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Thyroid Hormone Levels and Cigarette Smoking in Baboons[1] (42658)

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Abstract. Using a primate animal model, two studies were undertaken to examine the effects of cigarette smoking on thyroid hormone levels. In study 1, mean total triiodothyronine (total T_3) and mean total thyroxine (total T_4) levels were measured in two groups of baboons (*Papio cynocephalus*) who were taught to smoke cigarettes using operant conditioning techniques. The smokers were divided into established and naive smokers according to pack-years of exposure. A control group of never-smoker baboons was included for comparison. Blood sampling was done after long-term cigarette consumption and again 1 week after cigarette deprivation. In the naive smoker group, mean total T_3 concentrations were reduced below control group values ($P < 0.05$). After cigarette deprivation for 1 week, mean total T_3 values returned to normal. No significant differences in total T_4 levels were observed in either group. In study 2, we assessed some other indices of thyroid function. The same groups of baboons were divided into good and poor smokers by plasma cotinine and blood carboxyhemoglobin (% COHb) levels during 28 weeks of cigarette smoking activity. Immediate fluctuations and reductions in total T_3 levels were observed that were not accompanied by reductions in total T_4. The animals were then cigarette deprived for 1 week and blood samples were obtained every other day during this period. Significant increases in total T_3 concentrations were observed in poor smokers immediately after cessation. Both groups also exhibited significant reductions ($P < 0.05$) in T_3 uptake and free T_4 index (FT_4I) when compared to control group values. These data suggest that poor smokers are more susceptible to thyroid hormone level shifts than more established smokers, since the established smokers become habituated to the compounds contained in cigarette smoke through repeated exposure.

Chronic cigarette smoking causes potentially harmful changes in endocrine balance. Reductions in testosterone, human placental lactogen, and urinary estrogens have been observed in heavy smokers (1–3). Subnormal levels of thyroid hormones have been reported in human cigarette smokers and in rats that were chronically administered *N'*-oxidative nicotine metabolites (4, 5). Short-term administration of nicotine metabolites has resulted in reductions in serum total triiodothyronine (total T_3) after a single week (6). It is important to determine whether these nicotine- or cigarette smoke-induced alterations in hormone balance are reversible following smoking cessation.

The most favorable experimental condition under which to study the reversibility of tobacco smoke-induced changes and the restoration of endocrine system homeostasis would be through the use of an animal model. Several models have been developed to study the effects of cigarette smoking, but all differ from humans in the method of exposure to smoke (7, 8). A baboon animal model for cigarette smoking has been developed that closely resembles human smoking behavior. The smoke is actively inhaled into the lungs rather than being retained via passive exposure (9, 10). Baboons (*Papio cynocephalus*) have been trained to puff on cigarettes in a human-like manner that produces a dosimetric profile similar to cigarette smokers. This active uptake of cigarette smoke produces concentrations of tobacco-specific compounds and volatile gases in the

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blood that are the equivalent of human absorption patterns (12).

The baboon smoking model has been used in studies of atherogenesis, chronic obstructive lung disease, and carcinogen metabolism and in urine mutagenicity studies (13–15). These primates make ideal experimental animals because of their close physiological similarity to humans, and they provide a model system that will eliminate or control dietary and environmental confounders.

In this study, we determined that levels of thyroid hormones decreased in our primate cigarette-smoking population. The animals were then used to determine whether the observed hormone level fluctuations were reversible following cessation.

Materials and Methods. Baboons were originally conditioned to smoke cigarettes by operant techniques using water rewards (9). After several improvements in training regimen, the primates achieved blood COHb levels and plasma cotinine concentrations comparable to those of human cigarette smokers. The smoking baboon model corresponds well with the average human smoker's topographical pattern and produces lung loads of particulate matter equivalent to those found in humans (11).

Study 1. Long-term smokers ($n = 20$) were equally divided into two groups according to pack-years. Blood samples were obtained during smoking and again 1 week after cessation. The samples were analyzed for blood carboxyhemoglobin (COHb), plasma cotinine, total T_3, and total T_4. A control group of baboons ($n = 7$) that never smoked was included for comparison.

Study 2. Animals that had not smoked for at least 18 months were selected for this study. Measurements of thyroid hormone activity were determined as the animals started smoking and continued for 28 weeks of smoking activity. After this time period, the animals were cigarette-deprived and samples were obtained every second day for 1 week beginning with the first sample 24 hr after cessation. The animals were divided into two groups, poor smokers (plasma cotinine below 200 ng/ml, $n = 8$) and good smokers (mean % COHb of 6.0% and plasma cotinine levels between 200 and 400 ng/ml, $n = 8$) based on blood COHb and plasma cotinine concentrations. A control group composed of nonsmoking animals ($n = 10$) was also included for comparison.

