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ORIGINAL ARTICLE

Platelet aggregation in whole blood is a paradoxical predictor of ischaemic stroke: Caerphilly Prospective Study revisited

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Abstract
The Caerphilly Prospective Study demonstrates a paradoxical association of increased ischaemic stroke risk with decreased whole blood adenosine diphosphate (ADP) induced platelet sensitivity. A reanalysis of this association examines whether other haematological indices and prevalent disease at baseline may explain this finding. There were 1506 men free of clinical cardiovascular disease at baseline, with 85 men manifesting a first ischaemic stroke event over 8.3 years of follow-up in this population-based prospective cohort study. Using two different approaches, the paradoxical findings are confirmed *and* associations are slightly stronger after accounting for red cell, platelet, and white cell indices. A U-shaped relation of stroke with platelet count is noted. These findings are consistent with the existence of sub-clinical endothelial disease and compensatory mechanisms down-regulating ADP-induced aggregation sensitivity. They support an allostasis model of causality for understanding the paradox. A public health approach to prevention could have measurable impact if intervention strategies can be developed to alter early stages of disease appropriate to such mechanisms of causation.

Keywords: *Platelet aggregation, stroke, thrombosis, adenosine diphosphate, epidemiology, allostasis*

Introduction

Clinical thrombosis, a platelet mediated process, is the proximate cause of myocardial infarction and ischaemic stroke. Because thrombosis is conceptualised as a 'clumping' process called aggregation, no single biochemical test adequately characterises derangement.

Platelet aggregation has been well studied in vitro, highlighting: (1) how agonists such as epinephrine and adenosine diphosphate (ADP) induce aggregation; (2) how receptor-mediated signals regulate the balance of aggregation and disaggregation with the endothelium; and (3) how this balance is perturbed by endothelial dysfunction.

Measuring platelet aggregation in population-based studies has been challenging. Three prospective cohort studies have attempted to directly measure aggregation as a predictor of cardiovascular disease [1–3]. Each study used optical density measures in platelet-rich-plasma (PRP), but with different methods and results. The Norwegian study suggested significant relations of 'greater reactivity' of ADP-induced aggregation and high platelet count with prospectively measured heart disease mortality [1]. Two Medical Research Council (MRC) studies, Northwick Park [2] and Caerphilly [3], showed no relations with incident ischaemic heart disease (IHD); although the Caerphilly study has demonstrated *low* platelet sensitivity predicting *high* stroke risk [4].

The Caerphilly study undertook the most extensive characterisation of platelet aggregation [4]. Because creating PRP can alter agonist-induced aggregation, a whole blood (WB) electrode impedance method was examined towards the end of the second examination [5–7], and fully utilised at Examination 3. Using this method, men with low sensitivity paradoxically had greater stroke risk [4]. However, this analysis did not account for potential confounding by platelet and red cell counts (PLT, RBC) and mean corpuscular and platelet volumes

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(MCV, MPV) with ADP sensitivity [7, 8]—including the observation that red cell and white cell (WBC) mass may be a significant source of ADP-ase and nucleotidase activity in limiting delivered dose to the platelet mass [9, 10]. A second issue was the inclusion of prevalent stroke or other IHD cases along with incident events, as the former may have abnormal aggregation secondary to existing clinical disease resulting in 'reverse causality'.

This re-examination of Caerphilly data takes into account these two issues. The analytical strategy and interpretations are influenced by an allostasis model of disease causation whereby recurring short term adaptive actions in a system leads to long term deleterious effects.

Methods

Established in 1979 as a cohort study of cardiovascular disease in Caerphilly, Wales, the Caerphilly Prospective Study (CaPS) recruited 2512 of 2818 (89%) eligible men, aged 45–59 years, identified from electoral rolls and records of National Health Service General Practitioners [11–13]. CaPS was approved by the South Glamorgan Local Research Ethical Committee. Data collection and clinical procedures followed MRC and Health Authority guidelines. Men signed informed consents.

