious challenge for oncologic treatment and follow-up. Our earlier studies on geo-location showed that UTT are the fourth in incidence among men in Argentina (South America), with intriguingly different patterns occurring in several countries of this region [6].

The causes of UTT are still scarcely understood. Because genetic background seems to play a minor role in their proneness, environmental factors become the main factors of suspicion [1,2]. The major environmental causes of UTT appear to be tobacco smoking [7], occupational risks occurring in the dye industries [8], chronic inflammation of the bladder by certain microorganisms [9–11], alcoholism [1,2,5,12,13], chronic arsenicism from drinking water in South America (mainly Argentina, Chile) [14–16] and China [17], accidental intoxication with melamine (as happened recently in China, as this compound has procarcinogenic capabilities in rodent urinary mucosa [18–20]), and possibly several artificial sweetners [5]. These risk conditions are probably worsened by the urinary tract infections and urolithiasis that often coexist with some of these conditions [9,21–23].

Although research into the possible associations between UTT and dietary factors has been limited, several food-protein derivatives exhibiting mutagenicity capabilities have been identified in the urine of humans and animals. These include nitrosamines as well as tryptophan metabolites, but evidence of their tumorigenic effects on the urinary tract (UT) mucosa is still vague [24]. Moreover, the role played by nutritional fats, mainly polyunsaturated fatty acids (PUFAs) in UTT etiology, is comparatively even less understood [25–27].

* Corresponding author. Tel.: +54 351-4334023; fax: +54 351-4334021.
E-mail address: aeynard@gmail.com (A. R. Eynard).

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The main experimental, epidemiologic, and clinical aspects of the relationship between UTT and PUFAs were covered in our previous articles [1,2,4,5,18,19,28,29], in which we discussed considerable data indicating that dietary PUFAs play a crucial role in UTT. Hence, we proposed that the UT in mammals, including humans, might have a proneness to develop tumors if a deficiency or perturbations of the PUFA metabolism is present. In rodents, chronic essential fatty acid deficiency (EFAD) induces both urolithiasis, transitional hyperplasias and displasias, followed by the development of UTT [30,31], with Zhang et al. reporting similar results [31]. A high intake of saturated fats or non-essential fatty acids (EFAs), conditions that may induce a subtle chronic EFAD, increased the risk for bladder cancer in case–control studies. In other cell populations, EFAs, mainly those of the ω-3 family, are beneficial as preventive and therapeutic nutrients for avoidance and treatment of cancer. Therefore, we suggested that an abnormal metabolism and/or nutritional deprivation of EFA, by inducing a chronic deficiency or a subclinical EFAD together with chronic inflammation (urolithiasis), might enhance the risk for UTT [4,28,29]. Further studies provide support to this proposal [32–36]. The aim of this article was to give a fresh, updated review based on our basic and epidemiologic investigations and from other authors about the relationship among dietary fatty acids (mainly PUFAs), urolithiasis, chronic inflammation, and risk for UTT (Fig. 1).

Brief reminder of the metabolism and physiology of essential PUFAs

Dietary fatty acids (FAs) are oxidized to provide energy, stored in adipose tissue, and selectively incorporated into the phospholipids (PLs) of all cellular membranes. Once ingested in food, FAs are desaturated and elongated to yield several PUFAs, which are long carbon-chain molecules having two or more double bonds of the *cis* configuration. ω-3 and ω-6 PUFAs cannot be synthesized by metazoan, but must be ingested through the diet and hence are EFAs. The PUFA ω-6 family derive from linolenic acid (LA; 18:2 ω-6) and those belonging to ω-3 arise from α-linolenic acid (ALA; 18:3 ω-3). In contrast, monounsaturated palmitoleic acid (POA; 16:1 ω-7) and oleic acid (OA, 18:1 ω-9) are synthesized by the body. Although all EFAs are PUFAs, not all PUFAs are EFAs (revised by Das [37,38]). However, in this work PUFAs and EFAs are used synonymously. Non-EFAs refer to monounsaturated POAs and OAs and their non-EFA long-chain PUFA derivatives. Nevertheless, often saturated fat, *trans*-FA, and cholesterol are included under this name.

