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THYROID FUNCTION IN THE ELDERLY AND SEX HORMONES IN ELDERLY MEN: REFERENCE INTERVALS AND ASSOCIATIONS WITH HEALTH

by

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Our life is what our thoughts make of it.

- Marcus Aurelius

Abstract

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Thyroid function in the elderly and sex hormones in elderly men: reference intervals and associations with health

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Annales Universitatis Turkuensis, Medica-Odontologica, 2008, Turku, Finland

Aims: The aims were to create clinically feasible reference intervals for thyroid-stimulating hormone (TSH) and free thyroxine (FT_4) and to analyze associations between thyroid function and self-rated health, neuropsychiatric symptoms, depression and dementia in the elderly. The second aim was also to establish reference intervals for sex hormones and to analyze associations between sex hormone levels and self-rated health, symptoms, depression and dementia in elderly men.

Subjects and methods: The study population comprised 1252 subjects aged 65 years or over, living in the municipality of Lieto, south-western Finland. Self-rated health, life satisfaction, symptoms, depression, and dementia were assessed with specific questions, clinical examination and tools such as the Zung Self-report Depression Scale and the Mini-Mental State Examination. Independent variables were dichotomized, and associations of these variables with TSH, FT_4 or sex hormone levels were assessed. Levels of TSH and FT_4 in thyroid disease–free women and women treated with thyroxine were also compared.

Results: Elevated concentrations of thyroid peroxidase antibodies (TPOAb) or thyroglobulin antibodies (TgAb) were found to have a marked effect on the upper reference limit for TSH among women, who were thyroid antibody positive more frequently than men. The derived upper reference limit for TSH was significantly higher than suggested in several recent guidelines. After age adjustment, there were no associations between TSH levels and self-rated health, life satisfaction, or most neuropsychiatric symptoms in the thyroid disease-free population. Although women with thyroxine treatment for primary hypothyroidism had far higher TSH levels than thyroid disease-free women, there were no differences between thyroid-disease free women and women with stable thyroxine treatment regarding self-rated health, life satisfaction or symptoms.

Age had a significant positive association with luteinizing hormone (LH), follicle stimulating hormone (FSH), sex hormone binding globulin (SHBG) and negative association with free testosterone (fT). Testosterone (T) and estradiol (E_2) had no age dependency. In clinical practice, one range in men aged 65 years or over can be used for T, E_2 and FSH measured with the AutoDelfia method, but two separate reference intervals should be used for fT, LH and SHBG. After adjustment for age, higher levels of T and fT were associated with better self-rated health (SRH) in the reference population. After adjustment for age and body mass index (BMI), there were no associations between sex hormone concentrations and self-rated health, life satisfaction or most symptoms in elderly men. However, diagnosed depression was associated with lower T concentration.

Conclusion: Age-specific reference intervals were derived for thyroid function and sex hormones based on comprehensive data from a community-dwelling population with a high participation rate. The results do not support the need to decrease the upper reference limit for TSH or to lower the optimal TSH target in levothyroxine treatment in older adults, as recommended in recent guidelines. Older age or being overweight (and, to a smaller extent underweight) were predictors of poor health or many symptoms among elderly men. The associations of single symptoms with T levels were inconsistent among elderly men, although the association of low T level with diagnosed depression might be clinically significant.

Keywords: Aged, andropause, elderly, hypothyroidism, life satisfaction, reference interval, self-rated health, sex hormones, testosterone, thyroid antibodies, thyroid function, thyrotrophin, thyroid-stimulating hormone

Tiivistelmä

Seija Eskelinen

Kilpirauhasfunktio iäkkäillä ja sukupuolihormonit iäkkäillä miehillä: viitevälit ja yhteydet terveyteen.

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Tavoitteet: Tutkimuksen tavoitteena oli luoda tyreotropiinin (TSH) ja vapaan tyroksiinin (T4V) viitevälit ja selvittää TSH - ja T4V tasojen yhteydet koettuun terveyteen, neuropsykiatrisiin oireisiin, depressioon ja dementiaan iäkkäässä väestössä. Kilpirauhasen primaaria vajaatoimintaa (hypotyreoosia) sairastavien laboratorioseurannan ja hoitosuositusten yhdenmukaisuutta tutkittiin. Lisäksi määritettiin sukupuolihormonien viitevälit ja sukupuolihormonitasojen yhteydet koettuun terveyteen, neuropsykiatrisiin oireisiin, depressioon ja dementiaan iäkkäässä miesväestössä.

Aineisto ja menetelmät: Aineisto koostui 1252 iäkkäästä (keski-ikä 74 vuotta) henkilöstä, jotka asuivat varsinaissuomalaisessa Liedon kunnassa. TSH ja T4V viitevälit laskettiin 502 miehen ja 584 naisen aineistosta, josta oli ensin poissuljettu henkilöt, jotka olivat kilpirauhasvasta-ainepositiivisia tai joilla oli TSH tai T4V tasoihin vaikuttava sairaus tai lääkitys. Sukupuolihormonien ikäryhmäspesifiset viitevälit laskettiin 466 miehen aineistosta, josta oli ensin poissuljettu henkilöt, joilla oli sukupuolihormonitasoihin vaikuttava sairaus tai lääkitys. Koettu terveys, tyytyväisyys elämään, neuropsykiatriset oireet, depressio- ja dementiadiagnoosit selvitettiin validein kyselyin, kliinisellä tutkimuksella ja diagnostisia kriteerejä käyttäen.

Tulokset: Kilpirauhasvasta-ainepositiivisuus oli yleisempää naisilla kuin miehillä. TSH:n viitevälin yläraja laski naisilla, kun vasta-ainepositiiviset suljettiin pois. TSH:n viitevälin yläraja oli selkeästi korkeampi kuin mitä viimeaikaisissa kansainvälisissä suosituksissa on ehdotettu ylärajaksi. Kun ikä vakioitiin, TSH tasoilla ei ollut tilastollisesti merkitsevää yhteyttä koettuun terveyteen, tyytyväisyyteen elämään, dementiaan, depressioon tai suurimpaan osaan neuropsykiatrisia oireita. Vaikka tyroksiinihoidossa primaarin hypotyreoosin vuoksi olevien naisten TSH tasot olivat selkeästi korkeampia kuin kilpirauhasen suhteen terveiden naisten TSH tasot, tilastollisesti merkitseviä eroja koetun terveyden, elämään tyytyväisyyden tai neuropsykiatristen oireiden suhteen ei tullut esiin.

Vapaan testosteronin (fT), follikkelia stimuloivan hormonin (FSH), luteinisoivan hormonin (LH) ja sukupuolihormoneja sitovan globuliinin (SHBG) tasot olivat riippuvaisia iästä, kun taas testosteroni (T) ja estradioli (E_2) tasot eivät olleet iästä riippuvaisia yli 65-vuotiailla miehillä. Yhtä viiteväliä voidaan käyttää Autodelfialla määritettyihin T, E_2 ja FSH hormoneihin , ja kahta viiteväliä fT, LH ja SHBG tuloksiin 65 vuotta täyttäneillä miehillä. Kun ikä vakioitiin, korkeammat T ja fT tasot olivat yhteyksissä paremmaksi koettuun terveyteen. Kun ikä ja myös kehon painoindeksi vakioitiin, sukupuolihormonitasoilla ei löytynyt tilastollisesti merkitseviä yhteyksiä koettuun terveyteen, elämään tyytyväisyyteen tai suurimpaan osaan neuropsykiatrisista oireista. Diagnosoidun depression ja matalien testosteronitasojen välillä oli merkitsevä yhteys.

Päätelmät: Tämän hyvän osallistumisprosentin omaavan väestötutkimuksen perusteella lasketut kilpirauhasfunktiokokeiden viitevälit iäkkäillä ja sukupuolihormonien viitevälit iäkkäillä miehillä ovat nyt käytettävissä. Nykyisiin kansainvälisten suositusten ehdotuksiin laskea TSH:n viitevälin ylärajaa tai kilpirauhasen vajaatoiminnan vuoksi tyroksiinihoitoa saavien TSH tavoitetasoa tulee suhtautua varauksella 65 vuotta täyttäneillä. Korkeampi ikä ja ylipainoisuus (ja vähäisemmässä määrin alipainoisuus) oli yhteyksissä huonommaksi koettuun terveyteen ja moniin oireisiin 65 vuotta täyttäneillä miehillä. Yksittäisten neuropsykiatristen oireiden yhteys testosteronitasoihin tai muihin sukupuolihormonitasoihin ei ole suinkaan selkeä, joskin matalan testosteronitason ja diagnosoidun depression yhteydellä saattaa olla hoidollistakin merkitystä.

Avainsanat: Andropaussi, hypotyreoosi, ikääntyneet, iäkkäät, koettu terveys, kilpirauhasfunktio, kilpirauhasvasta-aineet, sukupuolihormonit, testosteroni, tyreotropiini, elämään tyytyväisyys, viiteväli

Contents

Abbreviations

ATC	Anatomical Therapeutic Chemical Classification of drugs
BAT	bioavailable testosterone
BMI	body mass index
CDR	Clinical Dementia Rating
CHD	coronary heart disease
CI	confidence interval
DHEA-S	dehydroepiandrosterone sulfate
DSM-IV	Diagnostic and Statistical Manual of Mental Disorders, fourth edition
E_2	estradiol
FSH	follicle stimulating hormone
fT	free testosterone
FT_4	free thyroxine
GP	general practitioner
ICD-10	International Statistical Classification of Diseases and Related Health problems, version 10
LH	luteinizing hormone
LOH	late-onset hypogonadism
MMSE	Mini-Mental State Examination
NHANES III	Third National Health and Nutrition Examination Survey
NHI	National Health Insurance
SD	standard deviation
SHBG	sex hormone-binding protein
SRH	self-rated health
T	testosterone
TgAb	thyroglobulin antibodies
TPOAb	thyroid peroxidase antibodies
TSH	thyroid-stimulating hormone, thyrotrophin

List of original publications

I Eskelinen S, Suominen P, Vahlberg T, Löppönen M, Isoaho R, Kivelä S-L, Irjala K. The effect of thyroid antibody positivity on reference intervals for thyroid stimulating hormone (TSH) and free thyroxine (FT_4) in an aged population. Clinical Chemistry and Laboratory Medicine 2005; 43:1380-1385.

II Eskelinen S, Vahlberg T, Isoaho R, Löppönen M, Kivelä S-L, Irjala K. Associations of thyroid-stimulating hormone and free thyroxine concentrations with health and life satisfaction in elderly adults. Endocrine Practice 2007;13:451-457.

