Psychological well-being on thyroid hormone replacement

by

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Declaration: I, Dr Ponnusamy SARAVANAN, declare that the publications, except *publication 9* have not been previously submitted or are currently being submitted whether published or in unpublished form, for a degree, diploma, or similar qualification at any university or similar institution. This work was carried out between Aug 1999 and Sep 2003.

Publication 9 was a follow-on work that has been submitted towards a PhD degree for Dr V Panicker, who has worked on the DNA samples collected from my original work.

Word count: 9299

Index **pages**

Section 1: Commentary linking the published material for PhD by publication

Section 2: Nominated material from the field of study

Partial substitution of thyroxine (T4) with tri-iodothyronine in patients on thyroxine replacement therapy: Results of a Large community based randomized controlled trial

Section 3: All the relevant publication in the field of study

Summary:

Despite 100 years after the discovery of thyroxine, controversy still exists regarding optimal thyroid hormone replacement therapy. Several anecdotal reports suggest that *thyroxine alone therapy does not normalise psychological wellbeing*. My cross-sectional study (n=1922) provided the first evidence in support of the hypothesis that a small proportion of patients on thyroxine alone therapy have increased psychological morbidity despite having normal TSH *(publication 1)*. My second study was the largest randomised placebo controlled study to date to compare the effects of thyroxine alone and combined T3/T4 therapy over a 12 months period. This *categorically proved that thyroxine alone therapy should be the first choice for hypothyroid patients (publication 2).* Further genetic analysis of the deiodinase genes showed that a sub-group of hypothyroid patients with an SNP on D2 gene do have reduced psychological wellbeing on thyroxine alone therapy and improve on combined T3/T4 therapy compared to those without *(publication 5)*. Both these findings were shown only by our study and were possible because of the large size (n=700). Detailed analysis of the various thyroid hormones and their ratio from our study showed that *in addition to TSH, free T4 should be taken into account when treating hypothyroid patients (publication 4).* My pharmacokinetic study provided the crucial and *first evidence of the profiles of thyroid hormones on once a day combined T3/T4 therapy,* highlighting the need to use either slow-release T3 or multiple doses of T3 in a day *(publication 3)*. Our invited commentary and review *(publications 6-8 & 10)* have highlighted the importance of "individualised set points" for thyroid hormones, the complexity of thyroid hormone transport and actions as well as an algorithm for approaching hypothyroid patients. My other work *(publication 9)* was the first to test the possibility and provided the first evidence of deiodinase gene polymorphisms affecting circulating thyroid hormone levels and their possible role in psychological wellbeing in normal population. Thus, my work in the area, "Psychological wellbeing in patients of thyroid hormone replacement therapy" has provided several landmark findings, resulting in 10 publications including 4 in JCEM, 2 in Lancet and 1 in Clinical Endocrinology.

Background:

Up to 3% of general population are on thyroid hormone replacement therapy in the western countries (1). The most common indication for such replacement therapy is primary hypothyroidism (2), but in a significant number of individuals thyroid hormone replacement follows destructive therapy for hyperthyroidism, nontoxic goitre or thyroid cancer with either radioactive iodine (RAI) or surgery. Autoimmune thyroid disease is one of the commonest endocrine disorders affecting up to 10% of general population in iodine-sufficient countries. Of these, around 1-3% has overt hypothyroidism and the remaining has untreated subclinical hypothyroidism (3-5).

A normally functioning thyroid gland produces both thyroxine (T4) and tri-iodothyronine (T3), containing 4 and 3 iodine atoms respectively. A majority of the thyroid hormone production is in the form of T4. Thyroxine does not have direct action on the tissues and has to be converted to T3, by deiodination, which acts on the thyroid hormone receptor (THR). Out of the daily requirement of T3 (around 30 mcg), approximately 24 mcg is generated by peripheral deiodination of T4 and the remaining 6 mcg is produced directly by the thyroid gland. Such deiodination is carried out by not one but three selenium dependent deiodinases (D1, D2 and D3) each with different catalytic specificity, tissue distribution and sensitivity to extracellular influences. For example, tissues expressing higher concentration of D2 are more sensitive to circulating T4 than T3 and those with higher expression of D1 are more sensitive to T3 than T4. As a result, the amount of intracellular T3 derived directly from circulating T4 rather than T3 can vary up to 10-fold between tissues (6). Indeed, the pituitary gland has much higher expression of D2 making it more sensitive to circulating T4 than T3.

Despite these complexities, the standard thyroid hormone replacement comprises only T4. This is because it has a much longer half-life and therefore can be used as once a day preparation. Thus, it yields more stable levels of serum T3 over 24-hours compared to T3

alone therapy, which has to be used 3 times a day. In addition, it was perceived that peripheral deiodination should provide adequate levels of T3 that is required for the tissue action.

In the past four decades, there have been significant changes in standard practice for thyroid hormone replacement. In the 1960s synthetic T4 replaced the use of desiccated thyroid extract as the latter posed a challenge for standardization (7). Then in the 1980s, following the introduction of sensitive TSH assays, it became established practice to adjust the dose of T4 to achieve a TSH level within the laboratory normal range (8). It was argued that thyroxine doses that normalize the TSH level must result in physiological thyroid hormone replacement as the hypothalamo–pituitary axis perceives such doses as satisfactory. This resulted in significant dose reductions for many patients, in some cases by up to 100 μg per day, to achieve TSH levels in the “normal range”.

In recent years, however, there appears to be an increasing number of patients who express dissatisfaction with their thyroid hormone replacement. Subsequently, several anecdotal reports suggested that treated hypothyroid patients began to complain that “they do not feel right”, especially reporting reduced psychological wellbeing despite their biochemical euthyroidism – referred as “euthyroid dysphoria”. In response to our article in the newsletter of the British Thyroid Foundation (BTF), 204 respondents reported persisting psychological symptoms following treatment for thyroid disease and 54 mentioned specifically not feeling normal despite their thyroid function being in the laboratory normal range (9). Many patients also found their physicians to be unsympathetic and dismissive of their symptoms. In this context, Carr et al. (10) had previously observed that patients’ visual analogue scales of well-being were highest on doses of thyroxine that resulted in low TSH levels (<0·2mU/l), doses that the authors considered to be 50μg higher than ‘optimal’ replacement.

In response to such expressions of dissatisfaction, a group of general practitioners and private

practitioners have advocated titrating the dose of T4 against the clinical status of individuals rather than the TSH levels (11) and doubt has been cast on the true value of TSH measurements in patients undergoing thyroid hormone replacement (12). Some endocrinologists agree with a role for clinical assessment in addition to measuring TSH (13), though many were not convinced. There was only one small study directly compared symptoms in patients on thyroxine with a normal TSH and controls and no significant differences were observed (14).

However, this is a difficult area, as many of the symptoms of hypothyroidism are nonspecific and could be confused with low mood, stress-related illness or depression due to other causes. As both depression (15, 16) and thyroid hormone replacement (2) are common in the general population a causal relationship between them could easily be wrongly inferred. On the other hand, evidence from rodent studies suggests that T4 replacement alone does not provide normal T3 levels in all tissues and suffer “tissue hypothyroidism” (17, 18). Indeed, as approximately 15% of circulating T3 in healthy human beings is produced by thyroid gland (rest generated peripherally from T4), and if we extrapolate the rodent data, such “tissue hypothyroidism” could exist in humans. This in turn could be at least one of the causative factors for the dissatisfaction of some of the hypothyroid patients on T4 alone replacement with normal TSH (“euthyroid dysphoria”).

Around the time of designing our baseline study, a small study (n=33) was published in the New England Journal of Medicine (19) suggesting combined T3/T4 therapy (both given once a day) is superior to T4 alone therapy for hypothyroid patients. However, there were several limitations, which are described in detail later. Thus, there were several unanswered questions in this common endocrine problem.

These were:

1. Does “euthyroid dysphoria” exist in treated hypothyroid patients with normal TSH on

thyroid hormone replacement therapy (T4 alone)?

2. Does combined T3/T4 therapy for hypothyroidism improves patients' psychological wellbeing?
3. Can adequate 24-hour thyroid hormone profiles (T4, T3 and TSH) be achieved on once a day T3/T4 replacement therapy?

We designed our baseline study to answer the first question. To address this, and to avoid selection bias as far as possible, we decided to study the psychological wellbeing of patients on T4 in a community-based setting. This was my first project and my first published work in this field.

