Extended results of the Alzheimer's disease anti-inflammatory prevention trial

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Abstract

Background: Epidemiologic evidence suggests that nonsteroidal anti-inflammatory drugs (NSAIDs) delay onset of Alzheimer's dementia (AD), but randomized trials show no benefit from NSAIDs in patients with symptomatic AD. The Alzheimer's Disease Anti-inflammatory Prevention Trial (ADAPT) randomized 2528 elderly persons to naproxen or celecoxib versus placebo for 2 years (standard deviation = 11 months) before treatments were terminated. During the treatment interval, 32 cases of AD revealed increased rates in both NSAID-assigned groups.

Methods: We continued the double-masked ADAPT protocol for 2 additional years to investigate incidence of AD (primary outcome). We then collected cerebrospinal fluid (CSF) from 117 volunteer participants to assess their ratio of CSF *tau* to $A\beta_{1-42}$.

Results: Including 40 new events observed during follow-up of 2071 randomized individuals (92% of participants at treatment cessation), there were 72 AD cases. Overall, NSAID-related harm was no longer evident, but secondary analyses showed that increased risk remained notable in the first 2.5 years of observations, especially in 54 persons enrolled with cognitive impairment—no dementia (CIND). These same analyses showed later reduction in AD incidence among asymptomatic enrollees who were given naproxen. CSF biomarker assays suggested that the latter result reflected reduced Alzheimer-type neurodegeneration.

Conclusions: These data suggest a revision of the original ADAPT hypothesis that NSAIDs reduce AD risk, as follows: NSAIDs have an adverse effect in later stages of AD pathogenesis, whereas asymptomatic individuals treated with conventional NSAIDs such as naproxen experience reduced

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AD incidence, but only after 2 to 3 years. Thus, treatment effects differ at various stages of disease. This hypothesis is consistent with data from both trials and epidemiological studies.

1. Background

As populations age, Alzheimer's dementia (AD) presents an enormous threat to public health [1]. The pathogenesis of AD includes presymptomatic and prodromal stages that together last a decade or more before onset of dementia [2]. Functional neuroimaging data suggest that presymptomatic AD is characterized by changes in synaptic function [3], possibly induced by oligomers of the Alzheimer amyloid peptide Aβ [4]. The familiar pathologic hallmarks of AD—senile plaques, neurofibrillary tangles, and neuronal degeneration—become preponderant later, typically in the prodromal and dementia stages [5,6]. This evolution in AD pathogenesis suggests that interventions may vary in their effects at different stages of disease. Such a varied response to treatment has been observed, for example, in the transgenic R1.40 mouse model of AD, in which the nonsteroidal anti-inflammatory drugs (NSAIDs), ibuprofen and naproxen, suppress new neuronal ectopic cell cycle events induced by Aβ oligomers, but fail to reverse existing events [7].

In many observational studies, users of NSAIDs have been found to develop AD with reduced frequency, but no such association has been found with exposure to these drugs shortly before onset of dementia [8,9]. Several randomized trials have assessed the possible neuroprotection suggested by these observational study results. With the single exception described in this study, however, those trials have all been conducted in symptomatic individuals. The results have been disappointing. Trials of NSAIDs in patients with established dementia showed no mitigation of symptom progression [10,11]. Even in patients with milder cognitive symptoms, incidence of AD was found to increase in those who received the cyclooxygenase-2-(COX-2)-selective NSAID rofecoxib [12]. Thus, a synthesis of the laboratory, observational, and trial data suggests that NSAIDs may provoke contrasting effects at different stages of AD pathogenesis, with neutral or adverse influence on the risk of dementia onset in people with symptoms, but possible protection against onset in those with healthier brains.

2. Methods

2.1. Approach

We extended the observation period of the previously reported randomized, controlled Alzheimer's Disease Anti-inflammatory Prevention Trial (ADAPT), designed to test the hypothesis that sustained use of naproxen or celecoxib would reduce incidence of AD in a healthy elderly population [13]. Concerns about safety had led to termination of the ADAPT treatments late in 2004, after an average 24 (standard deviation [SD] = 11) months of treatment assignment [13,14]. At that point, an analysis of 32 incident cases of AD (the trial's primary outcome) had suggested a possible increase in risk of dementia with either NSAID, as reflected by a hazard ratio (HR), versus placebo of 1.99 (95% confidence interval [CI]: 0.80–4.97; $P = .14$) for celecoxib and 2·35 (CI: 0.95–5.77; $P = .06$) for naproxen [13]. Following the principle of intention-to-treat (ITT), the primary analysis at that point included seven individuals who had been randomized before we learned that they had dementia at baseline. Because these seven were not at risk of incident dementia, we excluded them in a secondary analysis, planned before completion of data collection and unmasking of treatment assignment. The latter analysis produced a stronger suggestion of harm, with HRs of 4.11 (CI: 1.30–13.0; $P = .02$) for celecoxib and 3.57 (CI: 1.09–11.7; $P = .04$) for naproxen [13].