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1. INTRODUCTION

Changing levels of several hormones in the aged compared with younger individuals have been reported. The role of multiple hormonal changes in the whole aging process is not clear. It is uncertain whether it is possible to alter the aging process markedly by giving one single hormone to gain the same levels as younger adults have, and whether the benefits would then exceed the risks.

Growing awareness of age-related changes in the endocrine system, changing measuring methods, growing public interest (partly influenced by the pharmaceutical industry), the current trend to create consensus statements and guidelines by expert panels (including no or a minimal proportion of geriatricians or general practitioners) on the diagnosis and treatments of endocrine disorders, have led to controversy in clinical endocrinology. In particular, this concerns suggestions to decrease the upper reference limit for thyroid stimulating hormone (TSH) and thus the grounds for thyroxine treatment for elderly adults (Brabant et al. 2006; Surks et al. 2005; Wartofsky and Dickey 2005), and testosterone treatment for elderly men (Vermeulen 2001).

Overt and subclinical primary hypothyroidism is the most common pathological hormone deficiency (Canaris et al. 2000). If the upper reference limit for TSH is lowered in clinical practice, thyroxine treatment will become more common, especially for elderly women, and more subjects will be diagnosed with subclinical hypothyroidism without consensus on if/how to treat them after diagnosis (Surks et al. 2005; Surks et al., 2004). Testosterone replacement therapy has become more common for elderly men (Bhasin et al. 2003; Vainionpää 2007), although data regarding the long-term safety and effectiveness of this "substitution" treatment is not available.

In this situation, there is a need for data on empirical distributions and age-related reference intervals for hormones based on an elderly community-dwelling population, and evidence concerning the associations of hormone concentrations with symptoms and quality of life among the elderly. With this data, future expert panels will be more aware of the possible effects/consequences of changing the reference intervals and target levels of treatments of endocrine disorders in older populations. This evidence will have important consequences for primary care physicians who have the primary responsibility for health maintenance in most elderly adults.

2. REVIEW OF THE LITERATURE

2.1. Thyroid function

2.1.1. Reference intervals for thyroid stimulating hormone (TSH) and free thyroxine (FT_4)

Currently, measurement of serum TSH is the most sensitive and most common used indicator of the endocrinological function of the thyroid gland (Demers and Spencer ed. 2002). The development of TSH assays from "the first generation" radioimmunoassays (RIAs) (Utiger, 1965) to "the third generation" assays in the 1990s has lowered the upper reference limit to the level of 4-5 mU/L measured by immunoradiometric (or non-isotopic labels) methods. The improvement of the sensitivity of TSH assay has made it possible to determine the lower reference limit for TSH and thus also to screen for hyperthyroidism with the TSH test. At the present, there is a general acceptance of the lower limit of normal for TSH, which is about 0.3-0.5 mU/L, but no consensus on the upper limit. In particular, the characteristics of the reference population, the influence of thyroid autoimmunity for TSH and the statistical methods used when calculating the reference intervals (e.g. due to skewed distributions of TSH, TPO and Tg antibodies) have been questioned by practicing and academic endocrinologists and researchers (Brabant et al. 2006; Surks 2005; Wartofsky and Dickey 2005).

Out of the third National Health and Nutrition Examination Survey (NHANES III) population (n=17 353, 12 years or over in the United States), individuals who reported thyroid disease, goiter, or taking thyroid medication were excluded from the total population, leaving 16 533 people classified as the disease-free population (Hollowell et al. 2002). People who had antithyroid antibodies, those who were pregnant, or taking estrogens, androgens, or lithium, and those with laboratory evidence of overt hypothyroidism or hyperthyroidism, were further excluded from the disease free population, resulting in a reference interval for TSH of 0.45-4.12 mU/L (n=13 334).

TSH levels in the reference population of NHANES III showed a skewed distribution with a long "tail" towards higher levels from around a level of 2.5 mU/L. The consensus statement of the National Academy of Clinical Biochemistry (NACB) stated that those subjects with TSH over 2.5 mU/L still have subclinical thyroid disease which might have been detected by the use of ultrasound and more sensitive assays of thyroid antibodies, thus the "true" upper reference limit would be 2.5 mU/l (Demers and Spencer ed. 2002). NACB guidelines also recommend that despite the wider serum TSH variability in older individuals, there is no justification for using a widened or age-adjusted reference range. On the other hand, the disease-free population of NHANES has been considered the most appropriate for assessing the range of TSH that is relevant to clinicians in practice, who do not generally measure TPO antibodies before measurement of TSH (Surks et al. 2005). Only 81.5% of the disease-free group and 85.8% of the reference group had TSH levels below 2.5 mU/L in the NHANES-population, as Surks et al remarked.

The only study (Volzke et al. 2005) providing direct data to support the upper reference limit for TSH of 2.5 mU/L is from a population in a previously iodine-deficient area in Germany (4298 individuals, 20-79 years of age). A reference population was selected comprising 1488 persons (825 men) by excluding subjects with known thyroid diseases, TPOAb seropositivity or unknown thyroid disorders such as goiter, inhomogeneous thyroid pattern, nodules and hypoechogeneity examined by thyroid ultrasound. Reference intervals for serum TSH and FT_4 were 0.25-2.12 mIU/L and 8.3-18.9 pmol/L, respectively. TSH and FT_4 were measured from nonfasting blood samples by immunochemiluminescent procedures that had a low reference interval of 0.3-3.0 mU/L as stated by the manufacturer.

In a Danish study, a TSH reference range of 0.4-3.6 mU/L was found in 3174 participants aged 18-65 years without previous thyroid disease, TPO antibodies and with a normal thyroid according to ultrasonography (Knudsen et al. 2000). An other Danish study (n=1473, age 17-66 years) where the NACB recommendations were evaluated (Jensen et al., 2004), showed no difference in serum TSH concentrations with respect to age, gender, or medication for individuals who fulfilled NACB criteria (n=987) i.e. who had no thyroid antibodies or other risk factors. TPOAb alone or in combination with TgAb were associated with an increased TSH level. The common reference interval for TSH established in that study, 0.58-4.1 mIU/L, was much higher than expected based on the NACB guidelines.

The estimate of the prevalence of TPOAb positivity depends on the sensitivity and specificity of the method employed. The previously mentioned NHANES III reported detectable TPOAb levels in 12 % of subjects using a competitive immunoassay method, and antibodies were more prevalent in women than in men (Hollowell et al. 2002), in accordance with previous studies (Bjoro et al. 2000; Canaris et al. 2000). Previous studies have also shown that the reference interval can be shared (=common reference interval for men and women) after removing those with thyroid antibodies (Tunbridge et al. 1977; Vanderpump et al. 1995). Although there is a rising prevalence of thyroid autoimmunity with higher age (Bjoro et al. 2000; Canaris et al. 2000; Hollowell et al. 2002), the prognostic significance of positive anti-thyroid antibodies with or without increased TSH concentrations in the aged appears less predictive of later hypothyroidism than in middle-aged or young subjects (Huber et al. 2002; Lazarus et al. 1984; Sundbeck et al. 1995). The 20-year follow-up study of the Wickham cohort showed that TPOAb appears in the circulation long before a change in thyroid function can be observed through changes in TSH values, and antibodies can also disappear from the circulation (Vanderpump et al. 1995).

Because TSH is currently the most sensitive and most commonly-used indicator of the function of the thyroid gland, the introduction of more sensitive TSH into clinical practice was expected to reduce the number of free T4 measurements. The labeled antibody approach is currently the favored free hormone testing approach on most automated platforms. Current methods measure free hormone as a function of the fractional occupancy of hormone-antibody binding sites. Reference intervals for FT_4 immunoassay methods approximate 9-23 pmol/L (Demers and Spencer ed. 2002).

2.1.2. The influence of medication and morbidity on thyroid function (TSH and FT_4 levels)

2.1.2.1 Medication

TSH level is less affected by medication than any thyroid hormone concentration. Glucocorticoids in large doses (Samuels and McDaniel 1997) and dopamine (Kaptein et al. 1980) in sick hospitalized patients can inhibit TSH secretion. Several NSAIDs in normal therapeutic doses can lower serum thyroid hormone concentrations, principally by interfering with the binding of T4 and T3 to serum carrier proteins; patients taking these drugs remain euthyroid (Bishnoi et al, 1994). Propranolol can cause an elevation in TSH as a result of the impaired conversion of T4 to T3 (Geffner and Hershman, 1992). The iodide-containing anti-arrhytmic drug amiodarone has complex effects on thyroid gland function that can induce either hypothyroidism or hyperthyroidism (Daniels 2001; Harjai and Licata 1997; Martino et al. 2001) as does lithium also (Lazarus 1998; Oakley et al. 2000). Phenytoin and carbamazepine cause a 20-40% decrease in total and FT_4 concentrations (Isojärvi et al. 1989). Large intravenous doses of furosemide (Stockigt and Topliss 1989) or intravenous heparin administration (Mendel et al. 1987) can acutely increase FT_4.

2.1.2.2 Morbidity and increased TSH

Overt untreated hypothyroidism has been found to be associated with cardiovascular disease in the first case-control study with relatively small amount of autopsied subjects (Vanhaelst L 1967). In the population-based Wickham survey, a cohort study of 2779 men and women in the United Kingdom, no association with positive thyroid antibodies or raised TSH levels at first survey and subsequent mortality or development of ischemic heart disease was found after the 20 years follow-up (Vanderpump et al. 1996).

Recent studies have focused on a possible link between subclinical hypothyroidism and cardiovascular disease, resulting in inconsistent findings. Subclinical hypothyroidism (TSH > 4 mU/L with FT_4 in the reference interval) was associated with a greater age-adjusted prevalence of aortic atherosclerosis (odds ratio, 1.7) and myocardial infarction (odds ratio, 2.3) in 1149 women with mean age of 69 years (Hak et al. 2000). However, cross-sectional data from 3410 subjects over 65 years revealed no differences in the prevalence of angina, myocardial infarction, transient ischemic attack, stroke or peripheral artery disease, when compared with those with subclinical hypothyroidism and controls with normal TSH **(**Ladenson et al. 1994). The effect of mild thyroid deficiency on the lipid profile has been found to be relatively minor in a study with 49 patients aged 18-50 years (Caraccio et al. 2002).

