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Gastrointestinal Malabsorption of Thyroxine

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Levothyroxine, a largely prescribed drug with a narrow therapeutic index, is often a lifelong treatment. The therapeutic efficacy of thyroxine may be marred by behavioral, pharmacologic and pathologic issues acting as interfering factors. Despite a continuous search for an optimal thyroxine treatment, a significant number of patients fail to show a complete chemical and/or clinical response to this reference dose of thyroxine. Gastrointestinal

malabsorption of oral thyroxine represents an emerging cause of refractory hypothyroidism and may be more frequent than previously reputed.
In this review article we aimed at examining the pharmacologic features of thyroxine preparations and their linkage with the intestinal absorption of the hormone. We have stressed the major biochemical and pharmacologic characteristics of thyroxine and its interaction with the putative transporter at the intestinal level. We have examined the interfering role of nutrients, foods, and drugs on thyroxine absorption at gastric and intestinal level. The impact of gastrointestinal disorders on thyroxine treatment efficacy has been also analyzed, in keeping with the site of action and the interfering mechanisms. Based on the evidence obtained from the literature, we also propose a schematic diagnostic workup for the most frequent and, often hidden, gastrointestinal diseases impairing thyroxine absorption.

ESSENTIAL POINTS

- Gastrointestinal malabsorption of thyroxine may account for a significant fraction of refractory hypothyroidism
- pH is a major determinant of thyroxine fate in multiple metabolic step and even in the absorption of oral thyroxine
- The most frequent conditions that must be taken into account are Helicobacter pylori infection, lactose maldigestion and celiac disease
- Unawareness of thyroxine malabsorption cause may lead to repeated adjustments of dose and monitoring
- The individualization of treatment helps in detecting gastrointestinal malabsorption of thyroxine
- The schematic diagnostic workup to detect gastrointestinal disorders should start based on the clinical features and the prevalence of gastrointestinal disorders
- Thyroxine treatment may be used as a tool to reveal occult gastrointestinal diseases

INTRODUCTION

The efficacy of a drug depends on several factors including the appropriate dose, mode of ingestion, absorption and potential interfering factors (1-4). This is particularly true for drugs with a narrow therapeutic index, which means significant variations of effectiveness for small variations of dose (5). Thyroxine (T4) is one such drug with its treatment having been significantly changed over the years, based on the availability of more sensitive indexes of efficacy (2). The availability of more sensitive TSH assays, in fact, led to a progressive general reduction of thyroxine dose for both replacement and TSH suppressive purposes (2). A significant reduction of the harmful effects of both under treatment and overtreatment has been observed over the years (1, 3, 6), with particular emphasis on thyroxine treatment during gestation (7), at doses that are adequate for both the fetus and successful progression of pregnancy. The continuous search for an optimal daily thyroxine replacement has led to the 1.6-1.8 µg/Kg body weight/day consensus dose, a posology that is able to restore TSH into the normal range in most hypothyroid patients (4). However, the number of interfering issues rendered often inadequate for a patient a dose that is satisfactory for another (8). It has been reported that 20% to 50% of patients fail to show a complete chemical and/or clinical response to a reference dose of thyroxine (9). The consequence of that is the need for an increased dose, care and monitoring (10). The resulting repetition of unnecessary diagnostic workup represents a substantial hidden cost for the national health systems. So far, several psychological, nutritional, and pharmacological events may be responsible for the increased requirement for thyroxine in these patients (see 2-4, 11-13 for reviews) with some of such events deserving mention. Because of poor compliance with the prescribed regimen, certain

patients do not take thyroxine regularly, a condition known as pseudomalabsorption (14) and once reputed to account for most of the increased requirement of thyroxine. Thyroxine dose has to be sometimes increased when different T4 preparations are available and a patient switches one to another (see 2 for review). Once pseudomalabsorption has been excluded, impaired intestinal absorption of thyroxine caused by nutrients, drugs and gastrointestinal disorders represents a major cause of refractory hypothyroidism. In the era of precision medicine (15), treatment should be highly individualized and all the characteristics of a drug should be taken into account during a chronic and sometimes lifelong treatment.

In this review we aimed at examining the pharmacologic features of thyroxine preparations, and their linkage with the clinical evidence of a reduced intestinal absorption of the hormone.

How the pharmaceutical forms of oral thyroxine are prepared.

Typically, leaflets of medicines and websites of drug companies fail to give technical information on manufacturing of their products, and thyroxine makes no exception. In general, tablets, which are most often disc-shaped, are a solid dispersion of the active ingredient and disintegrable excipients, pressed or compacted from a powder into a solid dose. Excipients have several functions, and the same excipient may serve more than one function (Table I) (16). Examples of such functions are to improve powder flowability (gliding), to favor cohesion of different substances (binding), to facilitate tablet disintegration in the gastrointestinal tract or to prevent the tablet from sticking (lubrication) (17). To ease swallowing and to resist to environmental conditions, the tablet is coated with a polymer. Examples of glidants are magnesium stearate and talc; examples of binders are lactose, starch, cellulose or polyvinyl pyrrolidone; examples of disintegrants are poly vinyl pyrrolidone or sodium carboxymethyl cellulose (croscarmellose sodium); examples of lubricants are talc, silica or magnesium stearate (16, 17). Clearly, these ingredients must be granulated prior to compression to assure even distribution of the active ingredient and to contain the appropriate amount of active ingredient in each tablet (17). In one report (18), some intolerance to the excipients used to prepare tablet thyroxine has been observed. Recently refractory hypothyroidism linked to improper storage of T4 tablets has also been shown. This suggests that in addition to proper mode of ingestion, patients should be instructed on proper modalities of thyroxine storage, as well (19).

As summarized elsewhere (20), softgel capsules are composed by an outer gelatin shell and by a fill formulation, and the highly specialized manufacturing process is divided in five different steps. In the sole T4 softgel capsule available thus far, T4 is first dissolved in glycerine and then injected into a gelatin shell. This outer shell protects the active ingredient from degradation.

The brand liquid preparation of T4 was a 20 ml bottle containing 100 µg/ml sodium T4, being each ml equal to 28 drops, and each drop containing 3.57-µg sodium T4 (21). Drops are to be dissolved in water and swallowed. Excipients are ethanol and glycerol (21). Noticeably, the Food and Drug Administration (FDA) approved an alcohol-free oral solution in February 2017. Bernareggi et al (22) demonstrated the stability of this T4 oral solution when added to breakfast beverages. The bioequivalence of these alternative thyroxine formulations has been accurately described (21, 23). Concerning tablet T4, which represents the choice treatment of hypothyroidism worldwide, there was a concern about switching between brands, from a brand to a generic preparation and from a generic to another generic. In 2008, the Endocrine Society has published a position statement on this issue to ensure pharmacologic homeostasis of patients (https://www.endocrine.org/advocacy/priorities-and-positions/bioequivalence-of-sodium-levothyroxine). There is, however, general consensus that switching between different tablet T4 preparation should be avoided (2, 9).

How oral thyroxine is ingested

Thyroxine tablet is the most used preparation to treat hypothyroidism worldwide (1, 2). Patient's compliance is, of course, a key factor for the proper achievement of thyroxine therapeutic goal. Nonadherence to medications is a major challenge in the management of thyroxine treatment. When patients do not take T4 regularly, or do not comply with timing in relation to food ingestion, and fail to report such behaviors to the endocrinologist, the clinician may wonder about the cause of a persistently elevated TSH, despite a high T4 dose (14). The term T4 pseudomalabsorption has been used to describe this specific situation. Initially the clinician might suspect a decreased T4 gastrointestinal absorption (24), and an evaluation for diseases causing malabsorption, or drug interaction should be performed. Some authors suggest that an oral T4 absorption test (by giving a total amount of 600 to 2,000μg of thyroxine) could be used to demonstrate pseudomalabsorption (4, 25, 26).