ALA and LA, and eventually non-EFA from the ω-7 and ω-9 families, compete for a common delta-5 and delta-6 desaturase. In this "race," 18:3 ω-3 ALA is desaturated preferentially, followed by 18:2 ω-6 LA, thus avoiding the conversion of OA to the more highly unsaturated ω-9 metabolites, of which one of them is considered a reliable "marker" of EFAD, namely 20:3 ω-9 (Mead's acid) [39]. Thus, under the normal dietary habits prevailing in Western countries [24,40,41], tissue lipids will contain considerable amounts of OA, a modest quantity of POA but not their long-chain non-EFA/PUFA derivatives. Although a dietary lack of EFA is rarely seen in developed countries, this abnormality may be subtly induced by the long-standing ingestion of foods enriched in non-EFAs (OA, *trans*-FA, hydrogenated and/or saturated fats, and cholesterol-rich foods). Indeed, one useful experimental approach to induce a fast EFAD is the supplementation of dietary formula with OA, cholesterol, or saturated fats [18,19,42]. When the dietary amounts of OA and/or other non-EFAs are abnormally high, the activity of delta-6 desaturase is progressively stimulated. Thus, OA ω-9 becomes preferred for further elongation and desaturation. This process is considered an ineffective attempt to replace with the long-chain highly unsaturated metabolites (mainly 20:3 ω-9), the missing EFAs both in the "structural" membrane PLs and as substrates for eicosanoids and other bioactive lipid metabolites (BALs). Despite 20:3 ω-9 not being an adequate precursor for eicosanoids and other BALs, this PUFA still may be converted to abnormal, prostanoids, several eicosanoids, hydroperoxy FAs, and active leukotrienes (LTs), with this being perhaps one of the causes for a disbalanced functioning of the eicosanoids in EFAD [25,28,43,44].

PUFAs are essential molecules for PLs, which are major components of all cell membranes including urothelium. Hence, PUFAs per se will give to membranes particular properties such as fluidity/viscosity, and in turn modulate the dynamics and biophysical properties of biomembranes [45,46], ligand-receptor interactions, and also many activities of membrane-bound enzymes, ions channels, glycoproteins, and proteoglycan receptors of immune cells [46–48]. Long-chain highly unsaturated PUFAs (as arachidonic acid [AA], docosahexaenoic acid [DHA], eicosapentaenoic acid [EPA]) are highly flexible molecules compared with those more rigid areas of the bilayer caused by large amounts of monoenes and saturated FAs. The relative enrichment of cholesterol promotes lateral segregation of protein and the gathering of certain lipids in the bilayer, thereby forming more permanent clustered microdomains known as "rafts."These can prevent the movement of big protein complexes in the membrane, as happens with a peculiar variety of rafts, the uroplaquins of the mammal urothelium, whose structure is heavily modified by dietary PUFAs [49–53].

When PUFAs are released from PLs by the activities of several phospholipases [54,55], they are further processed through the activities of two main enzymatic pathways: the cycloxygenases (COX) and lipooxygenases, which lead to wide varieties of prostaglandins, eicosanoids, endocannabinoids, lipoxins, nitrolipids, neuroprotectins, maresins, resolvins, hydroxyeicosatetraenoic acids (HETEs), nitrolipids, and hepoxilins, among other BALs [37,38,56,57]. However, most of these BALs are very short-lived molecules, a fact that warrants accurate tissue homeostatic balance. BALs are produced locally when needed and are then almost instantaneously destroyed. Due to their intense activity at very low concentrations (even at values of nM or μM), it is easy to understand why there are no a pools of eicosanoids or BALs already formed in the body. Moreover, most eicosanoids arising from the same substrate (i.e., AA) exhibit an agonistic/antagonistic behavior, with their equilibrated balance being a key function as homeostatic cell controllers [37,38,58,59].

As mentioned previously, the relative availability of the ω-6 PUFA substrates in foods [24,40,41] tilts the synthesis to ω-6 BALs derivatives. However, if the ω-3 are progressively eaten, the BALs belonging to this family will be increased. The well-known beneficial activities of ω-3 PUFAs and its derivatives with regard to ω-6, but mainly in contrast to non-EFAs ω-9-BALs, have been consistently shown [58,60,61]. Simplistically speaking, taken as a whole, the PUFA derivatives from ω-3 exhibit anti-inflammatory and antineoplastic properties [62], with these comparatively beneficial properties having been demonstrated in several chronic diseases that have in common a long-standing "bed" of low-grade chronic inflammatory processes (LGIC), such as metabolic syndrome, obesity, type 2 diabetes, dislipidemias, stroke, coronary heart disease, lithiasis, endothelial dysfunction, atherosclerosis, and hypertension [38,61,63]. These complex and interlinked diseases have led UN Das to propose that endogenous anti-inflammatory PUFA derivatives (mainly from the ω-3

