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Inositol(s) in thyroid function, growth and autoimmunity

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Abstract Myo-inositol and phosphatidylinositol(s) play a pivotal function in many metabolic pathways that, if impaired, impact unfavorably on human health. This review analyzes several experimental and clinical investigations regarding the involvement of this class of molecules in physiological and pathological situations, with a major focus on thyroid. Central issues are the relationship between phosphatidylinositol and thyrotropin (TSH) signaling on one hand, and phosphatydylinositol and autoimmunity on the other hand. Other issues are the consequences of malfunction of some receptors, such as those ones for TSH (TSHR), insulin (IR) and insulin-like growth factor-1 (IGF-1R), or the connection between serum TSH concentrations and insulin resistance. Also covered are insulin resistance, metabolic syndrome and their allied disorders (diabetes, polycystic ovary syndrome [PCOS]), autoimmunity and certain malignancies, with their reciprocal links. Myoinositol has promising therapeutic potential. Appreciation of the inositol pathways involved in certain disorders, as mentioned in this review, may stimulate researchers to envisage additional therapeutic applications.

Keywords Myo-inositol · Phosphatidylinositol · TSH · TSHR · Insulin · IGF-1R

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1 Introduction

The family of inositol(s) (INS), which are hexahydroxycyclohexanes ($C_6H_{12}O_6$) deriving from cyclohexane, includes nine stereoisomeric forms. The molecule has a 6-carbon atoms ring with the hydroxyl group attached to each carbon of the ring; the isomers are obtained by epimerization of the six hydroxyl groups [1]. Of these stereoisomers, myo-inositol (MI) was the first to be identified. It was isolated from muscle extracts by the German physician and chemist Johann Joseph Scherer in the year 1850, and was called "inositol" because of its tissue origin and chemical composition [2, 3]. Later, this compound was found to be the main element of phytates [4]. MI constitutes almost all (>99 %) of the INS contained in the intracellular pool of most tissues. The interest for INS has waxed and waned, but in the last decades the scientific research has grown. Diet intake and endogenous biosynthesis are the sources of INS. In food, they are contained mainly in citrus fruits (with the exception of lemon), cereals with high bran content, nuts and beans [5]. In the human body MI is synthesized from glucose [6], with liver and kidneys being the main organs of synthesis.

MI is fundamental for our health, because it is one of the precursors for the synthesis of phosphatidylinositol (PI) polyphosphates, which accomplish relevant physiological functions [7]. MI is incorporated into cell membranes as phosphatidyl-MI, the precursor of inositol trisphosphate (InsP3), which is a second messenger participating in the transmission

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of numerous signals [8]. As important constituents of the structural lipids in cells, INS derivatives exert relevant cell and metabolic functions, such as morphogenesis, cytoskeleton rearrangement, regulation of cell proliferation and glucose metabolism [9, 10]. INS are deeply involved in signaling of hormones, such as insulin, gonadotropins (follicle stimulating hormone [FSH] and luteinizing hormone [LH], and thyroid stimulating hormone [TSH]). In thyroid, imbalances in the INS metabolism can impair hormone biosynthesis, storage and secretion.

Our review aims to offer an in-depth view on the metabolic pathways, primarily related to the thyroid gland, where MI and phosphatidylinositol(s) play a key role. Changes in the metabolism of MI and phosphatidylinositol(s) have consequences on health. However, a physiological state can be restored, because MI is a water-soluble molecule that is well tolerated and available as a nutraceutic.

2 Phosphatidylinositol and TSH

2.1 Thyroid hormone biosynthesis, storage, and secretion

Thyroid hormone biosynthesis, storage, and secretion require a series of highly regulated steps [11]. Thyroid hormone synthesis, starting with iodide uptake, is regulated primarily by TSH via binding to its cognate receptor (TSHR) (Fig. 1), the TSH/TSHR pair also being the master trophic hormone of the thyrocytes [11]. TSHR, which is expressed on the basolateral membrane of the thyrocyte, is a member of the G-protein coupled seven transmembrane receptor family, the same family to which, among others, the FSH, LH and chorionic gonadotropin (CG) belong to (Fig. 1). After iodide has been actively transported inside the thyrocyte by the sodium-iodide symporter (NIS), it is oxidized by TPO. This oxidation of iodide requires the presence of H_2O_2, which is generated by DUOX2, a calcium-dependent flavoprotein NADPH oxidase. The physiologic H_2O_2 production represents a limiting factor for the organification process, i.e., the reaction by which inorganic iodine is bound to tyrosine residues by thyroid peroxidase (TPO) [12]. Overproduction and/or lack of H_2O_2 degradation may favor inflammatory and neoplastic disorders of the thyroid, whereas impaired H_2O_2 production may cause congenital hypothyroidism [13]. Studies on certain naturally occurring inactivating mutations of the TSH receptor (TSHR) showed that such mutations do not impair cAMP generation but they decrease dramatically the efficacy of TSH for generating the inositol phosphates (IP) [14]. As a result of this impaired IP generation, the TSHR/IP/Ca^{2+} cascade is hypofunctioning and results in lower iodine organification [14].

In the follicular lumen, thyroglobulin (Tg) serves as matrix for the synthesis of both thyroxine (T_4) and triiodothyronine (T_3), a synthesis that requires TPO to catalyze the iodination (or organification) reaction (that is, to iodinate selected thyrosine residues of Tg to form mono- and di-iodothyrosines [MIT and DIT]), and then the coupling reaction (that is, to couple two iodothyrosine to form either T_4 or T_3). In thyrocytes, TSH dose-dependently stimulates inositol phosphate formation [15], with both the phosphatidylinositol 4,5-bisphosphate (PiP2) cascade and the cyclic AMP (cAMP) cascade controlling the synthesis of thyroid hormones.

2.2 TSH/TSHR/PKC/IP3 and TSH/TSHR/PKA/cAMP pathways

Binding of TSH to TSHR leads sequentially to coupling Gsα, subsequent activation of adenylate cyclase, formation of cAMP, phosphorylation of protein kinase A (PKA) and activation of downstream proteins in the cytosol and nucleus (Fig. 1). This cascade (or pathway) is the major regulator of thyroid hormones synthesis, growth and differentiation of the thyrocytes. At higher concentrations of TSH, again after TSH binding to TSHR, stimulation of $G\alpha_{q/11}$ and the phospholipase C (PLC)-dependent inositol phosphate Ca^{2+}/diacylglycerol (DAG) pathway (with formation of inositol 1,4,5-triphosphate [IP3]) activates H_2O_2 generation and iodination. IP3 increases the concentration of intracellular Ca^{2+} by favoring its release from the endoplasmic reticulum. H_2O_2 is a limiting factor in the iodide oxidation and organification, and the coupling reaction as well. Generation of H_2O_2 is activated by the TSH/TSHR/PKC/IP3 pathway (and agents that stimulates the PI pathway), and the TSH/TSHR/PKA/cAMP pathway. This is an example of the cross-talks between the two pathways of the TSHR signaling [15]. Of interest, the signaling pathway of phosphatidyl inositol 3 kinases (PI3K) is used by T_3 to upregulate the leptin gene expression in adipocytes [16].

Phospholipases also release arachidonic and linoleic acids that can be metabolized by lipoxigenase to form free radicals and lipid peroxides; these radicals and peroxides provide an inflammatory stress that can induce apoptosis, TSH, and cAMP agonists as well, protect thyrocytes from apoptosis that normally would be induced by H_2O_2 and other molecules [17]. In addition to PKA, this protective action of TSH involves PI3K, because inhibitors of the PI3-kinases promote thyrocyte apoptosis [11, 17].