The tablet formulations of levothyroxine contains a stable salt, sodium T4, together with a variety of excipients (27). After ingestion, a dissolution phase of the tablet is necessary for the subsequent intestinal absorption and, in the dissolution phase, a near physiologic gastric pH is required (28). After dissolution, and disregarding a hypothetical minimal absorption in the stomach, T4 is essentially absorbed in the small intestine (29, 30). Wenzel et al. (31) showed that the absorption of T4 is significantly reduced if the drug is taken after a meal. Indeed, it has been shown that certain foods or drinks (such dietary fibers, soybeans, coffee, or papaya, etc.) (32-35) reduce the absorption of T4. In addition, nonfasting regimens of T4 administration are associated with higher and more variable serum TSH concentrations (36). Under fasting conditions (in euthyroid subjects), the unidirectional absorption of thyroxine or peak values of T4 absorption (Cmax), occurs in the first 90 minutes following T4 administration, with a rapid increase in the first 60 minutes (37). The time to reach the maximum concentration (Tmax) of T4 is about 2 hours after T4 ingestion. Shortly after, absorption starts to plateau (37, 38). On the average, 60-80% of ingested T4 is absorbed and rendered bioavailable, the distribution volume of the hormone averaging 11.5 liters) (37). However, in a study on hypothyroid subjects the Tmax and distribution volumes were 3 hours and around 15 liters, respectively) (38). After a meal, the Cmax is decreased and Tmax is delayed, with resulting decreased T4 bioavailability. If food, is not postponed by at least one hour following T4 ingestion, delayed and decreased intestinal absorption of T4 may follow (37). However, bedtime intake of T4 significantly improved thyroid hormone efficacy, probably because the lower intestinal motility at night increases the exposure time of thyroxine to the intestinal mucosa (39) or because of a better patient's compliance with the treatment (40). Based on these results, postponing breakfast by one hour after ingestion of T4, has been suggested in different studies (28, 36, 40) to warrant an efficient absorption of thyroxine and to carry out properly studies on malabsorption (41). In fact, in the latter studies the daily dose of thyroxine required to obtain a serum TSH <0.5 and 2.5> mU/L was 1.3 μg/kg body weight (42, 43). This dose is significantly lower than the one recommended by an *ad hoc* ATA task force (1.6-1.8 μg/kg body weight) in adult patients with minimal endogenous thyroid function (4). In spite of these studies, leaflets of this hormone still indicate a lag time of 30'between the morning ingestion of thyroxine and breakfast, and the same interval, it is commonly recommended by the prescribing physicians. Weekly tablet T4 administration has been also proposed (44), suggesting that auto regulatory mechanisms may maintain near-euthyroidism. However, for complete biochemical euthyroidism, a slightly larger dose than 7 times the normal daily dose may be required (44). Nowadays, weekly tablet T4 administration is used only to detect non-adherence to treatment.

How oral thyroxine is absorbed

The daily dose required to obtain the therapeutic effect is not a linear function of the ingested dose of thyroxine, which is the main, but not the only decisive event. Various studies since 1933 investigated the percentage of oral thyroxine absorbed in man with different techniques: single isotope in feces, plasma and liver, double isotope, or using stable T4 and calculating the maximum increment in serum T4 or the AUC after serial plasma T4 measurement (45). In these studies, in which patients had been treated with different dosage and vehicle of oral thyroxine, the percentages of thyroxine absorbed had been estimated between 17 and 93% of the ingested dose with an overall value of 60-80% confirmed in a study published in 1991 using 131-I labeled thyroxine in 3 volunteers (mean value=71±3%) (30). These data have been confirmed, also using non-labeled T4, by more recent studies (37). This value seems to be increased in patients with overt hypothyroidism; however, this is still a debated issue (38, 46). Some life conditions may be associated to variations in the absorption rate of thyroxine. In particular, during pregnancy, the rise in progesterone leads to delayed gastric emptying and prolonged small bowel transit time, by ~30–50% (47). This change may contribute to the overall increased need for thyroxine during pregnancy (48). Also in the geriatric age, the absorption of T4 seems to be slightly reduced. In fact, it has been shown a decrease of tablet T4 bioavailability of about 4% by 10 years of age increase, suggesting that the percentage of T4 absorption is decreased in the elderly (49, 50). However, this issue has been recently questioned in an extensive study which failed to identify an effect of age on thyroxine requirement suggesting that the decrease in lean body mass observed in elderly may be more likely responsible for these age-related changes in levothyroxine pharmacokinetics (51).

Levothyroxine sodium is absorbed along the whole small intestine with different percentages in its different segments: it has been shown that the mean values of labeled thyroxine absorption were 15±5% in the duodenum, 29±14% in upper jejunoileum and 24±11% in lower jejunoileum. (30). A concomitant study of the mean transit time in the gastrointestinal tract revealed that it was 35±30 minutes in the stomach, 7±3 in the duodenum, and 31±8 in the upper jejunoileum. The time between the oral intake and the appearance in the plasma seems to minimize the possibility of T4 absorption in the stomach. Once ingested, levothyroxine sodium in tablet formulation undergoes disintegration, deaggregation and dissolution in gastrointestinal fluids (52): prerequisite for the successive absorption, in fact, is that active ingredient is in aqueous solution. Main factors affecting these processes are gastric juice pH and viscosity, type of excipients and structure, shape and dimension of active ingredient particles (53). Furthermore, to ensure molecule stability and higher aqueous solubility the pharmaceutical form is the sodium salt of levothyroxine: at 25°C, the aqueous solubility decreases as pH increases from 1 to 3, then reaches nadir values for pH between 3 and 7 and then increases again for pH higher than 7 (54). Recently, a study about crystalline conformation of sodium levothyroxine molecule revealed that it may exist in different polymorphs (different molecular conformation in the crystal form) which solubility changes at different pH values enlightening a possible role of the different molecule conformations on T4 solubility (55). In the Biopharmaceutics Classification System, levothyroxine is recognized as a Class III drug, i.e. a high solubility drug with a low intestinal permeability (56). However, it is important to underline that the solubility of a drug is tightly related to the pharmaceutical formulation and may vary among the different preparations (57).

Transport of thyroxine across the intestinal mucosa

In the early ‘60s, it was believed that the transport of thyroxine inside cells occurred through passive diffusion mechanisms based on the high lipophilicity of the molecule (58). Since then, evidence have changed our understanding on the mechanisms underlying thyroxine transport. First of all, thyroxine is an amphipatic molecule with a lipophilic aromatic ring and a hydrophilic side chain and possess three ionizable moieties, two acidic (phenolic -OH, and

carboxyl -COOH) and one basic groups (amino -NH_2). The relative dissociation constants are 6.71, 2.04 and 8.85, respectively (59) (Figure 1). Furthermore, it has been enlightened that at different pH, thyroxine molecule may exist at eight different micro species (60). In addition, it has been reported that the paracellular route of absorption may show only a negligible contribution for compounds larger than molecular weight of 250-300 D (61) and thyroxine is 774 D. For these reasons, iodothyronines are trapped and not be able to cross the lipid bilayer in the absence of specific carriers (62) and become constituents of the cell plasma membrane in vertebrates (62). Besides the amphotericity of the molecule, further hindrance to diffusion through the plasma membrane comes from the charge of thyroxine. In fact, in aqueous solution, thyroxine exists predominantly in ionized form and in particular in zwitterionic form (OH, NH_3^+, COO-) in the range of pH between 2.46 and 6.91 and in monoprotonate form for more basic pH (O-, NH_3^+, COO-) while the cationic and anionic forms predominate at extreme pH (63). As a matter of fact, the pH-partition hypothesis postulates that *"the absorption of ionizable drugs mainly takes place in compartment(s) where the local pH ensures a sufficient concentration of the non-charged form relative to the ionized form(s)"* (64). Nowadays, therefore these finding indicate the passive diffusion as a residual transport route (65).