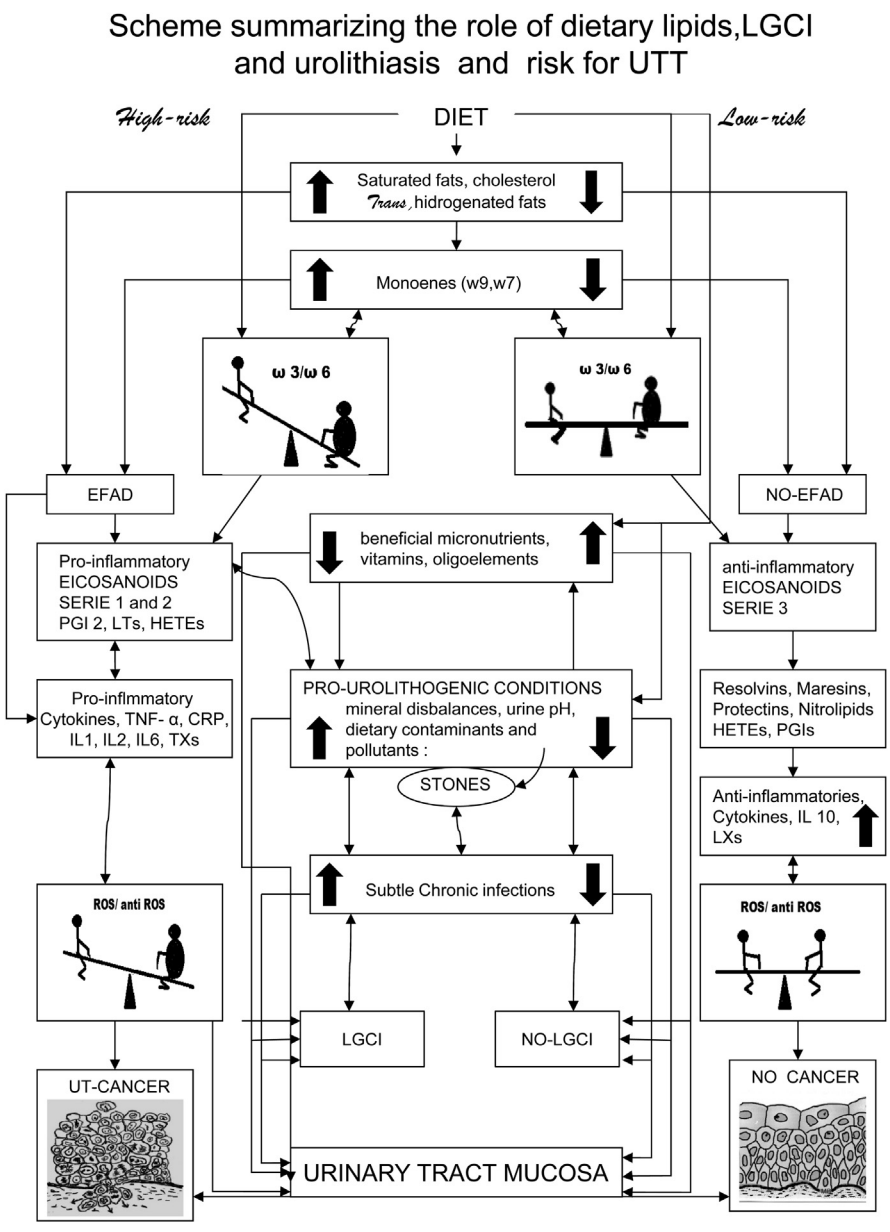

Fig. 1. The relationship among dietary PUFA, urolithiasis, and low-grade chronic inflammation (LGCI) and urinary tract tumor (UTT) risk. CRP, C-reactive protein; EFAD, essential fatty acid deficiency; HETEs, hydroxyeicosatetraenoic acids; ILs, interleukins; LTs, leukotrienes; LXs, lipoxins; PCIs, prostacyclins; ROS, reactive oxygen substances; TNF-α, tumor necrosis factor-alpha; TXs, thromboxanes.

family) have the capability to regulate inflammatory processes and to prevent on even suppress the LGIC conditions that are strongly linked to the illness conditions listed previously [37,38].

In many cancer cells, the metabolism of the PUFAs is clearly abnormal, insofar as there is a partial or complete loss of the enzyme delta-6 desaturase, which catalyzes one of the initial desaturation steps in the pathways involved in the synthesis of longer-chain PUFAs, whose sufficiency becomes progressively diminished. Additionally, cancer cells exhibit low levels of peroxidation and increased antiperoxidation strategies [25, 37,38,60, 64,65]. Therefore, considering that most experimental and epidemiologic studies show antineoplastic and anti-inflammatory capabilities for long-chain highly unsaturated ω-3 fatty acids [66,67], their beneficial role in preventing UTT and their function in the biology of UT deserve particular interest.