In cultured thyrocytes addition of somatostatin inhibits the proliferative responses induced by both TSH and insulin. Somatostatin also lessens TSH-mediated activation of adenylate cyclase, and inhibits PI3K in a PKA-independent mode [17].

Several cytokines alter thyrocyte growth and function, and such cytokine effects are influenced by TSH and cAMP. Once cytokine receptors in the plasma membrane are activated, they attract the cytoplasmic tyrosine kinase JAK (Janus kinase), creating docking sites for SH-2-containing proteins like the STATs (signal transducer and activator of transcription).

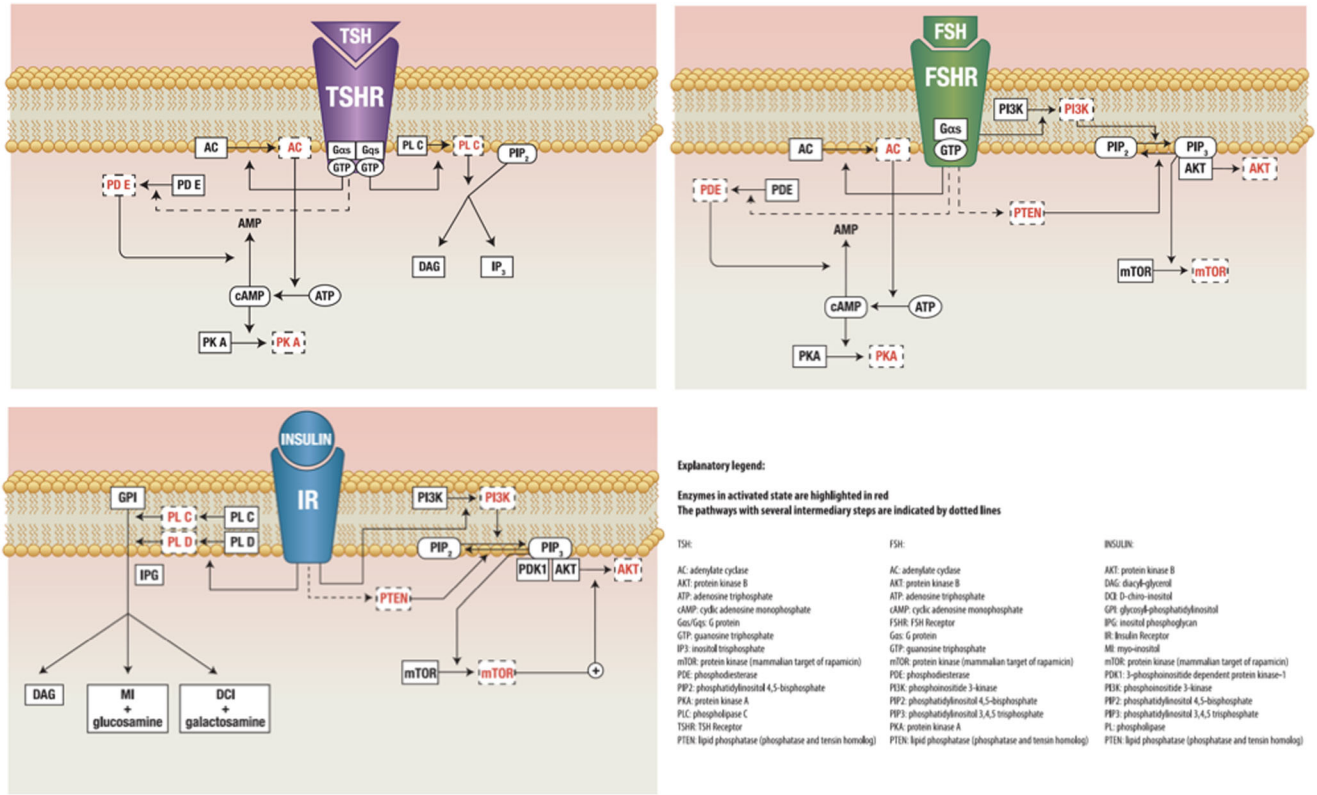

Fig. 1 Principal pathways of activation of the receptors: TSH receptor (TSHR), insulin/insulin receptor (IR), insulin-like growth factor-1 (IGF-1)/IGF-1 receptor (IGF1R) and follicle stimulating hormone receptor (FSH)

TSH phosphorylates STAT3, an action that can be blocked by inhibitors of protein kinase C (PKC), but not by inhibitors of PKA [17].

2.3 Two studies on molecular pathways in thyroid nodules

The ligand/receptor pair hepatocyte growth factor (HGF)/HGF-receptor (or c-met) induces cell growth, scattering and morphogenesis that, downstream, are mediated by STAT3, PI3K/protein kinase B (Akt) and Rho [18, 19].

In a study [18], which is pertinent in view of a subsequent section concerning Hashimoto's thyroiditis [HT], the immunohistochemical expression of the ligand/tyrosine kinase receptor pair HGF/c-met and three transducers of tyrosine kinase receptors (STAT3, PI3K, Rho) was evaluated in both the nodular tissue from 50 benign nodules (that is, colloid nodules) and paired extranodular tissue; 25 of the 50 nodules were associated with HT and 25 were not (control group). HGF/c-met and the two transducers PI3K and Rho were expressed in bening nodules, regardless of association with HT, with a higher rate of positive cases in HT-associated nodules compared to not HT-associated nodules (25/25 or 100 % vs 7/25 or 28 %; $P < 0.001$). HGF, PI3K and Rho expression was only stromal (fibroblasts and endothelial cells) in all 32 reactive benign nodules, while c-met localization was consistently epithelial (thyrocytes). Immunoreactions for HGF, c-met, PI3K and Rho were also apparent in the extra-nodular tissue of HT specimens, where HGF and PI3K were expressed not only in stromal cells but also in thyrocytes along with the c-met. Finally, a positive correlation was observed between the proportion of HGF, c-met, PI3K follicular cells and the grade of lymphoid aggregates in HT. In conclusion, HGF, c-met, PI3K are much more frequently and highly expressed in the extranodular tissue from thyroid glands affected by HT compared to the extranodular tissue from benign nodules of HT-free thyroid glands, and among all benign nodules in those present in the context of HT. HGF/c-met exerts a paracrine action on nodule development and expression of HGF/c-met/PI3K is regulated by the intrathyroid lymphocytic infiltrate [18].

In another study [19], the immunohistochemical expression of HGF, c-met, STAT3, phosphorylated-STAT3 (pSTAT3), PI3K, Akt and Rho was investigated in 83 benign thyroid nodules [30 colloid nodules, 18 hyperplastic nodules, 20 follicular adenomas, 15 oncocytic adenomas], and 46 thyroid cancers [20 papillary (PTC), 16 follicular (FTC) and 6 anaplastic (ATC)]. All seven proteins were expressed in 15 % of follicular adenomas (with HGF, PI3K and Rho having stromal reactivity) and 25 % of PTC. The combination HGF/c-met/STAT3/pSTAT3/PI3K was expressed by all PTC, each protein being expressed by the tumor cells. In contrast, 13/

16 FTC (81 %) expressed PI3K (with both epithelial and stromal localization), and 100 % of ATC expressed PI3K (both epithelial and stromal localization) and Rho (epithelial localization). Epithelial expression of PI3K correlated with the clinical behavior of histotypes. Within FTC, the proportion of PI3K+ cells correlated with both the clinical and pathological stage ($r = 0.95$; $P < 0.001$). As for the shared epithelial expression of PI3K, this concerned approximately one-fourth of tumor cells in FTC and ATC vs one-thirtieth in PTC [19].