Five years after inception a new 'baseline' study added men missed originally or new to the area, and optical densitometer measurements were conducted on PRP [14, 15]. Towards the end of this examination a pilot study introduced electrode-based impedance-change measures of WB ADP-induced aggregation [6, 7].

Examination clinics

Between 1989 and 1993 men were invited for a third examination [14]. Evening clinics collected information about social and life-style factors. Blood pressure was measured, and an electrocardiogram recorded. Within days men were scheduled for a morning clinic and fasting blood specimens were collected.

Laboratory tests

RBC, MCV, PLT, MPV, and WBC were measured using the Technicon HI cell counter on EDTA specimens. Plasma fibrinogen was measured nephelometrically. After expedited transport from Caerphilly to Frenchay Hospital, Bristol, all measurements where conducted on the same day as specimen collection.

WB aggregometry was done on 1886 of 2236 (84%) participants [4]. Between 30 and 120 min after collection into a 1/10th volume of 0.13 M Na_3 citrate, ADP-induced platelet aggregation was measured (Chronolog 560 Aggregometer) [5]. After dilution of an aliquot with an equal volume of saline, a sequence of ADP doses was rapidly added to a given and separate sample in which the electrode had first been placed, and the increase in impedance measured. The lowest ADP dose producing a 1.5 Ω or greater impedance increase within 2.5 min defined 'threshold' dose. This sequence involved 19 ADP concentrations varying from 0.1 to 21.5 μmol/l blood–saline. Some samples did not elicit the 1.5 Ω impedence increase within the time limit, even at 21.5 μmol/l and these are 'censored' observations. Concentrations increased in a logarithmic fashion. A high dose indicates low 'sensitivity' to ADP.

The dilution factor was not taken into account in any data analysis. However, all specimens were subject to the same procedure. Thus, its effect on the distribution of threshold measurements should be constant. Given that this ADP threshold measurement represents a consistent and valid device to capture variation amongst men in an ADP-induced aggregation mechanism, relations between this measurement and the constituent haematological indices would not be biased.

Determination of clinical events

Incident clinical stroke was determined using three sources of information: (1) self-report of hospital admission for stroke or a positive response to questions derived from the Oxford Stroke Study; (2) hospital admission lists with a discharge diagnosis of stroke; and (3) death certificates with any diagnosis of stroke. These sources were used to search hospital and general practitioners' records for stroke events. A committee of clinicians and cardiovascular epidemiologists managed by the Department of Social Medicine, University of Bristol, classified date of ischaemic or haemorrhagic stroke, or other (e.g., transient ischaemic attack, amaurosis fugax, etc.) using all available clinical, pathology, and imaging information [16]. Myocardial infarction events and deaths from any cause were also recorded.

At a given date it was feasible to determine a status of 'none', 'possible', or 'definite' stroke and/or IHD. Follow-up time was calculated as the difference between date of third examination morning clinic and date of a first clinically confirmed ischaemic stroke. Men without a stroke were censored either by the date of death from another cause, lost to follow-up, or 31 December 2000, if still alive.

Data analysis strategy

A CVD-free sampling frame was created from the 1886 men having ADP measurements (Figure 1) by a sequence of exclusions: prevalent stroke ($N = 55$); other prevalent cardiovascular clinical disease,

