Mahmoud Nasser, Ahmad Haider, Farid Saad*, Winfried Kurtz, Gheorghe Doros, Monika Fijak, Linda Vignozzi and Louis Gooren

Testosterone therapy in men with Crohn's disease improves the clinical course of the disease: data from long-term observational registry study

Abstract

Background: Crohn's disease is an inflammatory chronic bowel disease characterized by an imbalanced production of pro-inflammatory mediators (tumor necrosis factor-α) and an increased recruitment of leukocytes to the site of inflammation. Low serum testosterone is associated with an increase in inflammatory factors, while testosterone administration reduces them. There is evidence for an immunomodulatory effect of testosterone on differentiation of regulatory T cells.

Materials and methods: The research was carried out in clinics in Germany and Syria. The study was a cumulative, prospective, registry study with an increasing number of men over time receiving testosterone. While men diagnosed with Crohn's disease received appropriate treatment for Crohn's disease, they were tested for testosterone deficiency (cut-off point ≤12.1 nmol/L). In total, 92 men received parenteral testosterone undecanoate 1000 mg/12 weeks for up to 7 years. Fourteen men opted not to receive testosterone and served as a comparison group.

Results: In men receiving testosterone, the Crohn's Disease Activity Index declined from 239.36±36.96 to 71.67±3.26 at 84 months ($p<0.0001$ vs. baseline). C-reactive protein levels decreased from 12.89±8.64 to 1.78±1.37 mg/L at 84 months ($p<0.0001$ vs. baseline). Leukocyte count decreased from 11.93±2.85 to 6.21±1.01×10^9/L ($p<0.0001$ at 84 months vs. baseline). No changes were observed in the comparison group. There were no significant side effects of testosterone.

Conclusions: Normalizing serum testosterone in hypogonadal men with Crohn's disease had a positive effect on the clinical course, also evidenced by biochemical parameters. Testosterone administration appeared safe.

Keywords: Crohn's disease; Crohn's Disease Activity Index; highly sensitive C-reactive protein; inflammation; testosterone.

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Introduction

Testosterone traditionally has been regarded as a hormone serving male sexual and reproductive functions. Over the last decades it has become clear that testosterone is involved in a multitude of other biological processes, such as the health of bone and muscle, numerous metabolic processes and also the cardiovascular system. An even more novel aspect of the physiology of testosterone is its role in inflammation. Inflammatory processes play a significant role in the etiology of cardiovascular disease, and testosterone shows anti-inflammatory effects [1]. Several studies have documented that low testosterone levels are associated with an increase in inflammatory factors and that administration reduces their levels (for review [2, 3]). There is evidence showing an immunomodulatory and protective effect of testosterone in a model of chronic testicular inflammation and testosterone as a new factor in the differentiation of regulatory T cells [4].

***Corresponding author: Dr. Farid Saad,** Bayer Pharma, Global Medical Affairs Andrology, Muellerstrasse 178, 13353 Berlin, Germany, Phone: +49-30-46815057, Fax: +49-30-46895057, E-mail: farid.saad@bayer.com
Mahmoud Nasser: Department of Gastroenterology, University of Aleppo, Aleppo, Syria
Ahmad Haider: Private Urology Practice, Bremerhaven, Germany
Winfried Kurtz: Department of Internal Medicine, Klinikum Bremerhaven, Bremerhaven, Germany
Gheorghe Doros: Department of Epidemiology and Statistics, Boston University School of Public Health, Boston, MA, USA
Monika Fijak: Department of Anatomy and Cell Biology, Justus-Liebig University, Giessen, Germany
Linda Vignozzi: Sexual Medicine and Andrology Unit, Department of Experimental and Clinical Biomedical Sciences, University of Florence, Italy
Louis Gooren: VU medical Center, Department of Internal Medicine, Endocrine Section, Amsterdam, The Netherlands

Inflammatory bowel disease is a chronic inflammatory disorder that is comprised of both Crohn's disease and ulcerative colitis and is characterized by alternating phases of clinical relapse and remission. Although their etiologies are unknown, they are characterized by an imbalance in production of pro-inflammatory mediators, e.g., tumor necrosis factor (TNF)-α, as well as increased recruitment of leukocytes to the site of inflammation [5, 6].

