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Evidence Why Paroxetine Dose Escalation is Not Effective in Major Depressive Disorder: A Randomized Controlled Trial With Assessment of Serotonin Transporter Occupancy

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Dose escalation is often used in depressed patients who fail to respond to standard doses of selective serotonin reuptake inhibitors, but clinical efficacy is equivocal. We aimed to reassess the efficacy of paroxetine dose escalation and quantify whether paroxetine dose escalation increases occupancy of the serotonin transporter (SERT) more than placebo dose escalation in a randomized controlled trial. We recruited 107 nonpsychotic, unipolar depressed outpatients (18–70 years; Hamilton Depression Rating Scale ($HDRS_{17}$) >18) from primary care and psychiatric outpatient departments. After 6 weeks, open-label paroxetine 20 mg per day (T0), nonresponding patients ($HDRS_{17}$ decrease $<50\%$; $n=60$) were randomized to double-blind paroxetine (30–50 mg per day as tolerable) or placebo dose escalation (paroxetine 20 mg per day + placebo). Patients were followed until 6 weeks after randomization (T1). Forty-nine patients, drug free at study entry, underwent single-photon emission-computed tomography (SPECT) scanning before treatment and were scanned repeatedly at T0 and T1. Paroxetine serum concentrations and SERT occupancy were determined at T0 and T1 ($n=32$). We terminated the dose-escalation trial after an interim analysis. Thirty nonresponding patients were randomized to paroxetine (46.7 ± 5.5 mg per day), 27 to placebo dose escalation. Response rates were 10/30 (33.3%) and 10/27 (37.0%), respectively. Repeated measurement analyses showed no significant effect for treatment ($p=0.88$, exceeding *a priori* stopping rules for futility ($p>0.5$)). Overall dropout was higher for placebo (26.7%) than paroxetine (3.3%; $p=0.03$). Paroxetine dose escalation increased paroxetine serum concentrations ($p<0.001$). SPECT measurements (12 patients randomized to paroxetine (46.9 ± 4.8 mg) and 14 to placebo dose escalation) showed no significant increase of midbrain SERT occupancy ($2.5 \pm 26.4\%$, paroxetine; $3.1 \pm 25.8\%$ placebo; $p=0.687$) nor in diencephalon ($p=0.529$). Paroxetine dose escalation in depressed patients has no clinical benefit over placebo dose escalation. This is explained by the absence of significant increases of SERT occupancy by paroxetine dose escalation, despite increased paroxetine serum concentrations (ISRCTN44111488).

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INTRODUCTION

Major depressive disorder (MDD) is often treated with antidepressants, particularly selective serotonin reuptake inhibitors (SSRIs). Unfortunately, response and remission rates are modest (30–50%), which require additional strategies to gain remission (Kennedy *et al*, 2001; American Psychiatric Association, 2000). Switching (Rush *et al*, 2006) and augmentation (Trivedi *et al*, 2006a) have recently been evaluated. A third, and frequently applied option is dose escalation, recommended in treatment guidelines (Kennedy *et al*, 2001; American Psychiatric Association, 2000) and frequently used preceding other strategies. Only the recent NICE guideline is more reluctant in recommending dose escalation (NICE, 2004). Although an individual patient may improve after dose escalation, this could also represent a delayed drug response or reflect the natural course of the

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disease. Prolonged (up to 10 weeks), unaltered treatment with fluoxetine 20 mg per day improved the response rates of initial week 6 nonresponders (Quitkin *et al*, 2003). Theoretically, the concept of dose escalation assumes linear dose–response relationships which have not been proven for SSRIs (Adli *et al*, 2005; Dunner and Dunbar, 1992). Therefore, the efficacy of dose escalation of SSRIs has been questioned (Ruhe *et al*, 2006; Adli *et al*, 2005; NICE, 2004; Baker *et al*, 2003).

Previous studies did not show improved clinical effectiveness of dose escalation, but had serious methodological weaknesses (Ruhe *et al*, 2006; Adli *et al*, 2005; Baker *et al*, 2003; Fava *et al*, 1994, 2002; Licht and Qvitzau, 2002; Schweizer *et al*, 1990, 2001; Benkert *et al*, 1997; Dornseif *et al*, 1989). All previous studies increased dosages probably too early (mostly after 3–4 weeks) and too abruptly, which may have obscured true dose-escalation effects by delayed effect of the standard doses and selective early dropout of patients receiving true dose escalation (Ruhe *et al*, 2006; Baker *et al*, 2003). Moreover, no study provided a rationale why dose escalation was ineffective.

The primary molecular target of SSRIs is the serotonin transporter (SERT). Imaging techniques such as positron emission tomography (PET) and single-photon emission-computed tomography (SPECT) allow *in vivo* labeling of SERT in the brain, which can be used to study their occupancy. Till date, several imaging studies measured SERT occupancy after short or prolonged treatment with SSRIs (Klein *et al*, 2006, 2007; Voineskos *et al*, 2007; Parsey *et al*, 2006; Takano *et al*, 2006; Catafau *et al*, 2006; Herold *et al*, 2006; Cavanagh *et al*, 2006; Erlandsson *et al*, 2005; Suhara *et al*, 2003; Kent *et al*, 2002; Meyer *et al*, 2001b, 2004; Hiltunen *et al*, 1998). Particularly, Meyer *et al* (2004) showed 60–80% SERT occupancy after standard clinical doses of SSRIs, and demonstrated curvilinear dose–response relationships for SERT occupancy by SSRIs. However, high doses of SSRIs were rarely studied (Voineskos *et al*, 2007), and dose escalation was never studied.

Taking into account previous methodological criticisms and considering the molecular target of SSRIs, we have tested whether paroxetine dose escalation increases SERT occupancy and improves depressive symptoms more than placebo dose escalation. We performed a 6-week, multicenter, randomized study in depressed patients not responding to 6 weeks of paroxetine at 20 mg per day. As a novel extension to previous clinical trials, and in order to elucidate the neurobiological basis for an expected lack of benefit of dose escalation, we included a SPECT-imaging approach. Herewith, we quantified whether paroxetine dose escalation increased SERT occupancy more than placebo dose escalation. This enabled us to relate clinical findings to the neurobiological correlate of SERT occupancy.

