# Correlation between Tamoxifen Elimination and Biomarker Recovery in a Primary Prevention Trial[1]

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## Abstract

**We have shown previously that a reduction from the conventional dose of tamoxifen is associated with a comparable modulation of circulating biomarkers, including insulin-like growth factor-I and cholesterol. In the present study, we have correlated serum tamoxifen elimination with biomarker recovery in healthy subjects completing a 5-year intervention period. Tamoxifen, *N*-desmethyltamoxifen, and biomarker levels were measured at 0 (baseline), 2, 4, and 6 weeks after completion of treatment in 23 healthy postmenopausal women allocated to tamoxifen 20 mg/day and in 6 women allocated to placebo. Mean (±SD) serum tamoxifen and *N*-desmethyltamoxifen concentrations were, respectively, 141 ± 50 and 226 ± 77 ng/ml at baseline, 36 ± 19 and 99 ± 46 at 2 weeks, 20 ± 15 and 61 ± 37 at 4 weeks, and 12 ± 9 and 36 ± 26 at 6 weeks. Serum tamoxifen and *N*-desmethyltamoxifen half-lives were 9 and 13 days, respectively. Body mass index was associated positively with drug's serum half-life. Compared with baseline values, the percentage increase in total cholesterol, low-density lipoprotein cholesterol, and insulin-like growth factor-I 4 weeks after treatment completion was 5, 9, and 14%, respectively. No change during the 6-week period was observed in the placebo arm. Our findings indicate that the biomarker recovery is slower than serum tamoxifen elimination, suggesting that low tamoxifen concentrations may still exert a biological effect. In addition, the prolonged half-life of tamoxifen and metabolite provides the rationale for a weekly administration of the drug in a preventive context. However, the clinical implications of our findings remain to be defined.**

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## Introduction

Tamoxifen can halve the occurrence of breast cancer in women at increased risk, but toxicity remains a limiting factor, particularly in postmenopausal women, where a higher incidence of endometrial cancer and venous thromboembolic events has been observed in the tamoxifen arm compared with placebo arm (1). Importantly, the increased risk of endometrial cancer appears to be associated with time and cumulative dose (2–4). Recent data suggest that the use of tamoxifen for ≥5 years may be associated with an increased risk of poor prognosis endometrial cancers (5). Despite the world-wide use of tamoxifen for >2 decades and the evidence of its prolonged half-life in breast cancer patients (6), no dose-response studies have been undertaken. We have shown recently that a 75% reduction of the conventional dose, which resulted in an 80% decrease in serum concentration did not affect the drug's activity on biomarkers of cardiovascular and breast cancer risk and may in fact have a more favorable safety profile (7).

To provide additional insight into the activity of tamoxifen at low concentrations, we have studied the features of tamoxifen elimination and correlated its concentration with the recovery of some biomarkers in a group of unaffected subjects completing a 5-year intervention period.

## Materials and Methods

We selected a total of 29 consecutive women attending the Milan Unit of the League against cancer and who were completing the 5-year intervention period. A detailed description of the trial and its main preliminary findings has been published elsewhere (8). All subjects were healthy hysterectomized postmenopausal women. None of them had ever undergone hormone replacement therapy. No changes in body weight >10% occurred during the 5-year treatment period in all subjects. Concurrent medication and physical exercise were not modified during the 6-week study period. A single data manager independent of the present study provided the list of the subjects. Unblinding was performed after drug and biomarker measurements. Women were selected as follows: for every fourth subject receiving tamoxifen, 1 subject receiving placebo was chosen to ensure that no carry over effect was present in drug and metabolite measurements and to describe the biomarker trend over the 6-week study period. Thus, for a total of 23 women allocated to tamoxifen 20 mg/day, 6 were allocated to placebo. All subjects had their first fasting blood withdrawal between 7:30 and 9:30 a.m. of the first day after treatment completion (*i.e.*, ≤15 h after the very last tablet intake) between October 1998 and February 1999. Blood drawing was repeated in all subjects 4 weeks later. Fifteen subjects (odd-numbered cases according to patient ID number) had an additional blood with-

drawal after 2 weeks from drug completion, whereas the remaining 14 subjects (even-numbered cases) had their blood withdrawn 6 weeks later.