Biology of normal and neoplastic urothelium

The cell surface of the urothelium covering mammals' urinary passages consists of a particular thick (11 nm), angular asymmetric unit membrane (AUM), arranged in concave plaque zones alternating with hinged segments of thinner symmetric membranes [49,68,69]. The plaque zones of AUM are made up of a variety of grouped rafts of a two-dimensional crystalline conformation of polygonal areas of 16-nm glycoproteic particles, which are responsible for the asymmetric appearance of AUM [70, 71]. There are four major glycoproteic uroplakins (UP): Ia, Ib, II, and III [71], with the UPs being ordered in pairs of UPIa/II and UPIb/III [72]. It is generally believed that the urothelial AUM enlarges the luminal surface, thus preventing urothelial damage during fast bladder distention [73,74]. However, this does not

explain why the luminal surfaces of pelvices and ureters, which have identical AUM, do not appreciably distend by the constant slow dropping of urine through the lumen. Hence, a modified hypothesis about the role of AUM is necessary. Along with *zonulae occludens* and other cell–cell union complexes, the AUM builds the main morphologic component of the "permeability barrier" of the urothelium [49,50,70]. It is thought that the mammal transitional epithelium has a low, passive permeability to water and to small charged ions, compared with the high permeability of other mucous membranes. In this way, the leakage of putative mutagens and other toxic molecules from the urine to the body is halted [68,73]. However, the molecular basis responsible for the impermeability of the urothelium remains poorly understood.

Lipids are important for any barrier to permeability in outer luminal or surfaces, as has been demonstrated for essential PUFAs in the epidermis, capillary endothelium, enterocytes, and also on the surface of lung alveolar cells [29,75,76]. Pioneering ultrastructural research has shown that urothelium impermeability cannot be ascribed only to the highly ordered array of UPs [77–79], and the role played by lipids in healthy and diseased conditions of the UT should be carefully investigated. Perturbations, induced in the AUM by detergents that disrupts the lipid–lipid and lipid–protein hydrophobic interactions, induce a sudden flux of water and ions across the bladder wall, whereas the ultrastructure of the concave rafts remains almost unaltered [70,77]. We propose that there may be an increased risk for permeation of noxious substances, pollutants, and mutagens at several levels of altered surface membranes during subtle EFAD, such as in malphighian epithelia, small bowel epithelia, blood capillaries, and the UT itself [4,28,29], perhaps linked to perturbations of catenins, desmogleins, and e-cadherins at cell–cell junctions [80–83]. The "umbrella" cells located at the luminal surface of the urothelium have a fast turnover of AUM, with the new membrane being continuously synthesized in the Golgi complex and in some way being commuted up and down into the luminal surface from the discoidal vesicles present in the cytoplasm [68,73]. Thus, it can be assumed that umbrella cells have a constant high requirement for AUM, with the essential PUFAs being the rate limiting 'building stones" for the assembling of new, highly differentiated luminal plasma membranes.

AUM is unusually rich in diacylglicerol PLs. Ketterer et al. [78], found that PUFAs comprised more than 50% of the PL FA content of normal rat AUM, with LA, ALA, and AA contribuing more than 46% of this total. Intriguingly, this AUM also contains almost 6% of the unusual 20:3 ω-9 eicosatrienoic acid, which has been related to hyperproliferation of the skin, the altered synthesis of eicosanoids, and blocking of cell–cell adhesion molecules, such as e-cadherins [43,44,80,84,85]. We previously confirmed these results by revealing a significant percentage of 20:3 ω-9 in normal rat urothelium [86] bearing in mind that an abnormal level of 20:30 ω-9 is a reliable marker of EFA deficiency [39]. Coincidently, an in vitro study comparing the FA profile of normal and cancerous human urothelial cells in urinary sediments showed in the latter the typical changes of EFAD, which were an increase of non-EFA ω-7 and ω-9 monoenenes and a decrease in ω-6 and ω-3 EFA [87]. In further studies, we showed that isolated AUM exhibited more rigidity when olein-enriched diets (inducer of essential PUFA deficiency) were offered to rats, whereas the increased fluidity obtained with ω-3-rich diets was mainly due to increased values of ω-3 22:6 in the AUM PLs [45]. We concluded that dietary PUFAs strongly modulate not only the anisotropic properties of AUM, but also modify the associated UP glycoprotein moieties in the concave rafts, as previously described in ultrastructure studies [46]. Later, we reported that an EFAD-inducer diet, enriched in non-EFA ω-9 increased the rigidity of urothelial rafts and disorganized the UP structure, whereas more fluidity in the bilayer was gained when ω-3 and ω-6 were given to rats, thus confirming that essential PUFAs play key roles in the structure and functions of the urothelial plasma membrane [50].