Over time, studies have focused on the presence of specific carriers that facilitate the passage of thyroid hormones through the cell membrane. Different categories of transporters may act as T4 carrier the level of small intestine: monocarboxylate transporter (MCT) family, organic anion-transporting polypeptide (OATP) family, ATP-binding cassette (ABC) transporter superfamily and large neutral amino acid transporter (LAT) family (66).

Two MCT involved in thyroxine transport have been identified in the small intestine mucosa: in fact, MCT8 and MCT10 mRNA are expressed in both stomach and small intestine human mucosa (67). Both these transporters are constituted by 12 transmembrane domains with the amino- and carboxy-terminal intracellular domains, showing a high degree (49%) of homology of the amino acid sequence (65). Both the transporters are responsible for cellular influx and efflux of T4 (68). MCT8, the primary transporter of TH, is expressed in many tissues but its activity is mainly devoted to TH transport across the blood brain barrier and to the development of human brain, as proven by neurological deficits in patients with MCT8 gene mutations (69). A specific localization of MCT8 in human intestine has not been identified and the possible functions have not been extensively studied yet. However its involvement in T4 transport has been suggested by the increased need for thyroxine observed in athyreotic patients treated by sunitinib and imatinib which non-competitively inhibited this transporter *in vitro* perhaps by a direct binding (70). On the contrary, MCT10 is highly expressed at small intestine level and in particular in the basolateral membrane of mucosal cells leading to the hypothesis of its possible role in the intestinal T4 resorption (65). Some members of the organic anion-transporting polypeptide (OATP) family, able to transport T4 have been identified in small intestine. OATP1A2 has been identified in the apical brush border of duodenum enterocytes but in a small quantity (71, 72). This transporter carries a wide spectrum of substances in a Na+ and ATP-independent way (73), but its activity may be sensitive to medium pH (74). The study of the interferences on the OATP1A2 activity allowed defining its importance for the transport of different compounds including thyroxine: in particular, flavonoids as naringin and hesperidin, component of grapefruit and orange juice, respectively, may interfere with OATP1A2 (75). A study on the effect of naringin on OATP1A2 showed that its effect in reducing oral availability of these transporter substrates (i.e. thyroxine, acebutolol, fexofenadine etc.) lasted 2 to 4 hours. The extent of that effect was different for each substrate and more pronounced if the drug is hydrophilic and excreted unchanged and less when lipophilic and largely metabolized (75). It is not surprising that inhibition of OATP1A2 had only a minimal effect on AUC of thyroxine (35). The authors

postulated an effect on T4 elimination from enterocytes, and not on T4 absorption, that may impair the hormonal enterohepatic recycling and thus bioavailability (35). The evidence that β-blockers and tricyclic antidepressants may use this same transporter shed light on a novel site of interaction with thyroxine (76). A similar involvement in the process of recycling of thyroid hormones from ileum to the liver has been proposed also for OATP4A1, through the portal vein (77). In a recent study, Meyer Zu Schwabedissen et al (78) have studied the role of OATP2B1 in contributing to intestinal thyroxine absorption. They concluded that thyroid hormones are in one time substrates and transcriptional regulators of this transporter in enterocytes (78). P-glycoprotein is a membrane protein that belongs to the superfamily of ABC carriers that transports mono-directionally multiple substrates including thyroxine out from cells (79, 80). The inhibition of this protein seems to be related to the increased AUC of levothyroxine when co-administered with rifampin (81). LAT1 transporter, that when associated with CD98 transports T4, has also been detected in Caco-2 cells, a model expressing many morpho-functional characteristics of the absorbent epithelium of the small intestine (82). However, the rate of thyroxine uptake was low (83). Overall, the existence of a main or thyroxine-restricted transporter at the intestinal level is still an unanswered question. Moreover, it appears that substances and drugs impairing the activity of known intestinal T4 transporters mainly interfere with the outflow of the hormone from the enterocyte and with the enterohepatic recirculation of T4. In fact, once absorbed in the small intestine, thyroxine reaches the target organs where its peripheral metabolism takes place. Beyond deiodination, there are also alternative metabolic pathways as extensively reviewed by Visser et al. (84) and Wu et al (85). In the liver, thyroxine and triiodothyronine are conjugated with sulfuric and glucuronic acid which makes them more soluble, allowing their renal and biliary clearance (84). The presence of the intestinal microbial flora allows the binding of iodothyronine, thus creating a hormonal reservoir (85); on the other hand, due the presence of sulfatase (86) and glucuronidase (87) activities in the intestinal contents, intestinal microbiota help their deconjugation and the possible hepatic reuptake through the portal circulation. Changes in the intestinal microbiota, known as dysbiosis, may therefore be responsible for variation in these metabolic steps (88, 89).

Gastric disorders affecting thyroxine absorption

Plenty of conditions, either physiological, nutritional, pharmacologic or pathologic, may impair the intestinal absorption of oral levothyroxine (2, 3). However, an increased need for thyroxine not necessarily indicates gastrointestinal malabsorption of the hormone. Whether the serum TSH would be a reliable marker of hypothyroidism is still a debated issue (2, 90). However, in clinical practice, serum TSH levels is, yet, the easier and faster diagnostic tool to evaluate thyroxine treatment effectiveness (1, 2, 4).

In the past, the uncertainty about the appropriate thyroxine dose for the treatment of hypothyroidism, for both the general population and its relevant subclasses (i.e. children, adult, elderly persons, obese, pregnant, polypharmacy, thyroidectomized) did not help to focus on thyroxine malabsorption. The more recent efforts to standardize T4 treatment (see 1, 2, 4 for rev) and the seminal paper from Santini et al. (91), indicating that the dose of thyroxine is dependent on lean body mass, opened the door to more precise posology of thyroxine dose. Nowadays, the use of an individually tailored dose of T4 is advised to detect an increased need for thyroxine (41, 92 abstract).

Since there is no evidence that thyroxine may be significantly absorbed in places other than in intestinal sites, the observation that in patients with impaired gastric acid secretion a higher dose of T4 would also be required, widened the research on thyroxine malabsorption mechanism (28). Possibly confused with pseudomalabsorption in the past, the gastric-related malabsorption of levothyroxine represents nowadays an increasingly recognized issue in a

relevant number of hypothyroid patients (41). The acid producing machinery (i.e. the H+/K+ ATPase) is located in the oxyntic glands in the gastric fundus and its action is enhanced by the production of gastrin from the antral G cells) (93). Acid production is partially or totally abolished in patients with chronic gastritis and/or gastric atrophy (94), is blocked in those treated with proton pump inhibitors (PPI) (93) as well as partially blocked and counteracted by NH3 production in patients with Helicobacter pylori infection (95, 96). All these conditions have been related to increased thyroxine requirement (3, 28, 97, 98) (Figure 2).

Evidence *in vitro* and *in vivo* highlighted this novel role for the stomach in the subsequent intestinal T4 absorption (41, 99). *In vivo*, the role of gastric acid secretion has been demonstrated in treated goitrous patients with an impaired gastric acid secretion (28). In fact, daily T4 requirement was increased by 1/3 in patients with Helicobacter pylori-related gastritis, atrophic gastritis and maximal in those with both these conditions (28). In keeping with these results, hypothyroid patients with positive anti-parietal cell antibodies (PCA) showed an increased daily thyroxine requirement (100). These authors found an increase by 17% in PCA positive as compared with PCA negative patients and such an increase was even higher (+32%) in those patients with proven atrophic gastritis. In thyroxine-treated patients with recent H. pylori infection a significant TSH raise has also been observed. Such an effect was counteracted by increasing the dose of thyroxine, being only partially reversed by Helicobacter pylori eradication (28). Eradication of this infection, in fact, does not always re-establish the previous conditions of the mucosa, as an antritis or even a pangastritis may ensue with a possible evolution to gastric atrophy (101). Helicobacter pylori infection affects 30-to 50% of general population worldwide (102) and the high prevalence of this sole disorder may potentially increase the need for thyroxine in a higher number of patients than in those with supposed pseudo-malabsorption.