Commentary linking the work done:

Project 1 - Cross sectional study (publication 1):

Introduction:

My first project was a large community based study involving 1922 patients (publication 1). As a first stage of this project, in collaboration with a clinical psychologist, we designed a thyroid symptom specific questionnaire, TSQ (thyroid symptom questionnaire). This was developed using the feedback from the patients for an article in the British Thyroid Foundation newsletter. As the symptoms of T4 under replacement are poorly defined, we chose to use the General Health Questionnaire-12 (GHQ-12). The GHQ-12 asks subjects to describe how they currently feel compared with their normal expectation and was developed to detect psychological distress of an unspecified nature in the general population (20, 21). It is a well-validated questionnaire that detects 'caseness' in the general population with a sensitivity of 72 – 80% and specificity of 75 – 86%, when compared with complex, time-consuming interviews by a trained psychiatrist, such as the 'Clinical Interview Schedule and Present State Examination' (22). The TSQ was developed in a similar format to the GHQ-12. A pilot study on 100 patients was then conducted before the large community based survey. A trend towards less psychological wellbeing was observed in the study group (treated hypothyroid patients) compared to age and sex matched control group.

Methods:

This pilot study enabled us to design the main study requiring a study sample of 1000 subjects (500 controls and 500 study). Prescribing records from five general practices in the Weston-super-Mare area in South West England covering a population of 63,000 were used to identify the patients on thyroxine. A total of 961 patients aged between 18 and 75 years and who had

been taking thyroxine for at least 4 months were identified. Computer records were then used to identify a control subject from the same practice with the same age and sex as each of the patients. All 1922 individuals were sent the same two-page questionnaire, anticipating 50-60% returns.

Each questionnaire was accompanied by a covering letter and a prepaid return envelope. The covering letter, signed by the patients' general practitioner, explained that this was a survey into the 'effects of medication on people's general health'. It was stipulated that the completed questionnaires from those on *no medications* were also important and that the results of this survey will help to guide changes in the prescribing of certain medications. The first page of the questionnaire asked the individuals to indicate if they ever had any conditions or treatments from a list of 12 in order not to draw attention to just thyroid disease. These were as follows: diabetes, angina or heart disease, underactive thyroid disease, stroke, depression, radioactive iodine, high blood pressure, asthma, overactive thyroid disease, anaemia, epilepsy/fits and thyroid surgery. A list of present medication and doses was then requested to confirm that none of the controls and all of the patients were indeed taking thyroxine. This was also used to identify the use of psychotropic and other chronic disease medications.

The second page consisted of the GHQ-12. Respondents mark each question on a four-point scale from 'better than usual' to 'much less than usual'. Results were scored by the traditional bimodal GHQ scoring method (0, 0 and 1, 1) and the Likert scoring method (0, 1, 2 or 3) (20). The maximum score was therefore 12 with the GHQ scoring method and 36 with the Likert scoring method. A high score indicates high levels of dissatisfaction with the respondent's current mental status. The third page consisted of our devised TSQ. Answers were scored in the same way as the GHQ-12. This questionnaire has not yet been validated and hence was not used as a primary study endpoint.

Results:

The overall response rate was 60%. Five hundred and ninety seven patients on thyroxine (patient; P - 62%) and 551 controls (C 57%) responded. The responding populations were well matched for age (P : C = 59·96 : 59·35 years, P = 0·45) and sex (P : C = 84·7% : 87%, females). Though, as might be expected, the non-responders were younger than responders, there was no difference between the patient and control groups (non-responder P : C 56·20 vs. 55·52 years, P = 0·94) and the sex ratio was similar (P : C = 85·2% : 84·0%, females). The principal reasons for taking T4 was primary hypothyroidism. Out of the 597 patients, 462 (77·9%) had their TSH checked within the past 12 months and of these 85·9% (n = 397, nP group) were in the laboratory normal range (TSH – 0.2-5.5 u/l). Mean age and sex of the nP group were well matched to the controls (mean age nP : C = 59·73 : 59·35 years, P = 0·82, sex nP : C = 82·6% : 87%, females).

The GHQ-12 was completed by 535 out of 551 controls and 572 out of 597 patients. For the TSQ the equivalent figures were 534 and 583, respectively. Using the Likert scoring method, the mean GHQ-12 scores were significantly higher in the P than the C group (12·09 vs. 11·39, P = 0·028), indicating an increased incidence of dissatisfaction with current mental status. The differences persisted in the nP subgroup [12·11 vs. 11·39 (all controls), P = 0·017]. With the TSQ, a greater difference was seen between the P and C groups (12·55 vs. 11·52, P < 0·001), which also persisted in the nP subgroup (12·81 vs. 11·52, P < 0·001). To evaluate the clinical importance of these differences, the questionnaires were also scored via the 'GHQ method' and the results examined against different thresholds for "caseness". Of the control group 137/398 (25·6%) scored 3 or more using the GHQ scoring method, a cut-off level (GHQ 2/3) that has been used frequently in previous studies to indicate "caseness" (22). Using this threshold, a significantly higher proportion of both the total patient group and the group having normal TSH values would be classified as "cases" [P = 32·3% (p = 0·014), nP = 34·4% (p = 0·005)].

Figure 1: Odds ratio of “caseness” in patients compared to control population

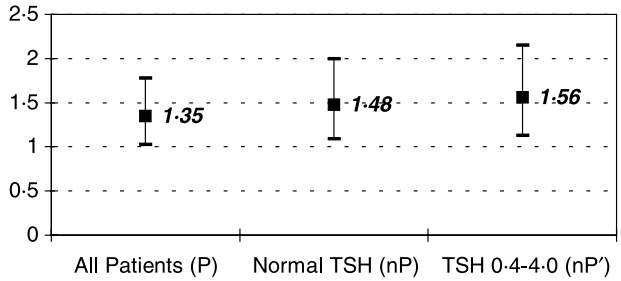

Figure 1: Odds ratios and confidence intervals for caseness (GHQ) comparing the patients and patient subgroups with normal TSH to controls. The Y-axis shows odds ratios for caseness using GHQ scores of 3 or more as a threshold. The X-axis shows groups compared with the control group: total responding patient group (P), patient group with TSH in normal laboratory range (nP) and patient group with TSH in a narrow normal range (0·4–4·0 mU/l; nP").

These differences persisted even after correction for age, sex, general practice, chronic disease and chronic drug use. Similar but somewhat greater differences were seen using the GHQ method to score the TSQ. Repeat analysis of the data using a subgroup of patients whose TSH values fell into a narrower normal range (TSH = 0·4 – 4·0 mU/ l, n = 296, nP*) gave similar, if not worse results.

Summary & significance of this publication:

Thus, the results show that patients on thyroxine with “normal” TSH had significantly increased morbidity compared to their age, sex and GP practice matched controls. Indeed, we identified a 6.7% (25.6% vs. 32.3%) absolute increase in caseness (odds ratio of 1.35-1.56) in patients on T4 with a TSH in the normal range compared with a matched control group. Such an increase, if true, could account for nearly 50,000 excess cases in the United Kingdom. This study had unique strengths, being the first to systematically study the psychological wellbeing in a large number of patients that is community-based, assessing everyone on thyroxine rather than a sub-group of patients recruited from secondary care (self-

presenting to specialists). *This was the first hard evidence that hypothyroid patients on T4 treatment do suffer from increased morbidity despite having their TSH in the normal range.* However, this did not prove a causal effect. This resulted in my second project.