In later studies, Grasso and Calderon observed that isolated discoidal vesicles, built with two opposed concave rafts of AUM, had abnormal leakage of fluorescent dye and its quencher when isolated from oleic-rich (EFAD) diets. As enriched linoleic vesicles behaved no differently to controls, it was concluded that non-EFAs strongly alter the pathway of endocytozed urinary fluids, where putative mutagens may be present [88]. These authors also reported that the same non-EFA–enriched formula induced an abnormal uncompling of the vacuolar-ATPase, which regulates acidification and the membrane traffic of endocyted material from bladder lumen [89]. Taken together, these experimental results make it clear that PUFAs play a pivotal role in the maintenance of the normal molecular structure and physiology of mammal urothelium. Indeed, the data showed that the urothelium plasma membrane, the barrier against putative noxious mutagens coming from urine, is highly sensible to EFAD inducers, such as non-EFA dietary fats, a risk worsened if a chronic inflammation by irritant agents is also present, thus favoring a proneness to UT tumorigenesis [4,28,29].

Chronic inflammation of UT and urolithiasis may be a favorable bed for UTT cancer development

Rodents are suitable models for the study of the relationship between diet and UTT because urolithiasis and carcinoma of the urinary bladder are generally uncommon in normal animals [90]. As in humans, UTT development in rodents is a continuous multifocal process that may progress from atypical hyperplasia to carcinoma in situ and even to invasive carcinoma. Progressive subcellular neoplastic changes induced by many urothelial carcinogens consist of a loss of the asymmetry of the AUM (which becomes thinner) and damage to the ordered array of UPs in the plaques (which become replaced by irregular microvilli covered by an altered glycocalix) [79,91,92]. Interestingly, we observed similar changes in the luminal surface of the urothelium of EFAD rats, comprising the loss of the normal asymmetry of the AUM of rafts and discoidal vesicles, the appearance of villous projections, and perturbations of the mannoside-binding sites to concanavalin A and the anionic dyes of the glycoprotein moieties of the membrane's concave rafts [31,46,93,94]. Briefly, histopathology of the UT of rats maintained steadily on an EFAD diet for 50 wk to 110 wk, showed that both EFAD and EFA-sufficient (corn oil) animals showed mild degrees of multifocal nephrocalcinosis, perhaps linked to a monotonous salt mixture composition. Small calculi in the fornices, ureters, and bladder frequently were seen in both groups, along with well-differentiated urothelial hyperplasias, but these were significantly more severe in the EFAD group. Only 35% of EFAD rats developed multifocal dysplasias, in situ carcinomas, and/or well-differentiated invasive transitional carcinomas (in more than 20% of the animals), located mainly in the pelvis and upper ureters. Severe congestion, intertubular hemorrhage, and other parameters of LGCI were significantly more severe in the EFAD group [30,31,94].

Burr and Burr, in their pioneering articles of 1929 and 1930 describing EFAD, showed that apart from the severe signs of inflammation in the skin and Reynauds-like tail necrosis, the most constant histopathology lesions were seen in the UT and kidney. They proposed that EFAD may be a useful experimental

approach to study chronic degenerative diseases of the UT such as urolithiasis [95,96]. Later, Borland and Jackson examined many aged rats from the experimental colony of Burr's, and found severe nephro-calcinosis, necrotizing papillitis with hematuria, and infiltration of all the cell components of chronic inflammation in the kidneys and UT of the EFAD rats. In the urothelium of 50% of the pelvis and upper ureters, several multifocal dysplasias and flat papillomas were recorded, usually associated with necrosis and calculi [97]. In agreement with these previous results, it was later reported that dietary PUFAs differentially modulate acrylamide-induced preneoplatic urothelial proliferation and apoptosis in mice. An EFAD status (induced by a rich ω-9 oil formula) exhibited promoting activity in this model, whereas a fish oil diet, rich in ω-3 fatty acids, attenuated this effect [32]. The anti-promoting effect of fish oil feeding was expanded by the observations of the chemopreventive effect of ω-3 in several induced tumors in rats [98].