An association between depression and subclinical or overt hypothyroidism has been described in several clinical studies (Gulseren et al. 2006; Larisch et al. 2004) in contrast to non-clinical settings. In a large unselected population (age range 40-89 years), no statistical association was found between thyroid dysfunction and self-rated depression, and the results remained identical when individuals with known thyroid disease were excluded (Engum et al. 2002). In that study, the group with biochemical

hypothyroidism had a significantly lower risk for depression compared with the reference group with normal thyroid function. In a prospective study of a population-based cohort of individuals aged 85 years (n=558), no consistent associations were found between thyroid status and cognitive performance or depressive symptoms, either in the cross-sectional or in the prospective analyses (Gussekloo et al. 2004). In a primary care practice setting (65 years of age or older) with a low participation rate, subclinical thyroid dysfunction was not associated with cognition, depression or anxiety (Roberts et al. 2006).

2.1.2.3 Morbidity and decreased TSH

The term nonthyroidal illness (NTI) is used to describe the subset of patients who are seriously ill and have abnormalities in their thyroid tests, mainly decreased TSH levels (Borst et al. 1983). TSH concentrations remain within normal limits in the majority of NTI patients. Patients recovering from severe illness may have a transiently raised TSH concentration (Hamblin et al. 1986).

There is more consistent evidence of the association of cardiac adverse effects with subclinical hyperthyroidism rather than with subclinical hypothyroidism. In the ten-year follow-up study of a cohort of 1191 subjects aged over 60 years, one single low TSH at screening was associated with increased mortality from all causes, and in particular with mortality due to circulatory and vascular diseases (Parle et al. 2001). A three-fold increased risk of atrial fibrillation in 10 years was found in men and women of at least 60 years of age with a TSH of 0.1 mU/L or lower with endogenous or exogenous (=subjects with thyroxine treatment) subclinical hyperthyroidism (Sawin et al. 1994) and a 2.8-fold increased risk in 2 years in subjects of at least 65 years (Tenerz et al. 1990).

Two meta-analyses reported significant declines in bone mineral density during prolonged subclinical hyperthyroidism in postmenopausal women (Faber and Galloe 1994;Uzzan et al. 1996).

A possible association between dementia and subclinical hyperthyroidism has been described in one study (Kalmijn et al. 2000), where incident dementia was especially common in those with positive antithyroid antibodies and was inversely related to T4 levels. The only large, population-based study (n=6884, aged 18-60 years) of an unselected, healthy cohort found no association between low TSH (lower than 0.21 mU/L) and physical or psychological symptoms of hyperthyroidism; nor were any differences between low TSH and concentration, depression or anxiety detected by means of validated instruments (Schlote et al. 1992).

2.1.3. Laboratory monitoring of patients with primary hypothyroidism

Thyroxine is the treatment of choice in primary hypothyroidism. Clinical criteria and thyroid function tests determine dosage adjustment. In addition to the guidelines in common textbooks (Tallis et al. 2003; Wilson et al. 1998; Välimäki et al. 2000), several consensus statements and treatment guidelines on hypothyroidism and/or hyperthyroidism have been published over past years (AACE, 2002; Roberts and

Ladenson 2004; Singer et al. 1995; Vanderpump et al. 1996). The guidelines are produced in order to provide an acceptable standard of care for patients with thyroid disease.

A statement from the American Association of Clinical Endocrinologists encourages treatment for patients whose TSH values lie outside the boundaries of a narrower margin based on a target TSH level of 0.3-3.0 mIU/L, but there is no argument justifying the statement (AACE 2002). In a seminar guideline on patients with primary hypothyroidism (Roberts and Ladenson 2004), a proposal was made to restore the TSH value in thyroxine treatment even to a level of about 1 mIU/L, but there are no original studies for the basis of that recommendation, either.

In the setting of a hospital endocrinology department with 385 patients over 55 years of age, 68 % of the subjects attained good control of the disease i.e. TSH within a reference interval of 0.4-5.0 mU/L, whereas 33 % showed inadequate control (Diez 2002). The 2-year follow-up of 56 newly diagnosed patients from the same study showed that an adequate control of hypothyroidism was attained in 63 % of the patients in 6 months, in 82 % during 1 year, and in 88 % during 2 years. In the cross-sectional Colorado Thyroid Disease Prevalence Study conducted in 1995 (Canaris et al. 2000), only 60 % of patients on thyroid hormone replacement or suppression therapy had serum TSH within the reference interval of 0.3-5.1 mU/L. Although 92% of subjects had seen a physician in the preceeding year, 40% of the patients on thyroxine therapy had an abnormal TSH level, including 22% with a low TSH.

In summary, with the present reference interval for TSH as the target in thyroxine treatment, researchers in several studies have noted that about a fifth of hypothyroid patients are receiving an inadequate thyroxine dose, and a fifth are being given an excessive amount of medication.

2.2. Sex hormones in men

2.2.1. Reference intervals for sex hormones in men

A decrease in T of approximately 1%/year with age in men from the fourth or even the third decade onwards has been shown in large epidemiological cross-sectional and longitudinal studies (Feldman et al. 2002; Harman et al. 2001; Morley et al. 1997). A greater decline in fT levels with ageing than that in T levels (due to rising SHBG levels with age) has been also shown in longitudinal studies (Feldman et al. 2002; Harman et al. 2001). A prospective cohort study from the Massachusetts Male Aging Study population showed that a 4 to 5 kg/m^2 increase in BMI was associated with a decline in T comparable to that associated with approximately 10 years of aging (Travison et al. 2007).

A substantial age-related increase in SHBG, the major serum carrier of sex hormones, is documented in several studies (Allen et al. 2002; Svartberg et al. 2003), as are the well-known associations between BMI and sex hormones, especially the decrease of SHBG with increasing BMI (Bjornerem et al. 2004; Muller et al. 2003). Furthermore,

total T and E_2 and BMI levels affect SHBG levels, especially in pathophysiological conditions, and the calculations of T and E_2 free portions.

Most researchers have reported that E_2 levels decrease slightly or remain constant with increasing age in men (Gray et al. 1991; Muller et al. 2003), or increase slightly (Bjornerem et al. 2004). LH increased by 1.3%/year and FSH by 1.9%/year in men aged 39-70 years (Gray et al 1991).

Published reference intervals of T in healthy eugonadal men have typically been 10.4-34.7 nmol/L (Braunstein and Glassman, 1997; Griffin 1998). A recent study (Boyce et al. 2004) using a radio-immunoassay method, reported a lower reference limit of T 7.41 nmol/L for 68 men aged 41-75 years and 10.07 nmol/L for 198 men aged 18-40 years (healthy with strict criteria, morning samples). The lower reference limit for fT was 33 pmol/L derived from a reference population of the 20-29 healthy non-obese men (Leifke et al. 2000). Previously published normal ranges of LH concentrations in textbooks for healthy men are 0.9-20.0 IU/L (Braunstein and Glassman 1997; Griffin 1998; Kratz and Lewandrowski 1998). In the study by Boyce et al mentioned above (Boyce et al. 2004), the reference interval for LH measured by an immunoassay method from morning samples was 0.9-7.0 IU/L (n=266, age 18-75 years, healthy by strict criteria). Age had a significant positive association with LH in that study: mean LH concentrations increased with age by 0.1% each year.

2.2.2. The influence/association of medication and morbidity on sex hormones in men

Medication

Of the commonly used medications among outpatients, digoxin (Neri et al. 1987), glucocorticoid therapy (MacAdams et al. 1986) and diazepam (Calvo et al. 1991) may decrease T levels. Finasteride (Gormley et al. 1990) may increase T levels. Carbamazepine, phenytoin (Isojärvi et al. 1990) and levothyroxine (de Bruin et al. 1993) may increase SHBG levels. Carbamazepine, valproic acid (Isojärvi et al. 1990), digoxin (Neri et al. 1987), and verapamil (Barbarino and De Marinis, 1980) may decrease LH levels. Doxepine (Linnoila et al 1977), spironolactone, (Tidd et al. 1978), and lithium (Sheard et al. 1977) may increase the LH level.

Morbidity

Data suggest that low level of T is correlated with chronic obstructive airway disease (Semple et al. 1980), active rheumatoid arthritis (Gordon et al. 1986), hepatic cirrhosis (Van Thiel 1981) and type II diabetes (Barrett-Connor et al. 1990; Mulligan et al. 2006). In patients with chronic renal failure, LH and FSH are slightly elevated with reduced T and fT levels and normal SHBG levels (Lim and Fang, 1976) and normal or low E_2 levels (Handelsman et al. 1981).

SHBG levels are reduced in men with type II diabetes (Barrett-Connor et al. 1990). Follow-up data of approximately 700 men in the Kuopio Ischemic Heart Disease Risk Factor Study showed that, after adjustment for age, non-diabetic men were twice as likely to develop diabetes or metabolic syndrome within an 11-year period if they were in the lowest quartile for T levels (Laaksonen et al. 2004). A recent systemic review of

35 cross-sectional studies concluded that the majority of studies reported that low T is associated with a higher prevalence of coronary artery disease (Jones et al. 2005). Bone mineral density has been found to be more strongly related to bioavailable E_2 than to T levels (Khosla et al. 2001), although there is also a weak positive correlation between bone mineral density and the T/SHBG ratio (Scopacasa et al. 2000).

There are conflicting data about the associations between low T levels and affective and cognitive deterioration in elderly men. In one cohort study of community-dwelling ambulatory men (n= 236, median age 75.3 years, participation rate only 47%) depressive symptoms assessed using the Geriatric Depression Scale (=GDS), age and the GDS score and all health assessment scores were significantly interrelated, but GDS scores were not related to T or fT levels (G. G. T'Sjoen et al. 2005). In the MMAS study with 1709 men aged 40-70 years (participation rate 52%), T levels were not associated with the self-reported depression inventory (Araujo et al. 1998). A 10-year follow-up of the Rancho-Bernardo Study (Barrett-Connor et al. 1999), 856 men aged 50-89 years completed the Beck Depression Inventory (=BDI). Men with lower bioavailable T levels had higher BDI scores, which is indicative of increased depressive symptoms. All these studies measured hormone concentrations using morning blood samples.

Few epidemiological studies have been conducted on the relationships between T levels and cognitive function specifically in older men. The prevalence of cognitive dysfunction in older adults is high. Estimates vary depending on the methodology and definitions used, which causes problems when making comparisons between the results. In a follow-up study of 574 men from the Baltimore Longitudinal Study of Aging (Moffat et al., 2004), a higher T/SHBG ratio, as an indicator of higher bioavailable T, meant a protective effect on the risk for Alzheimer's disease. Two population-based studies (Yaffe et al. 2002, Barrett-Connor et al. 1999) found that bioavailable T levels were positively associated with scores of cognitive ability, i.e. better cognition with higher hormone levels, the first study (n=310, mean age 73 years, part of a cohort study) on all the three tests used, and the latter (n=547 men, aged 59-89 years) on tests assessing mental control and verbal memory. Significantly poorer MMSE scores with increasing levels of total and bioavailable E_2 were found in the latter study.