Except for blood cell count, which was determined on fresh samples, all other measurements were determined simultaneously on frozen samples stored at −70°C. Blood cell counts were determined using an automatic instrument (Maxm; Coulter). Total cholesterol, high-density lipoprotein cholesterol, and triglycerides were measured by enzymatic methods with an Hitachi 911 (Boehringer, Mannheim, Germany). High-density lipoprotein cholesterol was determined in the supernatant after precipitation with phosphotungstate and $Mg^{2+}$ (Boehringer); LDL-C[3] was obtained according to the Friedewald formula (9). Plasma fibrinogen was measured using a fibrin polymerization assay (Fibrinogen Reagenz; Boehringer). Antithrombin III was assayed by chromogenic methods (Coamatic antithrombin; Chromogenix, Molndal, Sweden). Plasma IGF-I was measured by double antibody RIA using immunohistochemicals and [$^{125}$I] IGF-I provided by Medgenix (Fleurus, Belgium). The sensitivity of the assay was 0.02 nmol/liter; the intra-assay and interassay coefficients of variation were 6 and 7.5%, respectively. To avoid interference from binding proteins, plasma samples were treated with acid ethanol, according to Daughaday *et al.* (10). Normal IGF-I levels in postmenopausal subjects in the same age range is 13.1–45.9 nmol/liter (100–350 ng/ml).

Serum concentrations of tamoxifen and its main metabolites, *N*-desmethyltamoxifen and *N*-desdimethyltamoxifen, were measured, blinded as to treatment allocation, by high-pressure liquid chromatography, using the methods described previously (11). Briefly, tamoxifen and its metabolites were determined in an acetonitrile extract from serum and were separated by reverse-phase, low-dispersion liquid chromatography. The drug and its metabolites were detected after being converted to fluorophors by subjecting the effluent to UV light while passing a transparent coil. The within-day precision for the assay for tamoxifen, *N*-desmethyltamoxifen, and *N*-desdimethyltamoxifen were 0.7–5.6% for concentrations between 10 and 800 ng/ml.

**Statistical Methods.** On the basis of a previous observation showing a 10% decline in total cholesterol after 4 weeks of tamoxifen administration (12), the sample size of this study was calculated to yield 80% power to detect a full recovery in the individual level of total cholesterol 4 weeks after treatment completion (two-sided *t* test at 5% significance level). The assumed SD of the change was 35 mg/dl.

The concentration of tamoxifen, its metabolites, and biomarkers measured over time in each subject was analyzed as repeated measures data, and their log-transformed values were taken as the response variable. To keep into account the within-subject correlation between measures of the response variable at different time points, data were analyzed using a mixed model with the intercept and the time variable fitted as random effect at subject level. With this model, differences in the magnitude of the effect of time among subjects were assessed. The effect of individual covariates such as age and BMI was also investigated. The logarithms of the biomarkers were also described with a mixed model where the logarithm of tamoxifen was included as a predictor variable. Data were analyzed using the SAS procedure MIXED (SAS Institute, Cary, NC) and the SPLUS routine LME (SPLUS 2000; MathSoft, Inc., Seattle, WA).

[3] The abbreviations used are: LDL-C, low-density lipoprotein cholesterol; IGF, insulin-like growth factor; BMI, body mass index.

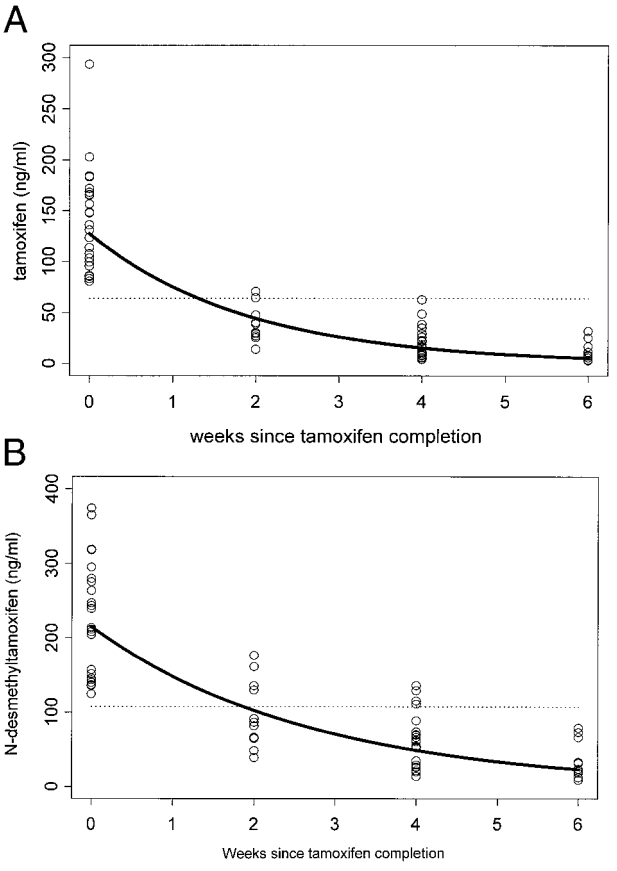


*Fig. 1.* Tamoxifen (*top*) and *N*-desmethyltamoxifen (*bottom*) elimination after 5-year treatment completion. ———, the values predicted by the model; ····, 50% tamoxifen or *N*-desmethyltamoxifen concentration at time 0.