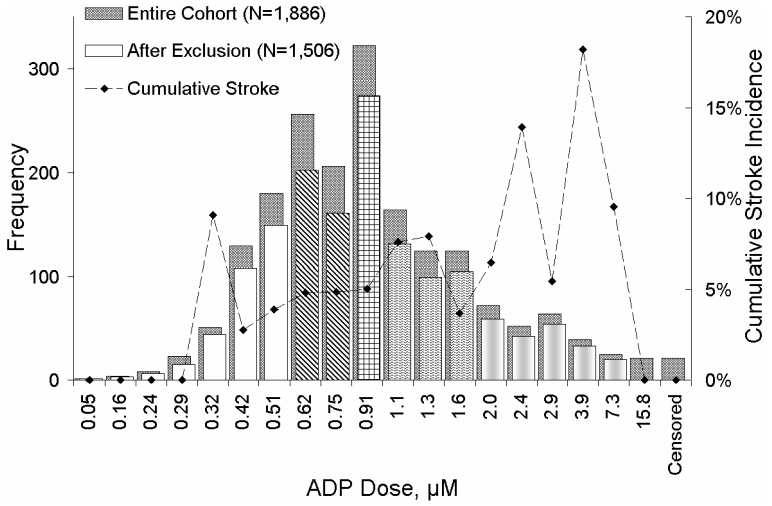

Figure 1. ADP dose distribution, eliciting 1.5 Ω impedance change from platelet aggregation. 'Censored' observations denote men who did not elicit the impedance increase even at the highest dose. Bars detail distributions of entire cohort and cohort after exclusions. Variation in bars after exclusions denotes quintile groupings. Line plot details cumulative stroke incidence, expressed after exclusion for prevalent CVD only ($N = 1572$). *x*-Axis is not scaled to a concentration metric.

an incident MI prior to a first stroke, or 'possible' cerebral vascular events ($N = 259$); very high ADP doses ($N = 36$, justification below); taking a physician—prescribed anticoagulant or antiplatelet drug at baseline ($N = 22$); missing data on primary covariates ($N = 8$). Men who met these criteria but who had a first MI during follow-up and no subsequent stroke contributed to person-years of observation up to the time of the MI. These exclusions left 1506 men.

Men with an exceeding high ADP dose ($N = 36$) were excluded based on four observations: (1) a progressive increase in cumulative stroke incidence (expressed as number of cases per 100 men or percent cases) was noted with increasing dose, *except* at the two highest doses having no cases; (2) the number of men in the two highest doses was disproportionate from that expected by a lognormal distribution; (3) extreme decreases were noted in platelet indices between these men and men at lower doses (PLT: 150 vs. 236×10^9/l, $P < 0.0001$; MPV: 7.29 vs. 7.83 fl, $P = 0.005$); and (4) during follow-up a higher proportion of deaths occurred (0.36 vs. 0.22, $P = 0.044$), by definition not CVD related. Further exploration indicated (a) an increased cumulative mortality from alcohol-related liver disease (3/36 versus 1/1506, $P < 0.0001$), and (b) an increased proportion with MCV ≥ 100 fl (19.4 vs. 6.0%, $P = 0.005$), suggesting that their aggregation measurements come from another distribution related to chronic alcoholism.

Prior to analyses each man's dose was altered to be the midpoint between the measured value and the next lower dose. This avoids a statistical bias which would otherwise inflate the intercept term in regression models [17, 18]. These are the ADP doses to which the Results and Tables I–IV refer. Because the distribution of ADP dose is skewed, logarithmic transforms were used in certain models with the other haematological covariates. Such a model assumes that variables act on a multiplicative scale. For other analyses ADP dose was categorised into five levels (quintiles) by grouping adjacent doses in ascending order and categorising the five groups to have roughly equal number of observations (Figure 1, Table II). These groupings are slightly different from those used previously [4].

Statistical methods

Age-adjustment and selected adjustment for haematological indices were done to calculate, along with 95% confidence intervals: (1) means, (2) proportions, and (3) incidence densities (incident cases/1000 person-years follow-up) and relative rates. Adjustments respectively used (1) analysis of covariance, (2) logistic regression, and (3) direct standardisation by person-years distributions within four age groups (<58, 58–62, 62–66, and ≥66 years) [19]. For analyses involving quintiles of the original ADP dose variable, linear trend tests among levels (Tables II and III) used orthogonal contrast coding; the linear contrast coefficients being adjusted by respective within-level mean ADP dose to reflect the unequally spaced intervals [20].