Animals. The healthy adult baboons (*Papio cynocephalus*) were cared for using procedures that comply with AALAC and FDA regulations. The animals were housed in single cages and conventional baboon chow was supplemented by one-half apple given at the end of the day. Access to food was restricted prior to blood collection.

The animals were bled using conventional squeeze cages and ketamine hydrochloride (10 mg/kg im) sedation. Venous samples were drawn by vacutainer containing EDTA as an anticoagulant. After determining blood percentage COHb, the samples were centrifuged and the plasma was separated and frozen prior to further analyses.

Biochemical analyses. Blood COHb was determined using an Instrumentation Laboratories IL-282 cooximeter with a microprocessor specific for baboons (16). The accuracy of this assay is 1.0% and the precision is 0.5%. Plasma cotinine was quantitated using a modification of the radioimmunoassay developed by Langone *et al.* (17). This method uses a specific antiserum produced by injection of trans-4-carboxycotinine bound to serum albumin in rabbits. The inter- and intraassay variations are less than 6% and the results compare favorably with those obtained by other methods (18).

Dunnett's procedure for multiple comparisons with a standard was used to compare treatment groups with control group values. All test of significances were conducted at a type 1 error of 0.05.

Total T_3 and total T_4 (Diagnostic Products Corp.) were measured by radioimmunoassay according to established protocols (19, 20). The *in vitro* T_3 uptake was assessed by the method of Coleman *et al.* (21). All samples from both control and experimental groups were treated identically and assayed simultaneously, in duplicate, to eliminate interassay variation. Two different human TSH radioimmunoassays were used to determine primate TSH levels (monoclonal NHS–TSH and polyclonal "ultra" TSH, Diagnostic Products Corp.). Analyses of samples were performed in duplicate and both a 3-hr and an overnight incubator time were used in the polyclonal RIA. Both methods proved unsuccessful. While values obtained in the ba-

TABLE I. THYROID HORMONE CONCENTRATIONS IN A BABOON COLONY THAT CEASED CIGARETTE SMOKING FOR 1 WEEK

	Plasma cotinine (ng/ml)	Blood COHb (%)	Plasma total T_3 (ng/dl)	Plasma total T_4 (μg/dl)
Established smokers (n = 10) (11.6 ± 5.2 packs/years)[a]				
Smoking levels	392 ± 106	4.9 ± 0.4	60.0 ± 5.8	4.4 ± 0.7
Cessation levels	6 ± 0.8	1.3 ± 0.1	67 ± 7.7	5.5 ± 0.5
Naive smokers (n = 10) (2.1 ± 0.1 packs/years)				
Smoking levels	174 ± 52	2.8 ± 0.5	56.0 ± 3.5[b,c]	4.6 ± 0.4
Cessation levels	3 ± 0.6	1.2 ± 0.1	75 ± 4.3	4.9 ± 0.3
Control (n = 7)	0	1.2 ± 0.3	70 ± 1.0	5.1 ± 0.3

[a] All values are equal to the means ± the SE of the mean for each group.
[b] Statistically different ($P < 0.001$) cessation levels.
[c] Statistically different ($P < 0.05$) from control group levels.

boon are similar to human values, human and primate TSH are not immunologically analogous (23).

The free T_4 index (FT_4I) was obtained in the following manner. The values of the *in vitro* uptake test (% T_3U) were multiplied by the serum total T_4 concentration to give the index value which has a numerical range similar to Total T_4 concentration values but no units.

Results. *Study 1.* Our established smokers (11.6 ± 5.2 pack/years) had overall higher mean plasma cotinine and blood COHb levels when compared to the naive smoker group (2.1 ± 0.1 pack/years, Table I). In naive smokers, smoking total T_3 values were reduced significantly below the control group, and after cessation for 1 week, returned to normal. In the established smoker group, no statistically significant changes in thyroid hormone concentrations were detected in smoking cessation or control group comparisons.

Study 2. Our smoking baboon population was divided according to blood carboxyhemoglobin (% COHb) and plasma cotinine concentrations. The good smokers maintained a mean percentage COHb value of approximately 6.0% throughout the study period (Fig. 1). The poor smokers had mean COHb levels of approximately 3.0%. The good smokers maintained plasma cotinine concentrations between 200 and 400 ng/ml for the first 12 weeks, while the poor smokers had cotinine levels below 200 ng/ml. Verification of smoking status by cotinine assessment was discontinued after smoking behavior was firmly established (12 weeks). Blood COHb monitoring was continued for the duration of the study.