This contribution reports our observations in hypogonadal men with Crohn's disease receiving testosterone treatment. While their hypogonadal status might be also explained by a number of etiological factors, inflammatory factors associated with Crohn's disease might have contributed to their hypogonadism [7, 8]. This has been found to be the case in not only elderly men but also younger men [9].

There is increasing evidence from both clinical and experimental studies showing that testosterone has marked anti-inflammatory effects. In a Russian randomized, placebo-controlled trial in hypogonadal men with metabolic syndrome (MetS), testosterone therapy resulted in significant reductions in highly sensitive C-reactive protein (hsCRP) ($p<0.001$), TNF-α ($p=0.03$) and IL-1β ($p=0.008$) [10]. In a controlled 2-year study from Italy in hypogonadal men with MetS, reductions of hsCRP ($p<0.001$) and carotid intima media thickness (CIMT) ($p<0.0001$) were shown [11]. In another controlled study by the same group of 3 years duration in hypogonadal men with MetS, hsCRP ($p=0.001$) was significantly reduced under testosterone treatment [12].

In a 12-month study in severely obese hypogonadal men undergoing lifestyle intervention by diet and exercise with or without addition of testosterone, there were significant reductions in hsCRP ($p<0.001$) and CIMT ($p<0.0001$) in the testosterone but not in the control group [13]. In a controlled 5-year study in hypogonadal men with MetS, significant reductions in hsCRP ($p=0.001$) and fibrinogen ($p=0.0001$) were observed [14].

In a wealth of studies, the anti-inflammatory effect of testosterone has been demonstrated [8, 15–21].

Several preclinical studies have demonstrated that androgens act as endogenous inhibitors of immune responses in several autoimmune processes, including those involved in non-alcoholic steatohepatitis (NASH) [22], benign prostatic hyperplasia [23] and autoimmune orchitis [4].

Moreover, the anti-inflammatory properties of androgens were shown already in several experimental animal models of autoimmune diseases such as experimental autoimmune encephalomyelitis, myasthenia gravis or diabetes [24–28]. At the cellular level, in vitro testosterone treatment leads to the shift from Th1 to Th2 response by reduction of TNF-α or IL-6 secretion, inhibition of proliferation of T cells and apoptosis [26, 29–32]. As shown recently, subphysiological levels of testosterone in androgen-deficient male rats lead to decrease in IL-2, IL-6, IL-10, IL-12 and IL-13, while testosterone supplementation has an opposite effect [33].

Recently, activation of androgen receptor (AR) by androgens has been demonstrated to reduce proliferation of CD4+T clones and to markedly suppress the inflammatory response of human non-professional antigen presenting cells culture – such as those present within the prostate – to inflammatory stimuli [such as TNF-α and the lipopolysaccharide (LPS)] or to co-incubation with activated CD4+T lymphocytes, therefore suggesting that androgens could play a broad anti-inflammatory role in T cells. Interestingly, supplementation of reduced serum testosterone levels in a rat model of experimental autoimmune orchitis led in the testis to reduction of disease severity, down-regulation of TNF-α, IL-6 and MCP-1 mRNA expression, inhibition of macrophages recruitment as well as increase in the number of regulatory T cells – responsible for aggravation of autoimmune responses. In vitro, testosterone stimulated directly the expression of regulatory T cell-specific transcription factor Foxp3 in rat splenic T cells. Moreover, at the systemic level significant decrease of pro-inflammatory Th1 cytokines IL-2 and IFN-γ secretion by mononuclear cells from lymph nodes draining the testis was observed. Our recent results show that testosterone administration is able to inhibit LPS-induced TNF-α expression in testicular cells (Sertoli and peritubular cells), and androgens secreted directly by Leydig cells stimulate production of regulatory cytokine IL-10 by T cells [34]. Similarly, human monocyte-derived macrophages stimulated with oxidized low-density lipoproteins and subsequently treated with normal or supraphysiological concentrations of testosterone showed reduced expression and secretion of TNF-α and IL-1 beta; however, the expression of IL-6 and C-reactive protein (CRP) was not affected [35]. All these reports underline immunoregulatory and protective properties of androgens in immune tolerance.