Using multivariable linear regression methods, we modelled the joint multiplicative associations of the five haematological indices with ADP dose. The residual values from this model, which we call the 'corrected ADP dose' represent the variation in ADP dose from the predicted average that an individual demonstrates after taking into account the other haematological values. By this means we compensate for the impact of different platelet, red cell, and white cell masses amongst men and its impact on the dose of ADP. Corrected ADP values were then grouped

into quintiles with the same sample size as the original dose groupings.

A series of six hazard ratio models were constructed examining the association between dose and incident ischaemic stroke, accounting for a series of potential confounders or causal mediators. The original ADP dose was used as a continuous variable as it demonstrated a progressive linear dose–response association with incident stroke. Rather than using the scaled metric of 1 μM as the comparison unit in these models, the original ADP dose was rescaled to a metric of 2 μM as the unit simply by dividing the ADP dose value for each man by 2. This rescaled unit approximates a comparison between 10th and 90th percentiles of the original ADP dose distribution.

For corrected ADP dose the quintile grouping was retained. A single contrast involving a combination of these five levels, justified by the dose–response pattern described in Results, constituted the measure of association.

No time-dependent relations with incident stroke were noted for these two constructions of ADP dose. However, associations with systolic and diastolic blood pressure, and MCV suggested non-linear and time-dependent dose–response relations. They were respectively stratified into three and two levels, and incorporated as potential confounders by stratification and time-dependent proportional hazards methods [21].

Results

Compared to men with past ischaemic stroke at baseline ($N = 55$), men free of clinical CVD ($N = 1506$) were about 2 years younger (Table I). These men had lower blood pressures, serum total cholesterol, and triglycerides. Of the haematological

Table I. Descriptive features of Caerphilly Prospective Study cohort third examination.

Variable[a]	Included[b]	Excluded, prevalent stroke[b]	(*P*-value)[c]
N	1506	55	
Continuous variables[a]			
Age, yrs	62.2 (4.5)	64.3 (4.5)	0.0005
Systolic BP, mmHg	144.3 (19.6)	150.6 (18.9)	0.02
Diastolic BP, mmHg	83.0 (10.9)	87.8 (10.7)	0.002
Platelet count, $\times 10^9$/l	236 (55)	249 (46)	0.07
Mean platelet volume, fl	7.86 (0.94)	7.75 (0.73)	0.38
Red cell count, $\times 10^{12}$/l	4.93 (0.40)	4.87 (0.46)	0.23
Mean corpuscular volume, fl	92.0 (8.8)	92.3 (4.9)	0.79
White cell count, $\times 10^9$/l	6.37 (1.68)	6.98 (1.85)	0.009
Total cholesterol, mmol/l	6.21 (1.13)	6.50 (1.13)	0.06
Triglycerides, mmol/l[d]	1.63 (0.52)	2.16 (0.63)	<0.0001
ADP dose, μM[e]	1.15 (1.02)	0.87 (0.62)	0.04
Categorical variables[a]			
Hypertension, by questionnaire			<0.0001
No	68.9% (1038)	29.1% (16)	
Uncertain	4.0% (60)	1.8% (1)	
Yes	27.0% (406)	69.1% (38)	
Incident myocardial infarction[f]			
No	93.8% (1413)	75.0% (36)	<0.0001
Yes	6.2% (93)	25.0% (12)	
Smoking habits[g]			0.052
Never	18.7% (281)	7.3% (4)	
Past	46.8% (705)	63.6% (35)	
Cigar/Pipe	6.9% (104)	5.5% (3)	
Current	27.0% (407)	21.8% (12)	
Unknown	0.6% (9)	1.8% (1)	
Alcohol intake habits[g]			0.29
Never	1.9% (29)	0.0% (0)	
Past	5.5% (83)	9.1% (5)	
Current	91.8% (1383)	85.5% (47)	
Unknown	0.7% (11)	5.5% (3)	
Aspirin			<0.0001
No	91.1% (1372)	61.8% (34)	
Yes	8.9% (134)	38.2% (21)	