To evaluate the longer-term effects of the ADAPT treatments, particularly including harm, we followed up participants off-treatment for another 18 to 24 months. Then, in post-treatment months 21 to 41, after completion of clinical follow-up, we collected cerebrospinal fluid (CSF) from 117 nondemented volunteer ADAPT participants for evaluation of treatment effects on biomarkers of AD pathogenesis.

2.2. Standard protocol approvals, registrations, and participant consents

All research procedures with human subjects for ADAPT (clinicaltrials.gov identifier: NCT00007189) were approved by the relevant Institutional Review Boards of the University of Washington (VA Committee, for the ADAPT Chairman's Office), the Johns Hopkins Bloomberg School of Public Health (Coordinating Center), and each of the six ADAPT field sites. All participants were capable of providing informed consent at the time of enrollment and at each subsequent point of data collection, and did so. We obtained separate consent from the 117 volunteers for lumbar puncture and measurement of AD biomarkers in CSF.

2.3. Methods of data collection

ADAPT data gathering methods have been described previously [13,15,16]. Briefly, 2528 healthy elderly individuals
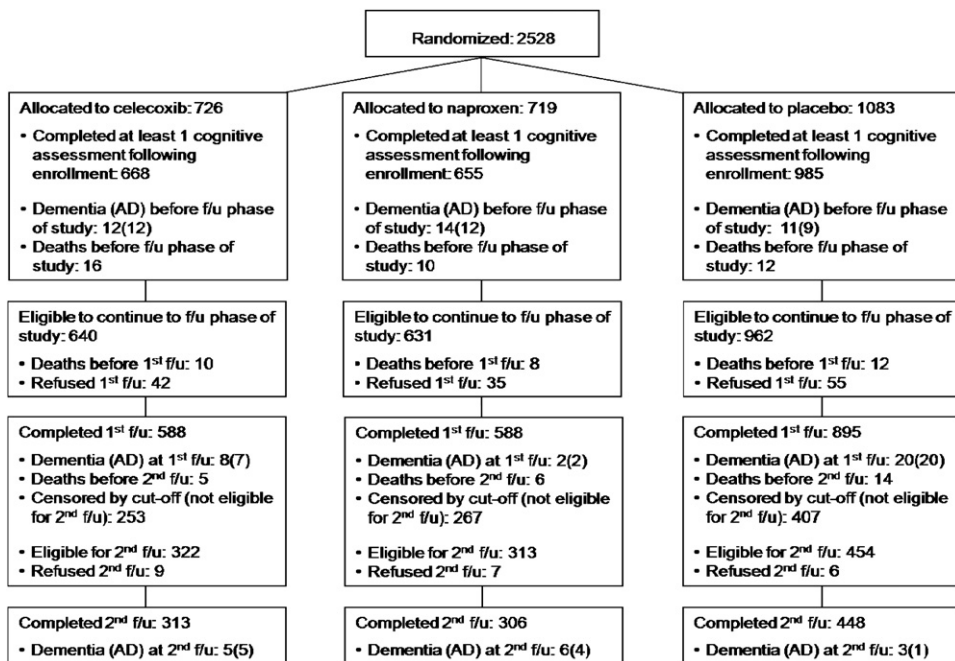
Fig. 1. Flowchart showing numbers originally randomized to the three treatment groups as well as numbers eligible to continue to follow-up observation after the end of the treatment interval. The latter include subjects whose first annual follow-up visit occurred during the follow-up period, and therefore differ from the final numbers remaining in the Consolidated Standards of Reporting Trials - style figure of Reference 13.

with a family history suggesting increased risk of AD were enrolled if they met entry criteria that included a minimum score on a brief cognitive screening battery. Enrollees were randomized to naproxen sodium 220 mg twice daily, celecoxib 200 mg twice daily, or placebo [17]. They then underwent more thorough cognitive screening and a complete evaluation for dementia when indicated, at baseline and then annually thereafter. The primary efficacy outcome throughout was the onset of AD, which was diagnosed using standard research criteria by multidisciplinary expert consensus panels at each of the six field sites. However, in one change of procedure, the present analyses dated outcome events to the screening cognitive assessment or clinical report that triggered a dementia evaluation resulting in the diagnosis. Figure 1 extends our previously published Consolidated Standards of Reporting Trials - style chart [13], showing participant flow during the period of follow-up data collection. Throughout these observations, investigators and participants remained masked to treatment assignment.

Over a 21-month period after completion of cognitive follow-up, we collected CSF from volunteer dementia-free ADAPT participants (Fig. 2A). The CSF donors' treatment assignments remained unknown to the staff of the field sites. Lumbar punctures were performed on participants after an overnight fast, using techniques identical to those described elsewhere [18]. Sequentially numbered 0.5 mL aliquots of CSF were promptly frozen and shipped on dry ice to storage facilities located in Seattle, where they were stored at $-80°C$ until analysis.

