P = 0.31) between TNF-α –308 GG and GA/AA carriers. The percentage difference of corrected TNF-α concentrations between the B2B2 and the B2B1+BB1 genotypes of the LTA Ncol marker was 19% (15–66%).

The mean (SD) monocye CD14 density of TNF-α –863 CC carriers [57.0 (20.4) × 10^3 abc] and carriers of an A allele [56.5 (19.7) × 10^3 abc] did not differ significantly (P = 1.0). The difference in monocyte counts was 0.46 (0.14) × 10^3 cells/μL for C homozygotes vs 0.39 (0.13) × 10^3 cells/μL for CA/AA carriers (P = 0.11).

The allele frequencies found in our study for the three TNF polymorphisms were in agreement with those reported in previous studies (11, 16). We also demonstrated that the –863 C/A polymorphism is associated with the TNF-α response of human whole blood of healthy volunteers to endotoxin stimulation. In particular, C homozygotes had significantly higher TNF-α values. Although they are located on the same chromosome, we found no linkage disequilibria between the TNF-α –863 and the TNF-α –308 or the LTA Ncol sites. We observed a linkage disequilibrium between the TNF-α –308 and the LTA Ncol polymorphisms, in agreement with a previous report by our group (16).

CD14, a mediator of endotoxin activity and monocyte count, was measured to exclude that these factors could influence the results. No difference in CD14 density was observed among the TNF-α –863 genotypes.

Interestingly, our data obtained with human cells are in line with previous reports (11). These authors used reporter gene assays and found remarkably higher transcription of the reporter gene assays and underlines the need of studies using human whole blood. The allele frequencies found in our study for the three TNF-α –863 genotypes, in agreement with those reported in previous studies (11, 16). We also demonstrated that the –863 C/A polymorphism is associated with the TNF-α response of human whole blood with various TNF polymorphisms studied using the expectation-maximization algorithm. Heredity 1996;76:377–83.

In summary, our findings suggest that the TNF-α –863 C/A polymorphism is a genetic factor influencing TNF-α synthesis that is not in linkage disequilibrium with the TNF-α –308 or the LTA Ncol polymorphisms. The genotyping assay described here is rapid, accurate, and suitable for routine laboratories with a high sample throughput.

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References

Effect of Thyroxine Replacement on Creatinine, Insulin-Like Growth Factor 1, Acid-Labile Subunit, and Vascular Endothelial Growth Factor, Christoph Schmid, Michael Brändle, Cornelia Zwimpfer, Jürgen Zapf, and Peter Wiesli (Department of Internal Medicine, Division of Endocrinology and Diabetes, University Hospital of Zurich, CH-8091 Zurich, Switzerland; * author for correspondence: fax 41-1-255-4447, e-mail peter.wiesli@dim.usz.ch)

Hypothyroidism is associated with endothelial dysfunction, arterial hypertension, and impaired kidney function.
(1–3). An increased serum creatinine and decreased glomerular filtration rate and renal blood flow have been described (2, 4, 5). These deleterious consequences may result from several mechanisms, including direct and indirect effects of thyroid hormones on blood vessels. Insulin-like growth factor 1 (IGF-1) and vascular endothelial growth factor (VEGF), growth factors with both local and systemic effects, may be involved as potential mediators. Hypothyroidism causes low concentrations of IGF-1, which can be normalized by thyroxine replacement therapy (6). IGF-1 is known to increase forearm blood flow and creatinine clearance in humans (7–9). VEGF increases endothelial nitric oxide synthase activity, contributing to the relaxing capacity of the renal vasculature (10–13). Thus, both IGF-1 and VEGF may improve endothelial function and renal blood flow.

The purpose of this study was to test the effect of thyroxine therapy on serum creatinine, IGF-1, and VEGF in hypothyroid patients. Patients with newly diagnosed primary hypothyroidism who had been referred to the Division of Endocrinology and Diabetes at the University Hospital in Zurich between February 1998 and July 2002 were included in this prospective case series. Oral informed consent was obtained from all patients. Patients with neoplastic disease, secondary hypothyroidism, and thyroid cancer were excluded from the study because VEGF is often increased in patients with tumors, including tumors of the pituitary gland and the thyroid (14, 15).