Our previous study demonstrated the effects of 2 years of serum testosterone levels normalization in a small group of men with Crohn's disease [36]. Upon normalization of plasma testosterone, the Crohn's Disease Activity Index (CDAI) and serum levels of CRP declined in hypogonadal patients with Crohn's disease. Immunosuppressive effects of testosterone may have contributed to this improvement of the disease.

The present study is a prospective, cumulative, observational registry study of hypogonadal men with Crohn's disease who received treatment with testosterone.

Untreated hypogonadal men with Crohn's disease served as comparison subjects.

Subjects and methods

The study was carried out in the Department of Internal Medicine, Klinikum Bremerhaven, Germany, together with a private urology practice in Bremerhaven, Germany, and the Department of Gastroenterology, University of Aleppo, Syria, between the years 2004 and 2013. In patients diagnosed with Crohn's disease, serum testosterone was measured, and if below normal (see below) they were referred to the urology practice for possible testosterone administration. In Syria, if hypogonadal, they received testosterone treatment in the Department of Gastroenterology, University of Aleppo. The study was a cumulative, prospective, registry study of 92 men with Crohn's disease with testosterone levels ≤12.1 nmol/L. Patients received standard treatment for Crohn's disease by their internist/gastroenterologist and also standard treatment for hypogonadism: parenteral testosterone undecanoate (Nebido®, Bayer Pharma, Berlin, Germany) 1000 mg/12 weeks following an initial 6-week interval for up to 7 years. New patients were consecutively entered into the study once they had completed 1 year of treatment. So, drop-outs in this first year of testosterone treatment have not been included in the registry. In total, 92 men were treated for at least 12 months, 74 for at least 24 months, 50 for at least 36 months, 30 for at least 48 months, 25 for at least 60 months, 17 for at least 72 months, and 12 for at least 84 months. The declining numbers do not reflect drop-out rates but are a result of the registry design. Fourteen hypogonadal men of similar age with Crohn's disease in the Aleppo clinic who opted not to receive testosterone were followed up for 30 months and served as an untreated comparison group.

The CDAI has been developed to assess the severity of the disease and to monitor the effects of interventions on the course of the disease. The CDAI consists of eight factors, each summed after adjustment with a weighting factor. Index values of 150 and below are associated with quiescent disease; values above that indicate active disease, and values above 450 are seen with extremely severe disease [37]. The severity of Crohn's disease was assessed every 3 months with the CDAI. In addition, hsCRP [38] and leukocyte count were measured. Liver enzymes were determined since the liver is the source of TNF-α [39].

The CDAI was assessed every 3 months [40]. Anthropometric parameters (waist circumference and weight), total testosterone, fasting glucose, lipids (total cholesterol, LDL cholesterol, HDL cholesterol and triglycerides), liver transaminases (aspartate and alanine aminotransferase, AST and ALT), hemoglobin (Hb), hematocrit (Htc), systolic and diastolic blood pressure, and heart rate were assessed at each visit. T scores were obtained from those patients who had osteoporosis. Prostate parameters [prostate volume, prostate-specific antigen (PSA)] were measured at every other visit.

Exclusion criteria for testosterone administration included previous treatment with androgens, prostate cancer or any suspicion thereof: such as PSA levels >4 ng/mL, International Prostate Symptom Score >19 points, breast cancer, a history of congestive heart failure or recent angina, history of cerebral vascular accident or severe untreated sleep apnea.

All initial serum testosterone samples had been obtained between 7:00 and 11:00 a.m. Serum testosterone levels were measured before testosterone administration, then before the second injection at 6 weeks and subsequently before the next injection of testosterone undecanoate was due, as a rule 12 weeks later. Serum testosterone was measured by commercially available chemiluminescent radioimmunoassays.

Ethical guidelines as formulated by the German "Ärztekammer" (the German Medical Association) for observational studies in patients receiving standard treatment were followed both in Germany and Syria. After receiving an explanation regarding the nature and the purpose of the study, all subjects consented to be included in the research of their treatment protocol.

Statistical analysis

For continuous variables, the mean, median, standard deviation, range, minimum, maximum and sample size for the overall sample and various groups were reported at each time point. For categorical variables, the frequency distribution was reported. We tested the hypotheses regarding change in outcome scores across the study period by fitting a linear mixed effects model to the data. Time (to indicate follow-up interviews) was included as fixed effect in the model. A random effect was included in the model for the intercept. Estimation and test of change in scores were determined by computing the differences in least square means at baseline vs. the score at each follow-up interview. For the correlation study, Pearson's correlation was calculated between baseline changes in outcomes at various time points. The significance of each correlation was tested using Fisher's test.