Mean plasma total T_3 concentrations in our two groups of smokers are represented in Fig. 2. Immediate elevations in total T_3 above established control values were noted at the onset of smoking in poor smokers. In the good smokers, total T_3 concentrations dropped below 70 ng/dl by Week 10 of smoking. After observing some initial slight though not significant reductions during the first 2 weeks, mean total T_4 concentrations began to stabilize in both groups.

After 28 weeks, smoking was discontinued in our two groups of smokers. Samples were obtained from each group every other day

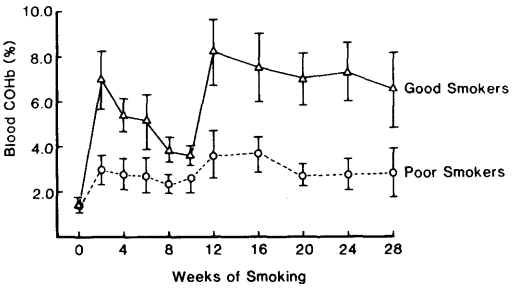

FIG. 1. Blood carboxyhemoglobin concentrations in two groups of cigarette-smoking primates (*Papio cynocephalus*). Each point is equal to the mean ± the standard error of the mean of 10 animals.

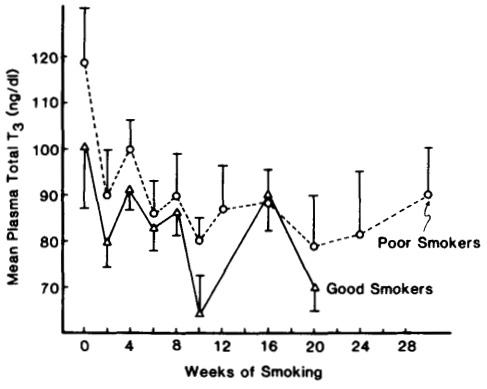

FIG. 2. Plasma total triiodothyronine (total T_3) concentrations in two groups of cigarette smoking-primates (*Papio cynocephalus*). Each point is equal to the mean ± the standard error of the mean of 10 animals.

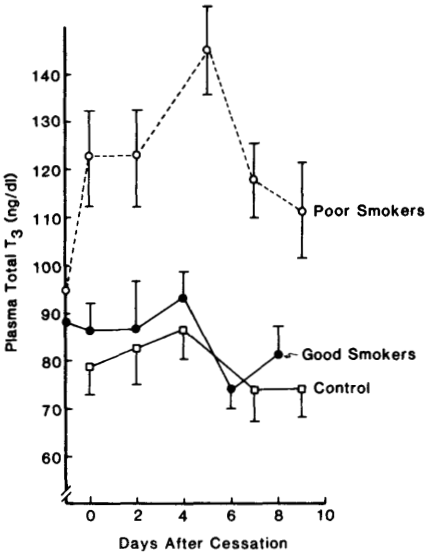

FIG. 4. Mean plasma total T_3 concentrations in poor and good cigarette-smoking primates (*Papio cynocephalus*) during 1 week of cessation. Each point is equal to the mean ± the standard error of the mean of 8 animals (control = 10 animals).

following ketamine sedation for 9 days after cessation. Mean plasma total T_4 concentrations were constant among the control animals. After 4 days, however, a modest but significant increase ($P < 0.05$) was noted in the poor smokers (Fig. 3). No compensatory changes in total T_4 were observed in the good smokers.

Mean total T_3 levels were compared during the same time period in the three groups (Fig. 4). No change was noted in either the control or the good smoker group. However, a significant increase in mean total T_3 ($P < 0.001$) was observed in the poor smoker group almost immediately after cessation. Levels continued to rise for the following 6 days. Even after 9 days, mean total T_3 values in this group remained well above control and good smoker values ($P < 0.05$).

The results of the percentage T_3 uptake analyses reveal that the protein-binding capacity for both smoker groups was significantly below control group values (Fig. 5). The FT_4 indices closely paralleled the changes in T_3 uptake in all groups (Fig. 5). Plasma TSH analyses were attempted using two RIA techniques (outlined under Materials and Methods). Our use of human TSH RIA to assess baboon TSH levels failed. Analyses of samples were performed in duplicate and both the 3-hr and the overnight protocols were used with the polyclonal kits. While plasma TSH was elevated using the "ultra" TSH assay, in the poor smoker group, these values were not confirmed by the monoclonal assay, and for this reason, TSH results are not presented.

All animals remained healthy throughout the duration of the study and there were no significant changes in weight between groups.