In the population-based, prospective Honolulu-Asia Aging Study (Geerlings et al. 2006), 2974 men aged 71 to 93 years were re-examined three times during six years for development of cognitive decline and dementia. T was not associated with the risk for cognitive decline and Alzheimer's disease, whereas higher E_2 levels increased the risk for both. No significant relationship was found between T, fT, LH, FSH or SHBG and cognitive functioning in 981 men aged 48-80 years in Massachusetts Male Aging Study by three tests of cognitive function assessing working memory, speed/attention and spatial ability after adjusting for age, education level and physical status (Fonda et al. 2005).

2.2.3. Definition and prevalence of hypogonadism in aging men and association of symptoms with T and/or fT levels in aged men

2.2.3.1 Prevalence of hypogonadism

The prevalence of hypogonadism in aging men (also known as late onset hypogonadism/ age-related hypogonadism/ andropause/ male climacteric /(partial) androgen deficiency of the aging male) is not known with certainty mostly due to the lack of a clearly defined standard for diagnosis. The operational definition of androgen deficiency/male hypogonadism: total T< 200 ng/dL=6.9 nmol/L, or ≥ 3 signs or symptoms of hypogonadism with a T level between 200 and 400 ng/dl (13.9 nmol/L) is based on statistically conventionally defined two standard deviations below the mean in a large cohort of men in their 20s (Araujo et al. 2004). For clinical purposes, testosterone deficiency has also been defined as a T concentration of <8.7 nmol/L (Snyder et al. 2000), <10.4 nmol/L (Basaria and Dobs, 2001; Mulligan et al. 2006), and even <12.1 nmol/L (Griffin 1998).

It is stated in the International Society for Andrology (ISA), the International Society for the Study of the Aging Male (ISSAM), and the European Association of Urology (EAU) Recommendations: about Investigation, Treatment and Monitoring of Late-Onset Hypogonadism in Males that LOH is a syndrome characterized primarily by approximately 15 signs or symptoms e.g diminished sexual desire and erectile quality, changes in mood, sleep disturbances, decrease in lean body mass and increase in visceral fat (Nieschlag et al. 2005). General agreement was achieved in that guideline on the following issues: T above 12 nmol/L or fT above 250 pmol/L do not require substitution whereas T below 8 nmol/L or fT below 180 pmol/L require substitution. In that recommendation it was assumed that symptoms of T deficiency become manifest between the concentration of 12 to 8 nmol/L, and trials of treatment may be considered in those in whom alternative causes of these symptoms have been excluded.

Estimates of the prevalence with which T concentrations reach levels that would be interpreted as hypogonadal in aged men varies from 30-40% in men over 65 years to 70% in men in their eighties (Ferrini and Barrett-Connor 1998; Harman et al. 2001; Morley et al. 1997). A French study (Szulc et al. 2003) reported that the prevalence of hypogonadism among men aged 50-85 years was 9% with a T cutoff of 257 ng/dl (8.9 nmol/L). In the study entitled "Hypogonadism in Males" (Mulligan et al. 2006) among men aged ≥ 45 years not receiving testosterone, the prevalence was 36.3% with a T cutoff of 300 ng/dl (10.4 nmol/L).

2.2.3.2 Questionnaires for screening androgen deficiency in aging men

The lack of a clearly defined standard for the diagnosis of androgen deficiency also complicates the validation of scales regarding psychometric test characters to screen for androgen deficiency and vice versa. The three scales presently used to screen for androgen deficiency follow different methodological concepts.

The Androgen deficiency in aging males (ADAM) questionnaire contains ten questions about complaints based on the clinical experience of researchers, i.e. what they had commonly observed in practice in older males with low bioavailable T (BAT) levels, e.g. decreased enjoyment of life, feeling sad and/or grumpy, lack of energy etc., including one item regarding loss of height (Morley et al. 2000). To validate the ADAM questionnaire for screening for low BAT, 25% of subjects with a BAT less than 70 ng/mL (20.2 nmol/L) were tested in 316 physicians aged 40-62 years. The ADAM questionnaire had 88% sensitivity and 60% specificity in that group. The coefficient of variation was 11.5% when administered twice, 2-4 weeks apart. Only three symptoms on the ADAM questionnaire, deterioration in work performance, decreased strength and/or endurance, and bothersome hot flushes were associated with low T and/or BAT in 370 men aged 55 to 75 years in Sweden (Spetz et al. 2007).

A screener from the Minnesota Male Aging study (MMAS) was developed from potential predictors of T deficiency from 34 variables administered in MMAS, resulting in an eight-item instrument based on age, BMI, diabetes, asthma, headaches, sleep patterns, dominance preferences, and smoking status, i.e. its questions mainly concern the presence/absence of chronic diseases, risk factors, and also age, weight and height, but to a minor extent on complaints (Smith et al. 2000). Because there was no gold standard for defining T deficiency, researchers conducted a brief mail survey of the members of the Endocrine Society. Based on the recommendations of 53 respondents, T deficiency was defined as total T below 12.1 nmol/L. The screener of MMAS was validated in 304 men aged 40-79 years presenting at a primary health care clinic for routine check-ups or minor medical problems. In that sample, the prevalence of T deficiency (<12.1 nmol/L) was 42.1%, and the screener had 76% sensitivity and 49% specificity.

The Aging Males' Symptoms (AMS) scale was originally developed to assess symptoms of aging between groups of males in different conditions, secondly, to evaluate the severity of symptoms/health-related quality of life over time, and, thirdly, to measure changes in pre- and post-androgen replacement therapy (Heinemann et al. 2004). Three dimensions of symptoms were identified in this 17-item Likert-response scale: a psychological (such as depression, irritability, anxiety, nervousness), a somatovegetative (such as sleep disturbances, impaired general well-being, decreased energy) and a sexual factor (such as the impression of having passed the zenith of life).

Although the AMS questionnaire was not developed as a screening instrument for androgen deficiency, it is widely used as such. The comparison of the AMS with the two screening instrument for androgen deficiency mentioned above showed sufficiently good compatibility. A positive predictive value of 92% and negative predictive value of 50% were found regarding the ADAM scale. The respective figures regarding Smith's screener were 65% and 49%, respectively (Heinemann et al. 2004). However, there was no correlation between the AMS (total and subscales) questionnaire and T levels among 81 consecutive self-referred patients aged 53-66 years (T'Sjoen et al. 2003), no correlation between the AMS questionnaire and T, fT or BAT levels among 161 healthy ambulatory men aged 74-89 years (T'Sjoen et al. 2004) and no correlation between the AMS questionnaire and T, fT, E_2, LH and FSH in 141 ambulatory men (Miwa et al. 2006). Increased BMI and low T and/or low BAT levels raised the risk of somatovegetative symptoms in AMS among 664 men aged 40-60 years (Kratzik et al. 2004). E_2, LH and SHBG were not related to symptoms in AMS.

Eight laboratory tests: T, BT, SHBG, LH, TSH, dehydroepiandrosterone sulfate (DHEA-S), prolactin (PRL) and insulin-like growth factor (IGF-1) were assessed in 59 men (mean age 58 ± 11 years) in one recent study (Morales et al 2007) investigating the value of the available questionnaires (ADAM and AMS) used for the diagnosis of T deficiency syndromes. Their sensitivity was considered high (>80%) but both lack adequate specificity. No difference was found in 6 out of 8 biochemical tests between a symptomatic and non-symptomatic group. Only IGF-1 and DHEA-S offered some discriminatory value, and their levels were significantly lower in the symptomatic than in non-symptomatic group. The newly developed Canadian Society for the Study of the Aging Make questionnaire was also evaluated in that study concluding that it is too time-consuming and lacks both sensitivity and specificity in several domains (masturbation, sexual interest and physical performance).

Statements from "The Self-report Depression Scale" by Zung (Zung 1965), which has often been used in assessing mood in people >65 years of age, also includes symptoms associated with hypogonadism such as depressed mood, anxiety, irritability, reduced cognitive capacity and loss of sense of well-being.

2.2.3.3 Testosterone replacement therapy in older men

To define accurately the indications for androgen supplementation and to identify the elderly to profit most of the treatment are the key issues still with no answer after fifteen years of trials on T supplementation for elderly men. Although testosterone treatment has been shown to be beneficial in younger men with overt hypogonadism with clearly low T levels, similar benefits in older men generally with only moderate androgen deficiency have not been as adequately and consistently assessed (Institute of Medicine 2003).

Reviews on age-related changes in testosterone, the role and the risk and benefits of replacement therapy in older men has found very few properly conducted randomized, double-blinded, placebo-controlled studies with inconsistent findings, involving cumulative approximately 900 men (Gruenewald and Matsumoto 2003, Allan and Mc Lachlan 2004). Different doses and formulations, usually short duration of T administration and cohorts with a wide range of baseline T levels made it difficult to compare the outcomes of these trials. As far as there is not a long-term placebo-controlled double-blind study of several thousand men to confirm the efficacy and safety of testosterone treatment of older symptomatic men, risk/benefit ratio of T supplementation cannot be evaluated (Institute of Medicine 2003).

2.3. Self-rated health and life satisfaction in the aged

Self-rated health (SRH) is a measure based on a single question. The role of SRH as a predictor of mortality and several other clinical outcomes implies that it has a biological basis (Emmelin 2003). SRH has been shown to have a graded association with frequently used biomarkers (albumin, white blood cell count, hemoglobin, HDL cholesterol and creatinine) in a large (4065 men and women) population sample aged 71 years or older (Jylhä et al. 2006). After adjusting for other indicators, hemoglobin and white cell count were significantly associated with fair or poor self-rated health.

When biomarkers and other indicators were adjusted for, SRH was still a significant predictor of mortality. That study did not include thyroid function tests. The impact of TSH, FT_4 or sex hormone concentrations on life satisfaction in a community-dwelling population is poorly known, although life satisfaction was the strongest predictor of poor perceived health in a primary care practice population (Al-Windi 2005).

3. AIMS

In detail, the aims were:

I To analyze the effect of the statistical methodology used to derive clinically feasible cut-off values for TPOAb and TgAb and to describe the influence of thyroid antibodies on TSH and FT_4 reference intervals among aged thyroid disease-free aged subjects.

II To assess whether there is an association between TSH or FT_4 concentrations and self-rated health, life satisfaction, symptoms, depression or dementia in a community-dwelling elderly population.

III To describe how consistently thyroid function tests, TSH and FT_4, are used when following up the treatment of older patients with primary hypothyroidism in primary care and to compare the practice with the recommendations in guidelines and textbooks.