2.4. Methods for analyses of CSF biomarkers

The methods for analyses of CSF biomarkers have been published elsewhere [19]. Briefly, CSF from the 4th or 5th mL collected was assayed for concentration of the Alzheimer amyloid peptide $A\beta_{1-42}$ and total *tau* protein (T-*tau*, there being no evident advantage in our experience for assays of individual phosphorylated *tau* species such as ^{181}P-*tau*.). We used Luminex reagents (Biosource Division of Invitrogen, Camarillo, CA) and X-MAP technology (LiquiChip workstation, Qiagen, Valencia, CA), following the manufacturer's instructions exactly. Standard curves were constructed using authentic standards included with each kit, 7 to 5000 pg/mL for both $A\beta_{1-42}$ and T-*tau*. The lower limits of quantitation were 20 pg/mL each for both $A\beta_{1-42}$ and T-*tau*. Each sample was analyzed in duplicate for both endpoints, and the average was taken for statistical analysis using GraphPad Prism (GraphPad Software Inc., San Diego, CA).

2.5. Statistical analyses

With the exception of individuals with dementia at baseline, outcome analyses were limited to participants who completed at least one annual cognitive assessment after enrollment. Analyses considered each participant's time at risk up to his or her last cognitive assessment. Ten participants with dementia not attributed to Alzheimer's disease were censored at the point their dementia was identified (five had vascular dementia; others had rarer conditions). Their
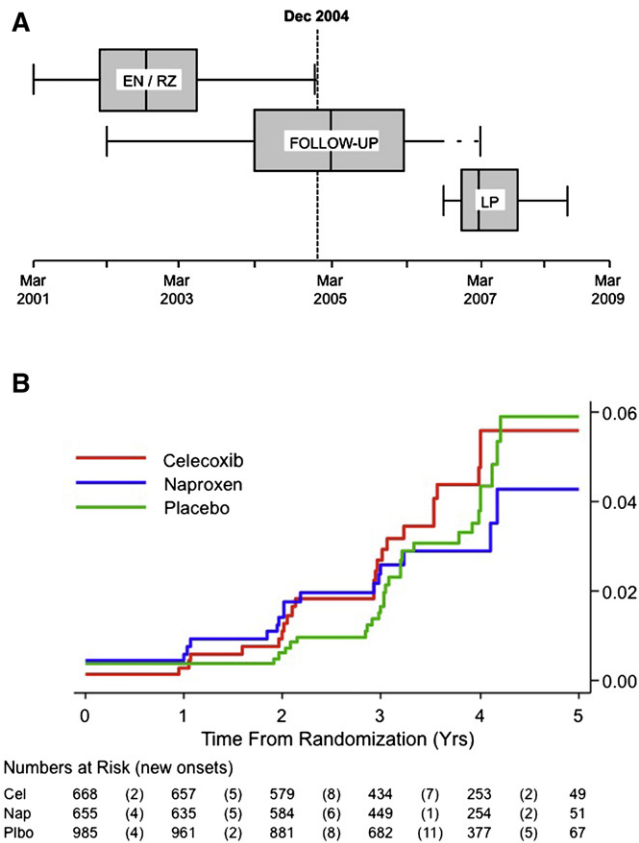
Fig. 2. (A) Box-plots showing periods of enrollment and randomization, follow-up evaluations, and lumbar punctures. Enrollment began in March 2001. Lumbar punctures commenced in September 2006 in participants for whom clinical follow-up was complete (dashed line). (B) Estimated cumulative incidence of Alzheimer's disease (AD; primary analysis). Only three new cases appeared among 447 naproxen-assigned individuals who remained after 3 years. The lines' non-zero origins reflect inclusion in this analysis of eight prevalent dementia cases.

treatment assignments (10 to placebo, 4 to naproxen, 1 to celecoxib) were proportional to the trial's randomization ratio (exact *P* > .5). By design, active treatments were compared with placebo and not with each other. Time-to-occurrence of AD was first evaluated using Kaplan–Meier plots and associated log-rank tests. We then used Cox proportional hazards regression models to seek semiparametric estimates of differences by treatment assignment. Such models assume a relatively constant ratio of incidence rates over the time of observation (proportional hazards assumption). We tested this assumption using Schoenfeld residuals, with *P* < .05 indicating that the HR varied more over the interval of observations than could likely be explained by chance. When the assumption was violated, we examined whether the effects of treatment varied systematically over time after randomization by evaluating the data for the presence of a statistical interaction with (continuous) time in Cox models. Provided that such interaction was present, we then examined whether a

similarly improved fit to the data was evident in more intuitive models that dichotomized the observation period into early versus later time intervals. The dichotomous models used maximum likelihood methods to estimate the optimum dividing point, concurrently using the statistical interaction term to estimate HR estimates for the early versus later time intervals. In each instance, we also verified that the early and the later interval data fulfilled the proportional hazards assumption. Following the data analytical plan of the ADAPT protocol, we adjusted all Cox models for stratification variables of 5-year age group and field site. Adjusted HRs were estimated with 95% CIs and Wald *P* values. All tests for statistical significance used a two-tailed alpha of 0.05.