MATERIALS AND METHODS

Participants

Following approval by the institutional ethical committee and written informed consent, we recruited outpatients (18–70 years) from primary care, our outpatient department, and public psychiatric settings between October 2003 and February 2007. Inclusion criteria were: MDD determined by the structured clinical interview for DSM-IV (First *et al*, 1999), and a Hamilton Depression Rating Scale (17 items; $HDRS_{17}$; Hamilton, 1960) score above 18. All participants were drug free or had undergone no more than one antidepressant treatment (other than paroxetine) at an effective dose for $\geqslant 6$ weeks for the present MDD-episode. By the latter criterion, we avoided treatment resistance as potential bias for inefficacy of dose escalation. Exclusion criteria, apart from pregnancy (or wish), were bipolar disorder, psychotic features, neurological cognitive impairments (ie dementia), primary anxiety and/or substance abuse disorders and acute, severe suicidal ideation. Contrary, we allowed secondary comorbid anxiety and/or substance abuse to increase applicability of our findings.

Interventions

Patients were treated by their referring physician or were referred to our outpatient department. After assessment at study entry, all patients were treated open label with paroxetine 20 mg per day for 6 weeks (Figure 1), because paroxetine is the most prescribed SSRI in the Netherlands. When severe adverse effects occurred, dosages were reduced to 10 mg per day and again increased to 20 mg per day after 1 week. We randomized all patients who did not achieve $\geqslant 50\%$ decrease in $HDRS_{17}$ score after 6 weeks, relative to study entry. They received a true paroxetine or a placebo dose escalation *added to* paroxetine 20 mg per day. Dose escalation was provided in blue capsules containing 10 mg paroxetine or placebo. Randomization was stratified for treatment setting (SPECT group, outpatient department AMC, primary care, public psychiatry), gender, and age. Within strata, we applied a minimization method to achieve a balanced distribution. We concealed allocation by using an independently operated computer program.

Dose escalation consisted of incremental steps of one capsule every 5 days toward a maximum of 50 mg per day (20 mg + 3 capsules). Patients were allowed to increase at a slower pace (eg by 7 days) or stop further escalation (eg 20 mg + 2 capsules) according to adverse effects (Baker and Woods, 2003). No dosage adjustments were allowed during the last 3 weeks of the study. We checked adherence by pill counts and anamnesis (Saunders *et al*, 1998).

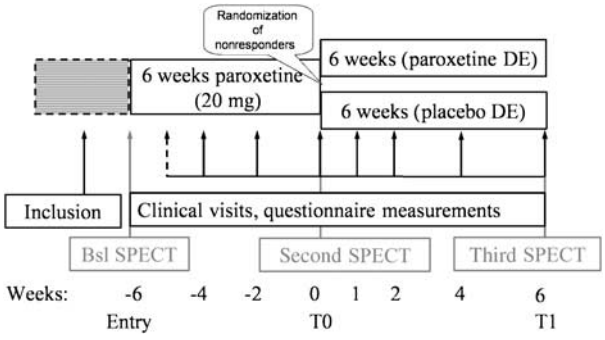

Figure 1 Design of the study; Bsl SPECT, baseline scan; DE, dose escalation.

Outcomes and Measurements

Primary clinical outcomes were $HDRS_{17}$ scores, and the proportion of patients achieving response ($\geqslant 50\%$ decrease in $HDRS_{17}$) or remission ($HDRS_{17} \leqslant 7$). Secondary outcomes were total and specific (adverse effects/inefficacy) dropout rates, the Maier and Bech 6 item subscales of the $HDRS_{17}$ (Ruhe *et al*, 2005; Faries *et al*, 2000), the Inventory for Depressive Symptomatology self-rated ($IDS\text{-}SR_{30}$; Rush *et al*, 1996) scores, the occurrence of adverse effects, and health-related quality of life (MOS-SF36; physical and mental component scales standardized to a general Dutch population; Aaronson *et al*, 1998).

We administered questionnaires at study entry, randomization (T0), and 6 weeks after randomization (T1). Depressive symptoms were also monitored at week 1, 2, and 4 using the Maier and Bech subscales and $IDS\text{-}SR_{30}$ (Figure 1). Three trained investigators administered clinician-rated questionnaires. Agreement between raters was good (intraclass correlation coefficient = 0.98). Raters and patients were blinded for treatment.

Subgroup for SPECT Imaging

From all patients who entered the trial, we recruited patients who were drug free (>4 weeks and $\geqslant 5$ half-lives of a previous antidepressant) as potential candidates for SPECT imaging. These patients were asked to participate in the SPECT substudy if their age was between 25 and 55 years to reduce variability in SERT measurements by age (van Dyck *et al*, 2000). A total of 49 patients could thus be recruited for a first SPECT scan. None of these patients reported past or present use of 3,4-methylenedioxymethamphetamine. We made a second scan in those patients who completed 6 weeks of paroxetine treatment ($n = 44$, including 12 responders), whereas only randomized nonresponders ($n = 32$) were invited for a third scan at the end of the study. We treated SPECT patients at the AMC outpatient department. Medication was supplied in pillboxes.

SPECT Imaging and Analysis

We performed SPECT imaging at study entry (baseline scan), T0 and T1 (Figure 1) between 14:00 and 22:00 hours, according to previously described procedures (de Win *et al*, 2005). We made all scans 230 ± 18 (SD) minutes after intravenous injection of approximately 100 MBq iodine-123-labeled 2β-carbomethoxy-3β-(4-iodophenyl)-tropane ([^{123}I]β-CIT), when the radioligand is at equilibrium for SERT binding in brain areas expressing high densities of SERTs (Pirker *et al*, 2000). To prevent thyroid uptake of ^{123}I, all subjects received oral potassium–iodide solution. We performed SPECT imaging using a 12-detector single-slice brain-dedicated scanner (Neurofocus 810, Strichmann Medical Equipment; Cleveland, OH) with a full-width at half-maximum resolution of 6.5 mm, throughout the 20 cm field-of-view (http://www.neurophysics.com). Blood for paroxetine serum concentrations (PSC) was collected at T0 and T1 immediately before scanning. Serum was stored at -20°C until analysis. PSC were determined in May 2007 using a validated HPLC-MS/MS method (therapeutic range 10–75 μg/l; Supplementary Appendix). The lower limit of quantification was 5 μg/l, the lower limit of detection was 0.3 μg/l.