Drugs interference at gastric level

A third way to impair the gastric acid secretion is the treatment with PPI. Firstly, the effect of omeprazole on thyroxine needs has been described in goitrous patients in whom the concomitant use of these two drugs significantly increased serum TSH (28). The effect was reversed by increasing the dose of T4 by 37% or by discontinuing the use of PPI. Similar results were obtained by Sachmechi et al. (103) using lansoprazole in patients with serum TSH >5 mU/l. In contrast, absorption kinetics of high dose of thyroxine seem to be unchanged by the concomitant ingestion of pantoprazole and esomeprazole for one week (104, 105). However, the effect of PPI on pharmacologic thyroid homeostasis has been confirmed by TEARS study (106). Noticeably, very recent data *in vivo* pointed out that the replacement dose of thyroxine is inversely correlated with gastric pH, supporting the hypothesis that variations in gastric juice pH, like during PPI treatment, may affect thyroxine dose (107). Beside PPI, other drugs seem to interfere in a pH-related fashion with thyroxine absorption (antiacids, calcium salts etc.), their effect having been extensively reviewed elsewhere (108,109). Despite the adsorption of thyroxine would be the main mechanism of interference for most of drugs (108), the role of gastric pH may be relevant for some of them (Figure 3). Beside the abovementioned effect of drugs in reducing gastric juice pH, a further pH dependent process may occur at gastric level (110). On this ground, the effect of calcium carbonate is a model. In fact, Singh and colleagues (110,111) have shown that both acute and chronic ingestion of calcium carbonate reduces the bioavailability of T4. A similar interference was also evident when using different preparation of calcium (112). In a first study (110), a supplementation of 1200 mg/day for 3 months led to a reversible increase of serum TSH in 2/3 of patients. Interestingly, in the same paper, a binding study revealed that at pH 2, but not at pH 7.4, a significant fraction of thyroxine was adsorbed to calcium carbonate in a dose dependent way, preventing the absorption at the intestinal level. In addition, this interference with oral thyroxine was confirmed by the evidence that calcium

carbonate acutely reduces the main pharmacokinetic parameters of T4 absorption (111). Some nutrients seems to act through a similar cooperative mechanism to reduce the absorption of thyroxine (113, 114); indeed, the papaya fruit contains proteolytic enzymes, including papain, which decreases histamine-induced acid secretion; but author suggested that the papaya fibers might also bind thyroxine in the intestine (113). Very recently, Chon et al have described that simultaneous milk ingestion decreases oral levothyroxine absorption (114). The pH of naïve cow's milk is around 6.6/6.7 and it contains more than 1 gram of calcium per liter, so that is not surprising that the pH sensitive fraction of T4 absorption might be affected (114). At the same time, naïve milk contains fat, proteins, lactose that may maintain thyroxine in the intestinal lumen preventing its absorption (29,115).

The overall mechanism by which intestinal absorption of thyroxine may be impaired in hypo-achlorhydric patients remains, however, unclear. Iodothyronines are themselves pH-dependent molecules (54). *In vitro* studies have been carried out and since 1971 Amirav Gordon indicated that blood pH may affect the partition between the intravascular and rapidly exchanging pools of thyroxine (116). Centanni M and Robbins J (117) have shown the role of pH in thyroid hormone uptake in skeletal muscle and several membrane transporters of thyroxine at the intestinal level are sensitive to pH changes (118). Beside the naïve thyroxine, the pharmaceutical preparation is also dependent on medium pH. Pabla and colleagues (99) have shown *in vitro* that the dissolution of tablet thyroxine is over 85% within 20 minutes only at low pH (<2.4). According with FDA, this time corresponds to the half-life of fasting gastric emptying for rapid release drugs (57). At increasing medium pH the dissolution of tablet T4 rapidly decreases at all times studied (30', 60' 120'), while the softgel T4 preparation seems to be less sensitive to that variable (99). However, the dissolution time does not fully explain the subsequent absorption rate. In fact, Kocic et al (119) have shown that the dissolution rate and the absorption rate in the first two hours may diverge, indicating that dissolution does not greatly influence the absorption of levothyroxine in the period of time (1.66 hours) in which the maximal absorption take place (37). Noticeably, a better absorption of thyroxine has been observed in patients simultaneously taking vitamin C (120, 121) which may lower gastric pH in patients with impaired acid secretion (109).

So far, it appears that the ionization status of thyroxine is important and that thyroxine ionizable moieties are dependent from ambient pH (122). It has been suggested that pharmaceutical T4 preparation, a hydrophilic sodium salt, may remain partly undissociated in a hypochlorhydric gastric environment and this event may impair the efficiency of subsequent intestinal absorption (28). Although the mechanism is not completely elucidated, the clinician must be aware of the widespread impact of these interfering conditions because of the very high prevalence of H. pylori infection (102) and the prescription rate of proton pump inhibitors (123). Therefore, in the past, the studies aimed at identifying an appropriate daily T4 dose may have been biased from the presence in the sample of an unknown number of infected patients.

Altered gastric motility and bariatric restrictive procedures

Gastroparesis is defined as delayed gastric emptying in absence of mechanical obstruction and its prevalence is about 0.04% in an area of United States (124). Idiopathic, postsurgical, and diabetic gastroparesis are the most frequent forms. Only few case reports deal with the correlation between gastroparesis and thyroxine absorption (125, 126). The first one described an old man with persistent hypothyroidism despite a high thyroxine dose (>2.7 mcg/Kg/day). In this case, upon the exclusion of the main causes of thyroxine malabsorption, the diagnosis of gastroparesis had been confirmed by gastric emptying study (125). In the second report, in a pregnant hypothyroid young female with type 1 diabetes, gastroparesis has been diagnosed (126). In this study, the link between T4 malabsorption and gastroparesis has been confirmed by a faint response of FT4 during T4 absorption test. On the contrary, the

weekly intramuscular injection of T4 led to the improvement of the thyroid hormonal profile. In addition to gastroparesis itself, the residual food in the patient's stomach, that may adsorb the ingested T4, might represent a further mechanism of malabsorption (31, 126). In this patient, the refractoriness to oral thyroxine treatment worsened during the pregnancy because of, besides diabetic gastroparesis, an even slower gastric motility, might be induced by progesterone (127). Reardon and Yoo (128) also reported a better performance of softgel thyroxine preparation in a hypothyroid patient with gastroparesis, who quickly corrected her thyroid function by using that formulation of thyroxine. A recent case report suggested that the motility of the whole gastrointestinal tract may affect thyroxine bioavailability (129). In fact, a severe refractory hypothyroidism was associated with systemic sclerosis, a chronic disorder characterized by muscular atrophy and fibrosis of gastrointestinal tract. In this case, the esophageal sclerosis reduced esophageal motility impairing the bolus progression of thyroxine (129). Authors suggested that the severe hypothyroidism was due to reduced thyroxine absorption, since TSH was progressively normalized upon switching to a liquid formulation of thyroxine.

