International Journal of Clinical Trials
McGowan NG et al. Int J Clin Trials. 2024 Feb;11(1):53-60
http://www.ijclinicaltrials.com
pISSN 2349-3240 | eISSN 2349-3259

DOI: https://dx.doi.org/10.18203/2349-3259.ijct20240043

Protocol

A randomized, placebo-controlled crossover trial to assess the influence of body weight on aspirin-triggered specialized pro-resolving mediators: protocol for the discover study

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Received: 20 October 2023
Revised: 09 January 2024
Accepted: 13 January 2024

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ABSTRACT

Background: Low-dose aspirin is ineffective for primary prevention of cardiovascular events in people with body weight greater than 70kg. While the prevalent explanation for this is reduced platelet cyclooxygenase-1 (COX-1) inhibition at higher body weights, supporting data are limited, thereby demanding further investigation of the reason(s) underlying this observation. We propose that aspirin-mediated cyclooxygenase-2 (COX-2) acetylation and the resulting synthesis of 15-epi-lipoxin A_4, a specialized pro-resolving mediator, is suboptimal in higher weight individuals, which may contribute to the clinical trial findings.
Methods: To test this hypothesis, we are conducting a double-blind, placebo-controlled, randomized, mechanistic crossover trial. Healthy men and women exhibiting a wide range of body weights take 81mg aspirin and 325mg aspirin for 3 weeks each, following 3-week placebo run-in and wash-out phases. Our target sample size is 90 subjects, with a minimum of 72 completing all visits estimated to be necessary to achieve power adequate to test our primary hypothesis. Our primary endpoint is the difference in change in plasma 15-epi-lipoxin A_4 occurring with each dose of aspirin. Secondary endpoints include lipid mediator profiles, serum bioactive lipid profiles, and other endpoints involved in the resolution of vascular inflammation.
Conclusions: Study enrollment began in November 2021 and is ongoing. The results of this study will improve our understanding of the mechanisms underlying aspirin's role(s) in the prevention of adverse cardiovascular outcomes. They may also lead to additional studies with the potential to inform dosing strategies for patients based on body weight.
Trial registration: This trial is registered with ClinicalTrials.gov identifier NCT04697719.

Keywords: Inflammation, Pro-resolving mediators, Obesity, Cardiovascular disease, Aspirin

INTRODUCTION

Approximately 75% of Americans are overweight or obese.[1] Obesity is characterized by chronic, low-grade inflammation, which contributes to an increased risk of developing atherosclerotic cardiovascular disease.[2] Despite substantial progress in preventive medical therapies over the past several decades, cardiovascular diseases remain the largest source of morbidity and mortality globally and are responsible for almost 70% of deaths in those with overweight and obesity.[3] Therefore, it is critical to improve prevention strategies to reduce this persistent risk.[4] Notably, although body weight and adiposity can influence both the availability and efficacy of cardiovascular medications, most are dosed without regard to body weight.[5] Low-dose (≤100 mg) aspirin is an important and widespread tool in the primary prevention of adverse cardiovascular outcomes.[6,7] Aspirin irreversibly acetylates platelet cycloxygenase (COX-1), inhibiting thromboxane production and leading to reduced platelet aggregation and associated thrombosis.[8,9] A recent meta-analysis of trials involving over 130,000 subjects investigated the impact of body weight on the efficacy of aspirin in primary prevention and found that low-dose aspirin exhibited no benefit in those with body weights above 70 kg. Furthermore, aspirin doses of at least 300mg reduced major adverse cardiovascular events (MACE) in those weighing above 90 kg.[10] The authors proposed that these findings were due to insufficient platelet COX-1 inhibition by low dose aspirin at body weights greater than 70 kg, but that higher doses of aspirin overcame this insufficiency.[10]

