Respiratory physiological perturbations after acute smoke-induced lung injury and during extracorporeal membrane oxygenation support in sheep

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Abstract

Numerous successful therapies developed for human medicine involve animal experimentation. Animal studies that are focused solely on translational potential, may not sufficiently document unexpected outcomes. Such studies often involve hastily developed methods, thereby leading to considerable amounts of archived data that could be used to advance veterinary science or to refine the base animal model. For example, sheep are increasingly being used as models of intensive care and therefore, any experimental data arising from such models must be interpreted and published. In this study, the hypothesis is that there is little information describing physiological data from multifaceted sheep models of intensive care and the author aimed to analyse such data to provide biological information that is currently not available for sheep that received extracorporeal life support (ECLS) following acute smoke-induced lung injury. Data from 19 mechanically ventilated adult ewes that were undergoing intensive care in a study that evaluated a form of ECLS (treatment) for acute lung injury were used to collate clinical observations. Eight sheep were injured by acute smoke inhalation prior to treatment (injured/treated), while another eight were not injured but treated (uninjured/treated). Two sheep were injured but not treated (injured/untreated), while one received room air instead of smoke as the injury and was not treated (placebo/untreated). The data were then analysed for eleven physiological categories and compared between the two treated groups. Compared with the baseline, treatment contributed to and exacerbated the deterioration of pulmonary pathology by reducing lung compliance and the arterial oxygen partial pressure to fractional inspired oxygen (PaO_2/FiO_2) ratio. The oxygen extraction index changes mirrored those of the PaO_2/FiO_2 ratio. Decreasing coronary perfusion pressure predicted the severity of cardiopulmonary injury. These novel observations could help in understanding similar pathology such as that which occurs in animal victims of smoke inhalation from house or bush fires, aspiration pneumonia secondary to tick paralysis and in the management of the severe coronavirus disease 2019 (COVID-19) in humans.

Introduction

During multifaceted experiments involving intensive care in large animal models in translational research, information related to animal monitoring is often collected with varying accuracy, scope, and end-user applications. Data collection can be manual, electronic, or both [1-3]. Manually input data can include subjectively scored end points, like the plane of anaesthesia, and objective data such as heart rate or breaths per minute. Depending on the goals of the study, certain information may be used to validate or test novel therapies, or to understand and refine existing treatments. In certain cases, experimental information may be collected for scientific curiosity or for 'classified' use, and outcomes may never be publicly available, particularly if the results are negative.

The source of data for this study was from a sheep model [2-5] in which sheep were treated for acute smoke-induced acute lung injury using veno-venous (VV) extracorporeal membrane oxygenation (ECMO) [2], a form of extracorporeal life support (ECLS) developed to complement the treatment of acute lung injury in humans [6-8]. During this type of ECLS, venous blood is carried from the patient to a gas exchange device where the blood is enriched with oxygen, has carbon dioxide is removed, and oxygenated blood is returned to the patient's circulation in the right atrium. This method can be used for treatment, as respiratory support during lung transplantation, and in critically ill patients with potentially reversible respiratory failure [8,9]. The multiple advanced cardiovascular [3], respiratory, patient point-of-care procedures and instrumentation associated with ECLS even in animal experimentation is highly data- and equipment-intensive. This platform is useful for developing research and methodological skills for *in vivo* animal instrumentation and for the processing of large, real-time clinical data sets from multifaceted animal studies that can be applied to similar intensive care scenarios. An opportunity to develop these skills arose within a source study conducted at Queensland University of Technology and The University of Queensland, which was an ongoing publicly funded animal experimentation study. While the objectives of the primary study had a separate focus, there were considerable amounts of redundant raw data with

potential use in veterinary science and other disciplines, once processed. The author hypothesised that there is little information describing physiological data from multifaceted sheep models of intensive care and the author aimed to analyse such data to provide biological information that is not currently available for sheep that receive ECLS following acute smoke-induced acute lung injury.

The overall goal was to provide useful information relevant to the sheep model itself as well as to those interested in broad animal experimentation and veterinary medicine in general. The specific objective was to utilise the raw data from the sheep ECMO model study and analyse that data to provide biological information that is not currently available for sheep that receive ECLS following acute smoke-induced lung injury to further understand the physiology of respiratory support.

Materials and methods

Ethics statement

Animals were obtained and treated in accordance with the Australian Code of Practice for the Care and Use of Animals for Scientific Purposes [10]. All studies were registered with institutional animal welfare and ethics departments; moreover, the Queensland University of Technology Animal Ethics Approval No. 110000053 was obtained and it was ratified by The University of Queensland.