All laboratory values were measured in the hypothyroid and the euthyroid state. Thyrotropin (TSH), free thyroxine (T4), creatine kinase, and creatinine were measured at the Central Laboratory of the University Hospital of Zurich with standard methods. For IGF-1 measurements, carrier proteins were removed by Sep-Pak® chromatography according to the instructions of the supplier (Waters Associates), and IGF-1 was measured by RIA (16) with a reference interval of 100–300 μg/L in adults. Acid-labile subunit (ALS) was measured by an active total ALS ELISA, an enzymatically amplified “two-step” sandwich-type immunoassay (Diagnostic Systems Laboratories) with a reference interval of 12–35 μg/L for individuals 16–60 years of age. Serum VEGF concentrations were measured with a commercially available ELISA (R&D Systems). The reference interval provided by the manufacturer was 62–707 ng/L, determined in serum samples of 37 healthy individuals. We measured VEGF in serum samples from 21 healthy individuals, and the values were between 42 and 1158 ng/L. Statistical analyses were performed using SAS, Ver. 8.2 (SAS Institute Inc.). Data are presented as means (SD). Differences between values before and after thyroxine replacement therapy were analyzed with the two-sided paired t-test except when the variables were not normally distributed, in which case the Wilcoxon signed-rank test was used. A P value <0.05 was considered statistically significant.

Fourteen patients (7 males and 7 females) with primary hypothyroidism were included. Mean (SD) age at diagnosis was 41 (16) years. Three patients were on antihypertension drugs; the treatment remained unchanged during the study period. At first presentation, all patients were symptomatic with low T4 and high TSH. Mean (SD) T4 was 3.8 (2.6) pmol/L (reference interval, 12–22 pmol/L), and TSH was 312 (277) mIU/L (reference interval, 0.27–4.2 mIU/L). All patients were treated with thyroxine and were, on average, euthyroid within 34 (20) weeks of replacement therapy. Mean T4 in the euthyroid state was 18.9 (4) pmol/L, and TSH was 2.7 (1.9) mIU/L. Creatine kinase concentrations, which were increased in 11 patients at baseline, returned to values within the reference interval. Mean blood pressure was 124/82 mmHg in the hypothyroid and 121/75 mmHg in the euthyroid state (P = 0.01 for diastolic blood pressure). Mean heart rate was 64 (10) beats/min at diagnosis of hypothyroidism and increased to 71 (10) beats/min after thyroxine treatment (P = 0.02).

The changes in serum creatinine, VEGF, IGF-1, and ALS after thyroxine replacement therapy are shown in Fig. 1. Serum creatinine was above the reference interval at diagnosis of hypothyroidism in 9 of the 14 patients and decreased from 116 (27) to 93 (17) μmol/L (reference interval, 60–105 μmol/L) after thyroxine replacement (P = 0.001). Serum creatinine decreased in all 14 patients but remained slightly above the reference interval in 3 patients.

IGF-1 was below the reference interval in eight patients at diagnosis of hypothyroidism and increased from 108 (62) to 159 (59) μg/L (P = 0.01). ALS was below the age-adjusted reference interval in five patients and increased from 14.2 (4.3) to 17.3 (4.3) ng/L (P = 0.008). IGF-1 increased in 12 and ALS in 13 patients. In the same period, VEGF increased from 326 (215) to 508 (456) ng/L (P = 0.005); these values were (at baseline) and remained (with treatment) within the reference interval of healthy individuals. VEGF increased in 13 of 14 patients.

IGF-1 concentrations are decreased in hypothyroidism and can be normalized by thyroxine replacement (17–20). We have now extended the findings to the ALS of the 150-kDa IGF carrier complex. On average, ALS concentrations increased significantly after thyroxine replacement therapy. To our knowledge, this is the first description of the influence of thyroxine replacement on ALS. In the setting of primary hypothyroidism, the data do not allow differentiation between an effect of thyroid hormones on the pituitary (on growth hormone secretion) or the liver, but it appears to be a coordinated up-regulation of IGF-1 and ALS. IGF-1 has effects in addition to growth, including effects on endothelial function and renal blood flow. NO mediates some of the effects of IGF-1 on glomerular hemodynamics. IGF-1 induces NO synthesis and release by cultured vascular endothelial cells (21). Coadministration of the NO synthase inhibitor Nω-nitro-L-arginine methyl ester blocked the IGF-1-induced increase in renal blood flow (22). A decrease in peripheral vascular resistance and in diastolic blood pressure may also be related to enhanced NO production and has been
found in hypothyroid patients treated with thyroxine (23–25).

To the best of our knowledge, the increase in VEGF (within the reference interval) in response to thyroxine replacement had not been described before. A limitation of the present study is that VEGF was determined in serum and not in plasma. However, this limitation was true for all individuals investigated. Serum VEGF concentrations have been found to be much higher than those in plasma because of the release of platelet- and leukocyte-derived VEGF during blood clotting (26). Therefore, possible explanations for the increase in serum VEGF after thyroxine treatment include effects of thyroid hormones on the amount of VEGF transported and/or released from blood cells, e.g., by affecting thrombopoiesis or platelet aggregation (27, 28).