As far as concerns the bariatric procedures involving gastric restriction (gastric banding and sleeve gastrectomy), they are considered procedures that alter drug absorption less than procedures involving intestinal diversion (130). The two mechanisms involved are the drug disintegration and the drug dissolution: the first step is necessary for a drug to become soluble with the gastrointestinal milieu and is the rate-limiting step in the absorption of most solid drug form because the gastric mixing promotes drug disintegration (52, 53). In sleeve gastrectomy a substantial part of the gastric fundus and body, the areas of the stomach containing most of the acid-producing cells, is removed. Therefore, the gastric pH increases and the solubility of some drugs would be reduced (130). Despite an expected increased need for thyroxine following bariatric surgery, the data are conflicting. Pharmacokinetic parameters of levothyroxine were, in fact, not decreased but rather increased in obese euthyroid patients before and after sleeve gastrectomy and gastric bypasses (131). In this study, however, the patients underwent thyroxine absorption test with an oral solution of levothyroxine that does not require the gastric dissolution (131). Aggarwal et al (132) have analyzed 19 hypothyroid patients that underwent sleeve gastrectomy and demonstrated that 13/19 patients improved their thyroid status with a lower dose of thyroxine, showing a correlation between percent excess of weight loss and the change in thyroxine dose. On the contrary, Sundaram et al (133) and Fierabracci et al (134) demonstrated a decrease or no change in total thyroxine dose but an increase of weight-based levothyroxine requirement, in patients after sleeve gastrectomy or gastric banding. To explain these contrasting results, authors have suggested time by time accelerate gastric emptying, modified gastrointestinal motility, alterations in bile acid and in gut microbiome composition without consistent data. The changes in lean body mass following bariatric surgery could also potentially contribute to a change in LT4 doses (91). In fact, only weight-based evaluation may lead to reliable results. Therefore, according to Gadiraju et al. (135), further controlled studies are needed before drawing definite conclusions.

Intestinal disorders IMPAIRING thyroxine absorption

Celiac disease (CD) has an estimated prevalence in the general population of 1% (136) but its prevalence rises to 2-5% of patients also bearing a thyroid autoimmune pathology (137), the main cause of hypothyroidism. Furthermore, being thyroid autoimmune disease the most frequent autoimmune disorder, a definite higher rate of Hashimoto's thyroiditis may be detected in patients with celiac disease than in the general population (138). The joint presence of these two diseases is one of the more frequent associations included in the polyendocrine autoimmune syndrome type 3 B (PAS 3) (139). The ratio between patients

with overt celiac disease and patients in which the disease is present but not diagnosed is about 1:8 (140). As celiac disease may also present without gastrointestinal suggestive symptoms (CD atypical presentation) (140), its presence should be suspected in hypothyroid patients with an increased need for thyroxine (42). To the best of our knowledge, the impairment of oral levothyroxine absorption in a patient with celiac disease has been reported since 1993 in a patient with milk protein allergy and celiac disease (141). Later, some case reports confirmed the occurrence of resistance to oral levothyroxine treatment in hypothyroid patients with celiac sprue (142, 143). In 2012 two studies (42, 144) have deepened the topic in 7 patients with untreated celiac disease and in 35 subjects affected by atypical celiac disease. In both groups, the gluten free diet reversed the increased hormone needs, only partially in the first study (144) and completely in the second one (42). The mechanisms leading to the increased need for thyroxine in patients with celiac disease may follow the progressive reduction of intestinal surface characterizing the histologic lesions of this disease: a) the villous shortening till total atrophy, owing to enterocyte's apoptosis and to inadequate cell regeneration in crypts (145) and b) the significant lymphocytic infiltration. This event, in turn, determines the loss of proteins and enzymes in the brush border, thus impairing the absorption of a large number of nutrients (146). In CD patients, the extent of impairment of drug efficacy may depend on the pharmacokinetic characteristics of each drug (147). In fact, beside the reduction of the absorbing surface, the following pathologic characteristics were reported in patients with celiac disease: increased intestinal permeability, increased gastrointestinal transit time and changes in intestine luminal pH (147, 148). An abnormal gastric emptying was even described, perhaps depending on an altered secretion of cholecystokinin and plasma polypeptide YY, hormones involved in the gastrointestinal motility (148). An increased prevalence of bacterial overgrowth in the small intestine has been also observed, usually in patients with clinical persistence of symptoms, even after removal of gluten from the diet (149). Similar to other nutrients and drugs, all these mechanisms may contribute to thyroxine malabsorption.

Lactose maldigestion is a condition, mostly symptomless, to which about 70% of adult human beings (lactase non persistent) is exposed (150). This condition is caused by the decline in residual lactase activity, due to the down regulation of the enzyme responsible for the hydrolysis of lactose to glucose and galactose (150). Lactose intolerance (LI), has instead a pleiotropic clinical picture, presenting with diarrhea, bloating, flatulence, abdominal pain, the intensity of them being related to: a) the amount of lactose ingested; b) intestinal microbial composition; c) gastrointestinal motor activity and, finally, d) the visceral sensitivity to fermentation products of lactose digestion (151). Beside the primitive form, lactose intolerance may follow any process able to destroy the mucosa of the small intestine (CD, infections, surgery, etc) (152, 153). At first, Muñoz-Torres (154) described a case of one patient showing an increased need for thyroxine induced by lactose intolerance, resolved by switching to lactose-free thyroxine formulation and starting a lactose free diet. A similar effect was observed following 8 weeks of lactose restriction confirming that lactose-free diet may improve thyroid pharmacologic homeostasis in patients with LI (155). Later, a systematic study confirmed the increased need for thyroxine in a larger group of patients with LI, as compared with a control group without signs or symptoms of malabsorption (+31%)(43). More than a mechanism may explain the effect of lactose intolerance on the need for thyroxine. When the hydrolysis of lactose does not take place, this sugar accumulates and attracts water in the intestinal lumen (151). Thus, an altered intestinal content may complex with thyroxine preventing absorption (43), or may even increase the intestinal motility, thus reducing the exposure of thyroxine to the absorbent surface (156). Moreover, LI is often associated with the small intestine bacterial overgrowth (SIBO) (157) which may also impair the enterohepatic recycling of thyroxine.

Lactose is also used as an excipient in some drugs and in some formulation of tablet thyroxine, and the amounts is not often disclosed in the leaflets (27). This fact led to the suspicion that lactose content in these thyroxine preparations may represent a problem: a study, however, provided evidence that ingestion up to 400 mg of lactose does not trigger gastrointestinal symptoms nor affect the lactose breath test (158). Noticeably, gastrointestinal symptoms are usually evoked by quantities greater than 5 g of ingested lactose (159). Therefore, the possibility that a very small amount of the disaccharide in the thyroxine tablet might be responsible for its malabsorption is unlike.

The parasitic infestation from *Giardia lamblia* (GL) has also been considered as a cause of thyroxine malabsorption. This parasitism is sporadic in Western countries where it seems more frequent in travellers (160). On the contrary, this flagellate protozoan is usually endemic in environments characterized by poor sanitary conditions (161). Clinical manifestations range from severe gastrointestinal derangement to asymptomatic forms (162). Only two case reports described an increased need for thyroxine due to the GL infestation (163, 164), both describing an elevation of serum TSH and an increased need for thyroxine that were reversed by appropriate antiparasitic treatment. The cause of thyroxine malabsorption in these patients is the inflammatory mucosal damage and epithelial apoptosis induced by the protozoan (165); furthermore, in these patients an increased intestinal permeability may be observed, due to the disruption of the intestinal tight junctions (166).

Besides the disorders directly affecting the absorbent mucosa, also diseases affecting organs involved in the digestion process may impair oral thyroxine efficacy. Indeed, pancreatic insufficiency may cause steatorrhea following the reduction of lipase secretion below 10% of normal levels (167). In an early paper on eight patients published in 1962 (168), the authors observed that pancreatic steatorrhea increased the elimination rate of the hormone, showing that fecal losses of the hormone were higher than normal. They hypothesized a defective hydrolysis of glucuronated thyroxine that impaired the enterohepatic circulation in these patients. A direct interference with compounds usually degraded by pancreatic enzymes, resulting in an organic iodine adsorption in the intestinal content was also suggested (168). Cystic fibrosis that frequently leads to pancreatic insufficiency has been reported as a cause of decreased thyroid hormone absorption (169). Furthermore, a recent paper described two cases in which an increased need for thyroxine has been shown in patients bearing liver cirrhosis (170). The authors speculated that the impaired bile secretion, besides the variation in Tiroxine Binding Globulin concentration, might explain this effect. On the same line, a paper by Sinha and Van Middlesworth (115) underlined the role of bile in reducing the binding of thyroxine to intraluminal plasma proteins, thus increasing the absorption of labeled thyroxine in a washed jejunal loop of rats. The authors postulated the presence in the bile of substances competing for T4 binding with plasma proteins (115).