IV To establish biochemical sex hormone reference intervals measured with a sensitive automatic non-radioactive immunoassay method for elderly men.

V To analyze the associations between sex hormone concentrations and self-rated health, life satisfaction, symptoms, depression or dementia in community-dwelling elderly men.

4. MATERIAL AND METHODS

4.1. Study population

4.1.1. Lieto study population

This study is part of the longitudinal Lieto study, an epidemiological population-based study of subjects aged 65 years or older. The purpose of the Lieto study was to investigate common illnesses among the aged. It was carried out in Lieto, a semi-industrialized rural municipality in south-western Finland. In this study, cross-sectional data collected between March 1998 and September 1999 were used. All residents born in 1933 or earlier living in Lieto on February 16th, 1998 (n=1596) were invited to participate in the survey in a random order. Of those eligible, 63 died before they could be examined, 190 refused, 4 had moved elsewhere, 69 did not respond to the invitation, and 10 could not be traced. The refusal rate was 15%, 22%, and 27% in subjects aged 65-74, 75-84, and 85 years or older, respectively. A total of 1260 individuals participated, 727 women and 533 men, i.e. 82% of those available. 63 (5% of all participants) were in long-term institutional care. The mean age of the population was 74 years (range 65-100 years). Samples for laboratory measurements were obtained from 1252 participants. Information on chronic health conditions and current medications was obtained through standardized interviews, a review of the medical records and clinical examinations. Figures 1 a and 1 b show the age distribution of the study population.

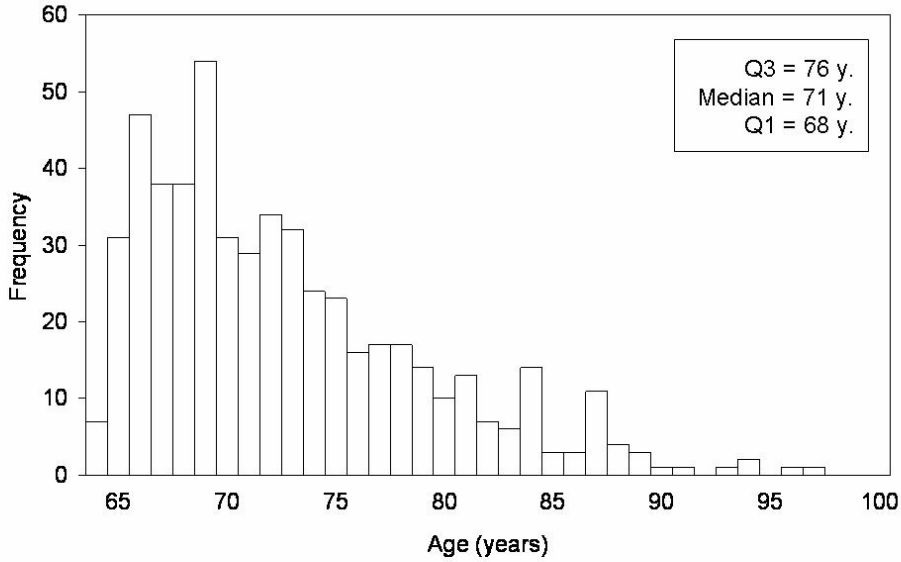


Figure 1a. Age distribution of the study population, men (n=533)

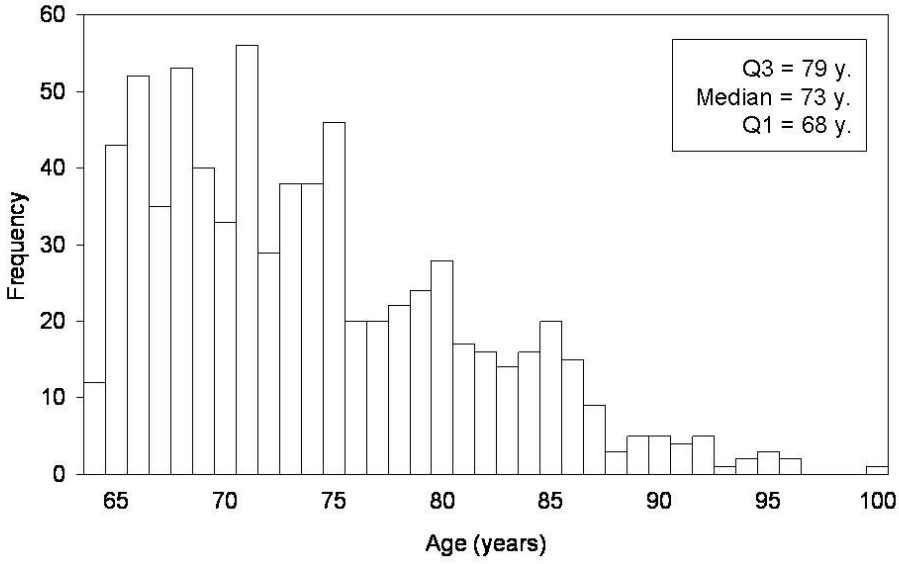


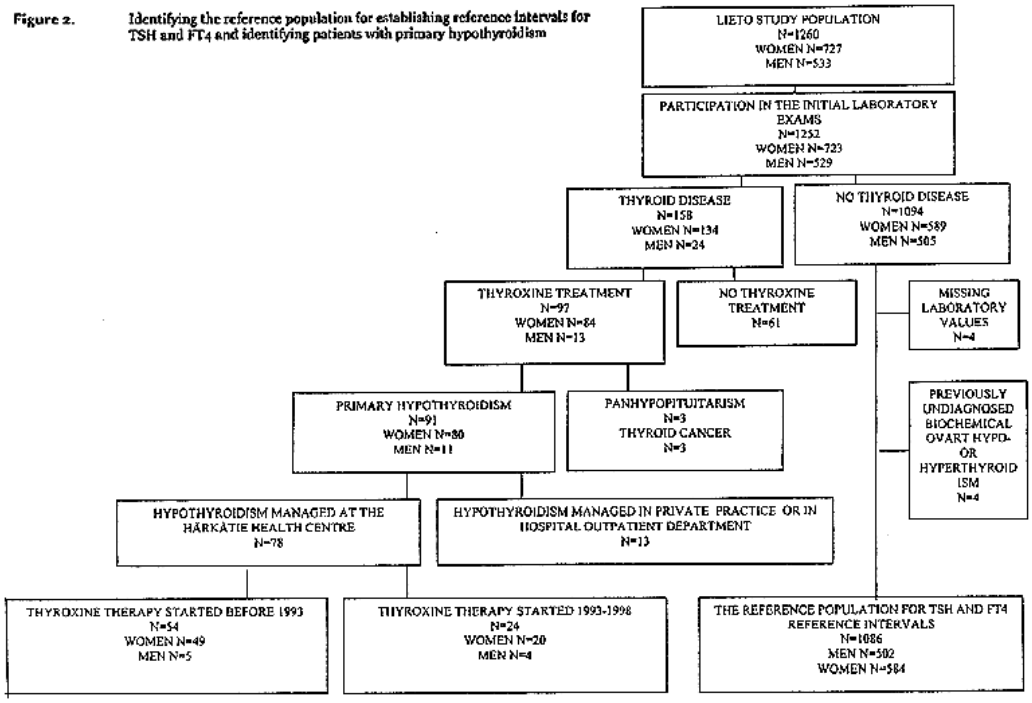
Figure 1b. Age distribution of the study population, women (n=727)

4.1.2. Identifying patients with primary hypothyroidism managed at primary care

To find patients with a previously diagnosed thyroid disease medical records were reviewed and data were collected on ICD 10 diagnosis codes for thyroid diseases, operation codes for thyroid operations, diagnoses giving entitlement to special reimbursement by the Social Insurance Institute of Finland (hypothyroidism and panhypopituitarism) and present regular medication with thyroxine or carbimazole. Patients with central hypothyroidism (n=3) or thyroid cancer (n=3) were excluded.

The group of elderly patients with primary hypothyroidism was divided into two subgroups: stable thyroxine users, whose thyroxine medication had started in 1955–1992 and initial phase thyroxine users, whose thyroxine medication had started in 1993–1998. Data on serum TSH and serum free T4 measurements and respective values cover a 4-year period between 1 March 1994 (when the Härkätie Health Center adopted the Effica laboratory database program) and 28 February 1998 (the Lieto study registration point). Laboratory results on serum TSH and serum free T4 measurements were collected in the autumn of 2003 from the medical records of the Härkätie Health Center Sinuhe-Effica database. Patients with primary hypothyroidism treated for more than 14 months in primary care before the laboratory data collection period (=stable users) were included in the study (Figure 2).

Figure 2. Identifying the reference population for establishing reference intervals for TSH and FT_4 and identifying patients with primary hypothyroidism



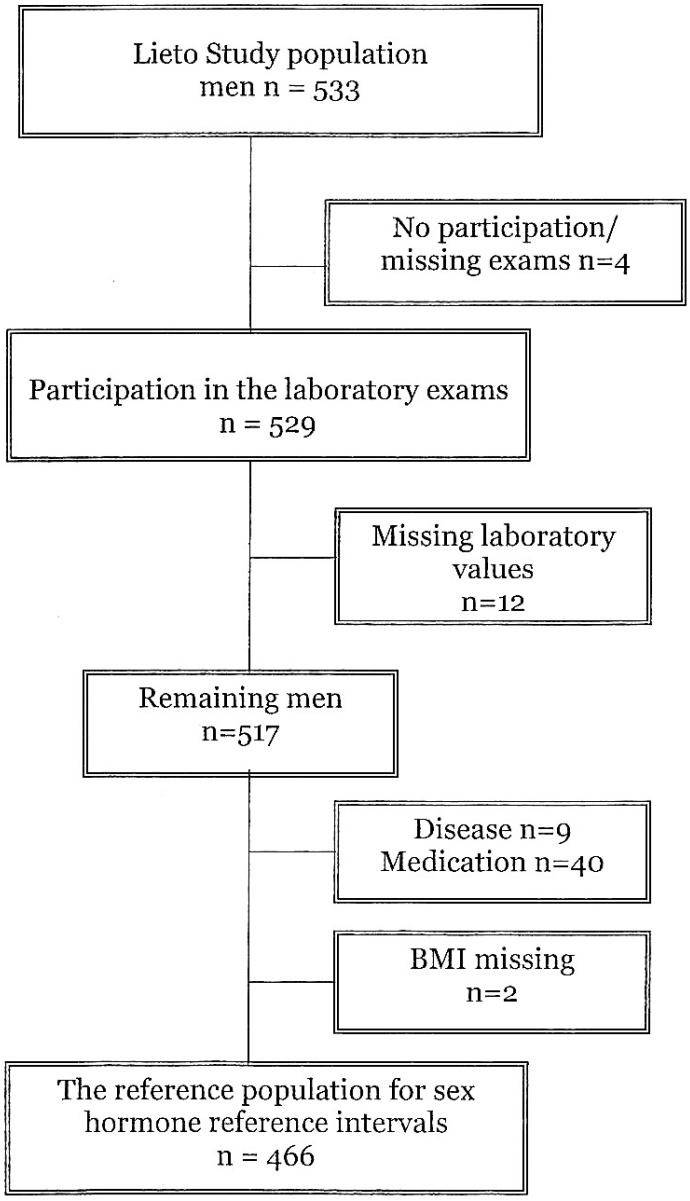
4.1.3. Identifying the reference population for establishing TSH and FT_4 reference intervals

Individuals with present thyroid disease or a history of thyroid disease or therapy, or those currently taking medication known to influence thyroid hormone levels were excluded (n=158), and an initial reference sample group (n=1094; 505 men and 589 women, mean age 73 years; range 65-100 years) was identified. From this population, the subjects with missing laboratory values (TSH one subject, FT_4 three subjects) and four subjects with previously undiagnosed biochemical overt hypo- or hyperthyroidism were also excluded. The final thyroid disease-free population (Figure 2) consisted of 1086 subjects (mean age 73 years).