While reasonable, the evidence cited in support of this suggested mechanism has significant limitations. Many of the studies that have found reduced platelet inhibition at higher body weights investigated enteric-coated aspirin, but in obesity, impaired systemic availability of aspirin from the enteric-coated formulation is often observed due to higher levels of intestinal esterases that metabolize aspirin.[10-14] Nonetheless, rates of MACE did not differ by aspirin formulation in the above meta-analysis.[10] In addition, obesity-associated comorbidities, such as diabetes, are known to influence platelet inhibition, but few studies demonstrate body weight or adiposity as the only factor driving reduced platelet inhibition.[15,16] Accordingly, we recently showed that in individuals without diabetes, neither body weight nor mass influenced platelet inhibition by either 81 mg or 325 mg of aspirin.[17] These observations together suggest that aspirin formulation may be responsible for the observed reductions in platelet inhibition, but that this mechanism is not wholly responsible for the observed differences in rates of MACE. Thus, to thoroughly dissect the robust findings of the meta-analysis, other mechanisms beyond aspirin-mediated COX-1 inhibition must be investigated.[10] Specialized pro-resolving lipid mediators (SPMs) are a family of lipids derived primarily from arachidonic acid (AA), eicosapentaenoic acid (EPA), and docosahexaenoic acid (DHA), and they are recognized as necessary for the active resolution of inflammation.[18,19] Both obesity and atherosclerosis are characterized by non-resolving inflammation; furthermore, deficient levels of SPMs may contribute to the chronic inflammation characterizing these conditions.[2,20,21] This is particularly relevant because in addition to inhibiting platelet COX-1, aspirin also irreversibly acetylates COX-2 within leukocytes and endothelial cells, but higher levels of acetylsalicylic acid are required for COX-2 modification.[8,18,22] After being modified, COX-2 is able to participate in the synthesis of a specific group of SPMs termed aspirin-triggered SPMs.[19] 15-epi-lipoxin A_4 (15*R*-LXA_4), also known as aspirin-triggered LXA_4, was the first SPM to be isolated and has been found to prevent atherosclerosis progression in pre-clinical studies.[23,24] While a single randomized controlled trial in humans suggested that higher doses of aspirin may lead to higher circulating levels of 15*R*-LXA_4, this study did not assess an influence of body weight or adiposity on 15*R*-LXA_4 generation.[25] Given these observations, we propose that insufficient generation of 15*R*-LXA_4 with low-dose aspirin as body weight increases may contribute to the medication's reduced efficacy in prevention of MACE in higher weight individuals.

METHODS

Study design

We are conducting a double-blind, placebo-controlled, randomized, mechanistic crossover trial to test our hypothesis that low-dose aspirin results in reduced synthesis of 15*R*-LXA_4 as body weight increases, whereas higher doses of aspirin maximally induce 15*R*-LXA_4 regardless of body weight. Subjects undergo four phases in the following order: a placebo run-in, an active treatment phase, a placebo washout, and a second active treatment phase. The study arm determines the order in which the subject receives aspirin (Figure 1).

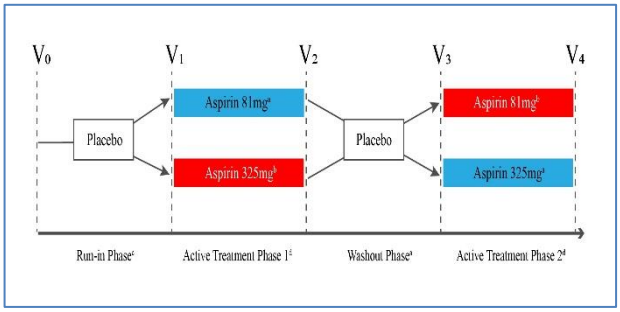

Figure 1: Crossover design with active treatment and washout phases ([a]Arm 1 receives 81 mg aspirin in first active treatment phase and 325mg aspirin in second active treatment phase, [b]Arm 2 receives 325 mg aspirin in first active treatment phase and 81mg aspirin in second active treatment phase, [c]Each placebo washout phase lasts for 21 -5/+9 days, [d]Each active treatment phase lasts for 21 -5/+9 days).

Table 1: Study inclusion and exclusion criteria.