According to the degree of development in rich and poor countries, between 10% to nearly 25% of cancers are related to infectious agents such as human papillomaviruses, hepatitis B and C viruses, and *Helicobacter pylori* in cervix, liver, and stomach malignancies, respectively [38]. Normally, UT inflammation is helpful in its acute phase as a response to infections, foreign bodies (as urinary stones), trauma, chemical agents for treatment bladder tumors, or other irritants. However, chronic inflammation may result deleterious by inducing genetic damage and cancer promotion [99,100]. Pro-inflammatory cytokines such as tumor necrosis factor (TNF)α, interleukin (IL)-1 and IL-6 are strongly increased in tissues of most cancer patients, and they induce per se cachexia, burning of adipose depots and sarcopenia, loss of appetite, anemia, and the whole histopathology and serum picture of LGCI not only in tumor tissues but in many other systems and biological fluids [101–103]. Increments of these cytokines have been reported in the UT in both UTT and LGCI, due to urotithiasis and cystitis [104,105]. High TNFα levels are reduced by both COX inhibitors and ω-3 and ω-6 PUFA supplementation (mainly AA, γ-linolenic acid [GLA], EPA, DHA), which in turn increases the levels of certain anti-inflammatory eicosanoids and BALs, such as lipoxins, protectins, and resolvins (which also ameliorate LGCI) [106].

In cancer tissues, coexisting with LGCI, the levels of free radical and reactive oxygen substances (ROS) are altered [107,108]. These short-lived molecules are necessary for the normal control of cell proliferation, differentiation, and apoptosis. However, when their synthesis or catabolism is unbalanced, they became harmful for cell control homeoestasis, as happens very often in several varieties of cancer. One of the main sources of ROS, peroxides, and other free radicals are the cell membranes PUFAs [109]. Lipid peroxidation in many cancer cells is blocked or heavily inhibited. However, the addition of ω-3 and ω-6 PUFAs to cancer cells decreases proliferation, triggers apoptosis, increases expression of cell adhesion molecules, and inhibits metastasis parameters.These desirable effects are accompanied and can be even preceded by a sudden release of ROS products, thus indicating that cancer cells are incapable of synthesizing the required levels of lipid peroxidation and other ROS to control the normal behavior of the cell neighbor society [110]. Impaired peroxidation in tumor cells may be caused by a complex combination of low levels of essential PUFAs in membrane PLs, glutathione, catalases, superoxide dismutases, along with higher levels of some natural antioxidants and free radical scavengers [37,38]. In this context, differences in the lipid metabolism of the cell and tissues of patients with benign or malignant diseases have been compared [110,111]. Plasma levels of PL FAs very similar to those typical of EFAD, such as the low levels of PUFA from ω-6 and ω-3 metabolites and abnormal increments of the non-EFA oleic ω-9, were reported by Horrobin and co-workers in almost 100 patients suffering from transitional bladder cancer [111]. These findings prompted the authors to propose that a deficit in EFAs may predispose humans to the development of urothelial cancer [111,112]. Several case–control studies showed a significant risk for developing bladder cancer linked to the high consumption of saturated fats and non-EFA monoenes (mainly oleic acid) [113,114]. In contrast, the high consumption of essential PUFA was associated with a lower risk. In a case–control study carried out in New Hampshire, the higher intake of ω-3 ALA exhibited a protective role against developing bladder cancer [27].

The association among the LGCI of the UT, urolithiasis, and cancer proneness is a recurrent and controversial issue. Some studies have reported a positive link between bladder cancer, history of urinary infection, and renal lithiasis [114,115]. Indeed, both epidemiologic and rodent models data pointing out that the chronic UT inflammation induced by lithiasis is a significant risk factor for the development of transitional cell tumors [18,19,116,117]. Additionally, participants having a high intake of fats exhibited an increased risk for bladder tumors [21,27,118,119]. A long-term increase of well-known markers of inflammation in biological fluids such as blood, plasma, urine, and kidneys and bladder tissues indicates that an LGCI state is present in the UT. Besides the usual increase in the counts of polymorphonuclear leukocytes [PMN]/lymphocytes/monocytes in blood and UT tissues, an LGCI condition is heralded by high levels of C-reactive protein (CRP), lipid peroxide products, ROS, TNFα, leukocyte myeloperoxidase (MPO), IL-1 and IL-6, among other molecules. Other relevant dosable mediators of LGCI are histamine, many lysosomal hidrolases, the coagulation/fibrinolysis system, and nitric oxide (NO) [37,38].