VEGF receptors are expressed on quiescent endothelia of glomerular capillaries in the kidney and therefore might have functions other than mediating endothelial growth (29). Our data demonstrate that IGF-1 and VEGF increase in response to thyroxine replacement in patients with primary hypothyroidism. IGF-1 and VEGF increased consistently in almost every individual treated, whereas serum creatinine concentrations decreased within the same period. In our data, we found a significant relationship between creatinine and VEGF after adjusting for IGF-1, age, and sex. In vivo and in vitro studies have shown that IGF-1 and VEGF may improve endothelial function and renal blood flow, whereas other studies have shown that endothelial function and renal blood flow are impaired in thyroid hormone deficiency (1, 10, 30). The increases in IGF-1 and VEGF in response to thyroxine may improve endothelial function and renal blood flow, thereby contributing to the observed reduction in diastolic blood pressure and decrease in serum creatinine in hypothyroid patients treated with thyroxine.

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References

Fig. 1. Changes in serum creatinine (A), VEGF (B), IGF-1 (C), and ALS (D) after thyroxine treatment in each patient. Values are shown at diagnosis of hypothyroidism and in the euthyroid state.
15. Tuttle RM, Flesher M, Francis GL, Robbins RJ. Serum vascular endothelial growth factor levels are elevated in metastatic differentiated thyroid cancer but not increased by short-term TSH stimulation. J Clin Endocrinol Metab 2002;87:1737–42.

Genomic Sequencing of a SARS Coronavirus Isolate That Predated the Metropole Hotel Case Cluster in Hong Kong, Stephen S.C. Chim,1 Yu-Kwan Tong,1 Emily C.W. Hung,2 Rossa W.K. Chiu,1 and Y.M. Dennis Lo1* (Departments of 1Chemical Pathology and 2Paediatrics, The Chinese University of Hong Kong, Prince of Wales Hospital, Shatin, New Territories, Hong Kong; * address correspondence to this author at: Department of Chemical Pathology, The Chinese University of Hong Kong, Prince of Wales Hospital, Room 38023, 1/F Clinical Sciences Bldg., 30-32 Ngan Shing St, Shatin, New Territories, Hong Kong; SAR; fax 852-2194-6171, e-mail loym@cuhk.edu.hk)

The epidemic of severe acute respiratory syndrome (SARS) swept across the globe, with reported cases in more than 30 countries. As of July 11, 2003, the number of reported probable cases was 8437, with 813 deaths (1). A novel coronavirus, SARS-CoV, was promptly implicated as the causative agent (2–4). Macaques infected with SARS-CoV subsequently developed respiratory symptoms and pathologies similar to SARS patients, thus fulfilling the Koch postulates (5). Efforts in sequencing the viral genome promptly followed, and the genomic sequence revealed little homology to previously characterized strains of coronaviruses (6,7). The complete genomic sequences of several SARS-CoV isolates have since been made publicly available (www.ncbi.nlm.nih.gov).

Several sequence variations exist among isolates. In general, based on these sequence variations, the majority of the isolates can be segregated into two groups: isolates that were obtained from individuals who were epidemiologically linked to and those who were not linked to the Metropole Hotel in Hong Kong (8–10). Ruan et al. (10) compared the genomic sequences of 14 SARS-CoV isolates and suggested that a haplotype comprising four nucleotide positions, namely, 9404, 17564, 22222, and 27827 [GenBank accession no. AY274119 (7)], clearly defined two distinct genotypes. Isolates that were epidemiologically linked to the Metropole Hotel cluster have the configuration T:T:T:T, as opposed to the sequence C:G:C:C seen in the unassociated strains. (Note: The usage of the DNA-based code for the designation of SARS-CoV haplotypes does not imply that this virus possesses a DNA genome.)

SARS was first reported in Guangdong Province, China, in November 2002 (11). Isolates that demonstrated the C:G:C:C haplotype were epidemiologically traceable to the early part of the epidemic (9). On the other hand, SARS was first reported in Hong Kong when a cluster of cases was noted among visitors to the Metropole Hotel. This case cluster comprised international travelers who subsequently brought SARS to other countries, including Vietnam, Canada, and Singapore (11). Epidemiologic investigations revealed that the cases were traceable to a nephrologist from Guangdong Province, China, who checked into the hotel on February 21, 2003 (8,11). These data suggest that since the emergence of SARS-CoV in