Parameters		
Inclusion criterion		
Age 40-70 years		
Exclusion criteria		
Medical and surgical history	**Use of concomitant medications**	**Subject characteristics**
ASCVD	Anti-platelet medication in the past 7 days	Recent change in body weight >5kg[c]
Prior gastric or bariatric surgery	Corticosteroids	Pregnancy
Known bleeding or clotting disorder	Anti-coagulants	Active smoking
Chronic inflammatory or connective tissue disease	GLP-1 agonists	-
Diabetes mellitus	Recent initiation or change in dose of statin therapy[a]	-
Immunological deficiency	Inhibitors of COX-1/COX-2/lipoxygenases	-
Aspirin intolerance or allergy	Omega-3 fatty acid supplementation[b]	-
Platelet count <100,000		-

ASCVD: Atherosclerotic cardiovascular disease; COX-1/COX-2; cycloxygenase-1/ cycloxygenase-2, [a]Statin dose must have been stable for the 3 months prior to Visit 0, [b]Must have not taken omega-3 fatty acids in the 3 weeks prior to Visit 0, [c]Body weight must have not changed more than 5kg in the 3 months prior to Visit 0.

Study medication is administered via identical-appearing capsules and taken once daily. Active treatments are non-enteric coated formulations of aspirin. Each active treatment period lasts 3 weeks (Figure 1).

To account for scheduling preferences, weekends, holidays, and unforeseen events, the window for each visit is 21 -5/+9 days. To ensure adequate effects from medication, medication adherence must be ≥90%. We are employing a crossover design because we are testing a hypothesized interaction between aspirin dose and a single-subject factor, body weight, as opposed to testing multiple variables, leading to a greater intra- than interindividual correlation of response. The active treatment duration period of 3 weeks ensures the detection of an impact of aspirin dose on 15*R*-LXA_4, as prior studies suggest 10 days of 81mg aspirin are sufficient to increase circulating levels of AT-SPMs.[9] Similarly, the 3-week placebo washout period between active treatment phases is adequate to eliminate any carryover effect given the half-lives of salicylate and circulating blood cells.[26]

Randomization

To minimize the risk of differences in baseline characteristics between active treatment pathways, we allocate subjects in a 1:1 ratio between the two arms. To ensure a broad body weight range within the study, randomization is stratified by weight (1:2:2 ratio between <70 kg:70-100 kg: >100 kg) and sex (1:1 male:female). The target demographics of subjects in the study are designed to correspond to those of the greater New York City region. If participants discontinue early, they are replaced with another participant of the same sex and weight stratum until at least 18 subjects in the <70 kg stratum and 36 subjects in the 70-100 kg and >100 kg strata have completed Visit 2. Inclusion and exclusion criteria are listed in Table 1. Inclusion criteria reflect the age range at which aspirin's use for primary prevention has the greatest clinical relevance.[27]

Study procedures

The procedures that occur throughout the study are listed in (Table 2). Subjects are fasting for at least 8 hours before the study visit to minimize potential confounding effects on SPMs. Blood sampling using a 21G needle without tourniquet occurs at each visit. Blood is immediately transported to the lab for processing and analysis. Assays performed include complete blood count and differential, serum and plasma isolation, flow cytometry, neutrophil isolation, and peripheral blood mononuclear cell (PBMC) isolation. Plasma, serum and supernatants from activated neutrophil specimens are mixed 1:2 with a volume of ice cold methanol, vortexed and immediately stored at -80°C for quantification of a diverse panel of bioactive lipids including SPMs, leukotrienes and prostaglandins using tandem mass spectrometry.[28]

Complementary measures of plasma 15*R*- LXA_4 are also made using ELISA (Neogen, Lansing, MI USA) as previously reported.[21,25] The Dietary History Questionnaire II (DHQII) is a validated method for dietary intake assessment.[29-31] Complementary intake data will be collected via a 3-day food diary and analyzed with Food Processor (ESHA, Salem, USA), a validated method for analyzing macro- and micronutrient content based on self-reported food intake.[32] Furthermore, the International physical activity questionnaire (IPAQ) is a validated measure for assessing levels of physical activity in adults.[33,34] Finally, assessment of the sublingual endothelial glycocalyx, a network of proteins on the

endothelial cells on the lumen, is performed using a handheld sidestream darkfield microscan video microscope (KK Technology, Honiton, England), then analyzed using Glycocalyx Measurement Software (GlycoCheck, Maastricht, the Netherlands), which is a validated way to assess capillary and endothelial glycocalyx function.[35-37]

Table 2: Study procedures by visit.