Again following the principle of ITT, the extended primary analysis considered all AD cases that were discovered after randomization. Also as described earlier, a planned secondary analysis examined rates of incident AD by excluding participants with prevalent dementia at baseline. Other secondary analyses, conducted ad hoc, examined outcomes in subgroups who had cognitive impairment—no dementia (CIND) at baseline (their randomized group proportions were similar to those in ADAPT; exact *P* = .26), and in the cohort after exclusion of participants with either prevalent AD or CIND. CSF levels of T-*tau* and $A\beta_{1-42}$ and their ratio were compared across randomized groups by analysis of variance using the same covariates as the Cox models. All analyses were conducted using STATA version 10.1 (Stata Corp., 2007, College Station, TX).

3. Results

3.1. Participant characteristics and cognitive outcomes

Demographics of the ADAPT participant cohort have been previously described [13]. Characteristics of the cohort at the beginning of the follow-up period are shown in Table 1. The primary ITT analysis results over the entire observation period are shown in Table 2, lines 1 to 7, and graphically in Fig. 2B. The crude rate ratios suggested no net effect of either NSAID. Corresponding log-rank *P* values were .73 for naproxen and .50 for celecoxib. The planned secondary analysis now excluded eight individuals with prevalent dementia rather than seven, because one additional enrollee had received a diagnosis of dementia after the cutoff date for our earlier analyses. Unlike earlier results, the secondary analysis of incidence now showed no increase in risk among the NSAID-assigned groups, but it was otherwise inconclusive (Table 2, lines 9–10; log-rank *P* values .66 for naproxen, .31 for celecoxib). However, the events represented in line 9 of Table 2 violated the proportional hazards assumption (for naproxen, *P* = .01; for celecoxib, *P* = .03). Subsequent analyses showed a significant interaction, with time entered as a continuous variable (for naproxen, *P* = .01; for celecoxib, *P* = .04), and similarly with time dichotomized into earlier and later observation intervals (Table 2,

Table 1
Demographics and selected characteristics of the ADAPT cohort at the time all had completed their first follow-up assessment off-treatment

	Naproxen	Celecoxib	Placebo	Total
Participants at treatment termination	640	645	957	2242
Completed first follow-up (see Fig. 1)	588	588	895	2071
Demographics for subjects who completed first follow-up				
Age, percentiles (years)				
50	78	77	78	78
25, 75	75, 81	76, 81	75, 81	75, 81
Range	71–92	71–94	71–95	71–95
Gender, %				
Female	46%	47%	43%	45%
Male	54%	53%	57%	55%
Ethnic group, n				
White, non-Hispanic	569	565	870	2004
African American	12	12	10	34
Hispanic	1	8	6	15
Other	5	2	8	15
Refused	1	1	1	3
Marital status, n				
Married	438	417	638	1493
Widowed	98	116	165	379
Divorced/separated	35	39	70	144
Never married	17	16	22	55
Education, n				
Less than high school	27	20	31	78
High school degree	95	112	176	383
College, no degree	160	164	224	548
College degree	99	116	189	404
Postgraduate	207	176	275	658
History of medical conditions, n				
Myocardial infarction	20	28	53	101
Diabetes	48	45	67	160
Hypertension treatment	249	245	374	868
Heart failure	4	8	5	17
Transient ischemic attack	21	18	37	76
Stroke	6	7	9	22

line 11). On the basis of inspection of Fig. 2B, the latter analysis divided the observation period into times before or after 2.5 years after randomization (this dichotomous approach was insensitive to the choice of dividing point, yielding identical results for points between 2.2 and 2.8 years after randomization). Within the dichotomized models, all data now satisfied the proportional hazards assumption (data not shown). The separate estimates for early and later HRs are shown in Table 2, lines 12 to 13. As in our earlier analysis, assignment to either of the treatments led to excess risk early in the trial (less conclusively so for naproxen), but the results of the naproxen-assigned group now suggested an opposite trend at later stages (Fig. 3A, $P = .07$).

Similar to our earlier analyses [13], the present results suggested that the NSAID treatments accelerated the onset of AD early in the trial. The onset of AD so early after randomization suggests that the early-affected participants had substantial Alzheimer neuropathology at the time of enroll-

ment. We tested this idea by examining treatment effects, specifically in the 54 participants whose baseline evaluations revealed the presence of CIND. A post hoc analysis of treatment effects in these individuals is shown in Table 2, lines 14 to 16, and in Fig. 3B. Complementing these results were HRs after the exclusion of individuals with either prevalent dementia or CIND (a post hoc simulation of a "true" primary prevention trial), as listed in lines 17 to 22 of Table 2, and in Fig. 3C. Unexpectedly, throughout the period of observations, many of the participants who developed dementia had not been identified as having CIND at their previous annual assessment [20].