[a]Continuous variables, mean (standard deviation); categorical, percent (*N*). Data missing on some variables. [b]See methods for exclusion criteria. [c]"Included" versus "Excluded"; continuous variables: 2-tail *T*-test of means; categorical: χ^2-test for variables with three or more levels, Fisher's exact test for variables with two levels. [d]Geometric mean, but standard deviation of $\log_e$ transformation. Statistical tests used transformed variable. [e]Dose of ADP needed to elicit a 1.5 ohm impedance change in whole blood. See methods. [f]New case of definite myocardial infarction during follow-up period. [g]For purposes of statistical tests of association, men with "unknown" status were excluded.

indices, PLT appeared to be marginally lower (236 vs. 249 $\times 10^9$/l, $P=0.07$) and WBC was significantly lower (6.37 vs. 6.98 $\times 10^9$/l, $P=0.009$). ADP dose was significantly higher (1.15 vs. 0.87 μM, $P=0.04$), indicating higher platelet sensitivity among men with a history of CVD at baseline.

Jointly adjusted mean values for the haematological indices by original ADP dose quintiles revealed a steady linear decrease for PLT, MPV, and WBC, while RBC and MCV demonstrated increases (Table II). No gradient was noted for plasma fibrinogen. Thus, men demonstrating less 'sensitivity' to ADP-induced aggregation had smaller and fewer platelets, but more and slightly larger red cells, while men with higher white cell counts seemed to require less ADP (more 'sensitivity') to induce aggregation onto the electrode.

A progressive trend of increasing age-adjusted incidence density with increasing dose was noted (Table III). Compared to the lowest level, men in the highest were 2.3 times more likely to have a stroke.

After correcting for joint relations of the haematological indices, the corrected ADP dose demonstrated an association with age-adjusted incident ischaemic stroke (Table III). Among the lowest three levels, incident densities of 5.1–6.4 strokes per 1000 person-years were observed. In the two highest levels incident densities of 9.0 and 8.6 were

Table II. Adjusted[a] mean values of haematological indices among strata of ADP-dose necessary to elicit a 1.5 ohm impedance change in whole blood.

	ADP-dose groupings (μM)						
Parameter	≤0.513	0.6215–0.753	0.9125	1.106–1.6235	≥1.966	Total	*P*-value[b]
Mean ADP-dose, μM	0.44	0.68	0.91	1.34	3.11	1.15	—
N	326	363	274	335	208	1,506	—
Platelet count[a], $\times 10^9$/l	262	245	234	221	204	236	<0.0001
[95% CI]	[257, 267]	[241, 250]	[228, 239]	[216, 226]	[198, 210]	[234, 238]	
Mean platelet volume[a], fl	8.01	7.96	7.85	7.81	7.56	7.86	<0.0001
[95% CI]	[7.91, 8.11]	[7.87, 8.05]	[7.74, 7.95]	[7.71, 7.91]	[7.44, 7.69]	[7.82, 7.91]	
Red cell count[a], $\times 10^{12}$/l	4.86	4.90	4.97	4.93	5.04	4.93	<0.0001
[95% CI]	[4.83, 4.90]	[4.87, 4.94]	[4.93, 5.01]	[4.90, 4.98]	[4.99, 5.09]	[4.92, 4.95]	
Mean corpuscular volume[a], fl	91.9	92.5	93.3	92.3	93.4	92.6	0.02
[95% CI]	[91.3, 92.4]	[92.0, 93.0]	[92.8, 93.8]	[91.8, 92.8]	[92.6, 93.9]	[92.3, 92.8]	
White cell count[a], $\times 10^9$/l	6.68	6.60	6.08	6.24	6.09	6.37	0.0004
[95% CI]	[6.51, 6.86]	[6.44, 6.76]	[5.90, 6.26]	[6.07, 6.40]	[5.87, 6.30]	[6.29, 6.45]	
Fibrinogen, g/dl	4.04	4.06	4.18	4.17	4.19	4.12	0.09
[95% CI]	[3.94, 4.13]	[3.97, 4.14]	[4.08, 4.28]	[4.08, 4.26]	[4.07, 4.31]	[4.08, 4.16]	

[a]For five indices, analysis of co-variance adjusting for other four indices and age; for fibrinogen, all five indices plus age. [b]Trend statistic based on *T*-test for linear trend, scaled to intervals of mean ADP-dose within a grouping.