Study visit	Visit 0	Visit 1	Visit 2	Visit 3	Visit 4
Informed consent	X				
Inclusion & exclusion criteria review[a]	X	X	X	X	X
Current and prior medical history[a]	X	X	X	X	X
Concomitant medication review[a]	X	X	X	X	X
Treatment-related AE monitoring[b]	X	X	X	X	X
Height	X				
Body weight	X	X	X	X	X
Pill count[c]		X	X	X	X
Dispense study medication	X	X	X	X	
Blood pressure	X	X	X	X	X
Waist and hip circumference	X	X	X	X	X
Blood draw[d]	X	X	X	X	X
Food assessment (DHQII)		X[e]			
Food diary		X[e]			
Activity questionnaire (IPAQ)		X[e]			
Bioelectrical Impedance Scale			X[f]		
DXA Scan			X[f]		
Glycocalyx microscopy[g]	X	X	X	X	X

DHQII: Dietary History Questionnaire II; IPAQ: International Physical Activity Questionnaire, [a]Assessed at each study visit as well as in between visits via check-in phone calls, [b]Adverse events are monitored and assessed throughout the study, [c]Subject returns pill bottle at each visit and pills are counted to assess medication adherence, [d]Subjects fast for at least 8 hours prior to blood draw, [e]Typically occurs at Visit 1, but can occur at any subsequent visit throughout the study, [f]Typically occurs at Visit 2, but can occur at any subsequent visit throughout the study, [g]Sidestream darkfield microscopy of sublingual microvasculature to evaluate the sublingual endothelial glycocalyx

Primary endpoints

Our primary endpoint is the difference in change of plasma 15*R*-LXA$_4$ occurring with each dose of aspirin. We hypothesize that as body weight increases above 70 kg, 81 mg aspirin will result in smaller increases in 15*R*-LXA$_4$ relative to 325 mg.

The slopes of the change in plasma 15*R*-LXA$_4$ concentration from placebo to each aspirin dose will be compared across the range of body weights of study subjects. Specifically, we hypothesize that response to 325 mg aspirin by body weight will exhibit a slope of m=0, whereas response to 81 mg aspirin across body weights will exhibit a negative slope.

Based on preliminary data, we hypothesize that for every 1kg increase in body weight there will be a 3.0% lower increase in 15*R*-LXA$_4$ to 81mg compared to 325 mg aspirin. The overall null hypothesis we will test is that there are no differences in the change of the selected study endpoints in association with body weight between the two doses of aspirin.

Sample size calculation and power analysis

Our preliminary data suggest medium and large effect sizes of obesity on 15*R*-LXA$_4$ in platelet releaseate and conditioned media of neutrophils incubated with platelet releasate, respectfully. Others have reported a medium effect size (d=0.7) of a difference in average body weight of ~45 kg on plasma 15*R*-LXA$_4$.[38] In the only published study of the effects of aspirin dose on 15*R*-LXA$_4$ in humans, 32 subjects yielded adequate power to detect a small effect of 81mg aspirin on plasma levels of this SPM.[25] Based on these observations, we conservatively estimate that for every 1kg increase in body weight, there will be a 3.0% lower increase in 15*R*-LXA$_4$ with 81mg aspirin compared to 325mg aspirin.

Recruitment will end when 90 subjects have completed Visit 2. We anticipate enrolling 125 subjects to achieve this goal, assuming an overall 28% rate of withdrawal. Assuming ≤20% attrition following Visit 2, our sample size of 90 subjects will result in at least 72 subjects completing both 81mg and 325mg active treatment phases. This achieves 80% power to reject the null hypothesis of equal slopes (15*R*-LXA$_4$ vs. bodyweight)

when the actual difference in population slopes is 0 vs. -0.026 in the 325mg and 81mg conditions, respectively. These calculations assume a standard deviation in body weight of 18kg and a standard deviation of residuals in 15*R*-LXA_4 of 1.