4.1.4. Identifying the reference population for establishing sex hormone reference intervals

Adequate samples for laboratory measurements of T, LH, FSH, SHBG and E_2 were obtained from 517 elderly men (mean age 72.5 years; range 64-97 years). For reference purposes, subjects were excluded if they used medication (n=40, mainly 5-alpha reductase inhibitors) or had diseases or conditions (n=9, mainly prostate cancer) known to influence sex hormone levels or if data for calculating BMI (the ratio of weight to the square of height, kg/m^2) was missing (n=2). This left 466 men (64-97 years, mean age 72 years) available for the calculation of reference intervals (Figure 3).

Figure 3. Identifying the reference population for sex hormones



4.2. Ethics

The study was conducted according to the guidelines of the Declaration of Helsinki. The Joint Commission of Ethics for the Hospital District of Southwestern Finland approved the study protocol. All participants or their representatives gave their written informed consent.

4.3. Methods

4.3.1. Measurements and diagnostic criteria

Trained nurses measured the height and weight of the participants. *Body mass index* (BMI) was measured as kilograms per square meter, kg/m^2.

One of the two research physicians (Minna Löppönen and Raimo Isoaho), both experienced GPs, performed a physical examination and registered the subject's diseases as stated in the medical records.

Coronary heart disease (CHD): the participant had such a diagnosis (I20-25) in the medical records, and/or had a history of coronary by-pass operation or angioplasty, and/or was entitled to reimbursements from the NHI for CHD medication, and/or had ischemic ECG findings (major/moderate Q/QS item as a sign of myocardial infarction (Marmot and Brunner 1991).

Dementia was identified in two-stages. In the first stage, the Mini-Mental State Examination (MMSE) was administered to evaluate cognitive functioning. Criteria for the second stage were: (1) an MMSE sum score of 24-30 (2) previous history of dementing disorder or any clinical suspicion of dementia in interview (the Clinical Dementia rating Scale (Hughes 1982) and the Hachinski ischemic scale (Hachinski V 1975) or clinical examination with an MMSE sum score of 24-30. Finally, dementia was assessed according to the DSM-IV criteria by experienced physicians.

Depression was diagnosed by research physicians if the person met the DSM-IV criteria for depression (including major depression, non-specific depression, dysthymic disorder and bipolar depression). "The Self-report Depression Scale" by Zung (Zung 1965) was used in assessing the severity of depression.

Items of the Self-report Depression Scale by Zung and Durham (Zung 1965) are presented as statements and respondents indicated whether each symptom is true "little or none of the time", "some of the time", "a good part of the time" or "most of the time". Ten of the items were negatively formulated (e.g. " I have crying spells or I feel like it") and 10 were positively formulated (e.g. "My life is pretty full"). For the analysis, answers were dichotomized as (1) "little or none of the time" and "some of the time", and (2) "a good part of the time" and " most of the time".

Life satisfaction was assessed by the subjects´ response to the question "Are you satisfied with life at present?" using the alternative answers: very satisfied, satisfied, more or less satisfied, unsatisfied, very unsatisfied. For the analysis, life satisfaction was dichotomized as (1) very satisfied, satisfied and more or less satisfied, and (2) unsatisfied and very unsatisfied.

Self-rated health (SRH) was measured by a single question: "How would you rate your health at present?" The alternative answers were very good, good, fair, quite poor and very poor. For the analyses, SRH was dichotomized as (1) very good, good and fair, and (2) quite poor and very poor.

4.3.2. Laboratory analyses

Venous blood samples were drawn at the Lieto Health Center between 08:00 and 10:00 after an overnight fast by applying light stasis and with the subject in the sitting position. Fresh serum samples of TSH and FT_4 were determined in 1998-1999 using the AutoDelfia automatic immunoassay system (Perkin Elmer/Wallac Turku, Finland). Samples of TPOAb, TgAb, T, LH, FSH, SHBG and E_2 were frozen immediately at -70 °C temperature and their serum levels were also determined using the AutoDelfia automatic immunoassay method in autumn 2001.

The Autodelfia hTSH Ultra assay is a solid phase, two-site fluoroimmunometric assay based on the direct sandwich technique in which three monoclonal antibodies are directed against separate antigenic determinants on the hTSH molecule. The AutoDelfia FT_4 assay is a solid phase time resolved fluoroimmunoassay based on the back-titration principle and using second-antibody separation. The AutoDelfia FSH, LH, and SHBG assays are solid phase, two-site fluoroimmunometric assays based on the direct sandwich technique, in which two monoclonal antibodies are directed against two separate antigenic determinants of the hormone molecule. T and E_2 assays are solid phase fluoroimmunoassays based on competition between europium-labeled T or E_2 and sample T or E_2 for polyclonal antiT- or anti E_2 antibodies.

The analytical sensitivity and inter-assay variance coefficients of the assays used were: for TSH: 0.005 mIU/L, 2.5% at concentrations of 0.90 mIU/L, for FT_4: 2 pmol/L, 3.9% (15.7 pmol/L), for TPOAb: 1 mIU/L, 6.4% (55.5 mIU/L), for TgAb: 1 mIU/L, 10.1% (52.7 mIU/L), for T: 0.3 nmol/L, 6.9% (11 nmol/L), for E_2: 0.05 nmol/L, 8.5% (0.18 nmol/L), for FSH: 0.05 IU/L, 2.3% (44.8 IU/L), for LH: 0.05 IU/L, 3.4% (42.4 IU/L), and for SHBG: 0.5 nmol/L, 2.4% (63.8 nmol/L), respectively.

4.3.3. Statistical analyses

Study I. The upper reference limits (97.5th percentile) for TPOAb and TgAb were calculated after exclusion of outliers with the iterative ± 4 SD method due to extremely positively skewed distributions. A more stringent "mode-method" was also employed, by summing up the mode + (mode-2.5th percentile) for exclusion of antibodies.

Reference intervals with corresponding 95% confidence intervals were calculated for TSH and FT_4 in the reference population. Due to the positively skewed distribution, TSH values were log-transformed before the analysis. Outliers were removed with the ± 3 SD criterion. After exclusion of subjects with elevated TPOAb and/or TgAb concentrations, the reference intervals for TSH and FT_4 were recalculated.

The difference in the proportions of positive TPOAb and/or TgAb subjects between men and women were tested with the chi-square test. The two-sample t-test was used

for FT_4 and the Mann-Whitney U-test for TSH, TPOAb and TgAb to test the differences between the genders. The associations between FT_4 and age were calculated with Pearson's correlation coefficient, and the associations between TSH, TPOAb or TgAb and age were calculated using Spearman's correlation coefficient.

Study II. Owing to the skewed distributions, the values of dependent variables TSH and FT_4 were Box-Cox transformed for statistical analysis. Independent variables were dichotomized. The Mann-Whitney U-test was used to test differences in age between the categories of these dichotomized variables.

The associations of dichotomized independent variables with TSH and FT_4 variables were evaluated with an age-adjusted analysis of covariance (ANCOVA) using a stepwise procedure. Variables were selected for the model according inclusion/exclusion criteria of $p < 0.05$.

Comparisons of dichotomized independent variables among thyroid disease-free women and women with stable thyroxine treatment were done using age-adjusted logistic regression.

Study IV. The associations between sex hormones and age, between BMI and age, and between sex hormones and BMI were calculated with Pearson's correlation coefficient. Pearson's partial correlations between sex hormones and age-adjusted for BMI were also calculated. Reference intervals using 2.5th and 97.5th percentiles with their corresponding 95% confidence intervals for sex hormones were calculated after exclusion of outliers with the ± 3 SD criterion, i.e. if any sex hormone in the subject was outside ± 3 SD. Because age correlated significantly with most sex hormones studied, the reference intervals were calculated in three age groups (64-69, 70-74, ≥ 75 years). Differences in mean sex hormone values between age groups were tested with a 1-way analysis of variance (ANOVA) followed by pairwise comparisons based on Tukey's HSD test.

Study V. Owing to the skewed distributions, the values of dependent variables T, fT, SHBG, E_2, FSH and LH were Box-Cox transformed for statistical analysis. Independent variables were dichotomized. The Mann-Whitney U-test was used to test differences in age between the categories of these dichotomized variables. The associations of dichotomized independent variables with sex hormone variables were evaluated with age- and BMI-adjusted analysis of covariance (ANCOVA) using a stepwise procedure instead of separate analysis due to controlling the amount of statistical tests. Variables were selected for the model by using $p < 0.05$ as the inclusion/exclusion criterion.

P-values of less than 0.05 were considered statistically significant. In all statistical computations, analyses were carried out with SAS System for Windows (version 9.1, SAS Institute Inc., Cary, NC, USA) and in study I also GraphROC for Windows (version 2.0) was also used (Kairisto and Poola, 1995).