Discussion. Cigarette smoking alters the levels of thyroid hormone in baboons. These findings agree with thyroid-related cigarette smoking effects observed under somewhat

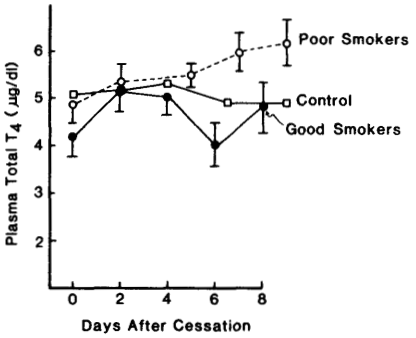

FIG. 3. Mean plasma total T_4 concentrations in poor and good cigarette-smoking primates during 1 week of cessation. Each point is equal to the mean ± the standard error of 8 animals (control = 8 animals).

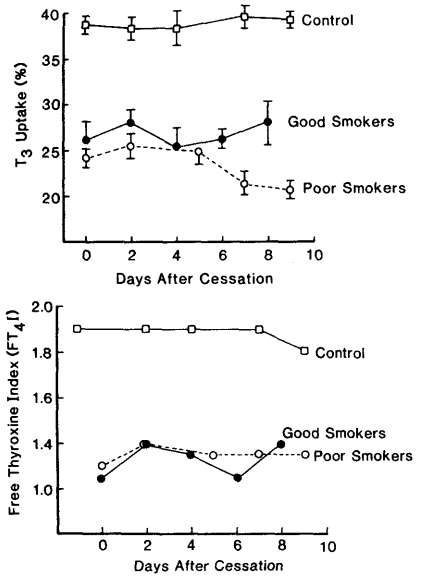

FIG. 5. Mean plasma T_3 BC levels and Free T_4 indices in poor and good cigarette-smoking primates (*Papio cynocephalus*) during 1 week of cessation. Each point is equal to the mean ± the standard error of the mean of 8 animals (control = 10 animals).

similar conditions in humans (4). Important data were derived from study 1 where the plasma from two groups of smokers were analyzed before and 1 week after smoking was terminated. First, the term "established smokers" is derived from a pack-year assessment and not from chronological age. Thus, more pack-years equates to more prolific and intensive cigarette consumption. This was verified biochemically by the observed differences in plasma cotinine and in blood COHb values between established and naive smokers. In the established smokers, no significant differences in total T_3 or total T_4 were observed. These values were not different from nonsmoking control group values. In the naive group, significant reductions in total T_3 were observed in smokers and these values returned to normal by 1 week post cessation.

In a second study, some additional thyroid hormone-related parameters were monitored as the baboons smoked for 28 weeks. We observed immediate fluctuations in total T_3 levels in the poor smokers. When the animals were cigarette deprived for 1 week, a compensatory elevation in total T_4 was observed in the poor smoker group that was not apparent in the good smokers. Concomitant with this change in total T_4, mean plasma total T_3 levels rose dramatically in the poor smokers. It should have been noted that most plasma T_3 is derived from intramuscular enzymatic conversion of T_4 to T_3. Thus, during cigarette deprivation, compensatory T_4 elevations may indeed have led to an increase in peripheral conversion of some T_4 to T_3.

Immediately after cessation, there was a decrease in percentage T_3 uptake. The total T_4 values remained similar in all groups, but both percentage T_3 uptake and FT_4I were depressed in the smoker groups relative to controls. While this situation occurs in a number of pathologies, one causal factor is acute liver disease. Possibly T_3-binding proteins that form a firm but reversible bond for circulatory transport are not synthesized in adequate concentrations in these smoker groups. Alternatively, some component present in cigarette smoke may be preferentially occupying specific binding sites, thus impeding T_3 transport. Some structural similarities do exist between nicotine and T_3 which may permit such a competitive mechanism. In humans, the main transport protein for T_3 is thyroxine-binding globulin (TBG). Direct determination of this protein is possible in humans, but immunoidentity has not been determined in primates. Numerous hepatotoxic compounds have been identified in tobacco smoke that alter liver function by inhibition of protein synthesis or by enhancing catabolic processes (24). We speculate that inhibition of TBG synthesis may play a role in the observed reduced binding capacity for T_3.

Taken together, the results of both studies indicate that poor smokers are more susceptible to thyroid hormone variation than better, more established, smokers. While total T_3 concentrations were depressed during cigarette smoking in both primate groups, only the poor smokers compensated with elevations in total T_3 and total T_4 concentrations after the inhibitory effects of smoking are removed. The good smokers exhibited no change in thyroid function after cessation. The good smokers possibly become habituated to the toxins through repeated exposure. The result may be increased efficiency

in detoxification of these xenobiotics by the liver. This has been observed previously (25–27). These same hepatic microsomal enzymes are also involved in the glucuronidation of triiodothyronine and possibly account for reduced concentrations of these and other hormones in smokers.

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