Project 2 – Prospective Randomised Placebo-Controlled Trial (publication 2):

Introduction:

My first project, the cross-sectional study, confirmed that there is strong association between treated hypothyroid patients and reduced psychological wellbeing. This supports the hypothesis, *"similar to animals, relative tissue hypothyroidism on T4 alone therapy may be present in humans causing hypothyroid symptoms related to certain tissues and cause reduced wellbeing".* However, it is not known whether combined T3/T4 replacement therapy (mimicking the normally functioning thyroid gland) corrects or improves the psychological wellbeing of the hypothyroid patients who are on adequate replacement of T4 judged by their TSH. Such improvement will confirm any such association is causal. There was increasing evidence from animal studies around the time of our cross-sectional study suggesting such association may indeed be causal. A series of elegant studies from Escobar-Morreale showed that unless the combination of T3/T4 is used for replacement in hypothyroid rats, several tissues lack adequate T3. However, evidence from humans was limited. The T3/T4 ratios are lower in hypothyroid patients on T4 alone therapy than those with endogenous thyroid function (23). This suggests some tissues especially the ones relying on circulating T3 may indeed have lower intracellular levels of T3 than required. The only clinical evidence to support the role of combined T3/T4 therapy came from a small study on 33 post thyroid cancer patients over 5 weeks showing the patients preferred combined T3/T4. However, there were several limitations of this study: 1) small number of highly selected patients who had thyroidectomy for previous thyroid cancer; 2) short duration (5 weeks of intervention); 3) no washout period during the crossover design and 4) use of a visual analogue scale that was not validated. To

resolve this issue, my second project was designed to test the effect of combined T3/T4 therapy for hypothyroid patients on adequate T4 only replacement (as per their TSH). Using the data from the cross-sectional study, we carefully powered and designed a large double blind, community based, randomised placebo controlled trial comparing the effects of T4 alone vs. combined T3/T4 therapy. *This was the largest (n=697) and of the longest duration (12 months) in the world to date testing the effects of combined* T3/T4 *therapy for hypothyroid patients (publication 2).*

Methods:

A sample size of 700 patients was needed based on our cross-sectional study data to detect a 0.7-point difference between the groups on the GHQ-12 Likert scale with 80% power at a significance level of 0.05. Potentially eligible subjects were recruited from 28 GP practices in the Bristol and Weston-super-Mare area, West of England, United Kingdom. Patients between 18 and 75 years of age on T4 dose of more than 100 mcg/d and their dose not adjusted for 3 months with a normal TSH level recorded in the last 15 months were included in the study. Those with a history of myocardial infarction, unstable angina or heart failure in the previous 3 months, thyroid cancer or secondary hypothyroidism, concomitant cholestyramine use, use of antidepressants in the previous 3 months or amiodarone in the previous 12 months were excluded. At randomization, patients T4 dose was reduced and substituted either by identical looking 10 mcg of T3 (T3/T4 group) or 50 mcg of T4 (placebo/T4 alone group). The remaining T4 dose (original dose minus 50 mcg) was given in open-label packs. Thus, in the combined T3/T4 group 50 mcg of T4 was substituted by 10 mcg of T3. Patients were assessed at 3 months (visit 2) and 12 months (visit 3). The following physical measurements were taken at each visit: weight, electrocardiogram, blood pressure (twice at 10-min intervals), resting pulse rate, and body composition. The patient's psychological well-being was assessed by the following scales: the GHQ-12, TSQ, the Hospital Anxiety and Depression questionnaire (HADS), and 23 visual analog scales of mood, cognitive behavior, and physical symptoms

used in the study of Bunevicius *et al.* (19). In addition, patients completed a satisfaction question on a five-point scale and a sleep and neuromuscular symptoms questionnaire. A serum sample was taken at baseline and 24-h post thyroid hormone dose (visits 2 and 3) for the following estimations: creatinine kinase, total cholesterol, alkaline phosphatase, calcium, free T3, free T4, TSH, SHBG and antithyroid peroxidase (anti- TPO) antibodies. Samples for thyroid function and SHBG from all three visits were analyzed together. The GHQ-12 and the TSQ were scored both by the Likert method (0–3 per question, maximum score 36-most dissatisfied, linear method) and by the GHQ method (0, 0, 1, 1, maximum score 12-most dissatisfied) to assess "caseness" (using a threshold score of 3 or more, categorical method). The changes in the GHQ-12 scores at 3 months, controlling for baseline scores, represented the primary outcomes. The secondary endpoints were changes in TSQ at 3 months as well as both the GHQ and TSQ scores at 12 months. The other secondary endpoints were the changes HADS, visual analogue scales, sleep and neuromuscular symptoms questionnaires and various biochemical markers are all used secondary endpoints. Results were analyzed by intention to treat. The last observation was carried forward to replace missing values at 3 months and at 12 months follow-up if the patients were withdrawn from the study.

Results:

Out of the 37 GP practices contacted, 28 expressed interest in taking part in the study. Of the initial 3621 on T4, 1868 patients were contacted after the inclusion and exclusion criteria. 1460 replied, and 1014 showed interest in taking part in the study. On further telephone screening and records review, 242 of these patients were excluded, mainly due to abnormal TSH or recent use of antidepressants. Of the 772 finally attended the screening visit, 697 patients were randomized.

Baseline characteristics were similar between the two groups including the TSH, Free T4 and Free T3 levels. Baseline free T4 levels (20.99 +/- 3.66 pmol/liter) were in the upper part of the

reference range (10.0 –24.0 pmol/l) whereas free T3 levels (3.85 +/- 0.7 pmol/ liter) were in the lower part of their reference range (2.8–7.1 pmol/liter). Nearly 95% were followed up at 3 months (primary end point) and 88% at 12 months. Three months after intervention, the mean free T4 in the T3/T4 group had fallen from the upper part to the lower part of the reference range and was significantly lower than in the T4 alone group [(13.73 *vs.* 19.59 pmol/liter), $p<0.001$]. Mean basal free T3 levels were unchanged in both groups, but a 132% rise in median TSH was seen in the T3 group (2.28 *vs.* 0.728 U/l, $p<0.001$).

Figure 2: Thyroid hormone levels at baseline, 3 and at 12 months

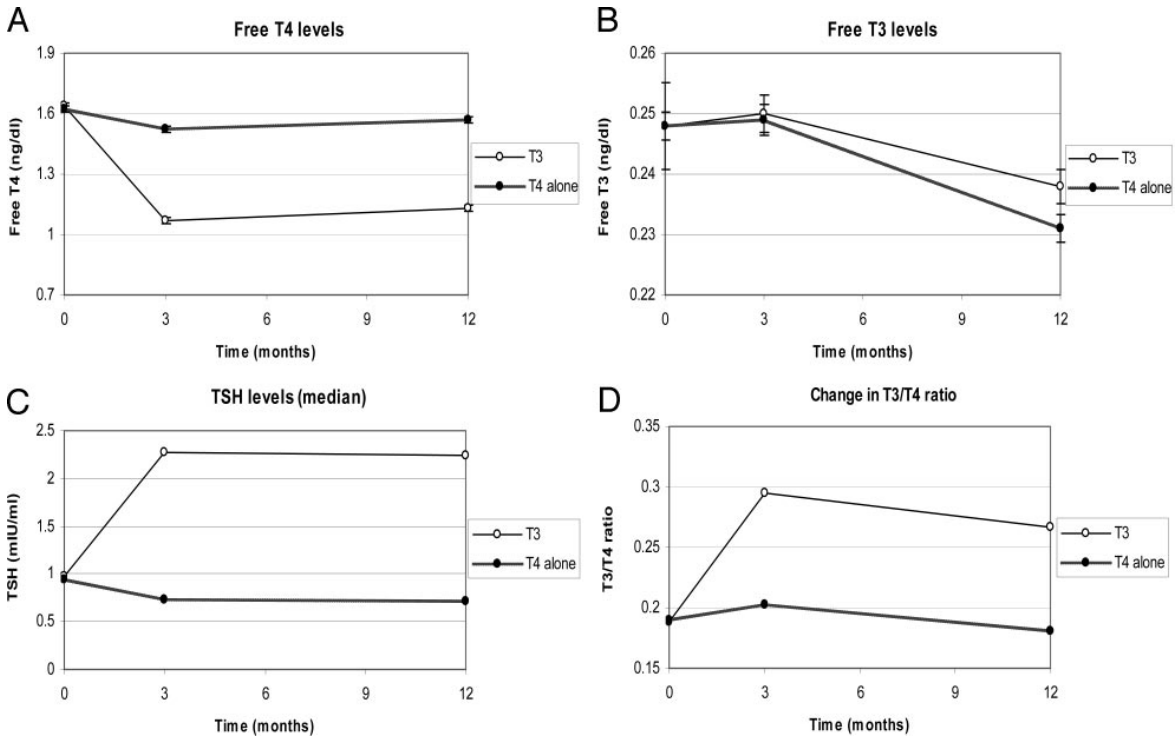

Figure 2: TSH, free T4, and free thyroid hormone levels at 0, 3, and 12 months. Median levels of TSH are shown. For conversion into SI units, multiply by: T3, 15.55; T4, 12.87

Though this was initially surprising, our pharmacokinetic sub-study (*publication 3, discussed later)* revealed the reasons behind such a raise in TSH.

At 3 months, the GHQ scores by the Likert method improved markedly in both the placebo (T4 alone) and the intervention (T3/T4) groups compared with baseline (baseline to 3 months: T4 alone, 13.48–11.13, $p<0.001$; T3/T4 group, 13.42–10.67, $p<0.001$) with a 39% relative improvement in caseness in the placebo group (43.9% to 26.6%). These changes are consistent with a marked placebo effect, although improved compliance with medication in the placebo group as evidenced by a significant fall in the serum TSH levels (baseline to 3 months: T4 alone, 0.94 – 0.728 U/l, $p<0.05$) could have contributed.