Statistical analysis

We will specifically test the interaction between weight and treatment efficacy of 81mg and 325mg aspirin using

$$Y_{ij} = \beta_0 + \alpha_0 W_i + \beta_1 P_{ij} + \alpha_1 W_i P_{ij} + b_i + e_{ij}$$

Where W_i is the bodyweight of individual i as a continuous variable. In this model, $\alpha_0 W_i$ represents the efficacy of 81mg aspirin, in which α_0 specifically represents whether the efficacy is correlated with the subject's bodyweight.

$$\beta_1 + (\alpha_0+\alpha_1)W_i$$

represents the efficacy of the 325 mg dose (compared to 81 mg) where the interaction term α_1 specifically represents whether the slope of efficacy with bodyweight is the same between 81mg and 325mg aspirin, and β_1 represents the weight independent part of the efficacy of 325 mg aspirin. We will explicitly test the hypothesis of the interaction term:

$$H_0: \alpha_1 = 0$$

We hypothesize that the slope of efficacy with bodyweight is different between 81 mg and 325 mg aspirin. We will also explicitly test the hypothesis.

$$H_0: \alpha_0 = 0$$

We hypothesize that the relative efficacy of 81 mg aspirin decreases as bodyweight increases, yielding yielding a negative slope. If the relationship between efficacy and bodyweight is not linear, we will use a spline model of the above equation to determine the best fit relationship. All p values will be adjusted for multiple testing with an alpha of ≤ 0.05 set as the criterion for statistical significance. We will analyze the primary and secondary outcomes using an intention-to-treat principle, with a per-protocol analysis as a sensitivity analysis.

Secondary endpoints

Our secondary endpoints are the bioactive lipid (SPMs, leukotrienes, prostaglandins) profiles of plasma (except 15*R*-LXA_4), serum, and supernatants isolated from activated neutrophils. Additional secondary endpoints include platelet-monocyte aggregates and platelet-neutrophil aggregates. Lastly, we will assess leukocyte surface expression of receptors involved in inflammation resolution pathways. These secondary endpoints were chosen given their relationships with 15*R*-LXA_4 and relevance to resolution of vascular inflammation.

Tertiary endpoints

For our tertiary endpoints, we will assess the microvascular function in the sublingual endothelial glycocalyx in association with different doses of aspirin. This tertiary endpoint was chosen because the endothelial glycocalyx has been implicated in the progression of atherosclerosis and other vascular diseases.[39, 40] We will also assess associations of macro- and micronutrient content with primary and secondary endpoints.

Exploratory endpoints

Exploratory analyses will include analyses of primary, secondary, and tertiary endpoints based upon alternative measures of body composition, such as BMI, waist circumference, lean body mass, and adipose mass, rather than

body weight. These measures are assessed using bioelectrical impedance and a DXA scan (Table 2), validated techniques to assess body composition.[41-43]

Recruitment

Enrollment began in November 2021 and is ongoing. Recruitment is conducted through NYU Langone Health (NYULH) at clinics within the Leon H. Charney Division of Cardiology as well as the Bariatrics Surgical Associates and Weight Management Program. Additionally, NYULH's DataCore service is used to identify and contact large groups of NYULH patients who fit study criteria. Interested patients then voluntarily indicate their interest in being contacted to discuss the study further. Recruitment is also conducted via ResearchMatch, a national health volunteer registry. ResearchMatch has a large population of volunteers who have consented to be contacted by researchers about health studies for which they may be eligible.

Retention

Several measures are taken to enhance retention throughout the study. Subjects are compensated for their time in the study and reimbursed for transportation costs associated with study visits. Additionally, the study team conducts one check-in call between study visits to discuss medication adherence, assess for adverse events, and answer questions about upcoming study visits. To further medication adherence and to improve records of any adverse events, subjects are asked to keep a medication diary between visits. This diary can be kept either on paper or using MyCap, a patient-facing smartphone application through REDCap which gives the subject daily reminders to complete their medication diary. Subject-reported adverse events are assessed to determine their severity, relationship to study agent, and

expectedness. Adverse events which are severe, related, and unexpected are reported to the NYULH Institutional Review Board (IRB). Review and approval for this study and all procedures was obtained from the NYULH IRB.