3.2. CSF biomarker analyses

Numbers of CSF donors randomized to celecoxib, naproxen, or placebo (38, 25, and 55, respectively) were proportional to the randomization ratio of the parent project (exact $P = .22$). CSF donors did not differ substantially from the entire cohort on baseline scores for the modified Mini-Mental State Examination (3MSE) or the original Mini-Mental State Examination (MMSE), both tests of global cognitive function [21,22]. The mean 3MSE score was 93.82 versus 94.23 ($P = .21$), and mean MMSE scores were 28.83 and 28.94 ($P = .42$). Although both groups of NSAID-assigned CSF donors trended toward higher baseline scores than placebo-assigned participants on the 3MSE (means of 94.29 and 94.64 for celecoxib and naproxen, respectively, vs. mean 93.13 for placebo; $P = .10$ and .058), the two treatment groups showed no meaningful difference on this measure ($P > .5$). Comparable statistics for the MMSE were $P = .16, .62, .36$. CSF donors included proportionally more men (71%) than the entire sample (55%, $P = .001$), a difference that was driven principally by a larger proportion of men among CSF donors assigned to placebo (78%, exact $P = .002$ vs. sex distribution in full ADAPT cohort). Again, however, there were no meaningful gender differences between the two NSAID-assigned groups of CSF donors (63% and 68% men, $P > .5$). The age of CSF donors at the time of enrollment did not differ from that of the entire cohort ($P > .5$), but NSAID-assigned donors tended to be older than those assigned to placebo, with mean ages (SD) of 78.8 (3.3) years for placebo, versus 79.4 (4.2, $P = .42$) for celecoxib and 80.6 (3.4, $P = .03$) for naproxen. There was no notable difference in age between the two treated groups ($P = .25$). Finally, the donor group did not differ from the entire cohort in percentage with an $\varepsilon 4$ allele at APOE, the polymorphic genetic locus that encodes apolipoprotein E, nor were there any meaningful differences in this percentage among CSF donors by treatment assignment (all values of $P > .5$).

Figure 4 shows box plots for the biomarkers T-tau and $A\beta_{1-42}$ as well as their ratio, by treatment assignment from the CSF obtained after completion of clinical follow-up. Neither individual biomarker showed a strong treatment effect, although trends were consistently in the direction of

Table 2
Proportions, rates, and hazard ratios for ADAPT participants classified by treatment assignment and contrasting intervals of observation

Treatment assignment		Naproxen	Celecoxib	Placebo
Primary intention-to-treat analysis				
1	Number of participants (person-years)	655 (2220)	668 (2234)	985 (3336)
2	AD cases *(rate)*	18 *(0.008)*	24 *(0.011)*	30 *(0.009)*
3	*AD rate ratio versus placebo (CI)*	*0.90 (0.47–1.67)*	*1.2 (0.67–2.1)*	*–*
4	Deaths *(rate)*	24 *(0.011)*	31 *(0.014)*	38 *(0.012)*
5	*Death rate ratio versus placebo (CI)*	*0.94 (0.54–1.62)*	*1.23 (0.74–2.03)*	*–*
6	AD or death *(rate)*	50 *(0.023)*	60 *(0.027)*	77 *(0.023)*
7	*AD or death ratio versus placebo (CI)*	*0.89 (0.59–1.34)*	*1.1 (0.78–1.67)*	*–*
Secondary analyses				
8	A. Participants (person-years) after exclusion of prevalent AD	652 (2220)	667 (2234)	981 (3336)
9	AD cases *(rate)**	15 *(0.007)*	23 *(0.010)*	26 *(0.008)*
10	*AD rate ratio versus placebo (CI)*	*0.87 (0.43–1.70)*	*1.2 (0.72–2.41)*	*–*
11	*Improvement in proportional hazards models by interaction with time before or after 2.5 years following randomization*	*P = .013*	*P = .074*	*–*
12	AD cases early (person-years)	9 (1241)	10 (1289)	5 (1876)
	HR-early (CI)	*2.67 (0.88–8.07)*	*3.01 (1.02–8.94)*	*–*
13	AD cases late (person-years)	6 (979)	13 (945)	21 (1460)
	HR-late (CI)	*0.43 (0.17–1.08)*	*0.93 (0.46–1.87)*	*–*
14	B. Participants (person-years) with CIND at enrollment	15 (32)	16 (47)	23 (66)
15	AD cases *(rate)*	4 *(0.12)*	7 *(0.15)*	4 *(0.06)*
16	*HR (CI)*	*3.2 (0.72–13.8)*	*4.0 (1.00–15.6)*	*–*
17	C. Participants (person-years) with no cognitive diagnosis at enrollment	637 (2163)	651 (2169)	958 (3217)
18	AD cases *(rate)**	11 *(0.005)*	16 *(0.007)*	22 *(0.007)*
19	*AD rate ratio versus placebo (CI)*	*0.74 (0.33–1.60)*	*1.1 (0.53–2.15)*	*–*
20	*Improvement in model by term for interaction with time before or after 2.5 years following randomization*	*P = .016*	*P = .037*	*–*
21	AD cases early (person-years)	7 (1212)	8 (1252)	4 (1828)
	HR-early (CI)	*2.50 (0.72–8.7)*	*3.11 (0.92–11)*	*–*
22	AD cases late (person-years)	4 (952)	8 (917)	18 (1390)
	HR-late (CI)	*0.33 (0.11–0.98)*	*0.64 (0.28–1.5)*	*–*

NOTE. Raw data are shown in conventional font; estimated or derived results in italics.