After attenuation correction and reconstruction in 3D mode (http://www.neurophysics.com), we defined regions of interests (ROIs) for midbrain, diencephalon, and cerebellum by using validated templates (Supplementary Figure S1) (de Win *et al*, 2005). One examiner, blinded for scan session (baseline scan/T0/T1), positioned all ROIs in two series. Intraclass correlation coefficients were >0.97 for all ROIs. If the two series differed by >5%, scans were re-evaluated by a second investigator. In the analyses we averaged the counts for the two series.

Using activity in cerebellum as indicator of nondisplaceable activity (nonspecific binding and free radioactivity) (Laruelle *et al*, 1988), we calculated specific to nonspecific binding ratios per scan as $BP_{ND} = (Activity_{ROI} - Activity_{CER})/Activity_{CER}$. BP_{ND} is proportional to transporter number under equilibrium conditions (Innis *et al*, 2007). In a different study, we found high reproducibility of SERT imaging with [^{123}I]β-CIT SPECT after repeated scanning of subjects, using the same camera and scanning protocol (de Win *et al*, submitted for publication). As primary outcomes, we calculated SERT occupancies at T0 or T1 relative to untreated baseline scan SERT availability: $OCC_{T0\,or\,T1} = (BP_{ND\,Bsl} - BP_{ND\,T0\,or\,T1})/BP_{ND\,Bsl}$.

Power and Interim Analysis

We performed *a priori* power calculations for two coprimary end points: (a) to detect a difference of $\geqslant 5$ points in $HDRS_{17}$ scores between paroxetine and placebo dose escalation, although assuming a common standard deviation of 7 and using a one-tailed $\alpha = 0.025$ and $\beta = 0.05$, sample sizes of 60 per group were required; (b) for response rates (assumed to be 50% and 30% for paroxetine *vs* placebo dose escalation) a two-tailed $\alpha = 0.05$ and $\beta = 0.20$ required 110 participants per group. Because previous dose escalation studies indicated no benefits relative to placebo dose escalation, we planned an interim analysis after SPECT data for had been collected on at least 30 randomized patients in the SPECT subgroup. Stopping criteria, using the most informative continuous scores in a mixed model, were predetermined using the O'Brien and Fleming (1979) approach, were undisclosed although performing the interim analysis, and were $p < 0.0026$ in case of superiority and $p > 0.50$ for futility (see Supplementary Appendix).

Data Analyses

Analyses were performed although blinded for treatment allocation. We based end point analyses on intention to treat (ITT), with last observation carried forward. To examine the effectiveness of paroxetine *vs* placebo dose escalation, we compared the proportion of patients with response, remission, and dropouts at the end of study using χ^2 or Fisher's exact test. We examined differences in mean continuous end points by ANCOVA with treatment as factor and value at randomization (T0) as covariate.

We used linear mixed models to assess differences in trends over time between groups in Maier, Bech, and $IDS\text{-}SR_{30}$ scores. Mean scores for these questionnaires were

modeled as a function of the randomized group (paroxetine *vs* placebo dose escalation), score at randomization, and time since randomization (categorical, four levels). The interaction between time × group was added to the model to test whether trends over time were different between the two treatment groups. We used the Akaike Information Criteria to choose the best fitting variance/covariance structure (unstructured, compound symmetry or first-order autoregressive) for each outcome parameter.

To examine changes in SERT occupancy between T0 and T1, we used ANCOVAs with treatment as factor, and SERT occupancy at T0 and age as covariates. In order to obtain maximum information of dose escalation in these analyses, we excluded patients that were likely nonadherent to paroxetine at T0 or T1 (PSC $<5\,\mu g/l$). Thereafter, we plotted SERT occupancy against dose and PSC. We modeled dose–response in an E_{max} model as $OCC = a \times PSC/(b + PSC)$, in which a represents maximal SERT occupancy and b the PSC with 50% SERT occupancy (Parsey *et al*, 2006; Takano *et al*, 2006; Catafau *et al*, 2006; Meyer *et al*, 2004; Suhara *et al*, 2003; Kent *et al*, 2002). We calculated a and b by fitting a nonlinear regression model that minimizes the sum of squares of the residuals. For quantification of differences in SERT occupancy between final responders and nonresponders, we used ANCOVA models corrected for differences at T0, age, and baseline scan SERT availability in diencephalon (Kugaya *et al*, 2004). We performed all analyses in SPSS v15.0.1.1 (www.spss.com).

RESULTS

Patient Disposition

A total of 107 patients (mean age 43.8 ± 9.8) started open-label paroxetine (Figure 2). The response rate in the open phase was 27/107 (25.2%), and 60 nonresponding patients were randomized for the double-blind phase. Randomization over the two treatment arms resulted in comparable groups (Table 1). A total of 51 patients completed the 6 week randomization phase including 31 from the SPECT study. We obtained at least 1 after randomization $HDRS_{17}$ score for 57 patients.

$HDRS_{17}$ scores at study entry (−6 weeks) were comparable in T0 responders *vs* nonresponders (ANOVA, $F_{1,85} = 1.972$, $p = 0.164$). At T0, $HDRS_{17}$ scores (± SD) were 7.8 ± 3.62 (66.6 ± 14.3% decrease) in responders *vs* 20.5 ± 6.25 (17.7 ± 20.3% decrease) in nonresponders.

