Development of a Novel Clinical Trial Design to Evaluate the Effects of Joint Therapeutics on Cartilage Turnover in Healthy Subjects

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evaluate arthritis, the Osteoarthritis Biomarkers Network funded by the National Institutes of Health/National Institute of Arthritis, Musculoskeletal, and Skin Disease (NIH/NIAMS) proposed a classification scheme for biomarkers to provide a common format for communication of research in this area. This scheme is termed BIPED which is an acronym for Burden of disease, Investigative, Prognostic, Efficacy of intervention, and diagnostic [8]. These characteristics help to rank biomarkers as to their clinical utility in diagnosing and treating arthritis. Based upon these criteria, indicators of cartilage turnover (i.e. synthesis and degradation) have moved to the top of the list of biomarker candidates likely to be most useful. We chose to investigate c-terminal cross-linked telopeptide of type-II collagen (CTX-II), a marker of cartilage degradation, as an indicator of chondroprotective effects from joint therapeutics. Urinary CTX-II levels are known to be substantially elevated in those afflicted with articular joint diseases, but levels are also known to be elevated in a variety of healthy subsets of the population, as well. Urinary CTX-II levels have been shown to be elevated due to high-impact exercise in healthy college-aged endurance athletes such as cross-country runners by about 85% over age- and weight-matched controls but were not significantly elevated in lower-impact endurance athletes like swimmers and rowers [9]. Urinary CTX-II has also been shown to be about 2-fold higher in post-menopausal women versus age-matched pre-menopausal women and moderately elevated (~25%) in overweight people (BMI $\geq$ 25 kg/m^2) versus normal-weight controls (BMI<25 kg/m^2) [10].

The fact that large portions of the population develop arthritis combined with the fact that there are currently no approved disease-modifying or chondroprotective agents results in an obvious urgent need for safe and effective joint therapeutics. To our knowledge, there are presently no clinical models designed to evaluate chondroprotective joint therapeutics. The aim of this research investigation, therefore, was to develop a simple and rapid clinical trial design to aid in the evaluation of chondroprotective joint therapeutics, and we hoped to take advantage of the apparent sensitivity of the articular cartilage of post-menopausal women and/or overweight individuals to evaluate joint therapeutics through increasing joint strain *via* exercise while monitoring uCTX-II output.

Patients and Methods

Initial investigation of design variables

The initial critical variable investigated was to determine whether or not CTX-II production and urinary clearance would occur in a narrow enough time frame to be useful for our envisaged purpose. Individuals clear proteins at differing rates, so it was also important to determine if the clearance rate was sufficiently

alternating days for two consecutive weeks on a 14 degree inclined treadmill at an approximate pace of 1.7 miles per hour. Group B subjects performed exercise for a minimum of 7 minutes on alternating days for two consecutive weeks on a seated step machine (NuStep® brand) with a workload of 7.0 and a pace of 30-40 steps per minute. Group C subjects performed 3 sets of 8 li s each of 90 pounds (41 kg) on a seated leg press (Cybex® brand) in a maximum of 7 minutes on alternating days for two consecutive weeks. All subjects provided urine samples for basal CTX-II level determination and urine samples at the end of each week for comparison of CTX-II levels to baseline. Urine samples were obtained from the 2nd void of the morning collected within 12-24 hours a er each subject completed the nal exercise for the week. Samples were frozen (-20°C) immediately following collection until needed for assaying.

Adverse events

e participants'

subjects [11,12] and well within the expected normal range for women of their age, BMI and hormonal status [10].