Unmasking

Unmasking will occur once all participants have completed study procedures and all analyses for planned primary and secondary endpoints have occurred.

DISCUSSION

We hypothesize that insufficient COX-2 acetylation and resulting impaired 15*R*-LXA$_4$ synthesis is responsible for low-dose aspirin's lack of efficacy for primary prevention of MACE in people with body weights above 70 kg. The double-blind, placebo-controlled, randomized, mechanistic crossover trial described in this manuscript tests the effect of body weight and aspirin dose on circulating 15*R*-LXA$_4$, and its results have the potential to improve our incomplete understanding of the reason(s) behind the ineffectiveness of low-dose aspirin for primary prevention of cardiovascular events in people with body weights greater than 70 kg. Key features of our novel study include the application of a crossover design, inclusion of equal proportions of men and women with a diverse profile of race and ethnicity, collection of numerous complementary inflammation-related measures and biosamples, and use of exploratory measures of body composition. Despite the strengths of our study design, it is not without limitations. While ours is the first RCT to study the influence of body weight on the effect of aspirin dose in stimulating AT-SPM synthesis, it is still of relatively modest size and may not provide adequate power to detect a meaningful difference in all of our outcomes of interest. For these reasons, we chose measures which our preliminary data and other previous reports suggest are highly affected by body weight, reproducible, and translatable to clinical measurement in patients.[44] Similarly, a crossover design makes this study more prone to attrition due to the longer duration of subject participation however, recruitment and retention plans both account for and reduce the anticipated increase in the rate of withdrawal. Given aspirin's favorable side-effect profile, we expect a low rate of attrition due to side effects; still, in the event of subject dropouts, data for a subject who completes one active treatment phase can be used to test the effect of body weight on one dosage's impact on SPMs. Further, our mechanistic trial is performed in a population without known atherosclerotic cardiovascular disease (ASCVD); however, there is equipoise regarding the utility of preventive aspirin therapy for patients without ASCVD. Notably, similar associations of body weight with aspirin efficacy have been observed in secondary prevention of stroke.[10] However, rigorously testing our hypothesis in patients with established atherosclerotic disease would require washout of the medication and changing the daily dose, without adequate data to justify any potential risk of these therapeutic alterations. If our hypothesis is supported, it will justify further investigation in secondary prevention populations. The results of this trial may inform future aspirin dosing recommendations for different individuals based on body weight and improve our understanding of the mechanisms underlying aspirin's benefits in the prevention of MACE. Even if our hypotheses are not supported by the experimental data, our results will provide a wealth of novel data in an area that is relatively understudied despite immense clinical significance. If our hypotheses are supported, the results may lead to improvements in primarily prevention to eventually minimize the vast toll of death and illness caused by adverse cardiovascular events.

CONCLUSION

This article summarizes the protocol for a double-blind, placebo-controlled, randomized, mechanistic crossover trial to assess the levels of plasma 15-epi-lipoxin A$_4$ occurring with different doses of aspirin. The results of this trial may improve our understanding of how body weight influences the dose of aspirin that can most effectively prevent MACE, allowing clinicians and researchers to work towards a reduction in the associated morbidity and mortality of adverse cardiovascular events.

Funding: This trial is funded by the National Heart, Lung, and Blood Institute through the National Institutes of Health, R01HL157430
Conflict of interest: None declared
Ethical approval: The study was approved by the Institutional Ethics Committee

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Cite this article as: McGowan NG, Zhong JH, Trasande L, Hellmann J, Heffron SP. A randomized, placebo-controlled crossover trial to assess the influence of body weight on aspirin-triggered specialized pro-resolving mediators: protocol for the discover study. Int J Clin Trials 2024;11(1):53-60.