3 TSH/TSHR, insulin/insulin receptor (IR), insulin-like growth factor-1 (IGF-1)/IGF-1 receptor (IGF1R)

3.1 TSH and IGF-1 synergism in thyrocyte proliferation

PI3K can be activated both by G-protein-coupled receptors (like TSHR) and tyrosine kinase receptors (like IR and IGF-1R) (Fig. 1) [20, 21]. Due to this dual activation, and associated intersection of the two signals, differences in signal responses may arise, with PI3K transducing a number of actions of TSH in thyrocytes. TSH induces IGF-1R production in thyrocytes, and potentiates the IGF-1-mediated proliferation via pathways associated with PI3K, tyrosine kinase, and mitogen-activated protein kinase (MAPK) [17]. Thus, TSH and IGF-1 are synergistic in causing thyrocyte proliferation. In Wistar rat thyrocytes, PI3K is required for cAMP-stimulated mitogenesis, an action that takes place via both PKA-dependent and PKA-independent pathways [17]. However, IGF-1 blocks the TSH-induced increase in NIS mRNA, a block that can be eliminated by inhibiting PI3K. Although TSH amplifies insulin and IGF-1 signaling in primary cultures of canine thyrocytes, it does not change PI3K activity [17]. In dog and human thyrocytes, TSH or cAMP are mitogenic only if insulin is present [17].

A functional IGF-1R is required for the cell to progress through the cell cycle. Most importantly, cells lacking this receptor cannot be transformed by any of a number of dominant oncogenes, a finding that proves that the presence of the IGF-1R is important for the development of a malignant phenotype [22]. Consistent with this role, IGF-1 can protect cells from apoptosis under a variety of circumstances.

3.2 Overexpression of IR in cancer cells

The expression of IGF-1 and IGF-1R was evaluated in normal and neoplastic thyroids (50 adenomas and 53 carcinomas) [23]. Compared to normal thyroid tissue, increased expression of immunoreactive IGF-1 was found in 31 adenomas (62 %) and 38 carcinomas (72 %); increased expression of immunoreactive IGF-1R was found in 40 adenomas (80 %) and 42 carcinomas (79 %). A corresponding up-regulation of IGF-1 mRNA was documented in carcinomas, which contrasts with the corresponding down-regulation in adenomas. Immunoreactivity for IGF-1 and IGF-1R positively correlated with tumor diameter and wide intrathyroidal extension, but not with patients' gender and age or with the stage of the tumors and the occurrence of lymph node metastases [23].

In a study based on the establishment of cultures of follicular cell precursors as thyrospheres from three PTC and the corresponding non affected tissues, the expression of IR, IGF-IR, and their ligands was evaluated. Thyrospheres from normal tissue, but not from cancer tissue, could be induced to differentiate. Both IR isoforms (IR-A and IR-B), IGF-1R, IGF-1 and IGF-2, were expressed at high levels in thyrosphere, but they were markedly decreased in differentiating cells. IR-A was predominant in thyrospheres, especially from cancer, while IR-B was predominant in differentiating cells. Cancer thyrosphere growth was stimulated by insulin and the two IGF. These data suggest that IR isoforms and IGF-1R are involved in the biology of follicular thyroid precursors, and that cell differentiation is associated with marked changes in the expression of these receptors and cognate ligands. These data may provide insight for future differentiation therapies in thyroid cancer [24]. Other studies showed that IGF-1 increased the expression of vascular endothelial growth factor (VEGF) in thyroid cancer cells, via both activator protein-1 (AP-1)/hypoxia inducible factor-1α (HIF-1α)- and PI3K-dependent mechanisms, with obvious implications on the role of IGF-1 in peritumoral angiogenesis [25].

The involvement of the insulin/IR and/or IGF-1/IGF-1R in thyroid molecular oncogenesis has been reviewed in more detail elsewhere [22, 26–28]. Overexpression of IR in cancer cells may explain their increased sensitivity to hyperinsulinemia. Moreover, IR-A together with autocrine production of its ligand IGF-2 is emerging as an important mechanism of normal and cancer stem cell expansion, and is a feature of several malignancies. *De novo* activation of the IR-A/IGF-2 autocrine loop also represents a mechanism of resistance to anticancer therapies. Increasing knowledge of the IR role in cancer has important implications for cancer prevention, which should include control of insulin resistance and hyperinsulinemia in the population and meticulous evaluation of new antidiabetic drugs for their metabolic mitogenic ratio. Several anticancer treatments may induce or worsen insulin resistance that may limit therapy efficacy. Future anticancer therapies need to target the IR-A pathway in order to inhibit the tumor promoting effect of IR without impairing the metabolic effect of insulin [26–28].

3.3 Hypertrophic thyroid and insulin resistance

Argentinian researchers were the first to show an association between clinically relevant thyroid growth and insulin resistance [29]. In that study [29], 111 women (mean age

32.2 ± 7 years) were divided into four groups based on weight and insulin resistance: G1 (n = 42, obese with insulin resistance), G2 (n = 21, obese without insulin resistance), G3 (n = 17, normoweight with insulin resistance), and G4 (n = 31 control group, normoweight without insulin resistance). Ultrasonographically measured thyroid volume was relatively low in the absence of insulin resistance (G4 = 12.1 ± 2.4 mL, G2 = 13.8 ± 2.8 mL) and relatively high in the presence of insulin resistance (G3 = 16.2 ± 2.1 mL, G1 = 17.0 ± 3.0 mL). Statistically different ($P < 0.05$) were differences in thyroid volume between G1 and G2, and between G3 and G4. There was a similar G4 through G1 gradient for the prevalence of nodular thyroid disease, as it was also low in the two noninsulin-resistant group compared to the two insulin-resistant group: 16.1 % (G4), 23.8 % (G2), 61 % (G3), 50 % (G4). Again, the differences between G1 and G2 and between G3 and G4 were statistically significant ($P < 0.005$ and $P < 0.001$, respectively, for each comparison). The authors concluded that "the thyroid gland appears to be another victim of the insulin resistance syndrome", because the higher circulating levels of insulin increased thyroid proliferation, causing larger thyroid volume and formation of nodules [29].

These authors conducted another two interesting investigations [30, 31]. In one study, they evaluated the ability of metformin alone or combined with levothyroxine (L-T_4), either administered for 6 months, to reduce the nodular size in benign thyroid hyperplastic nodules (<2 cm in diameter) in 66 insulin-resistant women with such nodules. Women were stratified into four groups: group I (n = 14, metformin alone), group II (n = 18, metformin plus L-T_4), group III (n = 19, L-T_4 alone), and group IV (n = 15, untreated). L-T_4 treated patients had the expected decrease in serum TSH, and metformin-treated patients had a normalized homeostasis model assessment insulin resistance (HOMA-IR). The median baseline size of all nodules was 298 mm^3 (≈0.84 cm in diameter (range, 32–3616 mm^3). After treatment, patients of group I and II showed significant reductions in their nodule size [median reduction, 108.50 mm^3 (30 %) and 184.5 mm^3 (55 %), $P < 0.008$ and $P < 0.0001$, respectively]. Patients in group III and group IV (that is, the two groups that received no metformin) did not have a significant reduction of their nodules. The authors concluded that metformin produced a significant decrease in the nodular size in patients with IR and relatively small thyroid nodules, with the combination of metformin with L-T_4 being the best treatment [30].