Abbreviations: HR, hazard ratio; CI, 95% confidence interval; "early" and "later" identify times before and after 2.5 years following randomization; CIND, cognitive impairment, no dementia.

* Proportional hazards assumption violation; we did not calculate HRs for these analyses.

improvement (lower T-*tau* and higher $A\beta_{1-42}$) among naproxen-assigned participants. Stronger differences were noted in the T-*tau*/$A\beta_{1-42}$ ratio, which was reduced by 40% in naproxen-assigned participants compared with placebo ($P = .02$ in models adjusted for age, *APOE* ε4 status, education, and gender). No attenuation was evident in the naproxen effect on this ratio over the 20-month interval during which CSF was sampled (data not shown). As expected, *APOE* ε4 status was associated with an increased T-*tau*/$A\beta_{1-42}$ ratio ($P < .001$), but the naproxen effect was unchanged in models after adjustment for this and the other covariates (all $P > .5$).

4. Discussion

In a randomized, controlled trial of naproxen and celecoxib for primary prevention of AD, treatments were stopped after an average of 2 years, but treatment effects were evaluated over an extended interval including an additional 18 to 24 months. Extended findings on the safety of the treatments, and on the second declared cognitive outcome measure of cognitive trajectory, will be reported separately.

Although earlier ITT and, especially, secondary analyses limited to incident cases had suggested harm from either NSAID, both approaches now suggested no net effect. However, treatment effects were heterogeneous over time, and secondary analyses suggested that harm was limited to early events (similar to the previous analyses of the treatment interval). Inclusion of later events seemed to mitigate this treatment-related risk, especially among participants assigned to naproxen. Subsequent CSF biomarker analyses suggested reduced rates of ongoing AD pathogenesis in those treated with naproxen.

These data suggest a need for revision of the original ADAPT hypothesis of neuroprotection from conventional NSAIDs (much less was known about "coxibs" such as celecoxib). The revised hypothesis adds the notion that NSAIDs have an adverse effect on AD pathogenesis in its later stages. Thus, early in the ADAPT observation period there was an excess of dementia in both of the NSAID-assigned groups, possibly reflecting adverse effects of NSAID exposure on enrollees who, although not yet demented, had substantial Alzheimer pathology. Limited additional evidence supports this idea. For example, 10 of the
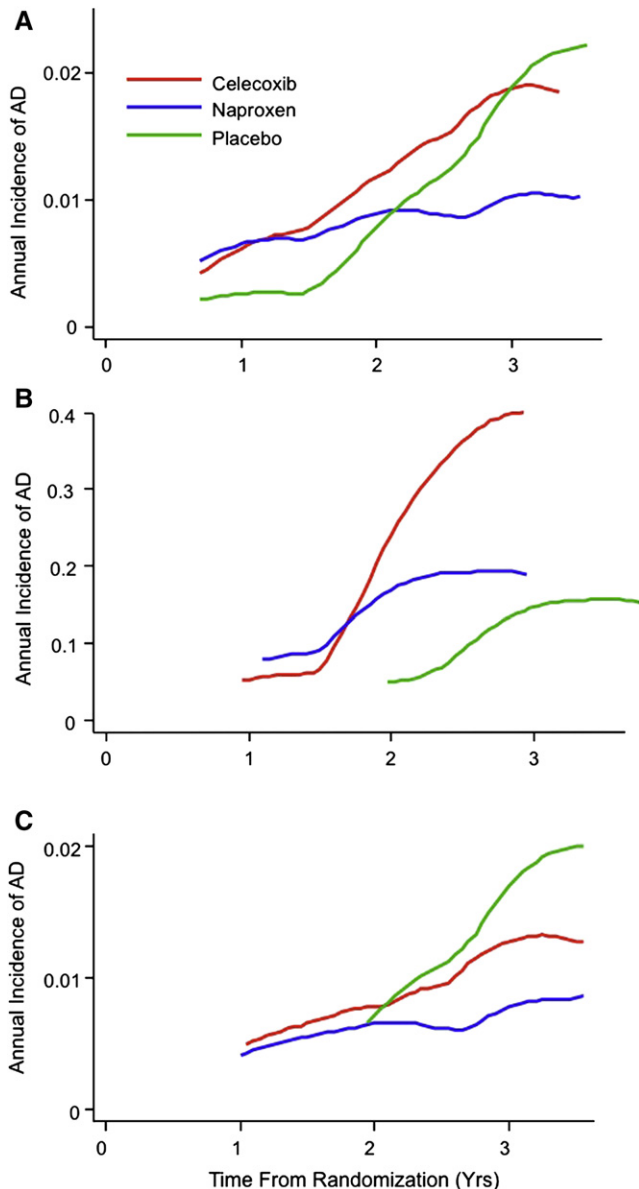
Fig. 3. (A) Estimated decimal annual incidence for the entire cohort (primary analysis). The smoothing program could not accommodate the sparse data from the final year of observations. (B) Annual incidence of AD among 54 participants with cognitive impairment—no dementia at baseline. Note the different scale for the ordinate versus A. The groups assigned to nonsteroidal anti-inflammatory drugs show acceleration of dementia onset by approximately 1 year, as compared with those who received placebo. The extreme ordinate values for the later observations among celecoxib-assigned participants should probably be ignored. (C) Similar to A, but after exclusion of eight enrollees with AD and 54 with cognitive impairment—no dementia at baseline. An early excess of dementia in both nonsteroidal anti-inflammatory drug-assigned groups is still evident, but no participants who were given placebo developed dementia for 22 months after randomization. New cases of AD increased rapidly thereafter in those assigned to placebo or celecoxib, but incidence among naproxen-assigned participants remained steady at about 0.01/year.