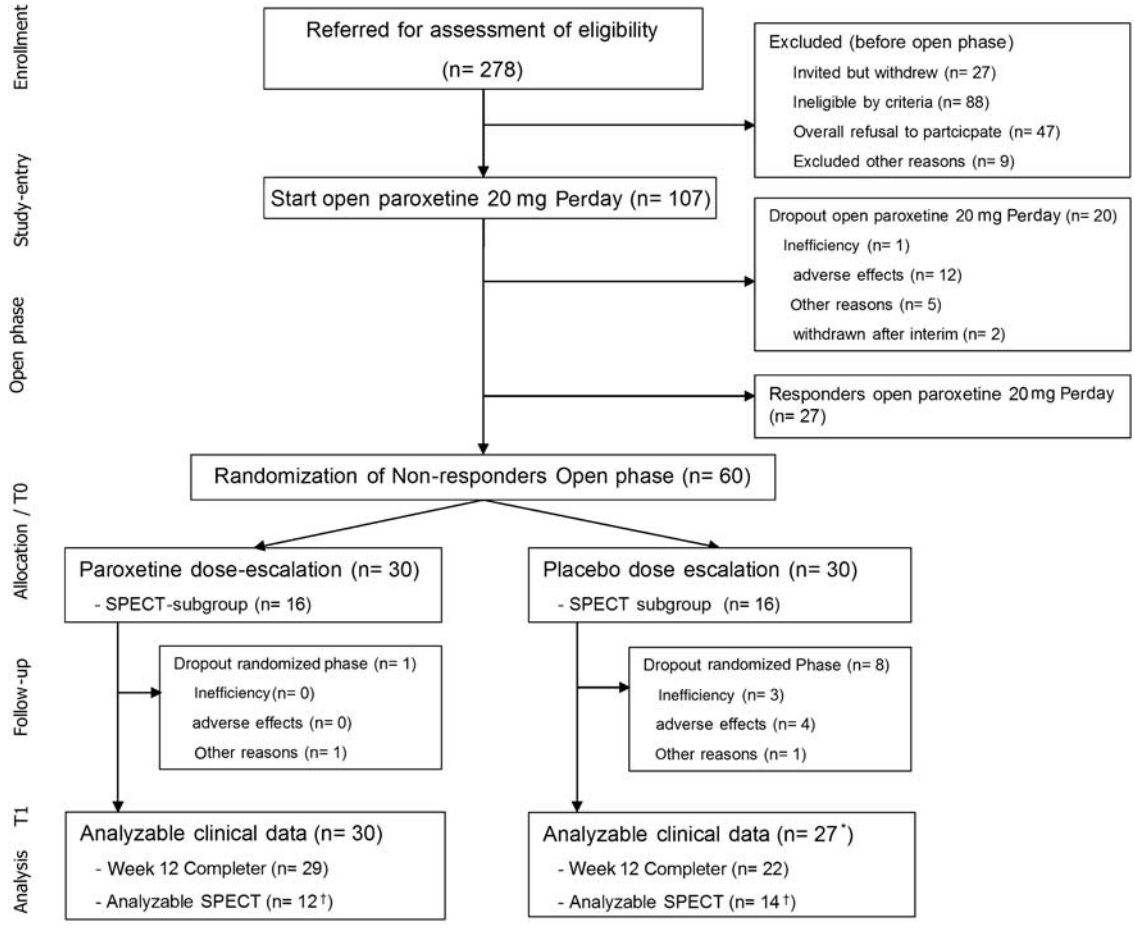

Figure 2 Recruitment and flow of participants. *Three patients who refused dose escalation after randomization, never ingested study drugs, and refused further questionnaires were excluded for end point analysis. †One single-photon emission-computed tomography (SPECT) patient dropped out early due to inefficacy, but for all SPECT patients' clinical data could be obtained. For SPECT analyses, six patients were excluded: one patient missed the T1 scan (placebo dose escalation), three patients were likely nonadherent at T0 (paroxetine serum concentration $<5\,\mu g/l$; all paroxetine dose escalation), and two were likely nonadherent at T1 (one paroxetine dose escalation, one placebo dose escalation).

Table 1 Characteristics of Nonresponding MDD Patients after 6 Weeks of Open Treatment With Paroxetine 20 mg per day (Trial Population)

	All patients ($n=60$)		SPECT subgroup ($n=32$)	
	Paroxetine DE ($n=30$)	**Placebo DE ($n=30$)**	**Paroxetine DE ($n=16$)**	**Placebo DE ($n=16$)**
Age at baseline (years)	41.9 ± 9.1	42.9 ± 10.3	42.5 ± 7.7	40.4 ± 7.7
Female sex, *n* (%)	21 (70.0)	19 (63.3)	11 (68.8)	10 (62.5)
Marital status, n (%)				
Single (never married)	15 (51.7)	12 (40.0)	6 (37.5)	5 (31.3)
Married	8 (27.6)	6 (20.0)	7 (43.8)	4 (25.0)
Divorced	4 (13.8)	11 (36.7)	2 (12.5)	7 (43.8)
Widowed	2 (6.9)	1 (3.3)	1 (6.3)	0
Educational level, n (%)				
Low	6 (20.0)	11 (36.7)	2 (12.5)	3 (18.8)
Intermediate	19 (63.3)	15 (50.0)	10 (62.5)	10 (62.5)
High	5 (16.7)	4 (13.3)	4 (25.0)	3 (18.8)
Unemployed, *n* (%)	6 (20.0)	11 (36.7)	4 (25.0)	4 (25.0)
Income /month (median; 25 and 75 quartiles)	1485 (875–1769)	1197 (767–1820)	1177 (715–1530)	1185 (610–2277)
Current smoking, *n* (%)	13 (43.3)	16 (53.3)	6 (37.5)	11 (68.8)
Alcohol use, n (%)				
⩽2 units per week	20 (66.7)	21 (70.0)	10 (62.5)	11 (68.8)
3–7 units per week	6 (20.0)	7 (23.3)	4 (25.0)	5 (31.3)
8–21 units per week	2 (6.7)	1 (3.3)	1 (6.3)	0
>22 units per week	2 (6.7)	1 (3.3)	1 (6.3)	0
Race, n (%)				
Caucasian	17 (56.7)	19 (63.3)	9 (56.2)	13 (81.2)
Creole	4 (13.3)	7 (23.3)	2 (12.5)	3 (18.8)
Asian	6 (20.0)	1 (3.3)	3 (18.8)	0
Other	3 (10.0)	3 (10.0)	2 (12.5)	0
MDD				
$HDRS_{17}$ at study entry (−6 weeks)	24.5 ± 4.7	25.5 ± 5.0	25.6 ± 5.0	24.4 ± 4.6
$HDRS_{17}$ (T0)[a]	20.1 ± 6.6	21.0 ± 5.9	21.3 ± 7.4	19.3 ± 5.1
Maier at (T0)[a]	10.0 ± 3.0	10.5 ± 3.1	10.4 ± 3.6	10.1 ± 2.9
Bech (T0)[a]	10.5 ± 2.9	11.3 ± 3.0	11.0 ± 3.4	10.8 ± 3.1
$IDS\text{-}SR_{30}$ (T0)[a]	38.1 ± 12.3	40.8 ± 12.2	40.3 ± 14.6	39.9 ± 11.8
First episode, *n* (%)	17 (56.7)	21 (70.0)	9 (56.3)	9 (56.3)
No. of episodes	1.6 ± 0.8	1.7 ± 1.8	1.6 ± 0.8	2.3 ± 2.4
Drug naive, *n* (%)	24 (80.0)	19 (63.3)	14 (87.5)[c]	8 (50.0)[b]
Used AD in current episode, *n* (%)	3 (10.0)	5 (16.7)	1 (6.3)	2 (12.5)
Melancholic, *n* (%)	23 (76.7)	19 (63.3)	12 (75.0)	12 (75.0)
Duration of episode, n (%)				
<5 months	9 (30.0)	5 (16.7)	7 (43.8)	3 (18.8)
Duration 5 months to 2 years	19 (63.3)	22 (73.3)	7 (43.8)	11 (68.8)
Duration >2 years	2 (6.7)	3 (10.0)	2 (12.5)	2 (12.5)
Age of first episode (years)	37.1 ± 9.1	38.1 ± 11.8	38.4 ± 8.8	34.4 ± 10.2