Serum tamoxifen concentrations were undetectable in 1 subject at 4 weeks and in 2 subjects at 6 weeks. The repeated measures analysis was conducted without these observations as the final results were not affected by their exclusion.

## Results

The mean ± SD age of treated subjects was 62 ± 6 years (range: 52–72 years), and their mean BMI was 24 ± 3 kg/m$^2$ (range 18–32 kg/m$^2$).

Mean (±SD) tamoxifen and *N*-desmethyltamoxifen concentrations were, respectively, 141 ± 50 and 226 ± 77 ng/ml at baseline, 36 ± 19 and 99 ± 46 ng/ml at 2 weeks, 20 ± 15 and 61 ± 37 ng/ml at 4 weeks, and 12 ± 9 and 36 ± 26 ng/ml at 6 weeks. The serum elimination of tamoxifen after 5 years of treatment showed an exponential trend with a mean ± SE half-life of 9.2 ± 0.5 days (Fig. 1*A*). *N*-desmethyltamoxifen had a similar trend, with a mean ± SE half-life of 13.1 ± 0.7 days (Fig. 1*B*). BMI was associated positively with both baseline drug levels and half-lives, indicating that heavier women had higher baseline levels and a slower decay of tamoxifen and *N*-desmethyltamoxifen (data not shown, $P < 0.01$). Because *N*-desdymethyltamoxifen elimination was faster, we could not fit our models to these data as the measured value was 0 in 39 and 58% of the women at 4 and 6 weeks, respectively. No

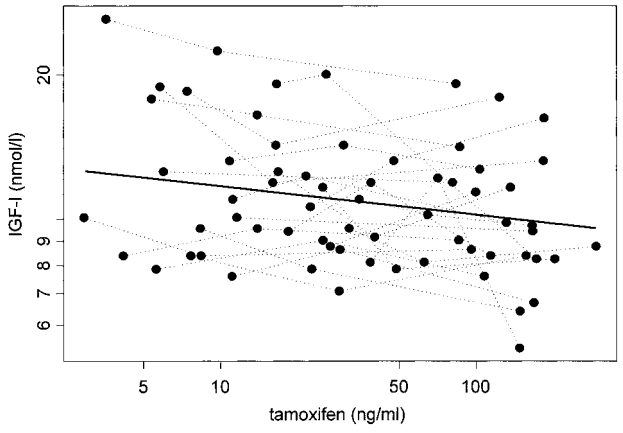


*Fig. 2.* IGF-I concentration *versus* tamoxifen concentration. ———, the values predicted by the fixed part of the mixed model; ····, the different concentration of the same subject during time.

tamoxifen or metabolite level was detected in the 6 subjects allocated to placebo.

At 4 weeks, the mean percentage increase in total cholesterol level was 6.4% (95% confidence interval, 2.9–9.8%) from a mean baseline value of 202 ± 28 mg/dl.

After drug cessation, total cholesterol, LDL-C, and IGF-I levels increased over time by an average weekly change ± SE of 1.3 ± 0.3%, 2.1 ± 0.6%, and 3.4 ± 1.0%, respectively ($P < 0.01$). For total cholesterol, LDL-C, and IGF-I, the estimated change within each subject was, respectively, 2.6 ± 0.6%, 4.3 ± 1.3%, and 6.8 ± 2.1% at 2 weeks; 5.3 ± 1.3%, 8.8 ± 2.6%, and 14.1 ± 4.6% at 4 weeks; and 8.1 ± 1.9%, 13.4 ± 4.1%, and 21.9 ± 7.3% at 6 weeks. No significant change over time was observed in the remaining biomarkers in the tamoxifen group, nor in any biomarker in the placebo group.

Within each subject, there was an association between the biomarker level and serum tamoxifen concentration. Fig. 2 illustrates the association between tamoxifen concentration and IGF-I. Compared with steady-state levels, a 50% lower concentration of tamoxifen was associated with a mean ± SE increase of 1.8 ± 0.4%, 3 ± 0.8%, and 4.1 ± 1.4% in total cholesterol, LDL-C, and IGF-I, respectively.

