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Response to the Comments of H.B. Ferraz et al.

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We have reported a steady and progressive improvement of motor capacity in 19 sporadic Parkinson's disease (PD) patients treated with high doses (30 mg orally every 8 h) of riboflavin plus elimination of dietary red meat for 6 months (1). The treatment was based on the following preliminary observations: I) 31 of 31 consecutively evaluated PD patients were found to have an altered enzyme glutathione reductase activation coefficient (EGR-AC) and low plasma levels of flavin adenine dinucleotide (FAD) compared to the internationally determined normal range of FAD (125-300) and to local non-PD patients suffering from other neurodegenerative conditions (dementia without a clinical history or computed tomography scan images compatible with stroke), and II) 19 PD patients were found to consume large quantities of red meat (2,044 ± 1,439 g/week) compared with 19 sex-matched controls of similar age from the same social environment (789 ± 509 g/week). Since all PD patients had a normal dietary content of riboflavin, we proposed that I) PD patients may be a subset of the large group of indi-

viduals (10-15% of the general population) expressing flavokinase (FK) with low substrate affinity (poor absorbers of vitamin B2; Ref. 2), and II) a high dietary red meat consumption might trigger PD in predisposed individuals (expressing altered FK). A review of the metabolic pathways requiring the participation of both vitamin B2 active forms (flavin mononucleotide and FAD) indicated that the association of low riboflavin absorption and high dietary red meat consumption (high dietary hemin production) might account for most (if not all) neurochemical changes reported in PD including glutathione depletion, impaired mitochondrial complex I activity, mtDNA mutations, disturbed iron metabolism, and 6(OH)dopamine formation (1).

By searching the current medical literature, Ferraz and associates might readily become familiar with countless preliminary studies which have been subsequently confirmed by larger and better controlled research. The two consecutive articles by Schoenen et al. (3,4) on the effects of high doses of riboflavin on migraine may afford an appropriate example. Preliminary reports are commonly submitted to communicate novel findings of potentially large benefit: further research by others is thus facilitated. Our initial report (1) was clearly presented as preliminary work being part of ongoing research.

A placebo effect is highly unlikely to account for our results. Goetz et al. (5) observed placebo-associated motor improvements in only 16% of 105 PD subjects evaluated at 4, 12 and 24 weeks of treatment, and demonstrated that none of them showed improvement at all three evaluations. Their observations contrast sharply with the initially rapid and then slowly progressive improvement found in all (100%) 19 PD patients in our own study (1). All patients were fully informed about the meaning of "red meat" and unequivocally advised to replace it with another protein source. The timing of levodopa administrations relative to meals did not change during treatment and would not explain the motor improvement found between pre- and post-therapeutic intervention. Now, after more than 18 months of sustained treatment, none of our 19 patients has experienced any deterioration of motor capacity, and some have experienced further motor improvement, although no additional patient (apart from the initial 3 subjects) has become symptom-free.

None of our initial 31 patients "dropped out" of the study: 12 were excluded (by the time the manuscript was submitted to this journal, 2 of them had bone fractures that prevented adequate motor evaluation, and one died of metastasis from thyroid carcinoma - all 3 before completing 3 months of therapy; the remaining 9 patients took erratic daily doses of riboflavin - confirmed by uncorrected EGR-AC and plasma FAD, or did not comply with full elimination of dietary red meat, or had recently initiated the treatment). There is long established evidence (6) that orally administered riboflavin is non-toxic (doses higher than 30 mg lead to decreased net absorption), and much higher doses (400 mg/day) have been safely given to humans (3,4).

Heterogeneous control groups (composed of patients suffering from different diseases) have long been employed in clinical research to establish whether a particular feature differentiates a specific disease from others. In addition, researchers may be dealing with a heterogeneous group of neurodegenerative diseases even when the currently employed clinical diagnostic criteria for dementia of the Alzheimer type are strictly fulfilled without a brain tissue biopsy. Our study has demonstrated that lower plasma concentrations of FAD differentiate PD patients from a clearly defined group of subjects with neurodegenerative disorders without Parkinsonism. Moreover, when the levels found in both groups of Brazilian patients were compared with the internationally determined

normal range of plasma FAD concentrations and EGR-AC levels, we observed deficient riboflavin status in all 31 consecutively evaluated PD patients but in only 3 of 10 control subjects. Therefore, the lack of population studies addressing the normal range of FAD among Brazilian inhabitants does not invalidate our data. In addition, none of our PD or control patients was malnourished or taking tablets containing riboflavin. Therefore, when analyzing the ethical issues involved in using placebo tablets in control groups of PD patients during blinded studies, one should consider the risk of aggravating permanent neurological deficits by postponing the correction of documented riboflavin deficiency.

The 5 consecutive stages reported by Hoehn and Yahr (7) are specifically based on the progressive motor disabilities of PD patients and on nothing else. Their validated scale has been widely used to characterize the degree of motor deterioration of PD patients. In our own percent rating system we followed the same sequence of events, although fragmented so as to detect motor improvements in a more sensitive manner. For instance, late stage IV (nearly full dependence for daily care) was differentiated from early stage IV (assistance required for only the few most delicate or difficult items like shaving and putting on socks).

Because only some of our patients were in the advanced disease stage exhibiting the "on-off" phenomenon, all patients were uniformly rated early in the morning, prior to the earliest administration of symptomatic drugs for PD. This timing usually coincides with the "off" state for those with advanced PD showing motor fluctuations and dyskinesias related to levodopa therapy.

All studies cited by Ferraz and associates (their Refs. 5 to 9), presented to contest our results, surprisingly contain no data on red meat consumption (as distinguished from the general protein consumption), laboratory evaluation of vitamin B2 status or the effects of high doses of riboflavin on PD patients. Two of them (their Refs. 5 and 6) did not even inquire about food habits, and in one (their Ref. 8) the authors confined their inquiry to "food and vegetables eaten raw, with seeds that are either swallowed or scraped with the teeth" in a search for a heat-labile component similar to that found in the seeds of the cycad plant which grows only in Guam and neighboring islands. Abbot et al. (their Ref. 9) reported a similar daily intake of riboflavin by PD patients and controls - a finding fully consistent with our own report on normal dietary content of riboflavin associated with low plasma levels of FAD and altered EGR-AC. The citations made by Ferraz and associates demonstrate that they have completely missed our point, even though it was clearly emphasized even in the title of our study (1). In addition to the chapter by Tanner (Ref. 37 of our preliminary report, Ref. 1), neglected by Ferraz and associates (while citing much older work by the same group: their Ref. 7), recent work has characterized the consumption of raw meat (8) and high intake of iron (9) as risk factors for PD.

Our inexpensive therapy (1) addresses the cause of PD and, rather than merely alleviating the symptoms (the major goal of the costly palliative treatments available), may provide partial disease regression even in the more advanced stages and, when administered to recent onset PD patients, may even lead to the asymptomatic state. In addition, nobody can register patent rights for riboflavin. Therefore, if properly considered by patients and colleagues, the confirmation of our results (allied to an early PD diagnosis and identification of predisposed subjects) may dramatically reduce the incidence of this disease, thereby alleviating the related burden on public and private health insurance systems. Ferraz and associates may best serve the interests of their questioning patients and colleagues by conducting their own study on this subject.

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