5. RESULTS

5.1. Thyroid function in the elderly

5.1.1. Reference interval for thyroid function (Study I)

The reference population consisted of 1086 subjects (502 men, 584 women, mean age 73 years). 27% of men and 33 % of women were over 75 years old. No significant associations were observed between age and TSH, TPOAb, or TgAb concentrations, whereas a slight and significant rising trend was found between age and FT_4. The cut-off points by 97.5th percentiles for TPOAb were 13.9 mIU/L and for 30.5 mIU/L for TgAb, and by the more stringent "mode-method" cut-offs were 7.3 mIU/L for TPOAb and 12.2 mIU/L for TgAb. The proportions of positive TPOAb and/or TgAb were higher in women than in men. The distributions of TSH among TPOAb-positive and TPOAb-negative women did not differ from each other. Women had higher TSH values than men (median 1.85 mIU/L vs 1.60 mIU/L), and after exclusion of antibody positive subjects by 97.5th percentiles the medians were 1.80 mIU/L vs 1.60 mIU/L. By "mode-method" the medians remained unchanged (1.80 mIU/L and 1.60 mIU/L).

The exclusion of subjects with elevated thyroid antibodies had no effect on the FT_4 reference interval in either gender or on the TSH reference interval in men. Among women, the exclusion of 34% thyroid antibody-positive subjects by 97.5th percentiles resulted in the lowering of the upper reference limit of TSH, from 7.2 mIU/L to 5.8 mIU/L. The reference intervals calculated after the exclusion of subjects with elevated TPOAb and/or TgAb based on the upper reference limit (97.5 percentiles) were 0.47-5.6 mIU/L for TSH and 9.8-17.6 mIU/L for FT_4. A more stringent "mode-method" by summing up mode + (mode-2.5th percentile) was also tried, resulting in the exclusion of 58% thyroid antibody-positive women, and the upper reference limit of TSH remained essentially the same.

5.1.2. Stable thyroxine users (Study III)

54 patients with primary hypothyroidism (49 women, 5 men) in stable thyroxine treatment were being managed in primary care. Thyroxine treatment had been started at an average age of 61 years (range 43 to 75 years). The average thyroxine dosage was 100 micrograms.

In most stable (=treated for more than 14 months) thyroxine users, both serum TSH (mean 1.4 measurements/year) and serum free T4 (mean 0.8 measurements/year) values were measured over a 4-year period of thyroxine treatment. 66 % of serum TSH values and 85 % of serum free T4 values were within the reference interval stated by the manufacturer. 42 % of serum free T4 determinations had been performed with TSH in the reference interval.

5.1.3. Associations of thyroid function with SRH, neuropsychiatric symptoms, depression or dementia (Study II)

After adjustment for age, there were no associations between TSH levels and self-rated health or life satisfaction or most symptoms in the thyroid disease-free population. No associations were found between diagnosed depression or the MMSE sum score and levels of TSH and FT_4. Dementia was associated with higher FT_4 concentration in men.

Although women with thyroxine treatment for primary hypothyroidism had TSH levels that were clearly higher than those in thyroid disease-free women, there were no statistically significant differences in self-rated health, life satisfaction or symptoms between thyroid disease-free women and women with stable thyroxine treatment.

5.2. Sex hormones in elderly men

5.2.1. Reference intervals for sex hormones (Study IV)

The reference population to establish sex hormone reference intervals consisted of 466 individuals between 64-97 years (26% over 75 years old) with a mean age of 72 years, and a mean body mass index (BMI) of 26.9 kg/m^2, range 15.4-41.2 kg/m^2. BMI correlated negatively with T, fT and SHBG and slightly negatively with LH. BMI and age had no correlation. Age had a significant positive association with pituitary gonadotropins LH and FSH and with SHBG, the major serum carrier of T, with the net effect that fT declined more rapidly and significantly with age than T. After adjustment for BMI, the associations between age and LH, FSH, SHBG and fT remained significant and the association between age and T became slightly significant.

The reference intervals were calculated for three age groups (64-69, 70-74, ≥ 75 years). Adjustment for BMI was not done because there were no significant differences in mean BMI between the age groups. The differences between the age groups in mean sex hormone values were significant for fT, LH and SHBG. In fT and LH, separate reference intervals should be used in men aged 64-74 and those aged 75 years or over. In SHBG two separate reference intervals by age (64-69 and ≥70 years) are also needed for aged men. Thus, in practice one age-specified range can be used only for T, E_2 and FSH in men aged 64 years or over.

5.2.2. Associations of sex hormones with SRH, neuropsychiatric symptoms, depression and dementia (Study V)

After adjustment for age, higher levels of T and fT were associated with better SRH in the reference population. After adjustment for age and BMI, there were no associations between sex hormone levels and self-rated health or life satisfaction or most symptoms. A lower level of fT was significantly associated with sleeping problems whereas a lower level of T was associated with less irritability. Diagnosed depression was significantly associated with a lower T concentration. No associations were found between diagnosed dementia or the MMSE sum score and the levels of T or fT. Higher LH and FSH level were significantly associated with diagnosed dementia.

In those, who considered their health was better, the proportion of digoxin users was 5% (18/395). In those feeling their health was poor, the corresponding proportion was 12 % (8/66). Digoxin users (n=26) were older than non-users (age median 73 vs. 71 years), and T and fT medians were : 19.0 vs. 19.1 nmol/L and 255 vs. 256 pmol/L, in consistency with age. So it seems that the inclusion of digoxin users in the reference population did not cause any markedly biased results. The number of other medicine use that are known to affect T levels was so small that it was also considered not to have biased our results markedly: 13 verapamil users (SRH better 2% vs. SRH worse 9%), 11 diazepam users 11 (SRH better 2% vs. SRH worse 4%), 9 prednisone users and no users of pravastatin and phenytoin.

6. DISCUSSION

6.1. Study population and reference groups

The population of Lieto is a typical population in south-western Finland, and its age distribution did not differ markedly from the average Finnish distribution (Tilastokeskus, 2005). This study population was characterized by a wide age range (64-100 years) and high participation rate (82%), thus offering a good opportunity to examine the possible relations of hormone levels with comprehensive data. The refusal rate rose with age and this may have skewed the results, but only to a small extent. Potential confounders are the progressive effect of age-related variables, such as chronic illness or cumulative exposure to medication, which are independent of the aging process itself (Gray et al. 1991).

To ensure that the data is representative of the general older population with wide BMI ranges and morbidity the reference material was not restricted to non-obese subjects with no use of medication. In selecting reference subjects, persons with clinical diseases or conditions or medications known to clearly influence thyroid function (studies I and II) or sex hormone levels (studies IV and V) were excluded. Morbidity was assessed by reviewing the medical records and special reimbursements for medications by the NHI and by clinical examination. These can be considered reliable sources of information compared to self-report only (Haapanen et al. 1997; Kriegsman et al. 1996).

One of the main focuses was to gain a representative aged reference population. For operative implementation, there is no exact definition of a healthy older person and the selection of appropriate exclusion criteria is a continuous topic of debate. Disabled and multimorbid persons were recruited actively, and the study population was thus considered to be representative of the older population in Lieto and not selected on the basis of good or excellent health.

In this outpatient setting, most preanalytical variables have a lesser effect on serum TSH, FT_4 or sex hormone concentrations compared to surveys involving hospitalized patients. Altogether, 88% of the subjects had used prescription drugs during the 7 days preceding the study interview (Linjakumpu et al. 2002). This study did not include subjects with severe non-thyroidal illnesses or subjects on medication used only in hospitalized patients (dopamine, intravenous heparin, large intravenous doses of furosemide etc.) that can influence thyroid function tests. There were no subjects with a diagnosis of hepatic cirrhosis or severe renal disease in the reference population. The associations of TSH and FT_4 with coronary heart disease or diabetes (common diseases among elderly) were examined. No association was found (data not shown).

Those, who felt their health to be worse and were unsatisfied with life, were older than those in a better condition, and older subjects used more medication than younger subjects. The number of users of medicines that might affect sex hormone levels (after excluding those clearly affecting endogenous hormone levels, like exogenous hormones and 5-α reductase inhibitors) was so small in the reference population for sex hormones that it did not bias the results to any great extent. Among commonly used medication in elderly outpatients, medication more often has a decreasing rather

than an increasing effect on T levels, but no single drug use had any significant effect in the results of this study.

Cross-sectional design of this study is a limitation. A longitudinal study of this Lieto study cohort would make possible to follow trends of hormone concentrations within individuals, and confirm directions of causal relations.

6.2. Laboratory measurements

In this study T and E_2 were estimated with an automated immunoassay method. Several different immunoassays of total T have been approved by regulatory agencies. The change in measuring method reinforces the need to establish reference intervals and age-specific reference ranges for adult males for each method, as has now been done with the automated immunoassay method AutoDelfia.

In a study by Wang et al (Wang et al. 2004), none of the four immunoassays tested were of sufficient accuracy at low serum T levels using liquid chromatography-tandem mass spectrometry as the gold standard, thus none of the assays tested was sufficiently reliable for investigation of women and children. A comparison of ten different immunoassays with isotope-dilution gas chromatography-mass spectrometry showed that conventional immunoassays performed reasonably well in men, but some immunoassays tended to underestimate and others overestimate total T over the range tested (Taieb et al., 2003).

Associations of E_2 with the variables studied should be judged with caution because of the poor precision and accuracy of the method at low serum E_2 concentrations (the analytical sensitivity of E_2 is 50 pmol/L with AutoDelfia). Despite the poor precision and accuracy of the immunoassay methods at low serum T and E_2 concentrations, economical and rapid automated immunochemical methods are widely used in clinical practice. Laborious reference methods are used mainly for scientific purposes, provided that low E_2 concentrations and low serum T concentrations are determined by immunoassay method (especially in sera from women and children) and should be verified by an accurate reference method.

The most reliable method for determining fT, equilibrium dialysis of undiluted serum followed by diethyl ether extraction, is time-consuming and complex (Törmä et al. 1995). Calculation of fT from T and SHBG, as used in this study, yields values in good agreement with values obtained by direct methods (Vermeulen et al. 1999).

6.3. Thyroid function in the elderly

In an unselected aged population, the clinical significance of (slightly) elevated thyroid antibody concentrations remains questionable. The effect of thyroid antibody positivity on reference intervals for TSH and FT_4 in an elderly population was found to be minor in this study. The effect was clinically negligible on FT_4 reference limits in both genders and on the TSH reference interval in men. Among women, the exclusion of thyroid antibody-positive subjects by conventional method (every third elderly woman) resulted in lowering of the upper reference limit of TSH. When the more stringent "mode-method" was used, almost sixty percent of women were excluded,

and the 97.5th percentile remained essentially the same. Furthermore, the distribution pattern of TSH among TPOAb-positive versus TPO-negative women showed no differences. The reference interval for TSH, 0.47-5.6 mIU/L, was much wider than that recommended in the NACB guideline, consistent with several recent studies (Jensen et al. 2004; Knudsen et al. 2000). Although subjects currently taking medication known to clearly influence on thyroid hormone levels were excluded, it may be argued that sporadic factors other than physiological aging and occult thyroid insufficiency due to autoimmunity are also responsible for the wide TSH reference interval in this study. These factors may include transient changes in TSH because of concurrent nonthyroidal illness and the effects of certain drugs widely used in aged patients (Keffer 1996; Surks and Sievert, 1995).