Figure 3: Change in GHQ Likert scores and % caseness by GHQ categorical scores

Fig 3a

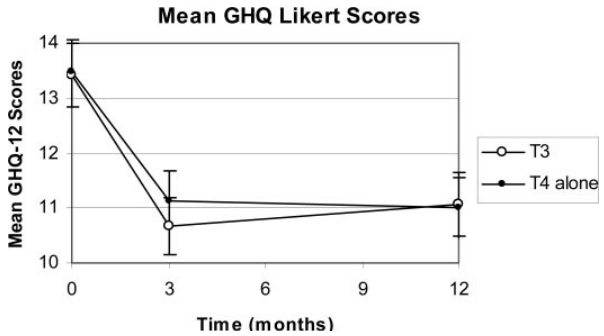

Fig 3b

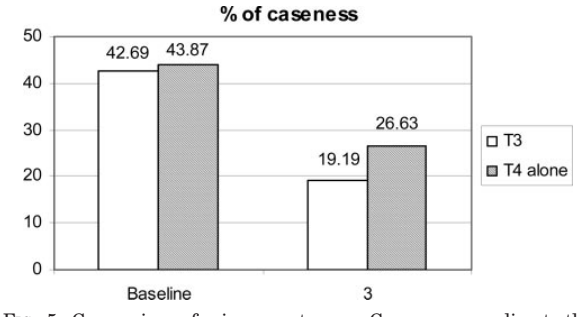

Figure 3a: Likert score on the GHQ at 0, 3, and 12 months. T3, Combined T3 and T4; T4 alone, placebo.

Figure 3b: Caseness according to the GHQ at 0 and 3 months. Percentages of cases are shown above the bars.

Comparisons between the groups revealed a difference of 0.47 points in the GHQ scored by the Likert method, which was smaller, than the difference used to power the trial (0.7) and did not reach significance at the $p<0.05$ level (95% CI, 0.26, 1.12; $p=0.218$). Using the categorical scoring methods with a threshold 2/3, a significantly greater reduction in caseness was seen in the T3/T4 group compared with T4 alone [19.2 *vs.* 26.6%, odds ratio (OR), 0.61; 95% CI, 0.42, 0.90; $p<0.01$). Improvement was also seen in the HADS anxiety score at 3 months (OR, 0.55; 95% CI, 0.32, 0.95; $p<0.033$). However, no difference was seen in the TSQ scores,

sleep, neuromuscular symptoms, HADS depression category, or visual analog scales, and the percentage of patients reporting that they felt better on direct questioning were not different. No significant differences were seen in any of the physical or biochemical measures other than a slightly lower diastolic blood pressure in the T4 alone group. The significance of these results was unchanged when controlled for age, sex, type of diagnosis, pre-study T4 dose, use of other chronic medication, baseline GHQ scores, anti-TPO positivity, and baseline thyroid function (free T3, free T4, TSH, and T3 to T4 ratio).

When the subjects were reassessed at 12 months, GHQ scores in the intervention group (T3) had risen (worsened, p=0.0034), and there was now no difference between the two groups (T3 *vs.* T4 alone, p=0.24). Interestingly, in both groups, the free T3 to T4 ratio fell significantly (T3 group, 9% reduction; T4 alone group, 6% reduction, both p<0.001) between 3 and 12 months. No change was seen in TSH levels over this period.

Summary & significance of this publication:

The results from this large community-based study categorically confirmed that for majority of hypothyroid patients T4 alone is the first choice thyroid hormone replacement. It did not provide conclusive evidence of specific benefit for all the patients on a fixed dose combined T3/T4 therapy over a 12-month period but showed possible benefits at 3 months. It remains possible that a small subgroup of individuals does benefit specifically from partial substitution, but parameters identifying such a group have yet to be clearly identified. In addition, this study showed several other important observations. Firstly, this study confirmed the observations of the cross sectional study that significantly increased psychological morbidity is observed in treated hypothyroid patients with normal TSH. Secondly, it showed that the pituitary gland is more sensitive to T4 than T3 (as observed by raise in TSH levels and fall in T4 levels in the T3/T4 group). Thirdly, contrary to belief, placebo effects can last for at least 12 months. Though the T3/T4 group were marginally better at 3 months, their wellbeing stayed at the

same level between 3 and 12 months. However, the T4 alone group continued to get better and were exactly the same as the T3/T4 group. Fourthly, the study group were better both at 3 and 12 months despite significantly higher TSH (more than 135%) suggesting a possible independent effect of T3. This also confirms the hypothesis that different tissues respond differently to T4 and T3 levels. Finally, the similar pre-dose T3 levels observed between the T3/T4 and T4 alone group, raising questions about adequacy and duration of action of T3 in the T3/T4 group. This was addressed in my third project – "Thyroid hormone profile study" (*publication 3)*. In order to identify a potential sub-group of patients who may respond differently to T4 alone and combined T3/T4 therapy, the DNA samples collected from this study were utilised to identify potential polymorphisms of the deiodinase enzymes and their correlation with well being *(publication 4).*

Project 3 – 24-hour profiles of thyroid hormones on combined T4/T3 therapy (publication 3):

Introduction:

Similar to our study, around the same time, six other studies were published on combined T3/T4 therapy. Five showed no benefit (24-28) and one showed patient preference for combined T3/T4 replacement (29). However, combined T3/T4 therapy is still widely used (30, 31) as once a day T4 and T3. Careful pharmacokinetic studies performed nearly 30 years ago showed that T3 if used as monotherapy for hypothyroidism, should be given at least three times a day to have a smooth 24-Hour profile of T3 (32). They also showed that thyroid hormone levels were stable over the 24-hour period on once daily T4 monotherapy. At the time of designing our pharmacokinetic sub-study, there was no evidence to show the 24-hour profiles of thyroid hormones on combined T3/T4 therapy. T4 has a half-life of 5-7 days. It is conceivable that combined T3/T4 therapy may result in smooth 24-Hour profile of both T3 as well as T4, as the peripheral deiodination of T4 is likely to provide continuous T3. On the

contrary, the half-life of T3 when used on its own is approximately 8-12 hours. The onset of action is quick and therefore it is possible that combined, once a day T3/T4 therapy could result in fluctuating T3 levels. The half-life of T3 when used in combination with T4 is not known. This was the rationale for our pharmacokinetic study designed to study the thyroid hormone levels over a 24-hour period on combined T3/T4 therapy. This study is likely to provide answer to the question *"can T3 be given once a day when used as a part of combined T3/T4 therapy?" (publication 3).* This was an important question, as slow release formulation of T3 is commercially not available, especially if combined T3/T4 therapy found useful.

Methods:

Twenty patients (10 patients on T4 alone and 10 patients on combined T3/T4 therapy) were randomly selected by the trial pharmacist, who was not in direct contact with the patients to be included in this pharmacokinetic study. This enabled the patients and the investigators to remain blinded. The patients had been on their study medication for a minimum period of 3 months at the time of investigation. 7 patients in the T4 alone group and 8 in the T3/T4 group were on thyroxine for primary hypothyroidism. The rest were on thyroxine after having radioactive iodine treatment for thyrotoxicosis. All patients were given written and verbal instructions to take all their study medication at 8.00 AM for a week prior to their 24-hour profile study. On the day of the study, baseline blood sample was taken at 8.00 am and subjects then took their study medication under supervision. Eleven more blood samples were collected at 0900, 1000, 1200, 1400, 1600, 1800, 2000, 2200, 2400, 0600 and 0800 hrs over the next 24-hours. Patients were ambulatory with normal daily activity although strenuous exercise was avoided. Subjects slept between 2200hrs and 0600hrs and the final blood sample was collected at 0800hrs on the following day. Eleven patients (T3/T4:T4=6:5) not on beta blocking or anti hypertensive drugs attended on a second occasion for monitoring of their 24-hour ambulatory blood pressure (BP) and pulse rate. Similar instructions were given prior to their attendance and the monitor was attached to the patients immediately after taking their

study medication. Thyroid function was monitored for the first 6 hours (5 samples at 0,1,2,4,6 hours post-dose). Patients' BP and pulse rate were monitored at the following intervals: half-hourly for the first 6 hours post-dose, hourly for the next 8 hours and then 2 hourly (at night time) until the following morning. Patients returned home after the first 6 hours and were advised to avoid strenuous exercise and take rest for at least 5 min before and during each BP measurement. All the blood samples were stored overnight at 4C and then centrifuged. The serum was then stored below − 70C for later analysis. All the samples were analysed for TSH, free T4 and free T3 levels.