early-affected cases in the trial had a baseline diagnosis of CIND, a condition often associated with neuropathologic characteristics of AD [6]. In ADAPT, assignment of participants with CIND to NSAIDs (especially to celecoxib) ac-

celerated their onset of AD dementia (Fig. 2B). Although cognitively "normal," the other 26 ADAPT enrollees without CIND, who nonetheless developed dementia within 3 years, had baseline cognitive function that was lower than that of the remaining cohort—for example, their mean adjusted score on the 3MSE was 90.1 (SD = 3.6) versus 94.3 (SD = 3.3) for others ($P < .0001$), and comparable results were observed with the MMSE (data not shown). If NSAID treatments do accelerate the expression of AD in people with early symptomatic or late-stage pre-symptomatic AD, this effect would add to concerns about the safety of long-term administration of NSAIDs to the elderly population [23,24].

The remainder of the restated hypothesis predicts, as before, that asymptomatic individuals treated with conventional NSAIDs such as naproxen will show reduced incidence of AD, but only after an interval of 2 to 3 years. The ADAPT data that suggest this hypothesis are shown in Table 2, line 22. These later results are also consistent with observational study results showing that the occurrence of AD in users of NSAIDs was reduced only if such use occurred 2 or more years before the observation period [25].

Other interpretations of the extended ADAPT results are possible. Notwithstanding the interaction P values, the contrasts between early and later rates may have occurred by chance. This is a particular concern, given the limited number of events and the post hoc nature of the interaction analysis. Even if "real," the differences in treatment effects may have resulted from the effects of treatment cessation, and not time after randomization. Thus, irrespective of the stage of disease progression, NSAIDs might be harmful when taken but leave a residual neuroprotective effect thereafter, perhaps resulting from selective removal of vulnerable individuals in the NSAID-assigned groups (although the latter part of this interpretation is challenged by the celecoxib results). Other limitations of the ADAPT results include their reliance on findings from an experiment in which treatments were stopped prematurely. Furthermore, the supporting analyses relied on relatively small numbers of events, and on post hoc stratification of observations based on a finding of statistical interaction with time. In particular, the later results with naproxen need verification by further observations.

Notably, however, the still-later CSF biomarker results do support the second portion of the restated hypothesis that naproxen can protect cognitively healthy individuals against AD neurodegeneration. The biomarker data came from an independent sample of 117 dementia-free participants studied 21 to 41 months after the treatments had been terminated. CSF *tau* increases in patients with AD, and in those with prodromal AD symptoms [26], whereas $A\beta_{1-42}$ is known to decline during the development of AD [27]. The ratio of T-*tau* to $A\beta_{1-42}$ robustly distinguishes normal elderly populations from those with AD or milder cognitive symptoms [2]. This ratio is also increased in normal elderly population with an $\epsilon 4$ allele at the *APOE* locus (a genetic risk factor for AD). More importantly, an elevated T-*tau*/$A\beta_{1-42}$ ratio identifies those individuals with mild cognitive symptoms
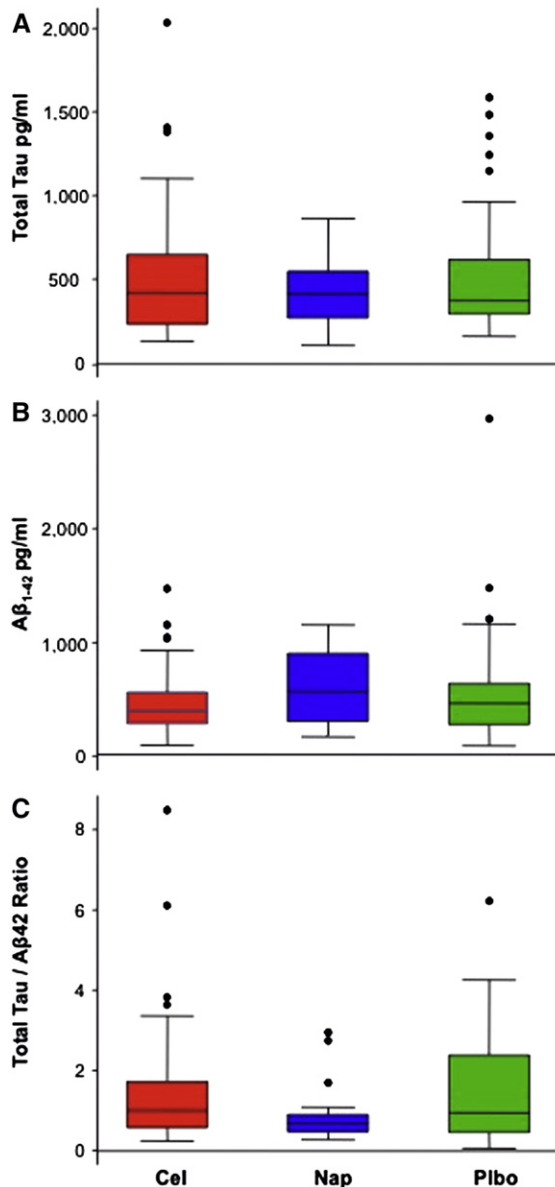
Fig. 4. (A) Box plots showing distribution of concentrations for total *tau* (T-*tau*) for each of the three treatment groups. The "boxes" indicate the range of values between the 25th and 75th percentiles. "Whiskers" indicate the range of values that did not lie outside the interquartile range (between 25th and 75th percentile) by more than $1.5\times$ the interquartile range. Although statistically anomalous, the apparent outliers were probably individuals whose elevated tau concentration was a realistic signal of neurodegenerative illness. All values were considered in the statistical analyses. (B) Box plots showing distributions of concentrations for cerebrospinal fluid $A\beta_{1-42}$ by treatment assignment. The meaning of the outlying values here is less clear. All values were again considered in the analyses. (C) Box plots showing distribution by treatment assignment for the ratio of T-*tau* to $A\beta_{1-42}$ concentrations. The ratios for celecoxib- and placebo-assigned participants were typical of other cognitively normal elderly subjects analyzed in the same laboratory [19]. Ratios for naproxen-assigned participants were reduced, as compared with placebo, by 40%.