What are the consequences of, or the rationale for narrowing the reference interval of TSH in older populations? If the suggestion for the TSH upper reference limit of 2.5 mIU/ in the NACB guideline is applied to the population of the Lieto Study, it would mean that 30% of women and 22% of men will be prone to further testing and the increasing disadvantages of overtreatment. Guidelines that suggest lowering the upper reference limit for TSH or considerably lowering the TSH target level in thyroxine treatment, cause difficulties in clinical practice, because thyroxine has a narrow toxic-to-therapeutic ratio. It is obvious that physicians will prepare for increasing problems in screening and management of primary hypothyroidism especially among patients with known ischemic heart disease (21% prevalence in population of the Lieto study), or in patients with previously undiagnosed ischemic heart disease, if the reference ranges narrows and, simultaneously, the optimal TSH target in levothyroxine treatment decreases.

Especially problematic in elderly populations is the concept and management of subclinical, usually asymptomatic, endocrinological disease. There were no associations between TSH levels and self-rated health, life satisfaction, or most symptoms in the thyroid disease–free population in this study. Where statistically significant associations were found, a higher TSH level was associated with a better condition. Even with present TSH intervals, controversy surrounds the necessity for the prompt diagnosis and treatment of subclinical hyperthyroidism, and especially the more common subclinical hypothyroidism (Surks et al. 2004). In older populations biochemical thyroid dysfunction is common (Canaris et al. 2000; Hollowell et al. 2002) even when based on current reference intervals for TSH. In the large Colorado Thyroid disease prevalence study, the difference in the prevalence of symptoms between subjects with subclinical hypothyroidism (13.7%) and euthyroid controls (12.1%) was marginal (Canaris et al. 2000).

Although women treated with thyroxine treatment for primary hypothyroidism had TSH levels that were higher than those in thyroid disease–free women, there were no statistically significant differences in self-rated health, life satisfaction or symptoms between these two groups in this study. This study shows that only 66 % of TSH values are within the normal range, a result that is quite similar to that reported in other studies (Canaris et al. 2000; Diez 2002). Textbooks, clinical guidelines and consensus statements on the management of primary hypothyroidism give somewhat varying and approximate instructions on how to monitor the adequacy of and compliance with thyroxine treatment through laboratory examinations. Physicians do

not readjust the dose of thyroxine rapidly enough even with the present reference interval (Canaris et al. 2000). The dosage adjustment will be even more complicated and the risks of overtreatment will increase, if the target level of TSH is lowered. It is quite a challenge to adapt the contents and implementation of the guidelines and recommendations to better meet everyday clinical practice. The implementation of strict reference limits derived from a general healthy population for an aged population may have several counterproductive effects.

When the sensitive method for determining TSH was taken into use, it was expected to reduce the number of free T4 measurements. The high number of free T4 measurements taken when TSH was in the reference interval shown by this study suggests that this aim has not been attained. It is highly probable that GPs are not very familiar with the sensitive TSH method and its properties, i.e. that free T4 measurement does not offer any advantage in managing primary hypothyroidism, especially when TSH is within the reference interval. The possibilities offered by present laboratory technology (e.g. instruction to measure free T4 automatically from the same sample when TSH is outside the reference interval) should be utilized in practice and should be included in the guidelines instead of saying that serum free T4 should be measured every now and then along with TSH.

6.4. Sex hormones in elderly men

In this study, the reference intervals for the pituitary gonadotrophins LH and FSH were wide and their levels increased significantly with age. Hypogonadism in older men usually has both primary and secondary components. It is possible that T levels in elderly men remained at quite adequate levels partly due to rising LH levels.

To ensure that the data is representative of the general older population with wide BMI ranges the material was not restricted to healthy non-obese subjects. Nevertheless, the lower reference limits of T and mean T were only slightly lower than those for a younger healthy age group given in the manufacturer's AutoDelfia kit insert information (9.4-33.5, mean 19.6 nmol/L; 21-66 years, n=147). Recent studies with T measured with a radioimmunoassay method (Boyce et al. 2004; Kehinde et al. 2005) with results of essentially lower reference limits for T than previously published suggest that reference ranges of T published earlier may be too high.

In aging and weight-gaining western societies, hormonal changes occurring with BMI, and mediated primarily via SHBG concentration (Bjornerem et al. 2004), encouraged the study of the influence of BMI as a mediator on associations of sex hormone levels and health among elderly men also. Older age or being overweight (and, to a smaller extent underweight) were predictors of poor health or many symptoms among elderly men. Age had a significant association with LH, FSH, SHBG, fT and, after adjustment for BMI, also T. Furthermore, BMI correlated negatively with T, fT, SHBG and LH. So it was not unexpected that, even though T and fT levels were clearly lower (or gonadotrophin levels higher) in univariate analyses of most negative responses of neuropsychiatric symptoms, these differences were not statistically significant after adjustment for age and BMI.

Most elderly men (87%) in the reference population had a T level above 12 nmol/L whereas 3% of men had T levels under 8 nmol/L, and according to recent guidelines (Nieschlag et al. 2005), require substitution. Thus 10% of the reference population of Lieto Study had their T levels in the grey area, between 8-12 nmol/L, where symptoms of T deficiency were assumed to be manifest according to that guideline, and trials of treatment may be considered in those for whom alternative causes of these symptoms have been excluded.

Most neuropsychiatric symptoms investigated seemed to have weak or no correlation with T or fT levels among elderly men after adjustment for age and BMI, presumably because of their nonspecific nature. Most previous studies where questionnaires for screening androgen deficiency (with cutoff levels for T) were evaluated have led to similar conclusions (Kratzik et al. 2004; Miwa et al. 2006; Spetz et al. 2007).

In previous epidemiological studies on depression (Araujo et al. 1998; Barrett-Connor et al.1999) or dementia (Barrett-Connor et al. 1999; Fonda et al. 2005; Geerlings et al. 2006; Moffat et al. 2004), heterogeneity in symptoms, different diagnostic assessments of depression or dementia, low participation rates and different study samples may have led to inconsistencies in the results. Exact comparisons between the results of this study and those of previous ones are difficult to make. In this study, diagnosed depression was significantly associated with lower T concentration, whereas a higher LH and FSH level was significantly associated with diagnosed dementia. These relations might have clinical significance, though clinical trials on the effect of T substitution in mood or cognition have not been convincing. The effects of T substitution in men with Alzheimer's disease have been evaluated in three small studies with inconsistent findings (Cherrier et al. 2005; Lu et al. 2006; Tan and Pu 2003). A systematic review on normative hypogonadism and depression (Seidman 2006) found only two placebo-controlled T replacement studies in which mood was assessed systematically (Sih et al. 1997; Steidle et al. 2003). In these studies the T placebo difference was not distinguishable with respect to mood.

In a recent double-blind, randomized, placebo-controlled trial of 237 healthy men between the ages of 60 and 80 years with a T level lower than 13.7 nmol/L (below the 50th percentile of the study population after screening 50 candidates), quality of life was measured with the Short-Form 36 Health Survey as a generic quality-of-life questionnaire and the Questions on Life Satisfaction Modules as a hormone-specific questionnaire. Supplementation with 160 mg of testosterone undecenoate orally during six months did not affect cognition or quality-of-life measures except for one portion, hormone-related quality-of-life measure that improved, especially on the item "resilience or ability to tolerate stress" (Emmelot-Vonk et al 2008).

6.5. Concentrations of thyroid and sex hormones and health among the elderly

In geriatric medicine and in family medicine, maintaining quality of life is more important than primary prevention of risks that will not become real for most elderly in their remaining lifetime. Despite the high prevalence of chronic conditions, most elderly people feel that they are healthy or are quite satisfied with their present life (Heikkinen 1983), as also in this study. Symptoms related to mild thyroid deficiency or

"andropause" are nonspecific for those disorders especially in older populations, but they are, however, frequent.

In the current situation, there is a need for more research based on an elderly community-dwelling population regarding the associations of hormone concentrations with conditions or end points which are meaningful for the elderly subjects themselves. There is also a need for calculations or at least evaluations of the risk/benefit ratios of possible treatments. It is questionable whether older people suffer from minimal biochemical changes in thyroid function or sex hormone levels to such an extent that they are willing to be exposed to unnecessary further testing and risks of treatment without certain knowledge of benefits.

7. CONCLUSIONS

The following major findings and conclusions were made:

1 The exclusion of subjects with elevated TPOAb and/or TgAb concentrations, regardless of the statistical methodology used, had no effect on the FT_4 reference interval in either gender or on the TSH reference interval in men, whereas the upper reference limit in women decreased from 7.2 to 5.8 mIU/L. The reference intervals calculated after the exclusion of subjects with elevated TPOAb and/or TgAb based on the upper reference limit were 0.47-5.6 mIU/L for TSH and 9.8-17.6 mIU/L for FT_4.

2 After adjustment for age, there were no associations between TSH levels and self-rated health, life satisfaction, diagnosed depression, dementia or most neuropsychiatric symptoms in the thyroid disease-free elderly population. Although women with thyroxine treatment had TSH levels that were clearly higher than those in thyroid disease-free women, there were no differences in self-rated health, life satisfaction or symptoms between thyroid disease-free women and women with stable thyroxine treatment. The results do not support the need to decrease the upper reference limit for TSH or to lower the optimal TSH target in levothyroxine treatment in older adults, as recommended in several recent guidelines.

3 The practice of following up stable thyroxine treatment by TSH and FT_4 determinations in elderly patients with primary hypothyroidism in primary care was quite inconsistent and differed considerably from clinical guidelines, especially the high number of FT_4 measurements/assessments. Some key issues in the clinical guidelines were difficult to interpret, and the patients' age or other main characteristics were not taken into consideration adequately.

4 Age had a significant positive association with SHBG, LH and FSH and negative association with fT. BMI correlated negatively with T, fT and SHBG and slightly negatively with LH. BMI and age had no correlation. In clinical practice, one range in men aged 64 years or over can be used for T, E_2 and FSH measured with the AutoDelfia method, but two separate reference intervals should be used for fT, LH and SHBG.

5 After adjustment for age and BMI, there were no associations between sex hormone levels and self-rated health, life satisfaction or most neuropsychiatric symptoms in elderly men. Diagnosed depression was significantly associated with a lower T concentration, although single neuropsychiatric symptoms commonly associated with androgen deficiency seemed to have weak or no correlation with T levels among elderly men.

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