3.4 Link between thyroid cancer and insulin resistance

In another study [31], the authors investigated whether patients with differentiated thyroid carcinoma (DTC) had a greater prevalence of insulin-resistance (HOMA-IR >2.5). Twenty women with DTC and 20 euthyroid individuals matched for age, gender, and body mass index (BMI) (control group) were investigated for insulin resistance. Twenty women (10 patients and 10 controls) had a BMI <25, whereas the other 20 had higher BMI values (overweight and obese patients). Insulin resistance was present in 50 % of the DTC group, precisely in 56.3 % of patients with PTC and 25 % of patients with FTC. In contrast, the rate of insulin resistance was only 10 % in the control group ($P < 0.001$). In the groups with BMI <25, insulin resistance was found in 30 % of DTC and 0 % (no cases) of controls, whereas in the groups with BMI >25 insulin resistance was present in 70 % of DTC and 20 % of controls. The authors concluded that such a high prevalence of insulin resistance would be an important risk factor for developing DTC, not differently from what it is known for a number of other nonthyroid carcinomas [31].

A Turkish study [32] investigated insulin resistance in patients with euthyroid nodular goiter (n = 63, none of whom with risk factors for insulin resistance) compared to healthy volunteers (n = 80). The two groups were similar with respect to age, gender, BMI, waist circumference, serum lipid levels, serum free triiodothyronine (FT_3), free thyroxine (FT_4) and TSH levels. Both HOMA-IR and thyroid volume were significantly higher in the patient group ($P = 0.007$ and $P = 0.03$, respectively). In the patient group, HOMA-IR correlated significantly with thyroid nodule volume ($P < 0.001$) but not with number of thyroid nodules. Thyroid cancer was diagnosed in 3 of 36 patients (8.3 %). The authors concluded that insulin resistance may induce increased thyroid proliferation, thyroid nodule formation and thyroid nodule volume. Therefore, insulin resistance may be a risk factor for euthyroid nodular goiter [32].

3.5 Metformin in thyroid cancer cells

The antimitogenic effect of metformin was studied in thyroid cells derived from goiters and in thyroid carcinoma cell lines by investigating cell growth, cell cycle progression, and apoptosis [33]. Furthermore, the influence of pretreatment with insulin or with chemotherapeutic agents on metformin-induced growth inhibition was investigated in thyroid carcinoma cells, in a doxorubicin-resistant thyroid carcinoma cell line, and in thyroid carcinoma-derived stem cells. Metformin showed an antimitogenic effect by inhibiting cell cycle progression and inducing apoptosis. In addition, metformin antagonized the growth-stimulatory effect of insulin, inhibited clonal cell growth, reduced thyroid cancer sphere formation, and potentiated the antimitogenic effect of chemotherapeutic agents such as doxorubicin and cisplatin on undifferentiated thyroid carcinoma cells. Remarkably, metformin inhibited proliferation even in a doxorubicin-resistant thyroid carcinoma cell line. The growth-inhibitory effect of metformin was, however, not restricted to differentiated thyroid cells and undifferentiated thyroid carcinoma cells but was also

observed in thyroid carcinoma cancer stem cells. The authors concluded that metformin could be an adjuvant treatment for thyroid cancer [33].

4 Association of serum TSH concentrations and thyroid hypofunction with insulin resistance

4.1 Metabolic syndrome induced by increased TSH levels

Even when comprised within the normal reference range, relatively higher TSH levels are associated with a worse metabolic profile. A Korean study recruited 2760 young female healthy volunteers (age, 18 to 39 years) with TSH levels in the normal range (0.3–4.5 mU/L) [34]. Setting the upper reference limit of TSH at 2.5 mU/L, as recommended by the National Academy of Clinical Biochemistry, volunteers were divided into high-TSH (n = 453) and low-TSH groups (n = 2307). Metabolic syndrome (defined using the 2007 International Diabetes Federation criteria) was looked for in both groups. The prevalence of metabolic syndrome was significantly higher in the high-TSH group than in the low-TSH group (7.5 % vs 4.8 %, P = 0.016). This 2-fold greater risk of metabolic syndrome remained after adjusting for age and BMI (odds ratio, 1.9). Central obesity (22.3 % vs 17.3 %, P = 0.012) and hypertriglyceridemia (8.0 % vs 4.2 %, P = 0.0007) also were significantly more frequent in the high-TSH group than in the low-TSH group. Waist circumference, systolic and diastolic blood pressure, and triglycerides were significantly associated with the TSH level after adjusting for age and BMI. The authors concluded that healthy young women with TSH levels >2.5 mU/L should be assessed for the presence of metabolic syndrome, even if their TSH levels are in the normal range [34].

Previously, another Korean study on 2205 Korean postmenopausal women found that TSH levels were associated with total cholesterol, LDL-cholesterol, triglycerides and diastolic blood pressure [35]. At multiple linear regression analysis, LDL-cholesterol, and triglycerides levels were independently associated with serum TSH. Multivariate logistic regression analysis determined that TSH levels strongly contributed to the metabolic syndrome. Compared with the lower most quartile (TSH, 0.3–1.44 mIU/L), the adjusted odds ratio for metabolic syndrome (defined by 2004 Adult Treatment Panel III criteria) was 1.95 in the upper most quartile (TSH, 2.48–4.00 mIU/L). The prevalence of metabolic syndrome increased as the TSH quartile gradually increased. The authors concluded that more attention should be focused on postmenopausal women with high normal TSH levels for the management of cardiovascular disease [35]. However, in another large cross-sectional study (n = 3148 Mexican subjects) in which the 2004 Adult Treatment Panel III criteria were also used for the definition of metabolic syndrome, the prevalence of the metabolic syndrome was similar in euthyroid and subclinical hypothyroidism (SCH) patients (31.6 vs 32.1 %, P = 0.89) [36]. In this study [36], the prevalence of SCH was 8.3 %. The euthyroid cohort consisted of 3033 patients (mean age 42.3 ± 10 years), of whom 1552 were women (51.2 %) and 1481 men (48.8 %). Total cholesterol was higher in patients with SCH (5.51 ± 1.19 vs 5.34 ± 1.05 mmol/l, P < 0.032). Serum TSH values showed a positive correlation (adjusted for age and sex) with total cholesterol, triglycerides, and waist circumference. In contrast, serum FT_4 showed a positive correlation with cholesterol, and an inverse correlation with waist circumference, insulin, and HOMA-IR [36].

Another cross-sectional study investigated a total of 2703 adult inhabitants of a middle-sized Dutch city [37]. Subjects who were not euthyroid, subjects who were taking thyroid and diabetic medications, and subjects for whom medication data were not available (n = 1122) were excluded. The metabolic syndrome was defined according to National Cholesterol Education Program's Adult Treatment Panel III criteria. After adjustment for age and sex, serum FT_4 was significantly associated with total cholesterol, LDL-cholesterol, HDL-cholesterol, and triglycerides. Both FT_4 and TSH were significantly associated with HOMA-IR. Median HOMA-IR increased from 1.42 in the highest tertile of FT_4 to 1.66 in the lowest tertile of FT_4. FT_4 was significantly related to four of five components of the metabolic syndrome (abdominal obesity, triglycerides, HDL-cholesterol, and blood pressure), independent of insulin resistance. The authors concluded that the found association between FT_4 levels within the normal reference range and lipids is in accordance with the earlier observed link between subclinical or overt hypothyroidism and hyperlipidemia. Moreover, low normal FT_4 levels were significantly associated with increased insulin resistance. Thus, these findings are consistent with an increased cardiovascular risk in subjects with low normal thyroid function [37].