Table III. Age-adjusted[a] Incidence Density (ID) and relative rates of ischaemic stroke events [95% Confidence Intervals (CI)] among strata of ADP dose[b]

	ADP dose groupings[b]						
Parameter	1	2	3	4	5	Total	*P*-value[c]
ADP variable							
N	326	363	274	335	208	1506	
Original ADP dose							
Stroke cases	13	17	14	22	19	85	
Person-years	2572	3001	2302	2873	1781	12 528	
ID, per 1000 person-years	4.6	5.4	6.2	7.9	10.4	6.8	0.04
[95% CI]	[2.1, 7.1]	[2.8, 8.0]	[2.9, 9.4]	[4.6, 11.2]	[5.7, 15.1]	[5.5, 8.4]	
Relative rate	1	1.18	1.35	1.73	2.27	—	0.02
[95% CI]		[0.57, 2.45]	[0.63, 2.89]	[0.87, 3.46]	[1.11, 4.63]		
Corrected ADP dose							
Stroke cases	16	14	14	25	16	85	
Person-years	2615	3023	2306	2757	1827	12 528	
ID, per 1000 person-years	5.6	5.1	6.4	9.0	8.6	6.8	0.06
[95% CI]	[2.9, 8.4]	[2.4, 7.7]	[3.0, 9.8]	[5.4, 12.5]	[4.4, 12.9]	[5.5, 8.4]	
Relative rate	1	0.90	1.14	1.59	1.53	—	0.03
[95% CI]		[0.44, 1.85]	[0.55, 2.34]	[0.85, 2.99]	[0.76, 3.06]		

[a]For ID and relative rate, age-stratified analyses were done using direct standardization weighted by person-years. [b]For the originial ADP dose, strata are described in Table II. See methods for corrected ADP dose. [c]For ID, Wald chi-square test; for original ADP dose, linear trend scaled to intervals of mean ADP dose within a level; for corrected ADP dose, contrast of lowest three levels versus highest two. For relative rates, age-adjusted proportional hazards model.

Table IV. Progression of relative rates and 95% confidence intervals of incident ischaemic stroke among two related categorizations[a] of ADP dose upon control for various confounders and mediators.

Model	Original ADP dose per 2 μM difference[a]	Corrected ADP dose Q123 vs. Q45[a]	Nature of co-variates
1	1.41 [1.05, 1.89]	—	Age only
2	1.54 [1.11, 2.14]	1.62 [1.06, 2.49]	Age, haematological indices[b]
3	1.56 [1.13, 2.16]	1.69 [1.10, 2.59]	Age, haematological indices, systolic and diastolic blood pressure
4	1.53 [1.10, 2.12]	1.62 [1.05, 2.48]	Age, haematological indices, serum total cholesterol and triglycerides
5	1.64 [1.16, 2.32]	1.54 [0.99, 2.40]	Age, haematological indices, usual alcohol and smoking habits
6	1.54 [1.11, 2.14]	1.64 [1.06, 2.54]	Age, haematological indices, OTC medications and dietary factors[c]

[a]Original ADP dose is rescaled to a 2 μM unit, approximating comparison between 10th and 90th percentiles. See methods. Corrected ADP dose represents five strata of the same sample sizes as original ADP dose strata (see Methods), the association contrasting the lowest three levels versus the highest two. [b]Red cell count, mean corpuscular volume, platelet count, mean platelet volume, and white cell count. [c]OTC: ''over the counter.'' Limited to non-steroidal anti-inflammatory drugs. Dietary factors are fish oils and garlic.