Table 1 Continued

	All patients ($n=60$)		SPECT subgroup ($n=32$)	
	Paroxetine DE ($n=30$)	**Placebo DE ($n=30$)**	**Paroxetine DE ($n=16$)**	**Placebo DE ($n=16$)**
Comorbidity, n (%)				
Anxiety disorder	5 (16.7)	7 (23.3)	0	4 (25.0)
Dysthymia	2 (6.7)	0	1 (6.3)	0
Drug (alcohol, cannabis, benzodiazepines) abuse/dependence	2 (6.7)	1 (3.3)	1 (6.3)	0
MOS-SF36				
Physical[a]	41.2 ± 9.0	40.3 ± 10.7	41.2 ± 8.6	43.9 ± 9.9
Mental[a]	26.1 ± 8.4	25.7 ± 10.2	25.3 ± 5.9	22.3 ± 6.7
SERT availability baseline scan (BP_{ND})				
Midbrain	N/A	N/A	0.553 ± 0.119	0.657 ± 0.217
Diencephalon	N/A	N/A	1.157 ± 0.226	1.134 ± 0.247
SERT occupancy (% of BP_{ND} in Bsl-scan)[a,c]				
Midbrain	N/A	N/A	73.7 ± 17.1	82.2 ± 18.5
Diencephalon	N/A	N/A	63.8 ± 15.4	70.3 ± 12.1

BP_{ND}, binding potential (non-displaceable); DE, dose escalation; MDD, major depressive disorder; PSC, paroxetine serum concentration.
Numbers represent means (± SD) unless specified otherwise.
[a]At randomization (T0).
[b]Significant difference between SPECT patients randomized to conditions (Fisher's exact test, $p=0.026$).
[c]$n=29$, excluding three patients who were likely nonadherent at T0 (PSC <5 μg/l).

Clinical Effectiveness of Paroxetine *vs* Placebo Dose Escalation

During dose escalation (T0–T1), 1, 8, and 21 patients reached final doses of 30, 40, and 50 mg per day, respectively. The placebo group escalated to a comparable number of capsules ($\chi^2=0.895$, df = 2, $p=0.639$). Adherence based on pill counts was comparable between both groups (Fisher's exact, $p=0.492$).

Paroxetine dose escalation did not yield better outcomes in depression severity and health-related quality of life compared with placebo dose escalation (ITT; Table 2). The robustness of this finding was confirmed in the longitudinal analysis (mixed model). Changes over time in the Maier subscale and IDS-SR_{30} scores (Figure 3), and Bech subscale scores (available on request) were comparable between the two groups. Overall dropout was higher with placebo (26.7%) than with paroxetine dose escalation (3.3%; $p=0.03$). Paroxetine dose escalation had significantly more adverse effects than placebo dose escalation, but this did not result in higher discontinuation rates due to adverse effects (Supplementary Table S1). Instead, adverse effects by paroxetine dose escalation moderately decreased over time, suggestive of habituation.

SERT Occupancy and Clinical Response

Out of 32 randomized patients in the SPECT subgroup, only 3 (9%) previously used mirtazapine or fluoxetine in the current episode (1 patient stopped mirtazapine 4 weeks before scanning, others stopped <2 months before scanning). One patient missed the T1 scan. On the basis of PSC at randomization or T1, five patients in the SPECT subgroup (four with paroxetine and one with placebo dose escalation; Fisher's exact test, $p=0.172$; see Figure 2) with PSC <5 μg/l were considered nonadherent, despite adherence according to pill counts. We excluded these five patients for analyses of changes in SERT occupancy after dose escalation, leaving 26 T1 scans analyzable for diencephalon occupancies after true or placebo dose escalation. Paroxetine dose escalation increased mean PSC from 36.2 to 154.3 μg/l (paired *t*-test, $p<0.001$), whereas mean PSC in placebo dose escalation remained unchanged (Supplementary Table S2). At randomization (T0), mean SERT occupancies (± SEM) in the paroxetine dose escalation group were 76.2 ± 4.70% in midbrain and 64.3 ± 4.60% in diencephalon. For the placebo dose escalation group these were 84.6 ± 4.95 and 72.2 ± 3.08%, respectively. Neither paroxetine nor placebo dose escalation significantly increased SERT occupancy further (Figure 4a). Plotting PSC *vs* SERT occupancy showed that PSCs >50 μg/l were not associated with further increases of SERT occupancy in midbrain or diencephalon (Figure 4b and c). Furthermore, individual changes in PSC (T0 to T1) were not significantly associated with changes in occupancy in midbrain ($F_{1,24}=0.101$, $p=0.754$) and diencephalon ($F_{1,27}=1.332$, $p=0.259$; Supplementary Figure S2).