PUFAs are the source of many BALs and have a dual role in LGCI. As already mentioned, under physiological conditions the synthesis and release of pro- or anti-inflammatory eicosanoids and other lipid derivatives are homeostatically maintained. However, if the nutritional sources of ω-3 and ω-6 are low and the dietary non-EFAs are increased together with chronic irritation in the UT, a shift to the production of pro-inflammatory PUFA-derived LTs, several HETEs and prostaglandin E2 (PGE_2) occurs [37,38]. These BALs are promitogens, which induce abnormal cell proliferation and differentiation, activate the anti-apoptotic Fkβ nuclear factor and block the expression of cell adhesion molecules such as e-cadherin and cytoskeletal desmogleins, hence favoring cell motility and eventually basal membrane permeation and metastases [80,81,83]. Thus, further integral strategies involving the chemoprevention of UTT by anti-inflammatory and pro-apoptotic measures through dietary modifications have been proposed [2,120]. In this regard, a life-span administration of a PPARα/γ agonist (Muraglitazar), in a dose-dependent way, increased urinary bladder tumors in male Harlan Sprague-Dawley rats, with urolithiasis development being a predisposing event [22]. The cell cycle regulatory mechanisms in UTT bladder calculi induced by terephthalic acid (TPA) was assessed in Wistar rats. This revealed that deregulation of the p16-cyclin D1/Cdk4-Rb pathway, but not of the oncogenic activation of *ras*, played a crucial role in bladder tumorigenesis induced by bladder stones [23]. In a further study, these authors showed that an increasing PGE_2 level (a pro-inflammatory eicosanoid highly augmented in the TPA-UTT model) involved the axis cytosolic phospholipases A2, COX-2, and thus concluded that PGE_2-synthase plays an important role in rat bladder carcinogenesis [104]. It was reported that

increased levels of thromboxane B_2, the major metabolite of COX_2 having proinflammatory activity, along with increased levels of the TPβ receptor occur in cells of patients with bladder cancer [121]. Furthermore, human bladder tumors were found to secrete substantial amounts of pro-inflammatory PGE_2, thereby promoting immunosuppressive phenotype of tumor-infiltrating myeloid cells, which in turn favored UT tumorigenesis [122]. Nephro- and urolithiasis, frequently linked to EFAD-induced [34,35,75,94,97,123] chronic irritation of the UT mucosa with the development of several degrees of hyperplasias, hyperemia, and other signs of chronic inflammation [9,10,75,94,124,125]. Thus, the UTT risk seems to be related to enhanced inflammation and is probably linked to a disbalanced eicosanoid metabolism.

Abnormal PUFA metabolisms are related not only to the illnesses included in the complex metabolic syndrome, such as diabetes and obesity among others, but also to the urolithiasis and infections of UT, which are closely linked to diabetes and PUFA perturbations. A significant increased risk for UT calculi among 12.000 new diabetes cases with diabetes mellitus in a follow-up population-based cohort study in Tawian was reported, providing consistent epidemiologic support that there is a causal association between diabetes and UT lithiasis. Interestingly, in diabetic women with UT infections, a greater rate of UT stones was found [11]. In patients with urolithiasis, an anomalous phospholipid metabolism of ω-6 PUFA was recorded: in plasma and the erythrocyte membrane PLs of stone subjects the observed AA/LA acid ratio was increased. Fish oil supplementation (rich in EPA) lowered calcium and oxalate urinary excretion, and normalized the erythrocyte oxalate exchange. Phospholipase A_2 increased the erythrocyte anion-carrier protein phosphorylation and the oxalate exchange, revealing that idiopathic nephrolithiasis is related to a perturbation in phospholipid- AA metabolism [33]. Additionally, an excess of AA in membrane PLs tilted the synthesis of eicosanoids and BALs and certain cytokines toward pro-inflammatory effects such as TXs, PGE_2, IL-1, IL-6, TNFα, macrophage migration inhibitory factor, and others [61,126]. The administration of palmitate, a non-EFA, induced IL-6 and monocyte chemoattractant protein-1 expression in human bladder smooth muscle cells, demonstrating additional links between diabetes and UT infections [127]. When the effect of dietary PUFA was studied in an urotithiasis model, calculi formation was significantly less severe in EPA-fed rats than in the groups administered olive oil or cholesterol, with both formula being EFAD inducers [34]. In conclusion, dietary supplementation with EPA in 88 patients with stones reduced urinary calcium excretion and decreased the pace of bladder stone formation [35]. Additionally, there is increasing experimental and epidemiologic evidence showing that calculi favors tumorigenesis in the UT [128,129].

As a consequence of repeated and chronic hematuria, such as that observed during urolithiasis, some infectious diseases, the chemopreventive drugs used in bladder neoplasms treatment, or EFAD, chronic hemorrhagic cystitis may appear. Curiously, when hematuria occurs, the urothelium develops active eritrophagocytosis with progressive infiltration of macrophages and PMNs [130]. Due to the slow lysis and digestion of cellular elements of blood (such as red blood cells), aggregated platelets and dying PMNs, there is a prolonged release of pro-inflammatory molecules, cancer cell chemoattractants, pro-mitotic cytokines, and eicosanoids, involving COX activation and being attenuated by nonsteroidal anti-inflammatory drugs [131–135]. The effects of these molecules on the control of urothelial cell cycles in the LGCI bladder as a concomitant factor for tumorigenesis risk deserves further research.