Results:

The thyroxine dose at the entry to the main study was similar between the 2 sub-groups. Baseline mean fT3 levels were similar between the 2 groups (T3/T4:T4 = 4.38:4.69 pmol/L, p = 0.208; range – T3/T4:T4 = 3.5– 5.2: 4.1–5.6) but as expected the baseline mean fT4 was lower (T3/T4: T4 = 12.05:17.9 pmol/L, p = 0.0001) and median TSH was higher (T3/T4:T4 = 3.5:0.7 U/L, range – T3/T4:T4 = < 0.01,15.3: < 0.01,2.7, p < 0.001) as a result of T3 substitution in the combined T3/T4 group.

In patients on T4 alone, a modest 16 % rise in fT4 levels peaking 2–4 hours post-dose was seen, with higher levels for 12–16 hours. No rise (indeed a slight fall) in fT3 levels was seen. However, in the T3/T4 group mean fT3 levels showed a marked 42% rise within the first 4 hours of medication ingestion, remaining above baseline for 16 hours and higher than the T4 alone group for 22 hours. The mean fT3 levels at 4 hours post-dose (T3/T4:T4=6.24:4.63 pmol/L, p=0.0007) as well as the overall area under the curve (AUC) were significantly higher in the T3/T4 group than the T4 alone (AUC: T3/T4: T4 = 1148:1062, p < 0.0001). 3 patients in the T3/T4 group but none in the T4 alone had fT3 levels above the laboratory reference range at some time over the 24-hour period. However these higher levels lasted only for a maximum of 2 hours. Despite greater T3 exposure in the T3/T4 group, this appeared not to alter the

pattern of TSH secretion. A similar circadian rhythm with a nocturnal rise persisted in both groups. 3 patients in the T3/T4 group and none in the T4 alone had a mean TSH higher than the lab normal range. One patient in the T3/T4 and 2 in the T4 alone had a suppressed TSH level throughout the 24-hour period.

Figure 4: Mean % change and actual levels of thyroid hormones over a 24-hour profile

Fig 4a Fig 4b

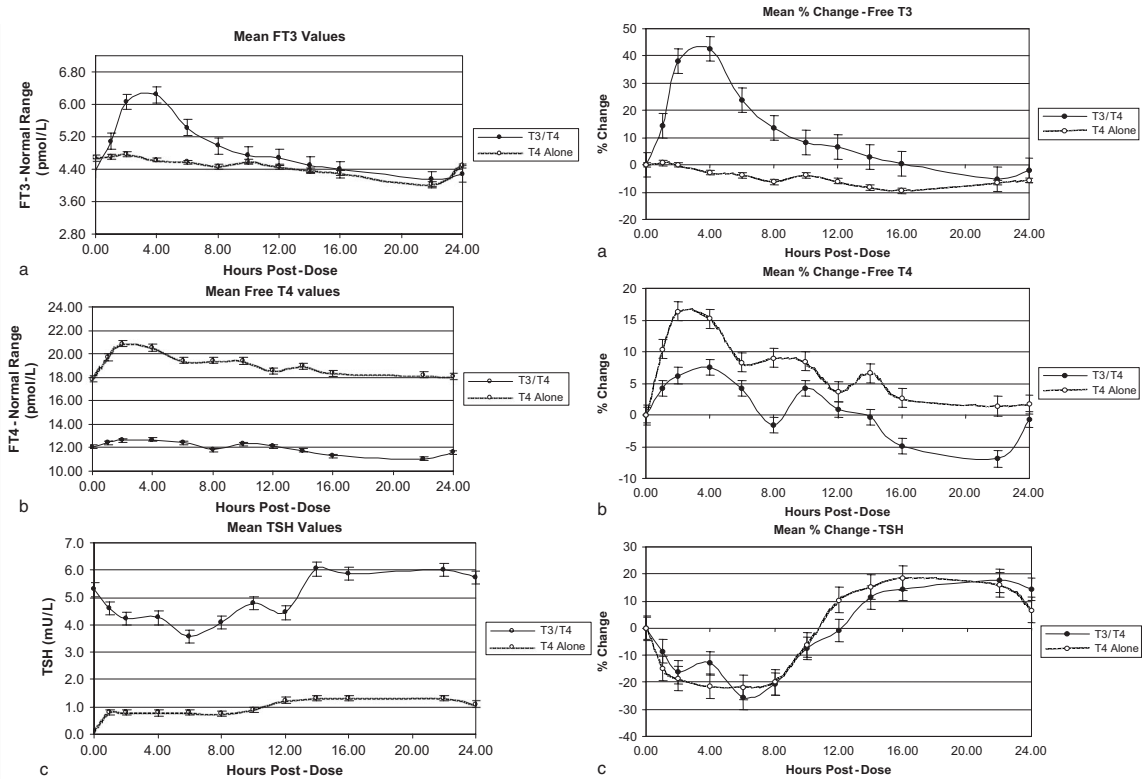

Figure 4a: a–c Mean values for free T3 (a), free T4 (b) and TSH (c) over the 24-hour period. Error bars show standard errors (SEM) for each value. (Normal ranges: Free T3: 2.8–7.1 pmol/L; Free T4: 10.0–24.0 pmol/L; TSH: 0.3–4.0 mU/L)

Figure 4b: a–c Mean values for the percentage change from baseline (time = 0) in free T3 (a), free T4 (b) and TSH (c) over the 24 h period. Error bars show standard errors (SEM) for each value.

In the patients who attended for cardiovascular parameter analysis (n = 11, T3/T4:T4 = 6:5), no difference was apparent in the 24-hour variation of the BP and pulse rate between the 2 groups. No post-dosing peak in heart rate or blood pressure that differed between the groups was discernable and both groups showed similar night-time falls in cardiovascular parameters.

Summary & significance of this publication:

In summary, we have confirmed that wide peak-to-trough variation in fT3 levels persist in once daily combination therapy with T3 and T4 after more than 3 months treatment. This variation makes interpretation of thyroid function tests on combined therapy more difficult as basal fT3 levels may underestimate total T3 exposure and peak fT3 levels may overestimate it. Furthermore, TSH levels cannot be used to indicate overall exposure to T3 as levels rose when T3 was substituted for T4 despite increased T3 levels. Thus, the pharmacokinetics of T3 in combination therapy in which T3 is also being continuously generated from T4 appears similar to that of T3 alone in hypothyroid patients and no adaptation to chronic therapy appears to occur (32). These observations have other important clinical implications. Firstly, our observations lead us to reconsider which features of the thyroid hormone profile on replacement therapy are most important. The baseline TSH levels rose on T3/T4 therapy despite a 42% rise in fT3 over the first 4 hours. There were no significant changes in cardiovascular parameters and patients did not appear to notice any particular symptoms over this period or report diurnal variation in symptoms as often reported on hydrocortisone replacement in hypoadrenalism (33, 34). Secondly, our observations raise the issue of the correct dosing interval for T3 on combined T3/T4 therapy. Even though we did not demonstrate any significant differences in the cardiovascular parameters, it would be logical to use a twice or three times daily dosing which may achieve smoother 24-hour T3 levels that resemble individuals with an intact thyroid axis more closely (35).

This was *the first and only study to date* to show the pharmacokinetics of thyroid hormones over a 24-hour period on combined T3/T4 therapy. The role of combined T3/T4 in thyroid hormone replacement remains controversial (36, 37). Many practitioners continue to use this combination (30, 31) and our own results in a large population are consistent with possible benefit in a subgroup of individuals (38). If a role is confirmed for combination therapy, it will be important to resolve the pharmokinetic issues raised by the current study in order to

achieve safe and optimal dosing. An alternative possibility is the formulation of a slow release T3. Hennemann et al recently showed that their in-house “slow release” version of T3 when used with T4 gives a smoother profile than the standard T3 for at least 9 hours post-ingestion (39). However, such slow release formulation is not currently available. In the mean time, it would be sensible to use both pre and 2 hours post dose levels of thyroid hormones for those on combined T3/T4 therapy.