In a previous section we have described the putative mechanism of thyroxine absorption at the small intestine level; the effect of the short bowel syndrome (SBS) on thyroxine absorption has been investigated since ‘80s (171, 172). This disease is usually the consequence of congenital malformations or acquired diseases that require surgical resection of small intestine, thus causing the reduction of intestinal absorptive surface along with motility disturbances (173). Moreover, the absence of last ileal loop and of ileo-cecal valve may lead colonic bacteria to rise in the residual small intestine prodicing a dysbiotic environment dominated by *Lactobacilli* (174). The study of radiolabelled thyroxine absorption in five patients with SBS as compared to two healthy subjects, revealed a net reduction of oral thyroxine absorption, independently from the lengths of intact bowel (171). It has been suggested that, beside the shortened intestinal surface, a key role in determining T4 malabsorption in these patients may be played by the accelerated intestinal transit (175).

Bariatric surgery

On a similar ground, the interference on thyroxine absorption exerted by bariatric surgery including intestinal bypass has been extensively analyzed, although with conflicting results (see above for bariatric surgery involving only the stomach). The three case reports about the effects of jejunoileal bypass technique consistently reported a significantly (from three to four fold than normal dose) increased T4 need, again suggesting the role of a reduced absorptive surface (176-178). This technique has been next abandoned because of the association with multiple severe short- and long-term complications. Nowadays, most of the paper published on this topic examined the need for T4 in patients who underwent Roux-en-Y gastric bypass (RYGB), a common procedure nowadays. By examining 23 patients, Raftopuolos et al. observed that 13 of them maintained the same daily requirement while eight showed a reduction of the dose (179). However, as mentioned above, Sundaram (133) and Fierabracci (134) described that, when normalized by weight, an increased thyroxine dose is necessary to achieve target TSH levels in most of these patients. These results are in keeping with the increased TSH values observed in patients treated with the same dose of thyroxine before and after surgery (180, 181). Two pharmacokinetic studies have been performed to investigate this issue (131,182). Despite an improvement of absorption parameters, Rubio et al. (182) observed a significant delay of T4 absorption in the surgical-treated patients. In the previously mentioned study (131), the pharmacokinetic parameters were similar before and after RYGB surgery. This latter study also examined 15 patients who underwent biliopancreatic diversion observing an improvement of thyroxine pharmacokinetic parameters (131). However, Fallahi et al (181), by using the same dose of tablet formulation of thyroxine before and after the surgery, observed that serum TSH worsened after surgery. While the results in patients exclusively treated with malabsorptive procedures agree to identify a clear increase in thyroxine requirement, results about thyroxine need in patients treated with procedures combining restrictive and malabsorptive techniques are conflicting. This may depend on the different schedule of thyroxine ingestion (often omitted) (i.e. lag time between food and therapy or other drugs) and on the diverse effects that surgery may have on patient's gastrointestinal physiology (i.e. altered gastric juice acidity, dumping syndrome, delayed gastric empting, different gut microbial flora, variations of lean and fat body mass ratio, etc)(183, 184). Moreover, the assessment of thyroxine needs not always normalized by weight does not allow a correct and complete comparison of the data. The heterogeneity of these studies in patient underwent bariatric surgery with mixed restrictive and malabsorptive techniques does not allow to draw definitive conclusions about the net effect on thyroxine requirement.

Nutrients and food interference

In 1977, using a double isotope method, Wenzel and Kirschsieper (31) clearly demonstrated that fasting condition ensures a better thyroxine absorption than in a feeding state. Over time, several papers have examined the interference exerted by different kinds of foods on the steps that bring to levothyroxine absorption.

As early as 1957, van Middlesworth (185) reported that cellulose- or bran-rich chow leaded to increased fecal loss of thyroxine in rats. In 1996, Liel et al (186) demonstrated a nonspecific, dose dependent adsorption of the hormone by wheat bran *in vitro*. This may explain the clinical findings of an increased need for thyroxine in 13 hypothyroid patients who had increased their fiber intake (186). On the contrary, a succeeding pharmacokinetic study in 8 healthy volunteers did not confirmed a variation in the absorption rate of thyroxine ingested with calcium polycarbophil or psyllium hydrophilic mucilloid (187). The possible interference of fibers on the enterohepatic recycling of thyroxine more than a direct effect on absorption has been suggested (188, 189) and may help to interpret these data. In fact, a similar mechanism has been postulated for the interference exerted by soy ingestion in thyroxine treated patients. In this first report in humans by Pinchera et al. (190), an athyreotic

cretin, fed soybean formula, showed a refractoriness to high doses of desiccated thyroid. Additional evidence were an increased fecal concentration of thyroxine and a higher fecal mass, so that they hypothesized an intestinal dysbiosis as a further cause of intestinal trapping of thyroxine (190, 191). Several studies on this topic have confirmed the effect of soy formula in hypothyroid infants (192-194) and one study reported a similar effect also in a thyroidectomized woman using soy protein supplement (195). In this latter case report, the efficacy of thyroxine treatment has been regained separating thyroxine from soy ingestion by 12 hours. A very recent pharmacokinetic study, performed in 12 patients on stable thyroxine treatment, demonstrated that bioavailability of thyroxine is superimposable in patients co-ingesting a fixed combination of soy isoflavones or with a six hours interval (196). A case report also described the impairment of pharmacological thyroxine homeostasis in a thyroidectomized patient after two weeks of large amount of papaya fruits consumption (113). After discontinuation of papaya consumption, the patients recovered the euthyroid state without increasing thyroxine dosage. Beside the effects on gastric acid secretion (see above), the authors hypothesized an effect on intestinal motility of a component of the fruit and an action on deconjugating bacterial enzymes that may increase fecal thyroxine loss (113). A brand new paper (114) has directly reported the effect of concomitant 2% fat cow-milk intake with thyroxine (see above), hypothesizing also an interfering role for milk proteins, based on evidence from *in vitro* studies by Hays (29) and Sinha and van Middleworth (115).

Even the espresso coffee may cause refractory hypothyroidism. The concomitant ingestion of coffee was examined *in vivo* and *in vitro* by Benvenga et al (33). The simultaneous ingestion of coffee and levothyroxine altered pharmacokinetic parameters also delaying the time of maximal incremental rise of serum T4. The *in vitro* study confirmed that coffee acts as a weak thyroxine sequestrant (33). The putative effects on thyroxine transporter activity by grapefruit juice have been discussed above.