Results

Serum testosterone

Testosterone levels at baseline were 9.68±1.09 nmol/L in the testosterone group and 9.57±2.96 in the comparison group. During treatment, testosterone increased to 16.4±1.96 nmol/L within 24 months and then remained stable. Serum testosterone slightly declined in the comparison group.

CDAI and inflammation parameters

In the testosterone group, the CDAI declined from 239.36±36.96 to 71.67±3.26 at 84 months (Figure 1). This decrease was statistically significant vs. baseline ($p<0.0001$) at the end of each year and vs. previous year for the first 3 years, after which levels stabilized. This decline was not observed in the comparison group.

hsCRP levels decreased from 12.89±8.64 to 1.78±1.37 mg/L at 84 months with statistical significance vs. baseline ($p<0.0001$) each year and vs. previous year for the

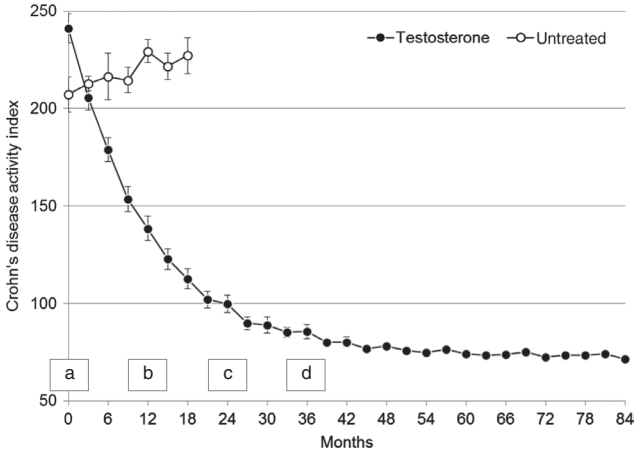

Figure 1: Effect of testosterone administration on CDAI in 92 men receiving testosterone compared to 14 men receiving no testosterone (mean±SEM). b vs. a, p=0.001; c vs. b, p=0.02; d vs. c, p<0.05.

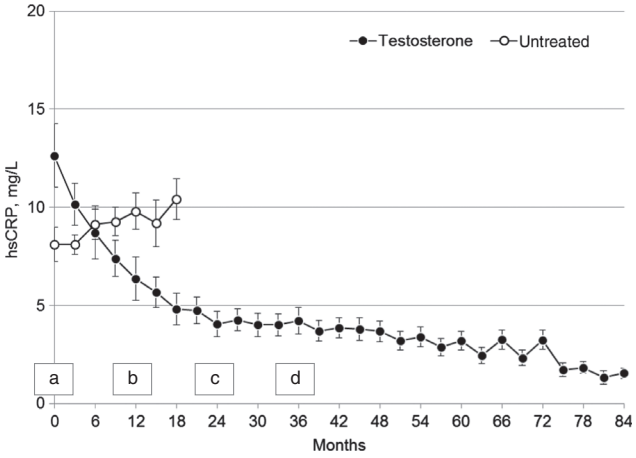

Figure 2: Effect of testosterone administration on hsCRP in 92 men receiving testosterone compared to 14 men receiving no testosterone (mean±SEM). b vs. a, p=0.001; c vs. b, p=0.02; d vs. c, p<0.05.

first 4 years in the testosterone group (Figure 2). There was a tendency to increase in the comparison group.

Leukocyte count decreased from 11.93±2.85 to 6.21±1.01×10^9/L in the testosterone group (Figure 3). This was statistically significant vs. baseline (p<0.0001). Leukocyte count remained unchanged in the comparison group.

Liver transaminases (AST and ALT)

AST declined from 43.75±17.93 to 20.08±3.58 U/L (Figure 4), ALT from 46.99±17.04 to 20.42±4.06 U/L in the testosterone group (Figure 5). Changes for both were statistically significant vs. baseline (p<0.0001) each year and vs. previous year for the first 3 years. Both enzymes showed a tendency to increase in the comparison group. Twelve patients had very high (>70 U/L) AST and 10 patients very high (>70 U/L) ALT levels at baseline, all in the testosterone group.