We explored whether SERT occupancy was related to clinical response at T1 irrespective of paroxetine or placebo dose escalation. Responders at T1 ($n=12$) had numerically higher SERT occupancy (± SEM) in midbrain (91.2 ± 5.8%) and diencephalon (69.2 ± 2.8%) than nonresponders ($n=14$; 77.8 ± 5.1 and 63.8 ± 2.6%, respectively; ANCOVA: $p=0.107$ and 0.178). These models used baseline scan

Table 2 Depression and Health-Related Quality of Life Scores After 6 Weeks Paroxetine *vs* Placebo Dose Escalation (T1); all Patients and SPECT Subgroup

	All patients ($n=57$)			SPECT subgroup ($n=32$)		
	Paroxetine DE ($n=30$)	Placebo DE ($n=27$)	p	Paroxetine DE ($n=16$)	Placebo DE ($n=16$)	p
Mean dosage (mg per day)	46.7 ± 1.00	NA	—	46.9 ± 1.20	NA	—
$HDRS_{17}$	16.1 ± 1.22	15.3 ± 1.28	0.650	15.8 ± 1.39	14.5 ± 1.39	0.519
Maier subscale	7.5 ± 0.61	7.5 ± 0.64	1.000	7.6 ± 0.69	7.4 ± 0.69	0.868
Bech subscale	8.1 ± 0.63	8.1 ± 0.66	0.996	8.2 ± 0.75	8.0 ± 0.75	0.832
Response[a], *n* (%)	10 (33.3)	10 (37.0)	0.788	4 (25.0)	8 (50%)	0.273
Remission[b], *n* (%)	4 (13.3)	2 (7.4)	0.673	1 (6.3)	2 (12.5)	1.000
$IDS\text{-}SR_{30}$[c]	34.8 ± 1.83	32.5 ± 2.05	0.406	39.2 ± 2.38	32.0 ± 2.38	0.042
MOS-SF36[d]						
Physical	41.8 ± 0.93	42.4 ± 1.06	0.679	42.5 ± 1.33	44.1 ± 1.33	0.399
Mental	29.6 ± 1.37	27.3 ± 1.57	0.264	27.2 ± 1.78	25.9 ± 1.78	0.620

DE, dose escalation.
Scores at end point of the study are based on intention to treat, with last observation carried forward for early dropouts. Values are means ± SD, corrected for (mean) scores at randomization (T0) (ANCOVA).
[a]$\geqslant$50% decrease in $HDRS_{17}$ with baseline score (−6 weeks) as reference; Fisher's exact test.
[b]$HDRS_{17} \leqslant 7$; Fisher's exact test.
[c]Owing to missing values: $n=29$, paroxetine DE and $n=23$, placebo DE for all patients.
[d]Owing to missing values: $n=29$, paroxetine DE and $n=22$, placebo DE for all patients.

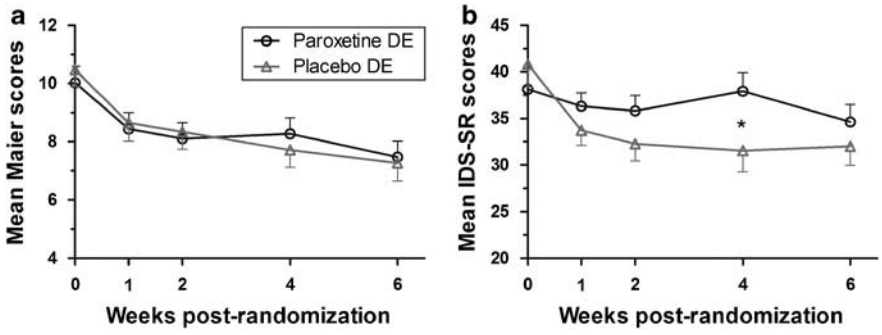

Figure 3 (a,b) Changes over time in Maier and inventory for depressive symptomatology self-rated (IDS-SR) scores after randomization. Points represent mean Maier (a) and IDS-SR (b) scores (± SEM) adjusted for scores at randomization (T0) for paroxetine ($n=30$) and placebo ($n=27$) dose escalation. Mixed model analysis (Maier: $n=57$, IDS-SR: $n=53$): overall difference between paroxetine *vs* placebo dose escalation for Maier scores $F_{4,101,400}=0.295$, $p=0.880$, and for IDS-SR $F_{4,45,519}=1.516$, $p=0.213$. *Mean IDS-SR score at week 4 differed significantly in favor of placebo dose escalation ($t_{49,161}=2.11$, $p=0.040$); DE, dose escalation.

diencephalon SERT availability as covariate as this accounted for a major part of variance ($F_{1,21}=4.831$, $p=0.039$ and $F_{1,22}=10.407$, $p=0.004$, respectively). Contrary, T1 SERT occupancy in midbrain or diencephalon did not significantly predict the percentage decrease in $HDRS_{17}$ in linear regression, or response status in logistic regression (neither when corrected for baseline scan SERT availability in diencephalon or age).

DISCUSSION

In this randomized trial, we examined clinical effectiveness of dose escalation in MDD patients, who were nonresponders to 6 weeks of 20 mg per day paroxetine, and explored potential underlying mechanisms. Despite markedly increased drug exposure, paroxetine dose escalation to 30–50 mg per day did not improve depressive symptoms more than placebo dose escalation, but was associated with more adverse effects. Concomitantly, increased PSCs were not associated with substantially greater SERT occupancy, indicating that standard paroxetine doses (20 mg per day) already resulted in maximum SERT occupancy.

Clinical Outcomes of Dose Escalation in Nonresponders

The dose–response relationship for paroxetine was previously examined in fixed dose, parallel group designs. A total of 20 mg per day and higher paroxetine doses yielded similar clinical improvements (Dunner and Dunbar, 1992). Similar findings were reported from parallel group, fixed dose studies of other SSRIs (Adli *et al*, 2005). Accordingly,