Drugs interference

Several drugs interact with intestinal absorption of levothyroxine. In most cases, these drugs are able to bind levothyroxine creating insoluble complexes that prevent successive thyroxine absorption process but this kind of interaction has not been proven for all of them. Some of these drugs seem to affect different steps of thyroxine absorption. Some clinical reports stated that aluminum hydroxide, active ingredient of antacid preparations, affects thyroxine pharmacological homeostasis (197). In particular, the impact of this drug on thyroxine absorption seem to involve both gastric and intestinal processes of absorption. In fact, beside the abovementioned increase of gastric pH and the slowing of gastric empting, a nonspecific adsorption of T4 *in vitro* (20% of the 50 mcg in the incubation system were removed by 1 ml of aluminum hydroxide) (198) may further hamper oral T4 efficacy. Conflicting results *in vitro* and *in vivo* where obtained with a further antiacid, sucralfate. In fact, in an early *in vitro* study the binding of thyroxine by sucralfate in gastric acid medium has been shown (199). Later on, one pharmacokinetic study, carried out on healthy volunteers, demonstrated that sucralfate reduces the maximum T4 absorption of about 70% with a significant delay in the absorption process (200). However, two clinical studies failed to confirm these findings (201, 202). This issue deserves clarification by further studies. On the contrary, the concomitant ingestion of ferrous sulfate has been proven to reduce thyroxine efficacy in hypothyroid patients (203-205). In fact, an *in vitro* experiment demonstrated that at pH 7.4 a ferric-ion binds three thyroxine molecules in an insoluble complex (203). A seminal paper published in 1969 (206) demonstrated that also cholestyramine resin, used as a lowering cholesterol agent (able to bind bile acids and cholesterol metabolites in the intestinal lumen), is able to bind large amount of thyroxine either at a pH 1 or 9 *in vitro*. By using ^{131}I labeled thyroxine in two patients, the authors demonstrated a two-fold increase in stool and a concomitant reduction in

urine radioactivity as compared to control values, thus proving a negative effect on thyroxine absorption (206). An interval of five hours from levothyroxine to cholestyramine ingestion was not at all sufficient to restore thyroxine absorption, and authors suggested that cholestyramine may act as an inhibitor of hormonal enterohepatic recycling (206). Because of this specific activity, this drug has been also used to treat acute hyperthyroid patients (207, 208, 209). The ability to impair the pharmacokinetic parameters of levothyroxine has been described in two studies for a further bile acid sequestrant, colesevelam (210, 211). This drug has been proven to bind thyroxine, *in vitro*, in an increasing fashion from a medium pH=1,2 (similar to gastric pH) to pH=6,8 (simulating intestinal fluid) (210, 212). An in vitro binding has been proven also for a cation-exchange resin, sodium polystyrene sulphonate (SPS) (213). Also this ability of SPS has been proven in both an acidic medium (pH 2) and a neutral one: the reduction of dissolved thyroxine in the supernatant was of 93% and 98%, respectively.

For some drugs, despite an *in vitro* binding study has not been performed, pharmacokinetic studies demonstrated a reduction of thyroxine absorption. This is the case for ciprofloxacin, sevelamer, chromium picolinate, lanthanum carbonate and raloxifene. Coadministration of these drugs with 1000 mcg of thyroxine in healthy subjects decreased the T4 AUC from 0 to 6 hours ranging from 17% to 50% as compared with the one observed in control test (81, 211, 214, 215). Finally, a possible interference of orlistat (216) and simethicone (217) with thyroxine absorption has been only mentioned in case reports.

The use of malabsorption of thyroxine as a tool to diagnose occult GI disorders

A tight schedule of thyroxine treatment represents a prerequisite to detect any increased need for thyroxine. Patients requiring a higher than expected dose of thyroxine to reach the target TSH may be encompassed in the definition “difficult patients” according to Ward LS (218). They are exposed to refractory hypothyroidism (3), but not all of them have GI malabsorption. Once ascertained the existence of an increased need for thyroxine, physician must firstly exclude the pseudomalabsorption, the recent and significant weight, BMI and/or body composition variations as well as the possibility of pregnancy. Then a careful nutritional and pharmacological anamnesis should be carried out (Figure 4). Once these issues have been positively excluded, and gastrointestinal disorders may be reasonably suspected, a diagnostic workup should be started (Figure 4). The clinical background should help in the diagnostic strategy, particularly when refractory iron-deficient anemia is concurrently present (219): this kind of anemia is, in fact, often associated to H. Pylori infection (220), celiac disease (221), and atrophic gastritis, prior to pernicious anemia (219). These latter are frequently associated with autoimmune hypothyroidism in the type III polyglandular autoimmune syndrome (98, 138, 222, 223) even in young patients (223, 224). A previous or active H. pylori infection must be screened as a first line as it is the most prevalent disorder (102). Either ^{14}C-Urea breath test or fecal H.pylori antigen detection and the measurement of antibodies against H. Pylori should be performed (102). If positive, following the appropriate treatment, the involvement of gastric mucosa should be investigated by endoscopy with multiple biopsies (225). Once the H. Pylori infection has been excluded, other possibilities should be investigated by measuring the anti-parietal cell antibodies (APCAb) and fasting gastrinemia (226). High serum gastrin levels and the presence of APCAb support the suspicion of an autoimmune gastric atrophy (227) and again performing endoscopy with multiple biopsies of gastric body and antrum is strongly suggested (94). In the presence of iron deficient anemia and/or abdominal bloating and pain and/or chronic diarrhea, celiac disease deserves attention (136). The assessment of anti transglutaminase antibodies (tTgAb) and total serum IgA levels may help to diagnose celiac disease (136) but, once again, only endoscopy may confirm or exclude the presence of this disease (228). If the suspicion concerns other intestinal disorders,

the diagnostic workup should include some breath tests (lactose, glucose, lactulose) able to detect lactose intolerance (229), bacterial overgrowth (230) and/or altered transit time in the gut (230), respectively. These breath test have been considered reliable, useful and safe tools in diagnose carbohydrate maldigestion, being also the least invasive tools to diagnose SIBO (231). Finally, the stool examination should be kept in mind when parasitism is suspected (e.g. Giardia lamblia) (164). Many of the described gastrointestinal disorders may occur in a hidden or even occult way (Figure 2). In this case, the diagnostic workup should be driven by the prevalence of each diseases, taking into account patient's and his/her familial medical history. A successful diagnostic workup may uncover the vast majority of gastrointestinal cause of poor response to thyroxine treatment, but would also be useful to diagnose and treat these silent, but not harmless, gastrointestinal disorders. In this view, malabsorption of T4 may represent a tool to diagnose occult gastrointestinal diseases.

ALTERNATIVE Pharmacologic Perspectives

While the tablet formulation of thyroxine remains the gold standard in the treatment of hypothyroidism, hormone paraphernalia evolved. The "difficult patient" as described by Laura Ward (218) is probably more than an exception in the treatment of hypothyroidism. The most recent advance concerning thyroxine treatment stems from the growing attention to alternative oral formulations: the liquid preparation and the softgel capsule. The soft gel capsule contains, in an outer gelatine shell, T4 dissolved in glycerine (23). This structure warrants a rapid dissolution in the acid gastric environment (232). The liquid formulation is composed of T4, glycerine and ethanol and its most important feature is that it does not require a gastric dissolution (16). A 2-way crossover, bioequivalence study in 84 healthy subjects has been carried out to compare T4 tablets, soft gel capsules and oral solution (21). Mean pharmacokinetic parameters were not statistically different between these formulations; however, a faster absorption of thyroxine solution was observed probably because of the lack of the dissolution phase (21). It is beyond the scope of the present review to analyze in detail the advantages and disadvantages of alternative preparations of thyroxine, which were, however, recently reviewed elsewhere (233). Here we analyzed the studies concerning the possible use of these alternative preparations in patients with gastrointestinal disorders. In a prospective study in 31 patients with gastric-related T4 malabsorption, stably treated with tablet thyroxine preparation and switched to softgel T4, the effective dose of this latter preparation was significantly reduced in about 2/3 of patients (234). More recently, very preliminary data suggested that the switch from tablet to softgel T4 preparation at unchanged dose improved TSH even in patients taking pill 30' before the breakfast (235). Furthermore, softgel T4 preparation helps to overcome the problem of coffee PPI interference on tablet T4 treatment (236, 237).