most likely to evolve to AD, and even those cognitively "normal" individuals who will shortly develop a syndrome suggestive of prodromal AD [2,19]. Thus, the ratio of T-*tau* to $A\beta_{1-42}$ in CSF is a likely indicator of ongoing AD

pathogenesis. Accordingly, the ADAPT biomarker results appear to constitute a separate endpoint that not only suggests a biological treatment effect but also may predict subsequent cognitive benefit from assignment to naproxen.

Mechanisms for a hypothesized disease stage-related contrast in the effects of the ADAPT treatments are unknown. The "classic" activity of NSAIDs is inhibition of cyclooxygenase (COX) isoenzymes that oxidize arachidonic acid to prostaglandin H2. The latter in turn activates G protein-coupled receptors, either directly or by acting as precursor for other eicosanoids that influence a variety of metabolic pathways [28]. One such pathway is COX-1- and COX-2-dependent activation of microglia resulting in release of pro-inflammatory and pro-oxidant factors that are injurious to nearby neurons or dendrites in experimental models [29]. The relative contributions of COX-1 and COX-2 to such microglial-mediated paracrine damage are not entirely clear. However, COX-1 suppression (e.g., by conventional NSAIDs such as naproxen) could protect neurons against such immune-mediated damage in the early stages of AD pathogenesis. By contrast, brain COX-2 is abundant in dendrites [30], where it is essential for transduction of postsynaptic signals from N-methyl D-aspartate-type glutamate receptors. Inhibition of COX-2 decreases the efficiency of such signaling [31], and could therefore provoke increased presynaptic stimulation through autoregulatory mechanisms. The latter could conceivably stress neurons that are already dysfunctional (e.g., in brains of patients with early AD, CIND, or late-stage presymptomatic AD). COX-2 inhibition, whether by conventional dual-inhibitor NSAIDs or, especially, by potent selective agents, could thereby be deleterious to patients with these conditions.

Although other activities have been proposed for some NSAIDs (e.g., some may be peroxisome proliferator-activated receptor, gamma-type agonists), the importance of these actions in vivo relative to COX inhibition is not established [32]. High doses of some, but not all, NSAIDs can modulate γ-cleavage of the amyloid precursor protein away from production of toxic $A\beta_{1-42}$ [33]. Neither of the ADAPT treatments modulates γ-secretase activity in this way however [34], and at present there is no human evidence for a preferential effect of the so-called Aβ-lowering NSAIDs in the treatment of AD [34], or in its prevention [35,36]. Although neuroprotective effects of NSAIDs have been demonstrated repeatedly in transgenic mouse models of AD [37], including one recent report that tested a strongly selective COX-2 inhibitor [38], the implications of these findings for AD in human beings are unclear.

The possibility of sustained effects of the treatments on cognitive outcomes in the ADAPT cohort is under continuous observation. At present, the results of the trial suggest a hypothesis that may offer improved understanding of an apparently conflicted literature of observational and clinical trial findings on NSAID use and AD. They appear also to indicate a need for further laboratory investigation of NSAID effects on AD pathogenesis.

Acknowledgments

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