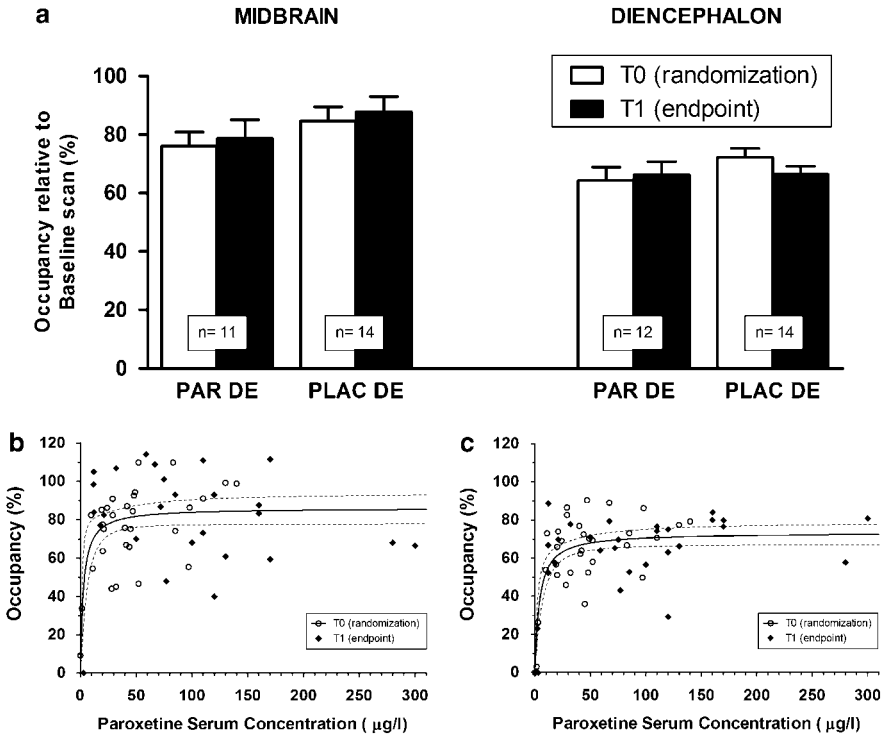

Figure 4 (a–c) Serotonin transporter (SERT) occupancy during randomized dose escalation of paroxetine. Changes over time and relation with paroxetine serum concentration. (a) Mean SERT occupancy (± SEM) for paroxetine dose escalation and placebo dose escalation at randomization (T0) and after 6 weeks of dose escalation (T1). SERT occupancy was calculated as percentage of initial available SERTs (expressed as BP_{ND}) at baseline (−6 weeks) scans (see text). Changes in SERT occupancy between T0 and T1 for paroxetine dose escalation and placebo dose escalation were nonsignificant in ANCOVA models correcting for age and differences in T0 SERT occupancy (midbrain: $F_{1,21} = 0.167$, $p = 0.687$; diencephalon: $F_{1,22} = 0.409$, $p = 0.529$). For one patient, insufficient midbrain was scanned at study entry to compute subsequent SERT occupancies. (b, c) Data for randomized patients used from both T0 (open circles) and T1 (diamonds). Dose—occupancy relationships are modeled as $OCC = a \times (PSC/(b + PSC))$. For midbrain (b): $a = 86.0 \pm 4.03$ (SE), $b = 2.65 \pm 1.39$ ($n = 30$ at T0 and $n = 27$ at T1); for Diencephalon (c) $a = 73.3 \pm 2.28$, $b = 3.97 \pm 1.07$ ($n = 32$ at T0 and $n = 29$ at T1). Dashed lines represent 95% confidence interval of fitting; DE, dose escalation; OCC, occupancy; PAR, paroxetine; PLAC, placebo; PSC, paroxetine serum concentration.

the usefulness of dose escalation in nonresponders to paroxetine and other SSRIs has been questioned (Ruhe *et al*, 2006; Adli *et al*, 2005; Baker *et al*, 2003). However, the underlying studies had methodological short comings, and dose escalation remains a recommended standard approach for nonresponders (Kennedy *et al*, 2001; American Psychiatric Association, 2000).

If dose escalation is applied too early (before week 6), randomization of 'late responders' will likely dilute the difference between true and placebo dose escalation, resulting in potentially false negative findings. Although one study reported randomized dose escalation of sertraline after 6 weeks of treatment, this was compromised by a nonrandomized dose increase 2 weeks before randomization (Licht and Qvitzau, 2002).

The benefits of dose escalation might be underestimated if actively treated patients dropout early due to adverse effects (Baker and Woods, 2003) or become more nonadherent. Our schedule for dose escalation did not increase dropout. Hypothetically, patients receiving a paroxetine dose escalation might have interpreted the increased level of adverse effects as subjective clue of greater drug effects, encouraging them to persevere in the trial. At first sight, a misbalance in adherence is suggested with four patients with paroxetine *vs* one patient with placebo dose escalation having low PSCs. However, this was not due to dose escalation. As mentioned in Figure 2, in three patients, low PSCs at T0 already classified them as likely nonadherent, with only one paroxetine *vs* one placebo dose escalation patient becoming likely nonadherent during dose escalation (T0–T1). Therefore, we think that neither adverse effects nor nonadherence account for the observed inefficacy of dose escalation.

Thus, the present study overcomes methodological limitations of previous SSRI dose escalation studies by using a randomized, placebo controlled, double-blind dose escalation in nonresponders to 6 weeks treatment with a standard dose of paroxetine. We also avoided treatment resistance as a factor for inefficacy of dose escalation by inclusion of patients who received no more than one effective antidepressant trial for the current episode. Under these conditions paroxetine was not superior to placebo in dose escalation. Moreover, our study was more inclusive than most previous ones and thus may be more applicable to 'real-world' first-line antidepressant treatment. This may also explain why we observed lower response and remission rates than previous studies.

Neurobiological Effects of Dose Escalation

Our pharmacokinetic and imaging measurements were designed to explore why paroxetine dose escalation would

or would not improve treatment outcomes. Our imaging of SERT occupancy bypasses potential bias by inclusion of patients with ultrarapid drug metabolism, which is often causally linked to nonresponse. Hypothetically, the clinical selection of nonresponders eligible for dose escalation might represent a selection of patients not reaching high levels of SERT occupancy.

Comparing SERT occupancies of different SSRI doses faces several methodological challenges. First, the assessment of occupancy requires knowledge on the available number of SERTs. Owing to interindividual differences in available SERTs, only assessments with individual drug-free baseline scans yield reliable data. Second, a given drug dose may yield a range of serum concentrations due to interpatient pharmacokinetic differences. Hence, associations based on serum concentrations are more reliable than those based on administered dose. Finally, intraindividual comparisons of occupancy changes following dose escalation are more powerful than those with historic data.