## Discussion

Several studies have shown that the endometrial effect of tamoxifen is associated with treatment duration, cumulative dose (2–5), and possibly daily dose (3).

On the other hand, recent data support the notion that the dose of tamoxifen may be lowered without affecting its activity. We have recently found a comparable modulation on circulating biomarkers by 10 mg every other day compared with 20 mg/day of tamoxifen (12). Because tamoxifen has a very high tissue per serum concentration (6, 13), the tissue level attainable with 10 mg every other day exceeds the growth inhibitory concentration of tamoxifen and *N*-desmethyltamoxifen in breast cancer cell lines, which is ~35 ng/ml (14–16). Moreover, *in vivo* studies in a spontaneous rat mammary tumor model showed that a dose equivalent to 1 mg/day in humans leads to a 94% inhibition of mammary tumor formation compared with control animals (17). Finally, a cross-sectional study has recently shown that 10 mg/day of tamoxifen can reduce the incidence of hip fracture among breast cancer women compared with nonusers and women taking 20 mg/day (18).

In the present study, we adopted a reversed and complementary approach of our previous experiment, namely, the study of the correlation of tamoxifen elimination with biomarker recovery. Our data indicate that 6 weeks after 5-year tamoxifen treatment, the biomarker recovery is far from complete despite tamoxifen concentrations in the range of 10 ng/ml, *i.e.*, ~15 times lower than the concentrations attained with 20 mg/day (7). Although the limited observation time and the specific drug pharmacokinetics prevented us from inferring the minimal active concentration, our data seem to suggest that biomarker recovery is slower than tamoxifen elimination from blood. This is in agreement with the observation that tamoxifen is retained in tissues for a long time (13) and suggests that tamoxifen may exert some kind of biological activity for weeks after treatment interruption.

However, our period of observation was too short to assess precisely the relationship between tamoxifen and metabolite disappearance and full biomarker recovery. Moreover, the kinetics of these biomarker changes are complex. Whereas no apparent change in weight or physical exercise occurred during the 6-week study period, we could not take into account for daily variations in food intake, which are known to affect some of these biomarkers as well. Taken together, these considerations underline the limitation of our study design in extrapolating inferences on the biological effect of lower tamoxifen concentrations.

The study also enabled us to assess for the first time tamoxifen half-life in healthy postmenopausal subjects after a prolonged treatment period. Consistent with breast cancer patients treated short term (19) or for longer periods (20), tamoxifen and *N*-desmethyltamoxifen serum concentrations were halved after 9 and 13 days, respectively. These findings underscore the importance of assessing the most appropriate schedule for preventive agents based on their pharmacokinetic characteristics, *e.g.*, antimalarial agents with prolonged elimination half-life such as mefloquine are generally administered on a weekly basis (21). Considering that the Cmax of a single dose of 20 mg of tamoxifen is ~25–30 ng/ml (22) and assuming linear pharmacokinetics, we anticipate that a weekly administration of 20 mg of tamoxifen leads to serum concentrations ranging from 15–30 ng/ml, a level which may still be active. However, our results suggest a considerable recovery of IGF-I after 4 weeks of drug cessation. A weekly administration of 20 mg of tamoxifen may indeed result in substantially lower tissue levels of tamoxifen and metabolites, which might lead to less favorable effects on IGF-I and other biomarkers. Because the impact of lesser IGF-I reductions on breast cancer risk is unclear, additional studies addressing this important issue are clearly warranted.

We observed a direct relationship between BMI and both baseline drug and half-life levels. This is not surprising because tamoxifen is a highly lipophilic agent which is stored and released by the fat tissue (13). Previous observations on the relationship between body weight and tamoxifen levels are still controversial (23), whereas it has already been shown that postmenopausal women exhibit higher tamoxifen concentrations than premenopausal women treated with the same dose (24, 25). Whereas the observed relationship may be explained by the decreased inhibition of cytochrome P450 activity after the drop of endogenous estrogens, our data support the notion that heavier women have higher tamoxifen levels and might consequently be at increased risk for adverse events. Indeed, our results are consistent with the data of Bernstein *et al.* (26) who showed that previous estrogen replacement therapy use

and BMI increase the risk of endometrial cancer associated with tamoxifen use in breast cancer patients.

In conclusion, our results suggest that biomarker recovery is slower than tamoxifen elimination, additionally upholding the notion that low tamoxifen concentrations may exert a biological effect. In addition, the prolonged half-life of tamoxifen and *N*-desmethyltamoxifen provides the rationale of a weekly administration of the drug in a preventive context. However, the clinical implications of our findings are yet to be defined.

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