In agreement with these data from an European population [37], there are data from an Asian population [38]. A total of 4938 Taiwanese subjects (2891 men and 2047 women; mean age of 50.1 ± 12.6 years) with normal serum FT_4 were enrolled. A modified National Cholesterol Education Program definition of metabolic syndrome was adopted, substituting BMI for waist circumference. Overall, 14 % of subjects had a high fasting glucose, 27 % had high blood pressure, 14 % had high serum total triglycerides, 8 % had low HDL-cholesterol, and 18 % were obese. Serum FT4 concentrations showed a statistically significant correlation with triglycerides and BMI, but not with blood pressure, glucose level, or HDL-cholesterol level. According to the presence of 0, 1, 2, and 3 or more features of the metabolic syndrome, age and sex-adjusted means of serum FT_4 were 17.8 ± 3.7, 17.6 ± 3.7, 17.5 ± 3.7, and 17.1 ± 3.3 pmol/L, respectively, resulting in a significantly decreasing trend (P < 0.05). When comparing subjects in the highest and lowest quartile of serum FT_4, the

former group displayed a 2-fold decrease in the odds ratio for metabolic syndrome with 3 or more metabolic features [38].

From the opposite perspective, it was examined the rate of SCH in patients with the metabolic syndrome. In 220 Turkish patients with the metabolic syndrome (167 women, 53 men; mean age of 48.5 ± 11.3 years) and 142 age- and sex-matched controls, the prevalence of SCH was 16.4 % and 5.8 %, respectively ($P = 0.001$), the female gender accounting for the difference [39]. In an Indian study on 420 patients with the metabolic syndrome (240 women, 180 men; mean age 51 ± 9.4 years) and 406 age- and sex-matched controls, the prevalence of SCH was 21.9 % and 6.6 %, ($P < 0.001$), while the prevalence and overt hypothyroidism was 7.4 and 2.2 % ($P < 0.001$) [40].

4.2 TSH, thyroid hormone abnormalities and type 2 diabetes (T2DM)

A recent study was performed in 490 euthyroid T2DM subjects, consecutively attending two outpatient diabetic units in Southern Italy [41]. Diabetic women had higher mean serum TSH levels and lower FT_4 concentration than diabetic men, while FT_3 levels were comparable in the two genders. Stratifying the study population according to quartiles of TSH levels, subjects in the highest TSH quartile were more likely to be female and younger, with higher values of BMI and waist circumference ($P = 0.05$ both), higher triglycerides ($P = 0.002$) and non-HDL cholesterol concentrations ($P = 0.01$), higher values of visceral adiposity index (VAI, which is an obesity-related index associated with the cardiovascular risk) ($P = 0.02$), and lower FT_4 levels ($P = 0.05$), when compared to those in the lowest quartile. At multivariate analysis, a younger age, female gender, triglycerides levels, and waist circumference were independently associated with higher TSH levels. In conclusion, in T2DM subjects with no evidence of thyroid disease, higher TSH concentrations within the normal range were more frequent in women and in younger subjects, and they were associated with visceral obesity and higher triglycerides concentrations, two well-known cardiovascular risk factors [41].

In an Egyptian study [42] on 147 prediabetic subjects and 150 healthy controls matched for age and sex, the prevalence of SCH and microalbuminuria was higher in the prediabetics (16.3 % vs 4 %, $P < 0.001$; and 12.9 % vs 5.3 %, $P = 0.02$, respectively). Prediabetic subjects with SCH had significantly higher HOMA-IR, TSH levels, urinary albumin-creatinine ratio, and prevalence of microalbuminuria compared to those with euthyroidism. TSH level was associated with total cholesterol ($P = 0.05$), fasting insulin ($P = 0.01$), HOMA-IR ($P = 0.01$), and UACR ($P = 0.005$). The authors concluded that prediabetic persons deserve to be screened for SCH [36]. In 34 Kuwaiti women with SCH, insulin levels were significantly higher in the SCH group compared to 20 controls (12.5 ± 2.67 vs 10.80 ± 2.01, $P < 0.05$) [37]. However HOMA-IR was not statistically different (2.85 ± 0.64 vs 2.54 ± 0.64) [43].

From the opposite perspective, in 100 Bosnian patients with SCH aged 51.7 ± 3.2 years, the rate of prediabetes and diabetes mellitus was 20 % and 38 %, respectively [44]. Fifty physically active patients were treated with low doses of L-T_4 (25–50 μg/d). After 6 months of treatment, patients had significantly decreased serum TSH (5.85 ± 0.92 vs 3.54 ± 0.55 mU/L), significantly decreased insulin levels (114.64 ± 24.11 vs 96.44 ± 17.26 pmol/L), and significantly decreased HbA1c (6.74 ± 1.01 vs 6.26 ± 1.12 %). The correlation between TSH and HbA1c was positive and significant ($r = 0.46$). The authors concluded that treatment with L-T_4 has a significant effect on the glycemic control in patients with SCH [44].

Both hyperthyroidism and hypothyroidism have been associated with insulin resistance, a major cause of impaired glucose metabolism in T2DM [45, 46]. As recently reviewed [45], the literature is punctuated with evidence indicating a contribution of abnormalities of thyroid hormones to T2DM. Thyroid hormones directly control insulin secretion. In hypothyroidism, there is a reduction in glucose-induced insulin secretion by beta cells, and the response of beta cells to glucose or catecholamine is increased in hyperthyroidism due to increased beta cell mass. Moreover, insulin clearance is increased in thyrotoxicosis [45]. Intracellular levels of T3 could contribute to aberrations in insulin sensitivity. It has also been reported an association between insulin resistance and the Thr92Ala polymorphism of the type 2 deiodinase (D2) gene product [47]. D2 converts T4 into the biologically more potent T3, and its Thr92Ala variant has a diminished enzymatic activity. The most probable mechanism leading to T2DM in thyroid dysfunction could be attributed to perturbed genetic expression of a constellation of genes along with physiological aberrations leading to impaired glucose utilization and disposal in muscles, overproduction of hepatic glucose output, and enhanced absorption of splanchnic glucose. These factors contribute to insulin resistance [45].

The importance of the intracellular levels of T3 are suggested by a very recent study in patients with type 1 diabetes (T1DM) [48]. Serum autoantibodies against thyroid hormones (THAb) were assayed in 52 consecutive patients with T1DM (53.8 % males; mean age, 37.4 ± 7.4 y; diabetes duration, 19.9 ± 8.2 y). The presence of autoimmune thyroid disease at baseline was associated with subsequent development of macroangiopathy (0 vs 33 % at baseline and follow-up, respectively; $P = 0.029$). Some THAb patterns, the majority having T3 binding in common, were associated with the progression and development of diabetes-related complications. THAb synthesis in T1DM might be driven by increased glycosylation of thyroglobulin. Anti T3-THAb may cause a relative "tissue hypothyroidism" by sequestering thyroid hormone extracellularly, this at least partially contributing to worsening diabetes-related vascular complications.

5 Correction of subclinical hypothyroidism (SHC) by MI

5.1 A clinical study on MI effect

Because of the effects of the inositols in the autoimmunity (see below), because of the described interactions of the TSH signaling with the insulin signaling and, more practically, the association of hypothyroidism with insulin resistance, it was logical to expect a study like the one [49] commented below. A single study prospective randomized double-blinded, evaluated the beneficial effect of inositol (as MI) on SCH associated with HT [49]. Unfortunately, the study was based on co-administration of MI with selenomethionine, the latter being an organic form of selenium that is able to decrease serum levels of thyroid autoantibodies (Ab) [50, 51]. The study population [49] consisted of 48 wowen (mean age 38 years) with TSH levels between 4.01 mIU/L and 9.99 mIU/L, normal FT_4 levels (0.6–1.8 ng/dL), TgAb and/or TPOAb above 350 IU/mL, as well as typical hypoechogenicity of the thyroid at ultrasonography. The primary endpoint of the 6-month duration study was restoration of TSH levels (<4.0 mIU/L). Secondary endpoints were decreased serum TPOAb and TgAb concentrations, and improvement of the thyroid hypoechogenicity and quality of life. Patients were randomized into two groups of 24 patients each, based on their initial TPOAb concentrations. Group A received orally 83 μg selenomethionine/day in a soft gel capsule. Group B received orally a combined treatment, namely, 600 mg MI contained in a 83 μg selenomethionine soft gel capsule. Two patients in group B were excluded from analysis because they become pregnant during the study period.