Hb: At baseline, Hb was 13.93±0.59 g/dL in the testosterone group and 14.43±0.23 in the control group (p=0.00269). Hb rose from 13.93±0.59 to 14.4±0.56 g/dL in the testosterone group (p<0.0001) with statistical significance vs. previous year for the first 2 years. Hb remained stable in the control group.

Htc: At baseline, Htc was 42.2±2.63% in the testosterone group and 45.5±1.61 in the control group (p=0.000014). Htc rose from 42.2±2.63 to 47.75±1.36% in the testosterone group (p<0.0001) with statistical significance vs. previous year for the first 4 years. Htc levels remained within the normal range (minimum: 46%; maximum: 51%). Htc showed a tendency to decrease in the control group.

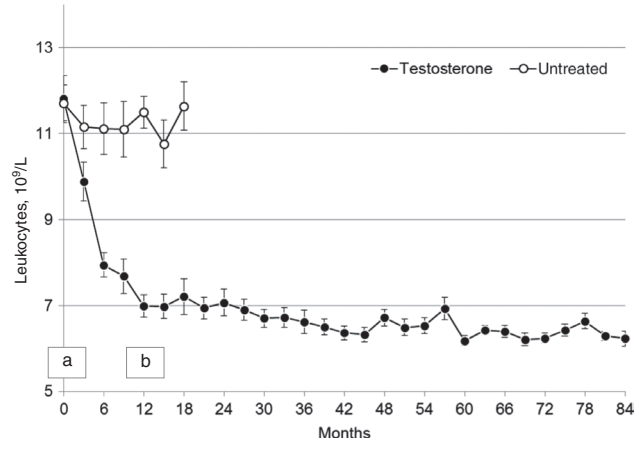

Figure 3: Effect of testosterone administration on leukocyte count in 92 men receiving testosterone compared to 14 men receiving no testosterone (mean±SEM). b vs. a, p=0.0001.

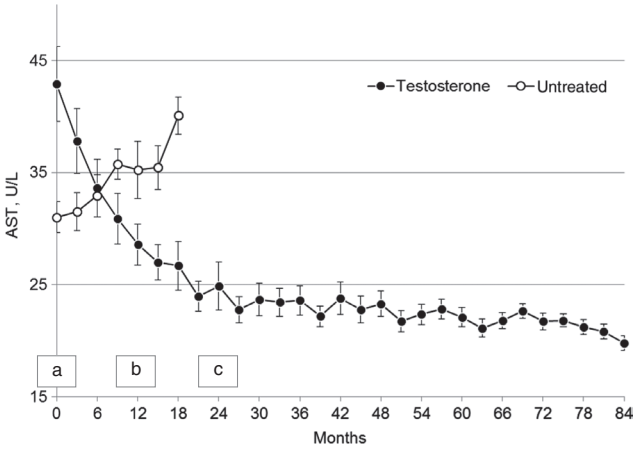

Figure 4: Effect of testosterone administration on AST in 92 men receiving testosterone compared to 14 men receiving no testosterone (mean±SEM). b vs. a, p=0.001; c vs. b, p=0.02.

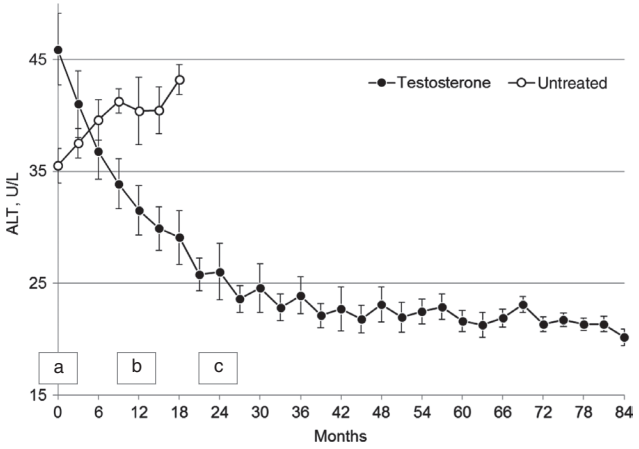

Figure 5: Effect of testosterone administration on ALT in 92 men receiving testosterone compared to 14 men receiving no testosterone (mean±SEM). b vs. a, p=0.001; c vs. b, p=0.02.