From our results and those of other workers it seems that PUFA deficiency, mainly due to an excess of non-EFAs, or BAL metabolites principally from the ω-9 family, appears to have a harmful effect on urothelial homeostasis, inducing progressive perturbations in the structure and biophysical properties of the AUM lipid bilayer, an alteration in the array of UP rafts, a severe rise in cellular proliferation, urolithiasis, vasodilation, and multifocal hemorrhages, with these alterations being a typical scenario of LGCI in the UT. There is increasing evidence that in many systems and organs, the conjunction of nutritional deficiencies in micro- and macronutrients, such as lipids, along with stone formation and chronic infections, constitute a LGCI environment that favors tumorigenesis [99,102,136]. In summary, we have shown a bulk of evidence, which points out that an abnormal PUFA metabolism, along with LCGI and urolithiasis, favor the development of UTT in rodents and perhaps in humans.

As discussed previously, several compounds having urolithogenic activity in humans, including drugs, and pollutants and environmental compounds, have been identified [137]. Furthermore, new risk agents for humans UTT still arise, as recently happened with melamine contamination. Since 2008, several articles led to the prevention about melamine, a synthetic nitrogenous compound widely used in domestic goods, being illegally added to powered infant milk formula to maliciously increase the nitrogen levels, a parameter related to the price of these milk preparations [138,139]. Once ingested, 90% of melamine (2,4,6-triamino-*s*-triazine), which is a resin, is excreted in the urine [140]. A high percentage of rodents chronically treated with low doses of melamine developed all the signs of LGCI, uroliths and transitional cell carcinomas [117]. In mice, the premalignant lesions developed mainly in the bladder, whereas hyperplasias, dysplasias, and carcinoma in situ of UT in melamine-exposed rats were localized predominantly at the proximal end of the UT (papilla and renal pelves). These findings are in agreement with those of Melnik and co-workers [141], who showed that in rats, melamine at low doses induced atypical proliferative lesions, mainly located at the proximal end of the UT. Hence, we used low doses of melamine plus different supplementations with ω-3, ω-6, and ω-6 oils, and observed that oleic-enriched EFAD diet increased, whereas essential PUFA (mainly ω-3) supplementation decreased the severity, progression, and frequency of the neoplastic lesions [18,19]. These results, together with consistent experimental, clinical, and epidemiologic findings showed a link between LGCI and urolithiasis by a low dose of melamine, led us to propose that an increased risk for UTT in thousands of surviving children exposed to melamine in their early childhood may occur when reaching adult age. Considering the observed preventive effects of ω-3 PUFAs and the deletereous effect of non-EFAs, as for ω-9, periodical screenings for the early urinary signs of UTT may be necessary in these children, together with acurate continuous ω-3 essential PUFA supplementations [20].

Conclusions

UTT is still a poorly understood multistage and multifocal process that includes initiation, promotion, progression, and eventually invasion and metastases, which can be influenced by promoting and antipromoting factors. Taking into account that cancer is conceived as involving both genetic alteration and its phenotypical expression, studies centered on the role of environmental factors, such as those present in the diet, may be potentially fruitful.

There is evidence of risk linking high fat consumption and the development of certain human cancers, such as of the breast, gliomas, and colon. However, the amount and type of nutritional lipids in humans remains to be fully elucidated [37,142]. The discussed results showing a crosstalk among PUFAs, urolithiasis, LGCI, and UUT risk have crucial research interest. Many data have been presented in this article showing that disbalances or deprivation of essential PUFAs, mainly ω-3, may play a role in UTT when associated with LGCI and lithiasis. An abnormal or deficient availability of essential PUFA in foods and/or membranes might indeed take place due to unhealthy dietary practices such as the consumption of foods very rich in fats and simple sugars, since chronic subclinical or borderline EFAD conditions may occur, as suggested half a century ago by Sinclair [143] and Holman [144]. On the other hand, the habit of eating foods extremely low in fats, in order to lose weight, primarily among young people, could lead to extreme states of bulimia, anorexia nervosa, and other mental illnesses. Another concomitant risk condition may be caused by food contaminants, due to the fact that even trace components in the diet might be harmful for the urinary tract [145], as discussed previously regarding melamine contamination. Most of the mechanisms by which dietary PUFAs could influence urothelial tumorigenesis involve, to some extent, modulations of gene expression. Inasmuch as increased cell proliferation, as observed in EFAD, is important in this process both for the fixation of altered gene information and for the promotion of tumor development, the determination of the genes controlling cell proliferation, differentiation, cell homing, and apoptosis, which are turned *on* or *off* by dietary FAs, will likely provide additional answers to explain the role of dietary essential PUFAs in UTT risk [146]. (see resumée in Fig. 1)

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