At entry, groups A and B were comparable for age (38.03 ± 1.63 vs 37.95 ± 2.16 years), serum TSH (4.33 ± 0.91 vs 4.43 ± 0.89 mU/L), FT_4 (18.75 ± 3.89 vs 18.87 ± 4.02 [unit of measure not given]), FT_3 (6.60 ± 1.66 vs 6.49 ± 2.36 [unit of measure not given]), TgAb (1080.80 ± 485.1 vs 1019.74 ± 374.21) and TPOAb (905.6 ± 401.6 vs 913.9 ± 543.9 IU/mL). Data for thyroid hypoechogenicity at entry were not provided, not were methodological details for its grading. By measuring plasma selenium and MI at entry (baseline) and at the end of the study, the authors could evaluate adherence to treatment. Plasma selenium values were similar in both groups at study entry (127.4 ± 15.3 μg/L, group A; 129.2 ± 15.1 μg/L, group B), and so were after treatment, precisely 223.5 ± 15.3 μg/L ($P < 0.01$ vs baseline) in group A, and 225.4 ± 12.5 μg/L ($P < 0.01$ vs baseline) in group B. Only final MI levels were reported. MI level significantly increased, only in group B (37.3 ± 4.5 vs 22.2 ± 4.1 μmol/L, P value not reported) while there were no differences between the two groups at baseline.

TSH concentrations in group B decreased by 31 % (4.4 ± 0.9 vs 3.1 ± 0.6 mIU/mL, $P < 0.01$), but they remained unchanged in group A (data not presented). TPOAb and TgAb significantly decreased in both groups. Particularly, TPOAb decreased by 42 % (905.6 ± 401.6 vs 522.6 ± 236.8 mIU/mL, $P < 0.01$) in group A and by 44 % (913.9 ± 543.9 vs 516.1 ± 315.4 mIU/mL, $P < 0.01$) in group B; TgAb decreased by 38 % (1080.8 ± 485.1 vs 670.1 ± 300.8 mIU/mL, $P < 0.01$) in group A and by 48 % (1019 ± 374.2 vs 533.9 ± 258.4 mIU/mL, $P < 0.01$) in group B. TgAb declined below 350 mIU/mL in 11 patients of group B (11/22 = 50 %) compared to 3 patients in group A (3/24 = 12.5 %). The difference between the 50 % and the 12.5 % rates was not statistically evaluated by the authors; however, it is significant by two-tailed Fisher's exact test ($P = 0.0096$; odds ratio 7.0 [95 % CI = 1.61–30.47]). Modifications of FT_4 or FT_3 were not described. Improvement of ultrasound echogenicity was observed in all patients in group B (22/22 = 100 %) vs 10 patients in group A (42 %, $P < 0.01$). [When analyzed by two-tailed Fisher's exact test, the difference between 100 % and 42 % is significant at $P < 0.0001$, with an odds ratio of 62.14 (95 % CI = 3.37–1145)]. Of note, thyroid echogenicity reverted to normal in the 11 patients group B and the 3 patients of group A in whom TgAb declined below 350 U/mL. The authors [49] also report that subjective well being revealed an improvement in 18 patients in group B compared with 8 in group A, no change in 6 patients in group B versus 14 in group A ($P < 0.05$); however, how well-being was evaluated is not described, and group B patients should have been 22, not 24, because two patients were excluded once they became pregnant.

5.2 Evidence from previous studies that used selenium only

In previous studies, selenemethionine was administered to HT patients (all or predominantly women) for 3, 6 or 12 months, and at daily doses of 100 or 200 μg, the reduction in serum levels of TPOAb being the fundamental outcome [50]. Of the 4 six-month duration trials on a total of 380 patients, 2 were conducted in Greece [52, 53], 1 in Hungary [54] and 1 in China [55]. Excluding the Hungarian study [54] because the magnitude of the TPOAb reduction was not reported, in the remaining three studies the decline of TPOAb (compared to baseline) ranged from 11 to 56 %.

In an Italian study [56], 77 TPOAb positive pregnant women were supplemented with 200 μg selenomethionine from the first trimester of gestation through month 12 postpartum. The average reduction in TPOAb, compared to baseline, was 62 % during pregnancy and 48 % during the 12 months postpartum. For

comparison, in the 74 TPOAb positive pregnant women treated with placebo (and with baseline values of TPOAb comparable to those of 77 TPOAb positive women treated with selenomethionine, i.e. 580 ± 39 vs 627 ± 42 U/L) reductions in pregnancy and postpartum were significantly lower (44 % and just 1 %, respectively). The rate of thyroid hypoechogenicity was significantly lower in the selenomethionine-treated group compared to the placebo group. At the end of the postpartum period, in the selenomethionine group, 10.4 % of women had normal echogenicity of the thyroid parenchyma (grade 0), whereas 62.3 % had mild thyroiditis (grade 1), 16.9 % moderate thyroiditis (grade 2), and 10.4 % advanced thyroiditis (grade 3). In the placebo group, 10.8 % of women had normal echogenicity of the thyroid parenchyma (grade 0), whereas 44.6 % had mild thyroiditis (grade 1), 35.1 % moderate thyroiditis (grade 2), and 9.5 % advanced thyroiditis (grade 3). Thus, at the end of the postpartum period, 72.7 % of women belonging to the selenomethionine group were grade 0–1 (not statistically different from 75.3 % at 10 week gestation or 77.9 % at delivery), whereas 27.3 % were grade 2–3 (not statistically different from 24.7 % at 10 week gestation or 22.1 % at delivery). By contrast, in the placebo group, 55.4 % of women were grade 0–1, and 44.6 % were grade 2–3, namely significantly worsened compared with the patterns at the beginning of pregnancy (77.0 % grade 0–1 and 23.0 % grade 2–3, $P < 0.05$) and at delivery (79.7 % grade 0–1 and 20.3 % grade 2–3, $P < 0.05$). Furthermore, when comparing the echogenicity patterns in the two groups, the selenomethionine-supplemented group displayed a significantly lower percentage of grade 2–3 thyroiditis at the end of the postpartum period (27.3 % vs 44.6 %, $P < 0.01$). Postpartum thyroid dysfunction and permanent hypothyroidism were significantly lower in the selenomethionine-group compared with the placebo group (28.6 vs 48.6 %, $P < 0.01$; and 11.7 vs 20.3 %, $P < 0.01$) [56].

6 Phosphatydylinositol and autoimmunity

6.1 The regulation of phosphatidylinositol (3,4,5) -triphosphate (PIP3) pathway

In mouse models, deletion of genes encoding for members of the inositol phospholipid signaling pathway cause defects in natural killer (NK) cell repertoire expression and effector function [57]. NK cells are an important defense against infection, malignancy and autoimmunity.

The PI3K family of lipid kinases regulates diverse aspects of lymphocyte behavior. Following antigen receptor engagement, activated PI3K generates 3-phosphorylated inositol lipid products that serve as membrane targeting signals for numerous proteins involved in the assembly of multiprotein complexes that are termed signalosomes, and immune synapse formation. In B cells, class IA PI3K is the dominant subgroup, whose loss causes profound defects in development and antigen responsiveness. In T cells, both class IA and IB PI3K contribute to development and immune function. PI3K modulates the function not only of effector T cells, but also regulatory T cells (Tregs); these disparate functions culminate in unexpected autoimmune phenotypes in mice with PI3K-deficient T cells [58].