Project 4 – Correlations of thyroid hormones and wellbeing (publication 4):

Introduction:

The occurrence of various abnormalities in brain function including cognitive and memory impairment in patients with overt hypothyroidism is now well established. Reduced levels of thyroid hormone appear to slow serotonergic neurotransmission in the brain (40), an effect associated with low mood. In addition, thyroid hormones are widely used to augment antidepressant treatment (41), although the trial evidence underlying this is controversial (42). Evidence for lesser variation in thyroid hormone levels affecting mood and psychological well-being remains more controversial. Some cross-sectional studies suggested that subclinical thyroid dysfunction is associated with depression, cognitive impairment, and memory loss (reviewed in (43). Carr *et al.* (10) reported that patients receiving thyroid hormone replacement appeared more content on higher doses of T4. The large HUNT (Nørd-Trondelag Health Study) community-based study failed to find an association, but the correlations were made with categories of TSH level rather than using T4 and TSH, as continuous variables. Interestingly, in the subgroup of patients on T4, a link with depression was reported (44). Including our study, several studies of thyroid hormone replacement therapy reported that the combination of T3/T4 is not superior to T4 alone. However, where thyroid hormone levels were raised, psychological well-being appeared to have improved (29).

In view of the large body of circumstantial evidence linking thyroid hormone levels and mood and the relative stability of endogenous thyroid hormone levels within a given individual over time (45, 46), we hypothesized that variation in thyroid hormone levels, even within the laboratory reference range, might represent an independent risk factor for low mood and depression. My fourth project was to test this hypothesis, by examining the relationship between thyroid hormone parameters and psychological well-being across a large cohort of patients (n=697) treated with T4 whose thyroid functions are in the laboratory normal range *(publication 4).*

Methods:

Baseline data from our large prospective study was used for this study. Patients' well-being was assessed at study entry by the General Health Questionnaire (GHQ)-12, which is a well-validated tool in predicting morbidity when compared with complex psychometric tests and detailed interview. The GHQ-12 has four responses for each question: "better than usual," "same as usual," "less than usual," and "much less than usual." In addition to the GHQ- 12, all subjects completed the Hospital Anxiety and Depression Scale (HADS) and an un-validated questionnaire, the Thyroid Symptom Questionnaire (TSQ) based on symptoms frequently reported by patients on thyroid hormone. All the questionnaires were scored both by the Likert (linear) and categorical scoring methods as described earlier. At the study entry, blood was drawn and stored for measurement of thyroid hormones and all the samples were analyzed as a single batch at the end of the study. The normal ranges were: serum TSH (NR 0.3– 4.0 U/l), free T4 (NR 10.0 –24.0 pmol/liter), free T3 (NR 2.8–7.1 pmol/liter) serum reverse T3 (rT3) (NR 0.14–0.34 nmol/liter) and anti-thyroid peroxidase (TPO) antibodies (+ve titre - >100). The relationships between psychological questionnaire scores and serum thyroid hormone measurements were ascertained using linear and logistic regression analyses for continuous and binary versions of the questionnaires, respectively. Multiple regression analysis was used when adjusting for age, sex, and anti-TPO antibody positivity.

Results:

The mean age of the patients was 57.3 yrs. Eighty-four percent of patients were women (n=586). The causes of hypothyroidism were autoimmune hypothyroidism (73.45%), post-radioactive iodine (9.33%), post thyroidectomy (15.78%), and post-thyroidectomy & post-radioactive iodine (1.44%). Forty-four percent of the patients (n=307) had a strongly positive titer for anti-TPO antibodies (titer>100).

Baseline fT4 showed a strong negative correlation to the GHQ-12 scores (correlation coefficient b: -0.155, *p*=0.005). The relationship persisted even after correcting for age, sex, and anti-TPO antibody positivity (b: -0.14, *p*=0.015) and was also present in the subset of patients with TSH between 0.3 and 4.0 IU/liter (b: -0.159, *p*=0.038, n=473). The same correlations were observed when the GHQ was scored as a categorical parameter (GHQ scoring). The relationship was in the expected direction (higher fT4 associated with lower GHQ scores implying improved well-being), and the slope indicated an *improvement of 1 GHQ point for a 0.51 ng/dl (6.5 pmol/ liter) rise in fT4.* A positive correlation was seen with log TSH and GHQ [b: 0.66, *p*=0.04; no change after controlling for age, sex, and anti-TPO antibody positivity (b: 0.68, *p*=0.04)]. This correlation was preserved in the subset of patients with TSH in the range 0.3–4.0 IU/liter (b: 2.3, *p*=0.006). In contrast, no correlation was seen among fT3, rT3 , rT3 to T4 and T3 to rT3 ratios, and anti-TPO positivity and GHQ scores. Similar results were observed with the TSQ. FT4 showed significant correlation with both the linear (correlation coefficient b: -0.11, *p*=0.03) and categorical scores of TSQ, and this persisted in the TSH 0.3–4.0 IU/l subset. Whereas no correlation was seen between linear TSQ and log TSH (b: 0.09, *p*=0.41), a relationship was seen between the categorical TSQ and log TSH (OR 1.4, *p*=0.007) but was lost in the TSH 0.3– 4.0 IU/l subset. Similar to GHQ, no other correlation was seen between TSQ and any other thyroid hormone parameters. No correlation was seen among any of the thyroid hormone parameters and the anxiety and

depression scales of the HADS (data not shown) with the exception of log TSH and HADS depression as a continuous variable (b: 0.562, p=0.004). However, this relationship was not seen when HADS depression score was used as a categorical variable (OR 1.2, p=0.54).

In the subgroup of patients with fT4 level above the reference range, no correlation between psychological well-being and the fT4 levels seen using regression model. However, the mean GHQ scores are significantly lower (improved well being) in this group as a whole, compared with the subgroup of patients with levels of thyroid hormones in the reference range (high fT4 *vs.* normal fT4: 12.33±4.79 *vs.* 13.72±5.45, p=0.007). By the categorical scoring method, the percentage of caseness was also less in patients with high fT4 levels (35.1 *vs.* 45.3%, p=0.03). Similar results were seen in TSQ scores (linear TSQ scores: high fT4 *vs.* normal fT4: 13.91±4.66 *vs.* 14.85±4.88, p=0.04; percent caseness: high fT4 *vs.* normal fT4: 56.1 *vs.* 66.0%, p=0.03). Similar *post hoc* analysis of patients according to anti-TPO antibody status did not show any significant difference in GHQ between anti-TPO-positive and negative patients (anti-TPO positive *vs.* anti-TPO negative: 13.71±5.42 *vs.* 13.12±5.26, p=0.147). Similar results were obtained when antibody status was used as an interaction factor in the regression model.

Summary & significance of this publication:

This is the first large study to explore the relationship between fT4, fT3, rT3 and the ratios of T3/rT3 & rT3/T4 with psychological well-being in subjects on thyroid hormone replacement. Improved psychological well-being was found to correlate with higher fT4 levels. The significance of the observation is supported by the finding of a relationship between psychological well-being and TSH with the opposite slope (higher TSH with reduced well-being as might be expected). A similar relationship with fT4 was found with an un-validated score of symptoms that relates more directly to thyroid status (TSQ) making a false positive association due to multiple testing less likely. Interestingly, no clear association was seen with

the HADS scale, which may suggest that the thyroid function influences parameters of psychological well-being not typical of anxiety or depression. TSH is often considered the most sensitive measure of thyroid function. However, it appears that the relationship between well-being and fT4 was as much if not more pronounced as with TSH. TSH levels reflect hypothalamopituitary sensing of circulating thyroid hormone levels, which may be different from thyroid hormone status in other tissues and the importance of fT4 measurement in addition to or distinct from serum TSH estimation to assess thyroid status has been emphasized by our study. Similarly, studies in pregnancy, maternal hypothyroxinemia in the first trimester and not raised TSH was associated with impaired psychomotor development in offspring (47), and fT4 but not TSH at 9 wk of pregnancy is directly proportional to the birth weight of the offspring (48). The failure to find a relationship between serum fT3 and GHQ/TSQ scores is also of interest. Many thyroidologists consider the T3 assay to be less technically reliable and less reflective of thyroid status, particularly in the hypothyroid range (49). Although T3 is the active hormone, free concentrations of T4 are five times higher, and many tissues obtain 30% or more of their intracellular T3 directly from circulating T4 (6). Hence, circulating T3 levels may not be directly reflective of intracellular levels. We measured serum rT3 levels as a possible measure of intracellular deiodinase activity (6, 50). The failure of rT3 levels or ratios with thyroid hormones to correlate with psychological well-being might relate to serum levels being more indicative of hepatic D1 and D2 activity, whereas intracellular levels are strongly influenced by local levels of membrane-bound deiodinases including D2 and D3 (6, 51). *Our findings provide the only support for the view that serum fT4 levels as well as TSH levels should be taken into account when adjusting dosages and that TSH may not be a perfect indicator of the adequacy of replacement, particularly with regard to psychological well-being*. However, large population-based studies of thyroid function parameters including fT4 and psychological well-being will be required to explore this further. This study provided the crucial pilot data for a later, large community based study by our group (DEPTH Study, ongoing).