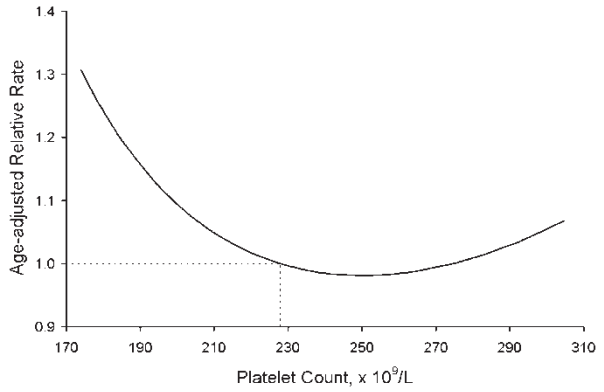

Figure 2. Relative rate of stroke from age-adjusted Cox model involving significant linear and square terms of $\log_e$[PLT]. Relative rate is referenced to median PLT, 228×10^9/l, and scaled to one-unit difference in $\log_e$[PLT] which is slightly less than PLT range. Relation is depicted between 10th and 90th percentiles of PLT.

noted. A contrast of the lowest three versus the highest two levels was statistically significant ($P = 0.03$).

Only minor changes in relative rates were noted after controlling for potential confounders and mediators (Table IV). For original ADP dose a 9.4% increase in age-adjusted relative rate, 1.41 to 1.54 (Models 1 and 2), was noted upon control for the five haematological indices. Respective but separate adjustments for various classes of covariates altered relative rates <10%. For corrected ADP dose the relative rate contrasting the three lowest and two highest levels is 1.62 (Table IV). Subsequent adjustment for covariates altered relative rates <4.9%.

The age-adjusted relation involving PLT was curvilinear. The best fit model included logarithm of PLT plus its square (LnPLT: $P = 0.021$; Ln^2PLT: $P = 0.024$; Figure 2). This U-shaped relation was confirmed by cross-tabulation methods (results not shown).

Discussion

Our more detailed analyses support the original observation that reduced platelet sensitivity is associated with increased stroke risk [4]. The paradox is thus noted in a sub-sample selected to be without baseline clinical disease and is evident even after statistical control for haematological constituents affecting ADP dose delivered to the platelet mass. Indeed, the contrast of *more* sensitive platelets (low ADP dose) among men prevalent for CVD at baseline, but *less* sensitive platelets (high ADP dose) among men free of CVD at baseline underscores the paradox at yet another level of analysis.

The paradox may be explained by known features of ADP-induced aggregation. Platelets become refractory to ADP-induced aggregation after incubation in ADP at in vivo concentrations [22–24]. The platelet possesses mechanisms limiting inappropriate activation leading to 'irretrievable damage' [25]. This refractory state may be receptor-mediated [26, 27], involving differential down-regulation of $P2Y_1$ and $P2Y_{12}$ [28].

Although no methods were available at the time of this study to measure endothelial function, from these observations and recently published evidence we hypothesise: (1) men going on to have ischaemic strokes have sub-clinical asymptomatic endothelial disease. (2) The compromise of endothelial function diminishes local antithrombotic activity, including roles for nitrogen oxide, prostacyclin, and ecto-ADPase [29, 30]. (3) With diminution of antithrombotic activity platelets become acutely susceptible to aggregation from a variety of agonists, including ADP and epinephrine [28, 31, 32]. For ADP that includes shape changes (0.1–0.5 μM ADP), a reversible aggregation phase (0.5–1.5 μM ADP), and irreversible aggregation releasing ADP granules [33]. (4) The source of the ADP-induced refractory state is the degranulation process of platelets

themselves, which enhances activation of agonist-induced aggregation [33]. (5) Because endothelial dysfunction is incipient some antithrombotic activity remains locally, limiting the full extent of potential aggregation. (6) The net *chronic* effect of altered interactions between endothelium and platelet is a new state of homeostasis which attempts to compensate for the effects of early stages of endothelial dysfunction. This compensation limits the acute enhancement of aggregation by ADP, evoking an *in situ* ADP-induced refractory condition [22–24].