Tregs play an important role in preventing both autoimmune and inflammatory diseases. Many recent studies have focused on defining the signal transduction pathways essential for the development and function of Tregs. Increasing evidence suggests that T-cell receptor (TCR), interleukin-2 (IL-2) receptor (IL-2R), and co-stimulatory receptor signaling are important in the early development, peripheral homeostasis, and function of Tregs. The PI3K-regulated pathway is one of the major signaling pathways activated upon TCR, IL-2R, and CD28 stimulation, leading to T-cell activation, proliferation, and cell survival. Activation of the PIP3 pathway is also negatively regulated by two phosphatidylinositol phosphatases: SH2 domain-containing inositol phosphatase (SHIP) and phosphatase and tensin homolog (PTEN). Several mouse models deficient for the molecules involved in PIP3 pathway suggest that impairment of PIP3 signaling leads to dysregulation of immune responses and, in some cases, autoimmunity [59]. PI3K also regulates both chemokine responsiveness and antigen-driven changes in lymphocyte trafficking. PI3K signaling is not a simple switch to promote cellular activation, but rather an intricate network of interactions that must be properly balanced to ensure appropriate cellular responses and maintain immune homeostasis [58].

A mechanism for intravenous immunoglobulins acting against autoimmune processes is to functionally silence autoreactive B cells that have evaded central tolerance checkpoints. Intravenous immunoglobulins suppressed, in *ex-vivo* stimulated human B-cells, PI3K signaling, which is crucial for determining the B-cell fate [60]. B lymphocytes express a variety of membrane molecules containing immunoreceptor tyrosine-based inhibition motifs (ITIMs) in the cytoplasmic region, such as FcγRIIB, FCRLs, CD22, mouse sialic acid-binding immunoglobulin-type lectin (Siglec)-G/human Siglec-10, Platelet endothelial cell adhesion molecule-1 (PECAM-1), mouse PIR-B/human LIRB1 and LIRB2PD-1 and CD72. When phosphorylated, ITIMs in these molecules recruit and activate phosphatases such as SH2 domain-containing protein tyrosine phosphatase 1 (SHP-1), SHP-2, SH2 domain- containing inositol 5-phosphatase 1 (SHIP1) and SHIP2

depending on receptors. These phosphatases then negatively regulate B cell antigen receptor (BCR) signaling. Because of their ability to inhibit BCR signaling, these ITIM-containing molecules are called inhibitory BCR co-receptors. Studies on mice deficient in an inhibitory co-receptor have demonstrated that the inhibitory co-receptors regulate B cell development, antibody responses and development of autoimmune diseases [61].

6.2 Inositol poly-phosphatases and their targets in T cell

Recent genetic and chemical evidences indicate that the inositol poly-phosphatases have important roles in both the effector and regulatory functions of the T cell compartment. The 5′-inositol poly-phosphatases SHIP1 and SHIP2 can shunt PI(3,4,5)P3 to the rare but potent signaling phosphoinositide species PI(3,4)P2. SHIP1 and SHIP2 and the inositol polyphosphate type I and type II enzymes (INPP4A and INPP4B) may modulate the PI3K signaling. Pharmaceutical manipulation of these enzymes for therapeutic purposes can be potentially efficient in disease settings where T cell function is a key *in vivo* target [62]. Mice genetically-deficient for the B isoform of the inositol 1,4,5-trisphosphate 3-kinase (or Itpkb) have a severe defect in thymocytes differentiation, and thus lack peripheral T cells. These Itpkb-deficient peripheral T cells have an increased capacity to secrete cytokines upon stimulation [63].

Glycosylphosphatidylinositols (GPI) are complex glycolipids that are covalently linked to the C terminus of proteins as a post-translational modification and tether proteins to the plasma membrane. One of the most striking features of GPI-anchored proteins (APs) is their enrichment in lipid rafts. Wang et al. [64] generated a post-GPI attachment to proteins 3 (PGAP3) knock-out mouse (PGAP3(−/−)) in which fatty acid remodeling of GPI-APs does not occur. They found that a significant number of aged PGAP3(−/−) mice developed autoimmune-like symptoms, such as increased anti-DNA Ab, spontaneous germinal center formation, and enlarged renal glomeruli with deposition of immune complexes and matrix expansion. The authors concluded that PGAP3-dependent fatty acid remodeling of GPI-APs has a significant role in the control of autoimmunity, possibly by the regulation of apoptotic cell clearance and Th1/Th2 balance [64].

Finally, in addition to the three major antiphospholipid antibodies (anticardiolipin, lupus anticoagulant, and β_2-glycoprotein), other autoAb of the antiphospholipid antibody syndrome (APLAS) that can be synthesized are those against phosphatidylserine, phosphatidylinositol, phosphatidic acid, phosphatidylethanolamine, phosphatidylcholine, and annexin V. Antiphospholipid antibodies are detectable in approximately 5 % of healthy individuals.

Antiphosphatydylinositol antibodies may well interfere with the intracellular PI3K signaling [65].

7 The association of polycystic ovary syndrome (PCOS) with greater serum concentrations of TSH and greater prevalence of positivity for thyroid autoantibodies

7.1 Clinical studies on PCOS patients

Janssen et al. [66] were the first to describe this association. Over a period of 30 months, 175 patients with PCOS and 168 age-matched women without PCOS (controls) were recruited to a prospective multicenter study to evaluate thyroid function and morphology 168 age-matched. Elevated TPOAb or TgAb were detected in 26.9 % of patients and 8.3 % of controls ($P < 0.001$). On thyroid ultrasound, 42.3 % of PCOS patients, but only 6.5 % of the controls ($P < 0.001$) had a hypoechoic tissue typical of autoimmune thyroiditis (AIT). Thyroid hormone levels were normal in all subjects, but PCOS patients had a higher mean TSH level ($P < 0.001$) and a higher proportion of TSH levels above the upper limit of normal (10.9 % vs 1.8 %; $P < 0.001$). This study demonstrated a 3-fold higher prevalence of AIT in patients with PCOS, correlated in part with an increased estrogen-to-progesterone ratio and characterized by early manifestation of the disease [66].

Of particular interest is the study by Garelli et al. [67] on 113 consecutive outpatients referred over 18 months and diagnosed with PCOS according to the Rotterdam criteria, and 100 age-matched healthy women as controls. The interest is because, in addition to thyroid tests (thyroid ultrasound and measurement of serum TSH, FT_3, FT_4, TgAb and TPOAb), tests for other autoantibodies were performed. These included autoantibodies against parietal cells, intrinsic factor, adrenal cortex, 21-hydroxylase, steroid-producing cells, 17-alpha-hydroxylase, side-chain cleavage enzyme, islet-cells, glutamic-acid decarboxylase, nuclei and mitochondria. The prevalence of non-thyroid autoantibodies in PCOS patients was not different from controls. In contrast, AIT was present in 27 % of patients compared with 8 % of controls ($P < 0.001$). Among the PCOS patients with AIT, the rate of SCH was 43 %, the remaining 57 % patients had normal thyroid function. Thus, only AIT, but no other autoimmune disease, was associated with PCOS [67].