Discussion

Crohn's disease is a chronic inflammatory bowel disease characterized by a relapsing-remitting clinical course. The disease is characterized by intestinal inflammation which leads to progressive bowel damage, increasing disability and a loss of quality of life, and the treatment is cumbersome. In recent years, a significant role of testosterone as an immune modulator [3] in a large number of disease entities such as cardiovascular disease [41], rheumatoid arthritis [42] or inflammatory processes of the central nervous system [43] has become apparent.

In our previous pilot study investigating the effects of normalization of serum testosterone in hypogonadal men suffering from Crohn's disease, we have shown a strong significant reduction of the severity of Crohn's disease as measured by CDAI and a reduction of levels of CRP and white blood cell count with an improvement of Hb levels [35]. The present study includes a much larger group of patients and contains also a comparison group without testosterone treatment. The obtained results are in agreement with our earlier study. The lack of changes in the above mentioned parameters in the comparison group not receiving testosterone confirms the importance of testosterone administration on the disease process.

The design of the study was a cumulative, prospective registry, and 50 men had received testosterone treatment for at least 36 months. The beneficial effects of normalization of their serum testosterone levels had lasted for the duration of 36 months; therefore, the effects of testosterone were not transient.

Hb levels and Htc increased significantly but remained well within safe margins. Fears that normalization of serum testosterone in elderly men will induce prostate cancer can no longer be credited [44], nor that proper use of testosterone induces cardiovascular disease [45].

However, our study design has a number of limitations: it was not a double-blind placebo-controlled study. Over 2 years only the testosterone treatment group could be compared to a smaller group of men who did not receive testosterone treatment.

An abnormal immune response plays an important role in the development and perpetuation of the inflammatory cascade in a variety of chronic inflammatory diseases, including inflammatory bowel disease [46]. Under most conditions, an immune competence of the gut would be beneficial to the host. However, in some situations a perturbation of the fine balance in immune regulation with a switch toward an effector Th1/Th17 phenotype might lead to the development of chronic intestinal inflammation, as observed in the pathogenesis of Crohn's disease [46]. An overt, or even a subclinical, bacterial or viral infection is thought to cause intestinal inflammation (first hit) that could be auto-sustained or exacerbated by the presence of an altered and abnormal Th1 and Th17 immune response.

TNF-α plays an important role in the pathogenesis of both Crohn and NASH, which indeed can be frequently seen in the same patient [47].

An anti-inflammatory effect of testosterone in the liver has previously been reported in preclinical [22] and clinical papers [48–51]. Androgens inhibit Th1 differentiation of CD4 T cells and are a factor for modulating actions of androgens to mitigate CD4 responses in disorders of autoimmunity [32]. Specifically, the importance of testosterone in down-regulating the systemic immune response by cell type specific effects in the context of immunological disorders has been recently reviewed [52].

The mechanisms by which testosterone exerts its anti-inflammatory effects are not completely understood. We recently demonstrated that androgens inhibit both TNF-α-induced NF-κB activation, a master transcription factor in inflammation, and, as an additional mechanism, TNF-α-induced overexpression of receptor for oxidized LDL (oxLDL receptor, LOX-1) [53]. LOX-1 plays important roles in pro-inflammatory signaling in several MetS-associated chronic inflammatory diseases, including NASH [22]. Interestingly, activation of the AR by dihydrotestosterone was demonstrated to blunt oxLDL-induced inflammatory response by reducing the expression of LOX-1 in human non-professional antigen presenting cells [53].

Our study contributes to the mounting evidence that testosterone deficiency is associated with systemic inflammation in an increasing number of diseases, such as cardiovascular disease [41], rheumatoid arthritis [42] and

the central nervous system [43]. Normalization of serum testosterone can ameliorate this situation. So, it seems mandatory that men with these conditions are tested for testosterone deficiency. However, physicians treating these conditions are often unfamiliar with the administration of testosterone and are unduly apprehensive of the side effects of testosterone administration which are unfounded [44, 45].

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Conflicts of interest: Farid Saad is an employee of Bayer Pharma AG, the manufacturer of testosterone undecanoate, the androgen used in this study. The other authors declare no conflict of interests.

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