Patients with *active* atherosclerotic disease have lower PLT and higher MPV compared to people with stable angina or no disease [34, 35]. Patients with advanced coronary calcification and acute coronary syndromes demonstrate increased micro-aggregation levels [36]. These findings suggest active atherosclerotic disease creates ongoing platelet consumption leading to increased platelet volume as a marker of a compensated response [35]. This seems to partially hold in our analysis. Men with low PLT are at high risk, but no compensatory relation involving MPV is noted. The exclusion from analyses of men with any indication of prevalent cardiovascular disease probably accounts for this negative observation.

The nature of association between the ADP threshold dose and WBC follows that of the platelet indices rather than the red cell indices, with higher WBC being associated with low ADP dose. This observation suggests, but does not prove, that white cells may be affecting the impedance signal, possibly by adhering to the electrode and thus creating the impression that a low ADP dose is aggregating platelets. This observation would also suggest that cell mediated nucleotidase degradation of ADP dose to the platelet mass is more a phenomenon of red cells, which exceed white cells in number by almost 800-fold. The statistical controls reflected in the various models of Table IV compensate for these potential effects.

This model of chronic adaptive change is consistent with the concept of allostasis [37]. Distinct from homeostasis, allostasis represents a chronic compensatory physiological response to repetitive insult or stress. Adaptive actions are protective in the short term, but long-term effects are damaging.

In this allostasis framework the paradox of high ADP dose associated with increased stroke risk would represent early stages of endothelial dysfunction interacting with platelets to limit potential for a catastrophic thrombosis-mediated clinical stroke. The 9.4% increase in magnitude of association between original ADP dose and incident stroke noted with addition of haematological indices (Table IV) and the U-shaped relation between platelet count and stroke risk (Figure 2) are consistent with this causal model.

In addition, the baseline observation of *more* sensitive platelets among men prevalent for CVD, but *less* sensitive platelets among men free of CVD (Table I) is consistent with an allostasis model. Men prevalent for disease at baseline probably have well developed atherosclerotic disease and attendant endothelial dysfunction. Physiologically mediated compensating mechanisms are probably no longer effective in these men, and they sit on the cusp of another catastrophe even if their disease is controlled by medications intended to limit potential for thrombosis. Indeed, 25% of men prevalent for stroke at baseline go on to have a MI during follow-up, compared to 6.2% of the 1506 disease-free men.

The allostasis model allows for compensating interacting systems to maintain function, but pays a price for increased risk of catastrophe when systems become chronically overloaded. With stroke this catastrophe may be precipitated by the interaction of epinephrine—mediated processes effecting aggregation in refractory ADP-induced states [28, 31, 32]. Sleep apnea could precipitate such catastrophe, being sufficiently prevalent and evocative of disturbed epinephrine-induced platelet activation [38].

The manifestation of such a systems view depends *intimately* on the distribution of stages of dysfunction and sub-clinical disease in cohort selection and data analysis sampling frames. This selection coupled with the allostasis framework can lead to conflicting results among studies [39]. The value of the allostasis model is that it can lead to standardised methods of framing a study design and/or data analysis by focusing on specific elements of the model appropriate to the pathophysiological question at hand.

These results and interpretation, although indirect, suggest elements in the early dysfunction of the endothelial/platelet axis are manifest in population-based settings. The technology and methods used to study platelet aggregation in the Caerphilly cohort do not lend themselves to be tools of clinical assessment, nor did this study have the means at the time to assess derangements in endothelial function independently of platelet function. However, if the allostasis model of causation extending from this work and interpretation is true, then the model suggests prevention could have measurable impact if intervention strategies can be developed to alter early stages of disease appropriate to this mechanism.

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