	Group A	Group B	Group C
Age (yrs)	58.2 ± 4.7	52.4 ± 6.2	57.8 ± 7.1
Weight (kg)	73.4 ± 11.1	83.5 ± 18.2	77.2 ± 12.2
BMI	26.8 ± 3.4	30.1 ± 4.4	28.6 ± 4.2
uCTX-II (ng/mmole Cr)	259 ± 102	219 ± 98	288 ± 84
Notes: Values are reported as mean ± standard deviation (n=10 per group). There were no statistical differences between treatment groups in any of the listed parameters. **Abbreviations:** BMI: Body Mass Index; calculated as weight in kilograms divided by the square of height in meters. uCTX-II: urinary c-Terminal Crosslinked Telopeptide of type-II collagen, reported as nanograms per millimole of Creatinine (ng/mmol Cr).			

Table 1: Period 1 participant baseline demographic data.

Table 2 contains urinary CTX-II results for the test groups of subjects (Group A not shown) at baseline and a er preforming the

(Period 2). e groups were not statistically di erent in any of the baseline demographic data using the non-parametric Mann-Whitney U Test for independent groups. It is also signi cant that their uCTX-II levels had returned to levels similar to what was found in Period 1 of the study following the 3-week resting period. Again, CTX-II levels remained consistent with being healthy post-menopausal females. Table 4 presents the uCTX-II results for the two groups of subjects at baseline and a er preforming the designated exercise regimen for two consecutive weeks.

	Group 1	Group 2
Age (yrs)	56.2 ± 8.0	56.1 ± 4.8
Weight (kg)	78.4 ± 12.1	77.7 ± 16.7
BMI	28.4 ± 3.7	28.6 ± 4.7
uCTX-II (ng/mmole Cr)	220 ± 97	174 ± 76

Notes: Values are reported as mean ± standard deviation (n=15 per group). There were no statistical differences between treatment groups in any of the listed parameters.
Abbreviations: BMI: Body MADermr
Telopeptide Æype-II collagen, reported nogramMper mM=MMM'

	Weeks Post-treatment	Treatment			Absolute Treatment Effect
		Untreated	NEM		
uCTX-II	Baseline (n=10, 10)	288 ± 84	237 ± 82		-
	1 (n=10, 10)	331 ± 117	227 ± 85		-19.10%
	2 (n=10, 10)	313 ± 108	230 ± 70		-11.7%[#]
Immediate	Baseline (n=10, 10)	0.1 ± 0.3	0.6 ± 1.0		-
Pain	1 (n=10, 10)	1.0 ± 0.8	0.8 ± 0.9		-867%[#]
	2 (n=10, 10)	0.8 ± 0.8	0.7 ± 0.8		-683%[#]
12-hour	Baseline (n=10, 10)	0.1 ± 0.3	0.6 ± 1.0		-
Pain	1 (n=10, 10)	1.0 ± 1.1	0.7 ± 0.9		-883%[*]
	2 (n=10, 10)	0.7 ± 1.1	0.4 ± 0.7		-633%[#]
Immediate	Baseline (n=10, 10)	0.3 ± 0.5	0.8 ± 1.1		-
Stiffness	1 (n=10, 10)	1.0 ± 0.8	0.9 ± 1.1		-220%[#]
	2 (n=10, 10)	1.2 ± 1.0	0.6 ± 0.8		-325%[*]
12-hour	Baseline (n=10, 10)	0.3 ± 0.5	0.8 ± 1.1		-
Stiffness	1 (n=10, 10)	1.2 ± 1.0	0.9 ± 1.3		-287%[#]
	2 (n=10, 10)	0.9 ± 1.1	0.6 ± 0.8		-258%[*]

Notes: Values are reported as mean ± standard deviation. Absolute Treatment Effect is the net difference of NEM treatment versus untreated for the change in mean treatment effect from baseline expressed as a percent. Negative values for pain or function indicate superior improvement in the treatment group.
Abbreviations: *p<0.05; #p<0.10 versus untreated

structural integrity of the articular cartilage results. Products of this degradation imbalance can be found in both blood and urine of arthritic patients. Initially cartilage breakdown occurs *via* slow but extensive proteoglycan loss in the ECM matrix resulting in loss of cartilage thickness. e process culminates in the breakdown of the brillar