Project 5: Identification of sub-group of hypothyroid patients who may have reduced psychological wellbeing on T4 alone therapy and respond better to combined T3/T4 therapy (publication 5)

Introduction:

Several studies using different combination of T3/T4 therapy, including our large community based study failed to show convincing benefit of such therapy over T4 alone therapy. However, the reduced psychological wellbeing in patients on thyroid hormone therapy has been confirmed by other studies in addition to our large cross-section study *(project 1).* Anecdotally several patients feel better on combined therapy. In addition, at least 2 of the combined T3/T4 studies suggest there may be a sub-group of hypothyroid patients who may respond better to such therapy. Indeed, if the sub-group represent <20% of the population, such patients may be too infrequent for their presence to be detected in the intervention trials but could still account for significant morbidity in patients on thyroxine therapy. *Our hypothesis is that such sub-group has inherited abnormality that becomes clinically significant only when they become hypothyroid.* The 3 deiodinase enzymes (D1, D2 & D3) represent possible candidate loci for such genetic variation. Our study *(publication 2)*, the largest to date, provided an opportunity to explore this hypothesis. We studied the role of the common polymorphisms in the 3 deiodinase genes in determining the psychological well-being of patients on T4 alone therapy and the response to combined T3/T4 replacement.

Methods:

Out of the 697 participated in the original randomised controlled trial *(project 2)*, 552 subjects provided their blood for extracting DNA. We used genotype data from the Caucasian European individuals in the International Haplotype Mapping Project (http://www.hapmap.org) to select a set of SNPs that capture the majority of common variation across the three

deiodinase genes (DIO1, DIO2, and DIO3) including 50 kb either side of the genes. We used a minor allele frequency of at least 10%. The 21, seven, and seven SNPs in the DIO1, DIO2, and DIO3 genes required nine, four, and six SNPs, respectively, to capture all common variants with an r^2 >0.8. These were: DIO1, rs11206237, rs11206244, rs2235544, rs2268181, rs2294511, rs2294512, rs4926616, rs731828, and rs7527713; DIO2, rs12885300, rs225011, rs225014, and rs225015; and DIO3, rs1190716, rs17716499, rs7150269, rs8011440, rs945006, and rs1190715. We used only SNPs that were in Hardy Weinberg equilibrium (p>0.05) and were genotyped in at least 97.5% of the samples in the final analyses. We examined the association between these SNPs and baseline (before randomization) psychological well-being by linear regression analysis (GHQ Likert score as dependant variable and genotype as independent variable). Repeated measures ANOVA was used to detect the response to therapy with treatment arm (T4 alone or T3/T4) and genotype were the between-subject effects. Baseline scores were adjusted for as covariates and included two-way interactions between treatment arm & genotype and genotype & baseline score.

Results:

Effect on psychological well-being:

Out of the 16 SNPs studied, only 2 SNP in the DIO1 gene (rs225014, rs225015) showed an association at *p*<0.05 level of significance. Because the two DIO2 SNPs are in strong linkage disequilibrium with an r^2 of 0.88 in this population, and rs225014 is also in linkage disequilibrium with the third SNP studied in this gene, rs225011 (r^2 of 0.59), further analysis is done only on rs225014 alone. In this SNP, each C allele (TT, TC, CC) was associated with an average increase of 0.71 GHQ points (worse well-being, *p* for trend = 0.02; exactly the same difference observed in our cross-sectional study – *project 1*). HADS depression score showed similar association reaching significance (p=0.01) and the others showed trend in the same direction without reaching statistical significance. Interestingly, this SNP did not have any

detectable effect on thyroid functions suggesting this effect *may be independent* of serum thyroid hormone levels.

Effect on response to therapy:

There was significant interaction between treatment arm and genotype on improvement in GHQ (p=0.03), TSQ (p=0.03) and satisfaction scores (p=0.02). This suggests an improved response to combined (T3/T4) therapy in this genotype (rs225014). This is the same sub-group who had the poorest psychological well-being at baseline (on T4 alone therapy). In the CC genotype, the mean improvement in GHQ score was 2.33 (95% CI: 0.38, 4.38) at 3 months and 1.44 (95% CI: -0.25, 3.12) at 12 months with combined T3/T4 therapy. The rs225014 genotype frequencies were not significantly different between the study groups (frequency of TT, TC, and CC genotypes: 40.6, 45.5, and 13.9%, in T4 /T3 group and 41.1, 41.1, and 17.9% in T4 only group, p=0.38). The prevalence of minor homozygous (CC) of the rs225014 is 14.1% in the overall cohort, confirming our initial hypothesis that if there is a small sub-group, they may not be detectable in the intervention trials. Indeed, despite being the largest study, we would estimate that only around 50 subjects would have had this genotype in the intervention group, who would respond to combined therapy. Given the large placebo effects seen, the differential change in these subjects was not detected in the initial analysis of the whole cohort.

Figure 5: Response to therapy by genotype (rs225014 vs. GHQ, TSQ & satisfaction)

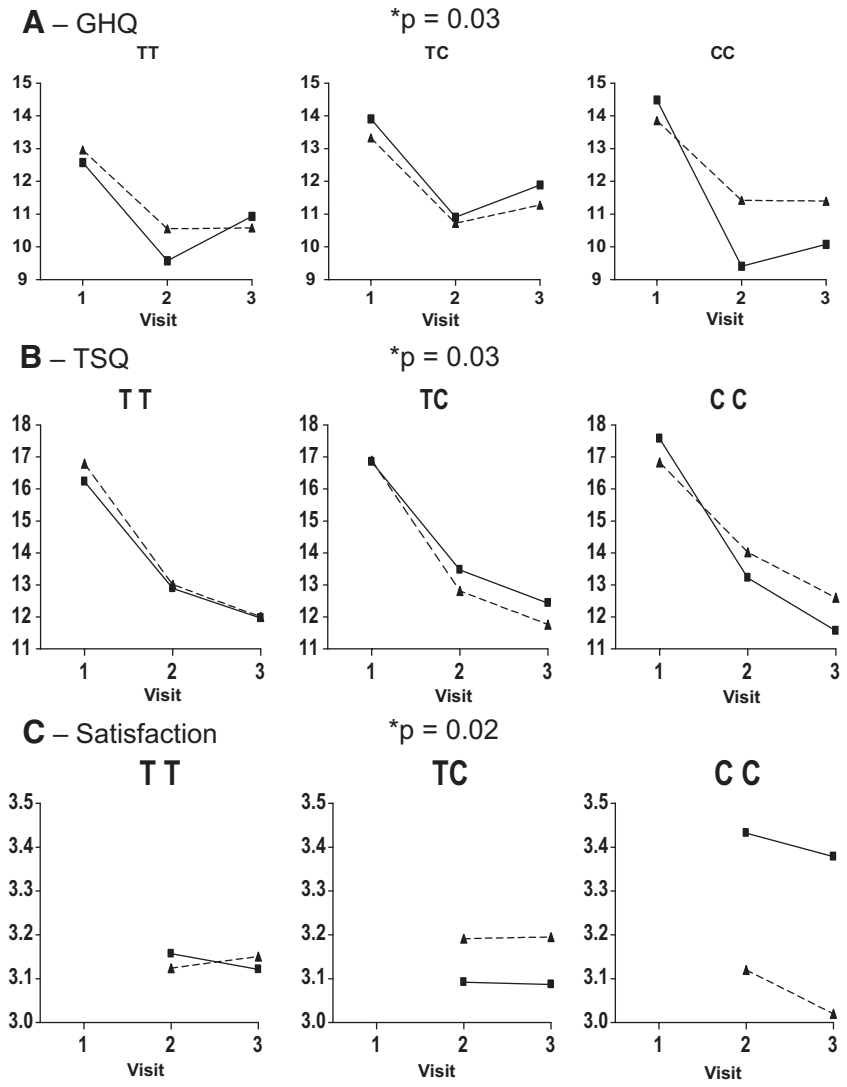

Figure 5: Response to therapy by genotype rs225014 as measured by GHQ (A), TSQ (B), and satisfaction score (C). Squares and continuous line, T4/T3 group; triangles and dashed line, T4-only group. P values reflect the significance of an effect of the CC genotype on difference in scores by treatment arm using repeated-measures ANOVA. *, P < 0.05. There was a significant effect of the interaction between the CC genotype and treatment arm at follow-up (visits 2 and 3) on GHQ scores, TSQ scores, and satisfaction. There are no baseline (visit 1) scores for satisfaction with therapy (C) because this was not assessed at baseline. For GHQ and TSQ scores, higher scores indicate worse well-being, whereas for satisfaction higher scores indicate more satisfied