7.2 A meta-analysis on six studies

A recent meta-analysis was conducted based on English-language articles searched on PubMed, Embase, Medline, Web of Knowledge and the Cochrane trial register [68]. A total of 6 studies, involving 726 PCOS patients and 879 controls, were eligible for the meta-analysis. The prevalence of AIT (tested in 3 of 6 studies), serum TSH elevation (tested in 5 of the 6 studies), TPOAb (tested in 4 of the 6 studies) and TgAb (tested in 3 of the 6 studies) positive rate in PCOS patients were all significantly higher than those in control

group. AIT was diagnosed on the basis of at least two of the following criteria: (a) TSH levels above the normal range; (b) TPOAb and/or TgAb positivity; (c) diffuse hypoechogenic pattern with high vascularization by ultrasonography. Additional data were analyzed. LH, FSH and LH-FSH ratio was presented in 3, 3 and 2 studies, respectively. The difference of LH and FSH was not significant between PCOS patients and controls. In one study [66], thyroid antibody-positive patients had a higher LH-to-FSH-ratio. Another study found trendwise higher TPOAb levels in clomiphene citrate-resistant women, and TPOAb were of value for predicting clomiphene citrate resistance with sensitivity, specificity, positive and negative predictive values of 97.4 %, 20.2 %, 38.3 %, and 93.9 %, respectively [69]. In sum, PCOS may be a kind of autoimmune disease which has close association with AIT. Accordingly, it will be helpful to assess thyroid function routinely in patients with PCOS and offer thyroid hormone replacement therapy if necessary [68].

In a cross-sectional study, 80 PCOS patients (defined according to the revised 2003 Rotterdam criteria) and 80 age-matched controls were evaluated [70]. The PCOS women had significantly higher prevalence of AIT (22.5 % vs 1.25 %), as confirmed by significantly raised anti-TPO Ab levels (28.04 ± 9.14 vs 25.72 ± 8.27 IU/mL, $P = 0.03$), and significantly higher TSH level than controls (4.55 ± 2.66 vs 2.67 ± 3.11 mU/L, $P < 0.001$). The PCOS group also had a significantly higher prevalence of goiter (27.5 % vs 7.5 %), and thyroid hypoechoic pattern at ultrasonography (12.5 % vs 2.5 %), which is also compatible with the existence of AIT.

There is now reasonably firm evidence that endocrine disrupting chemicals (EDCs) and in particular Bisphenol A are widespread pollutants evaluated as potential environmental contributors to PCOS and thyroid disorders pathogenesis [51, 71].

7.3 The tight connection between thyroid and ovary

In the discussion section of the said meta-analysis [68], the authors allude to a strong interaction between thyroid and ovary, as implied by many *in vitro* researches, both in humans and animals. For example, Tg and TSHR were detected in bovine luteal cells by immunohistochemistry [72]. Serum TSH is elevated, because of severe hypothyroidism, in the ovarian hyperstimulation syndrome [73]. One study showed that the 3′-untranslated region variant in gonadotropin-releasing hormone receptor (GNRHR) is associated with serum TSH concentration, insulin levels after oral glucose tolerance test and insulin sensitivity index, suggesting that such genetic variant also contributes to the phenotypic expression of PCOS [74]. Another contribution to both thyroid function (serum T_4, FT_4 and FT_3) and PCOS phenotype expression comes from the polymorphism of the CYP1B1 gene (which encodes an estrogen enzyme that oxidizes 17β-estradiol to 4-hydroxyestradiol) [75]. Ghosh et al. summarized the possible pathophysiologic mechanisms of ovarian cyst formation in patients with subclinical and overt hypothyroidism, which are similar to those occurring in PCOS [76]. In another study, basal ovarian size of patients with hypothyroidism (with or without polycystic ovaries) was significantly larger than controls. Hypothyroidism was found to produce ovarian cysts, and the polycystic appearance of the ovaries disappeared in all patients after T_4 treatment. These findings further suggest that the PCOS-like appearance of the ovaries can be caused by primary hypothyroidism. A decrease in ovarian volume, resolution of ovarian cysts and reversal of the PCOS-like appearance, together with improvement in serum hormone levels, occurred after euthyroidism was restored [77]. Finally, as we discussed in the preceding section of this review, there is a relationship between thyroid function and insulin sensitivity, as impaired insulin sensitivity is an important feature of PCOS.

From the opposite perspective, the prevalence of PCOS was evaluated in patients with AIT. A study from India evaluated 175 adolescent euthyroid girls with chronic lymphocytic thyroiditis (CLT) and 46 age-matched non-CLT girls [78]. Significantly higher prevalence of PCOS (46.8 vs 4.3 %, $P = 0.001$), higher mean values for BMI, waist circumference, and systolic blood pressure ($P = 0.001$), serum cholesterol, fasting and postprandial glucose ($P = 0.001$), HOMA-IR (4.4 ± 4.2 vs 2.3 ± 2.7, $P = 0.001$), higher mean number of menstrual cycles/year (8.4 ± 3.5 vs 10.1 ± 1.4) and mean Ferriman-Gallwey score (11.9 ± 3.5 vs 3.0 ± 2.4 ($P = 0.001$) were noted in patients compared to controls. The authors concluded that autoimmunity can be involved in the etiopathogenesis of PCOS [78].

7.4 MI and cytokines

Autoimmune thyroid diseases (AITD) result from an immune attack on the thyroid. Indeed, the common pathological feature of AITD is the presence of lymphocyte infiltrates within the thyroid. AITD are T cell-mediated organ-specific autoimmune disorders [79, 80]. Recent studies have shown the importance of cytokines and chemokines in the pathogenesis of systemic and organ-specific autoimmune disorders, including AITD [81–83]. In thyroid tissue, recruited T helper 1 (Th1) lymphocytes may be responsible for enhanced IFN-γ and TNF-α production, which in turn stimulates CXCL10 (the prototype of the IFN-γ-inducible Th1 chemokines) secretion from the thyroid cells, therefore creating an amplification feedback loop, initiating and perpetuating the autoimmune process [84].

Cytokines modulates MI in thyrocytes. Kung et al. [85, 86] investigated the action of IFN gamma on the production of IP and intracellular Ca^{2+} mobilization in primary cultures of human thyrocytes using the fluorescent Ca2+ indicator fura-2.

IFN gamma increased the production of inositol mono-, bis-, and triphosphates, and dose-dependently increased intracellular Ca^{2+}. The tyrosine kinase inhibitor genistein inhibited the production of IP and the elevation of [Ca2+] induced by IFN gamma, but had no effect on ATP. It was concluded that the mobilization of intracellular Ca^{2+} and the production of IP are two important signaling events for the action of IFN gamma in human thyrocytes [85, 86]. It remains to be elucidated whether MI modulates the cytokine-induced chemokine production in thyrocytes.

8 Future perspectives

Based on the literature provided and commented above, which concerns essentially nodular and autoimmune thyroid disease and PCOS as well, it seems that the therapeutic potential of inositols would be directed especially to the female gender. Insulin resistance is a common denominator between thyroid disease and PCOS, and thus it may not be coincidental that Hashimoto's thyroidits and PCOS coexist in the same woman. Improvement of insulin resistance, which is a major biochemical target of the inositols, represents a key rationale for the therapeutic usage of inositols themselves.

However, the male gender may also benefit from the administration of inositols. Indeed, male hypogonadism/infertility and erectile dysfunction have been associated with insulin resistance [87–90]. This association stems from the PI3K signaling being implicated in both testicular steroidogenesis and spermatogenesis [91–93]. Evidence exists for improvement of male hypogonadism upon administration of the classic insulin-sensitizer metformin [94]. As correctly pointed out recently, "Currently, there are limited prospective studies examining the effects of treating metabolic syndrome on male reproduction and these relationships will need to be a focus of further investigation." [88]. In the setting of the medically assisted reproduction, measurable improvements on certain indices of the spermatozoa have been observed [95].

Compliance with ethical standards

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