The obvious and most important advantage of the liquid T4 solution is the possibility of administration in pediatric age (238-240) or in patients who are not able to swallow tablets or capsules (233, 241). In hypothyroid newborns and infants the daily thyroxine dosage is calculated according to age and T4 tablets are usually crashed and given with liquids (239, 240). In these little patients, liquid thyroxine formulation is easily managed and administered and may is also better absorbed than the tablet formulation (240). The European Society for Paediatric Endocrinology (ESPE) guidelines acknowledged this alternative treatment in pediatric age (242). In adult hypothyroid patients, some studies analyzed whether the restraints of tablet thyroxine treatment may be overcome by the use of this preparation: i.e., whether the foods may interfere with liquid thyroxine absorption as they do with tablet formulation (243, 244). A double blind, randomized, placebo-controlled, crossover trial suggested that a liquid T4 formulation could be ingested directly at breakfast, potentially improving therapeutic compliance (244) by reducing one of the major issues in the treatment

with tablet T4: the negative impact of breakfast on the treatment efficacy (31, 36, 37, 40, 244, 245). Liquid thyroxine preparation was tested even in patients with gastrointestinal malabsorption. In fact, in a prospective observational study, the liquid preparation has been proven to overcome T4 malabsorption induced by the increased gastric pH due to PPI treatment (246) and by lactose intolerance (247). Similarly, in a case series, it has been shown that oral liquid formulation might bypass the pH alteration due to atrophic gastritis in patients who were still hypothyroid when treated with tablet thyroxine (248). As previously discussed above, drug malabsorption is a potential concern after bariatric surgery. In a recent study, hypothyroid patients, well replaced with thyroxine tablets before surgery (13 RYGB; 4 biliary pancreatic diversions (BPD), showed an increased TSH 3 to 8 months after surgery (181); the switch to liquid T4, at the same dose, significantly improved TSH values also in patients with BPD, confirming previous studies in patients submitted to RYGB (180).

Despite some encouraging results in patients with gastrointestinal malabsorption the overall analysis of these studies revealed some limitations: the entire field deserves further systematic studies before drawing definite conclusions (249).

Concluding Remarks

Gastrointestinal malabsorption of oral thyroxine is more frequent than previously reputed and may account for a significant fraction of refractory hypothyroidism. On this ground, an accurate individualization of hormonal treatment helps in the detection of gastrointestinal T4 malabsorption. Although the site of thyroxine absorption is the small intestine, gastric pH emerges as a major prerequisite for the efficacy of tablet thyroxine treatment. An increased need for thyroxine should induce endocrinologist to start a diagnostic workup, based on the clinical features and on the prevalence of gastrointestinal disorders.

Despite the growing number of studies, some questions are far from being clarified. The pathophysiologic mechanisms of thyroxine absorption at the intestinal level are not yet completely defined as shown in the real life by a number of patients in whom the cause of an increased need for T4 remain obscure. In particular, the intestinal transport pathways, the actual contribution of the enterohepatic recycling and the role of microbiota composition in the absorption of thyroxine in humans are issues for future studies.

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Figure 1. Different ionization status of the three ionizable moieties of the thyroxine molecule at different medium pH. pK_{a1}: ionization constant of the carboxyl group. pK_{a2}: ionization constant of the phenolic group. pK_{a3}: ionization constant of the amino group

Figure 2. Main pathologic and post-surgical conditions leading to thyroxine malabsorption. Arrows indicate the progressively lower frequency of these disorders. Diseases have been subdivided based on the primitive site involved (stomach and intestine).

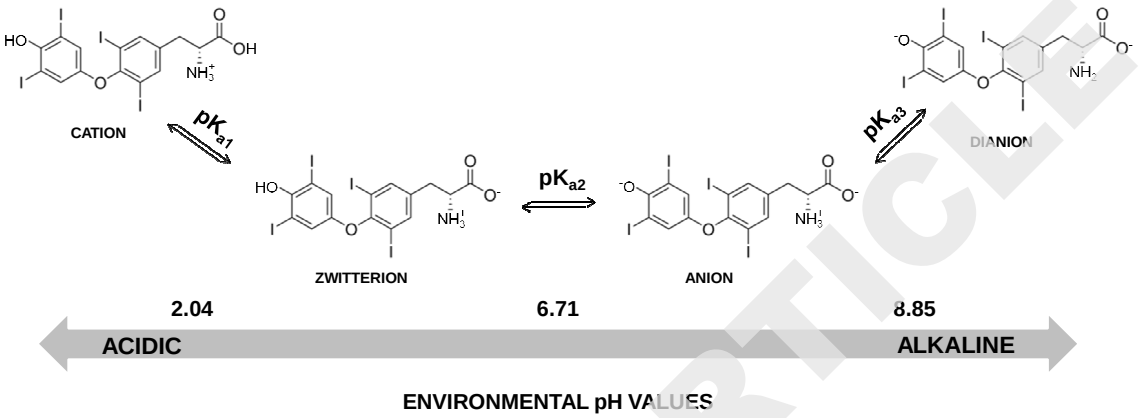
Figure 3. Nutrients, foods and drugs interfering with thyroxine absorption. In the yellow circle: gastric level as the prevalent site of interference. In the red circle: intestinal level as the prevalent site of interference. In the orange overlapping part: interference exerted at both gastric and intestinal levels.

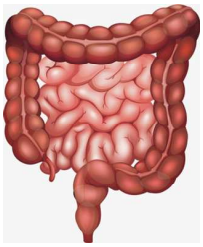
Figue 4. Diagnostic work up for the main pathologic gastrointestinal causes of thyroxine malabsorption based on the clinical ground and/or the prevalence of each disorder in the general population. Grey boxes: issues that must be excluded before starting diagnostic work up. Orange boxes: diagnosed disease. Green boxes: diagnostic method.

Table 1. Excipients in T4 tablet formulations.

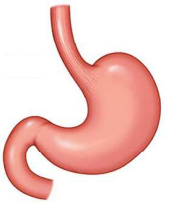
	Tablet, brand							Tablet, generic			
	1	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**
Cellulose	√	√		√	√		√			√	
Talc		√				√	√				
Povidone				√		√					
Colloidals silicone dioxide		√								√	
Starch		√	√			√	√	√	√	√	√
Acacia						√		√			
Gelatin			√								
Mg stearate ± stearic acid	√	√	√	√	√	√	√	√	√	√	√
Na croscarmellose	√		√		√						
Na citrate							√	√			
Na carboxymethylamide							√				
Ca phosphate				√			√				
Ca sulphate					√						
Bicarbonate					√						
Lactose	√		√			√		√	√		√
Dyes (color additives)	√				√	√					

***TABLET, BRAND*- 1= Bagothyrox** (Quimica Montpelier); **2= Eltroxin** (GlaxoSmithKline); **3= Euthyrox** (Merck); **4= Levothroid** (Forest/Sanofi Aventis); **5= Levoxyl** (Jones/King Pharmaceuticals); **6= Synthroid** (Abbott); **7= Tirosint** (IBSA Farmaceutici Italia srl);
***TABLET, GENERIC*- 8=** (Mercury); **9=** (Almus); **10=** (Sandoz); **11=** (Actavis)





GASTROINTESTINAL MALABSORPTION OF THYROXINE: DISEASES AND DISORDERS



FREQUENTLY OCCULT

LACTOSE MALDIGESTION

CELIAC DISEASE

SMALL INTESTINE BACTERIAL OVERGROWTH

PARASITIC DISEASES

MOTILITY IMPAIRMENTS

HELICOBACTER PYLORI INFECTION

ANTRITIS

PANGASTRITIS

GASTRIC ATROPHY

MOTILITY IMPAIRMENTS

CLINICALLY EVIDENT

SHORT BOWEL SYNDROME

BILIOPANCREATIC DIVERSION

RYGB

GASTRIC BANDING

SLEEVE GASTRECTOMY

LIVER CIRRHOSIS

PANCREATIC STEATORRHEA

NUTRIENTS, FOODS AND DRUGS: PREVAILING SITE OF INTERFERENCE WITH THYROXINE ABSORPTION