Summary & significance of this publication:

Our study is the only study to show the potential presence of a sub-group of patients (DIO2 gene SNP rs225014) who are dissatisfied with T4 alone therapy. Our study also showed that this sub-group of hypothyroid patients may respond better to combined T3/T4 therapy. We studied 16SNPs across 3 genes. Though our study is the largest T3/T4 trial available, it is still underpowered to reliably detect gene-treatment interaction. However, if replicated, it has huge implications for a large number of patients on thyroid hormone replacement therapy (estimated around 1.5 million people in the UK alone). This is likely to reflect an effect on local, tissue level deiodination of T4 by D2 in the brain as this do not have any effect on circulating thyroid hormone levels. Our observation that common genetic variation in the DIO2 gene but not the DIO1 or DIO3 genes could be relevant to psychological well-being is interesting because the D1 enzyme is not expressed in the brain and D3 is a deactivating enzyme. Therefore, D2 is the only enzyme able to convert T4 to T3 in the brain and is likely to play a key role in determining the ability of the brain to respond to circulating T4 levels. Indeed, common variation in D2 activity may represent the best available marker of intracellular T3 levels in the brain. The lack of effect of the DIO2 polymorphisms on serum thyroid hormone levels means that without performing genetic testing, it is impossible to select out the group likely to respond to combination therapy for subgroup analysis in intervention trials.

Other publications:

Publication 6&7: Whose thyroid function is normal – yours or mine?

Until recently, endocrinologists believed that thyroid hormone is the easiest hormone to replace. Treated hypothyroid patients thyroid hormone replacement is determined by their TSH. Traditionally, “normal or reference ranges” are derived either using epidemiological data

or using statistical method. The statistical method is the most commonly used as it is the easiest and in the absence of epidemiological data associating with outcomes. Such reference range is generally 2 standard deviations above and below the mean of apparently "disease-free" individual. As nearly 10% of the population might have sub-clinical thyroid disease, such reference range will therefore have limitations. Indeed, a 20-year longitudinal Wickham survey indicated that individuals with TSH values >2.0IU/l have increased risk of developing overt hypothyroidism over the next 20 years (3). An elegant study by Andersen et al showed that individuals' TSH revolves around a "set point" and there is a significant inter-individual variation (45). The variation for an individual is much narrower than the laboratory reference range. Our invited commentary highlighted the potential of being hypothyroid of a given subject despite their TSH in the "normal range". This also highlighted the importance of measuring the "relative change" in TSH along with clinical symptoms in considering initiating treatment with thyroid hormone replacement therapy.

Publication 8: Understanding thyroid hormone actions: A review

Following the publication of our initial work (cross-section study, *publication 1)* and the subsequent large randomised trial *(publication 2)*, several other groups throughout the world published works highlighting the relative complexity of thyroid hormone action. Several steps are involved from the time the thyroid hormones are released in the serum and the time of its action on the nuclear receptors. Thyroid hormones are taken into the cell by transporters, converted to its active metabolite by deiodinase enzymes before it exerts its function on nuclear receptors. There were also novel findings about actions of thyroid hormone transporters and the potential consequences of mutations involving such transporters. Understanding the complexity has opened a new avenue for further research in this area. This was comprehensively reviewed by us *(publication 7)* providing insights in the potential mechanisms of "euthyroid dysphoria".

Publication 9: Variation in deiodinase gene polymorphisms and thyroid hormone levels:

It is known that genetic factors influence circulating thyroid hormone levels. However, until recently the common genetic variants influencing the levels has not been identified. Data from our large randomised trial on hypothyroid patients *(publication 2)* was used to thoroughly examine the role of common variation across the three-deiodinase genes in relation to circulating thyroid hormones. The findings from our cohort (n=552) were taken forward to three other cohorts in people not on thyroid hormone replacement therapy (n=2513). The results showed an SNP in the DIO1 gene (rs2235544) was associated with T3/T4 ratio with genome-wide level of significance ($p=3.6x10^{-13}$). The C-allele of this SNP was associated with increased T3/T4 ratio and T3 levels and decreased T4 and rT3 levels. There was no effect on TSH levels. Thus, this study provided the *first* evidence that common genetic variation in D1 gene alters type 1 deiodinase function resulting in alteration in the balance of circulating T3 and T4 levels. This finding will therefore provide a valuable tool to assess the relative effects of circulating T3 and T4 levels on a wide range of biological parameters.

Publication 10: What is the optimal thyroid hormone replacement?

This invited editorial was written to practicing general practitioners and clinicians, reviewing the latest evidence on thyroid hormone replacement therapy and provides guidelines on deciding thyroid hormone replacement therapy for patients with hypothyroidism. This paper also provided simple algorithm for treating hypothyroid patients.

Acknowledgements:

I am grateful to my PhD supervisor, Dr Colin M Dayan, for his insight in spotting my abilities, his constant encouragement, enthusiasm and endless energy. I have learnt a lot under his supervision, which I treasure until today.

I am eternally thankful to the thousands of patients who have contributed their valuable time, efforts and unconditional support towards my three projects. I have learnt a lot from them.

I am thankful for my co-authors, in particular the statisticians, Dr R Greenwood and Prof T Peters for their invaluable advice.

I am thankful and indebted to my wife, Sophia, for her extraordinary understanding during my research time. I am grateful for putting up with me in my “highs and lows” as well as several late nights despite being pregnant during my RCT. I dedicate this work for her.

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Appendix 1 - Publication List:

1. **Saravanan P**, Chau F, Roberts N, Vedhara K, Greenwood R, Dayan CM Psychological well-being in patients on "adequate" doses of L-thyroxine: results of a large controlled, community based questionnaire study. **Clin Endocrinol** (Oxf). 2002 Nov;57(5):*p577-85*
2. **Saravanan P**, Simmons DJ, Greenwood R, Peters TJ, Dayan CM Partial substitution of thyroxine with tri-iodothyronine in patients on thyroxine replacement therapy: results of a large community-based randomised controlled trial. **J Clin Endo Metab** 2005 Feb 90(2): *p805*-12. Epub 2004 Dec 7
3. **Saravanan P**, Siddique H, Greenwood R, Dayan CM Twenty-four hour hormone profiles of TSH, free t3 and free t4 in hypothyroid patients on long-term combined T4/T3 therapy. ***Experimental and Clinical Endocrinology & Diabetes*** 2007 Apr;115(4):261-7
4. **Saravanan P**, Visser T, Dayan CM Psychological wellbeing correlates with free T4 but not free T3 levels in patients on "adequate" thyroid hormone replacement. **J Clin Endo Metab** 2006 Sep;91(9):3389-93. Epub 2006 Jun 27
5. **Panicker V*, Saravanan P***, Vaidya B, Evans J, Hattersley AT, Frayling TM, Dayan CM: Common variation in the *DIO2* gene predicts baseline psychological well-being and response to combination T4/T3 therapy in patients on thyroid hormone replacement **J Clin Endo Metab** 2009 May;94(5):1623-9. Epub 2009 Feb 3
6. Dayan CM, **Saravanan P,** Bayly G Thyroid-stimulating-hormone concentrations and risk of hypothyroidism. **Lancet** 2002 Dec 21;360(9350) *p2082*
7. Dayan CM, **Saravanan P,** Bayly G Whose normal thyroid function is better--yours or mine? **Lancet**, 2002 Aug 3;360(9330):*p353*
8. **Saravanan P**, Dayan CM Understanding thyroid hormone action and the effects of thyroid hormone replacement – Just the beginning not the end**. Hot Thyroidology** 2004 Oct: No:1
9. Panicker V, Cluett C, Shields B, Murray A, Parnell K, Perry JRB, Weedon MN, Singleton A, Hernandez D, Evans J, Durant C, Ferrucci L, Melzer, D, ***Saravanan P**, Visser TJ,

Ceresini G, Hattersley AT, Vaidya B, Dayan CM, Frayling TM: Common variation in the *Deiodinase 1* gene alters the balance of the thyroid hormones free T4 and free T3 with genome wide levels of significance **J Clin Endo Metab** 2008 Aug;93(8):3075-81. Epub 2008 May 20

10. **Saravanan P** What is the optimal thyroid hormone replacement and how far are we from individualising thyroid hormone therapy? – Editorial. **Thyroid Research and Practice** 2006 Sep-Dec; 3(3) *p67-70*