Against this background, Voineskos *et al* (2007) recently reported high SERT occupancies in striatum (~85%), thalamus (~79%), and midbrain (~98%) in 12 depressed patients exposed to >4 weeks of venlafaxine 225–450 mg per day, sertraline 150–200 mg per day, or citalopram 60–80 mg per day in a [^{11}C]DASB PET study. They concluded that high doses significantly increased occupancy compared with an average of 80% SERT occupancy determined in previous studies with standard SSRI doses (Meyer, 2007; Meyer *et al*, 2004), which would favor the concept of dose escalation. However, they did not determine occupancy relative to baseline scans of the same patients without medication, nor at standard doses. We performed drug-free study-entry scans, in addition, >90% of patients did not use antidepressants for the current episode of MDD. Furthermore, low therapeutic dosages of several SSRIs also yielded high SERT occupancy in most studies (Klein *et al*, 2006, 2007; Parsey *et al*, 2006; Takano *et al*, 2006; Catafau *et al*, 2006; Herold *et al*, 2006; Erlandsson *et al*, 2005; Suhara *et al*, 2003; Kugaya *et al*, 2003; Kent *et al*, 2002; Meyer *et al*, 2001b; Hiltunen *et al*, 1998). The present study resolves this controversy by showing that fourfold increases of PSC on paroxetine dose escalation did not significantly increase SERT occupancy. This offers an explanation for our findings: SERT occupancy is limited by a ceiling effect. A PSC achieved with a 20 mg per day paroxetine dose is sufficient to yield maximum SERT occupancy (Figure 4b and c). If low doses already yield maximum SERT occupancy, dose escalation cannot be expected to increase treatment efficacy, which is in line with our clinical findings. Our results do not necessarily challenge the relationships between dose, SERT occupancy, and clinical response but rather suggest that these relationships exist mainly at low and subtherapeutic doses. Furthermore, the relationship between SERT occupancy and response might be confounded by other factors such as SERT gene polymorphisms.

In a recent study, Owens *et al* (2008) showed increased SERT occupancy with increasing paroxetine CR doses (12.5–75 mg per day) in an *ex vivo* model using human transporter transfected cells, which might be at odds with our findings. However, validation of this *ex vivo* method (in cultured cells) with concomitant *in vivo* SPECT or PET SERT occupancy (the gold standard) is not yet available. In addition, Zitterl *et al* (2008) found a significant relation between SERT occupancy and clinical response in obsessive-compulsive disorder treated with clomipramine (150 mg per day), but did not study the effects of dose escalation in their study. Therefore, our study optimally quantifies the neurobiological effects of dose escalation of antidepressants in patients.

Critique of Methods

For logistic reasons, we used [^{123}I]β-CIT for SPECT imaging, which is a nonselective radioligand, and also binds to dopamine transporters (DAT; eg substantia nigra) and norepinephrine transporter (NET; eg locus coeruleus) (Neumeyer *et al*, 1991, 1996; Innis *et al*, 1991). Furthermore, imaging studies indicated increased striatal DAT binding after treatment with paroxetine (Booij *et al*, 2007; Kugaya *et al*, 2003), especially when the occipital cortex was used as a reference (Booij *et al*, 2007). Nevertheless, uptake in midbrain and diencephalon is considered to reflect predominantly SERT (Laruelle *et al*, 1993), as these structures are rich of SERT relative to DAT and NET. Therefore, although this nonselectivity might have concealed changes in SERT occupancies due to additional DAT or NET binding, we think our findings in diencephalon and midbrain mainly reflect SERT occupancy. Nevertheless, it would be challenging to replicate our study using a selective ligand for SERT like [^{11}C]DASB for PET or [^{123}I]ADAM for SPECT imaging. By the start of our study, [^{123}I]ADAM was not routinely available in the Netherlands.

Our study did not investigate secondary effects of paroxetine. Many adaptive pre- and postsynaptic effects of chronic administration of SSRIs have been documented, including neuroadaptive alterations in serotonin receptors and intracellular signaling pathways (Blendy, 2006; Kim *et al*, 2002; Meyer *et al*, 2001a; Davidson and Stamford, 2000; Benmansour *et al*, 1999; Blier and De Montigny, 1994), as well as time-dependent effects on neurogenesis (Martinowich and Lu, 2008; Djavadian, 2004; Malberg *et al*, 2000). These hypothetical additional effects of dose escalation remain to be investigated. Nevertheless, neither the results of our trial nor the findings from previous randomized controlled trials indicate that dose escalation is an efficacious strategy for SSRI nonresponders in MDD (Ruhe *et al*, 2006; Adli *et al*, 2005; Baker *et al*, 2003).

The fourfold increase of PSC after dose escalation from 20 to 50 mg per day may question adherence of patients in the open phase of the study. However, paroxetine inhibits the cytochrome P_{450} enzyme 2D6, also responsible for the metabolism of paroxetine. Therefore, nonlinear increases of PSC reflect normal paroxetine pharmacokinetics (Anonymous, 2008).

Our study was discontinued after an interim analysis with relatively small patient numbers. However, the criteria for premature trial termination regarding futility had been prespecified, making it highly unlikely that we overlooked clinically relevant differences. Moreover, the neurobiological parts of our study provide a rationale why even with much larger patient numbers no substantially different outcome can be expected. On the other hand, premature stopping reduced the power to examine whether subgroups of patients were more responsive to dose escalation.

This study was not designed to test the efficacy of paroxetine *per se*, as this is well established in patients with severe MDD ($HDRS_{17} > 18$) (NICE, 2004; Kennedy *et al*, 2001; American Psychiatric Association, 2000). Therefore, we did not include a pure placebo arm. Rather we investigated dose escalation, and accordingly only included placebo during dose escalation. This approach is similar to eg the STAR*D project, which interestingly reported similar response rates for open treatment with citalopram (Trivedi *et al*, 2006b).

CONCLUSION

Previous studies had failed to demonstrate a clinical benefit of dose escalation by SSRIs, but had methodological limitations (Ruhe *et al*, 2006; Adli *et al*, 2005; Baker *et al*, 2003). Addressing those limitations, our trial replicates that dose escalation of paroxetine above the 20 mg per day standard dose has no additional clinical benefit. As a novel extension, we revealed the underlying neurobiological mechanism for this inefficacy: maximum SERT occupancy was already reached with the standard dose. Similarly, high SERT occupancies reported with low doses of other SSRIs suggest that our conclusion may be applicable to the entire drug class. However, this does not exclude that dose escalation has clinical benefits for antidepressants with additional molecular targets, eg the norepinephrine transporter, such as venlafaxine. This drug has shown dose dependency of the clinical response in fixed dose studies (Thase *et al*, 2006; Rudolph *et al*, 1998).

If dose escalation is not promising for paroxetine and presumably other SSRIs, two clinical options remain for the treatment of nonresponders to standard doses. These are either continuation of treatment until 10 weeks while waiting for a potential delayed response, or a change to a different and potentially more effective treatment strategy. Both strategies will further improve response rates, but studies directly comparing these strategies have not yet been performed.

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DISCLOSURE/CONFLICTS OF INTEREST

All authors declared no conflict of interests.

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