Animal experimental groups

The study involved 19 sheep divided into 4 experimental groups (Table 1). The experimental groups were classified based on the following two aspects: the duration of treatment (24 hours of ECMO only—E24H); treatment after smoke inhalation (injury) (24 hours of ECMO after smoke inhalation—SE24H). Two additional groups included one group that received smoke inhalation injury but no treatment (24 hours of monitoring only after smoke inhalation and no ECMO; SC24H), and another group that inhaled room air only as the injury (placebo) and no treatment (24 hours of monitoring, no smoke inhalation and no ECMO; C24H). Robust data was acquired from 16 sheep (E24H and SE24H) and included fully in the study; however, data of three sheep from groups

SC24H and C24H was considered only as early observational data. A systematic approach was developed for processing the data. (All raw and processed data can be downloaded at doi:10.5061/dryad.3r2280gd5.)

Table 1. Characteristics of treated and untreated groups (control experiments) of sheep

Experiment Group	Date of experiment	Sheep No.	Age (Y)	Weight (kg)	Length of Sheep (cm)	BSA
E24H	06/10/2011	E24H-01/390	2	50	110	1.29
	20/10/2011	E24H-02	2	47.6	110	1.25
	17/11/2011	E24H-03	2	51	110	1.31
	01/03/2012	E24H/4616	2	50	110	1.29
	29/03/2012	E24H-05/4627	2	47	110	1.24
	04/04/2012	E24H-06/4146	2	40	110	1.11
	12/04/2012	E24H-07/4032	2	52.5	110	1.34
	03/05/2012	E24H-08/4630	2	53	110	1.34
SE24H	02/02/2012	SE24H-01/4139	2	44	110	1.19
	09/02/2012	SE24H-02/4542	2	53	110	1.34
	16/02/2012	SE24H-03/4280	2	45.5	110	1.21
	23/02/2012	SE24H-04/4624	2	50	110	1.29
	17/05/2012	SE24H-05/4458	2	55	140	1.38
	24/05/2012	SE24H-06/8461	2	46	140	1.22
	24/01/2013	SE24H-07/09C8032	3	52	130	1.33
	21/02/2013	SE24H-09A0142	2	50	140	1.29
SC24H	18/06/2013	SC24H-01	2	51	140	1.31
	27/06/2013	SC24H-02	2	57	140	1.41
C24H	08/08/2013	C24H-01	2	53	140	1.34

Table 1 legend: BSA = Body surface area; E24H = uninjured sheep treated with extracorporeal life support (ECLS) for 24 hours (uninjured/treated); SE24H = sheep with acute smoke-induced lung

injury treated with ECLS for 24 hours (injured/treated); SC24H = sheep with acute smoke-induced lung injury monitored for 24 hours without ECLS (injured/untreated); C24H = sheep subjected to room air injury as a control for smoke and monitored for 24 hours without ECLS (placebo/untreated).

Experimental procedures

Critical care of animals, VV ECMO setup and physiological data acquisition

The study was performed at the purpose-built Medical Engineering Facility of Queensland University of Technology (QUT-MERF) at the Prince Charles Hospital Campus of The University of Queensland as previously described elsewhere [1].

The details of animal selection, care, and pre-anaesthetic processes; anaesthesia technique; airway access and ventilation; instrumentation for VV ECMO; haemodynamic monitoring; respiratory monitoring; temperature, fluids, vasoactive drug administration, and electrolyte management; blood collection; physiological data acquisition; and the technique for euthanasia of the sheep after the experiments have previously been described in a detailed protocol that can be accessed at https://www.ncbi.nlm.nih.gov/pmc/articles/PMC3982457/ or doi: 10.1155/2014/468309 [1]. In brief, the sheep was restrained in a sling cage and the ventral neck region was aseptically prepared to enable intravenous access. For VV ECMO implementation, venous blood was accessed from the right jugular vein of the animal and then oxygenated and returned to the right atrium of the heart after it was made to pass through an oxygenator. For the combined purpose of blood sampling, administration of medications, and fluid administration, a multi-lumen central venous catheter was inserted into the left jugular vein of the animal under local anaesthesia. The left jugular vein was also cannulated with an 8G sheath for the insertion of a pulmonary artery catheter for haemodynamic monitoring. In addition, an 11G sheath catheter was then inserted proximally into the left jugular vein for intra-cardiac echocardiography catheter insertion. The right jugular vein was cannulated both proximally and distally with single lumen central lines to aid insertion of return and access ECMO cannulas, respectively. All animals were intubated and received mechanical ventilation as previously described [1]. Briefly, the initial ventilator tidal volume was set to approximately 10

mL/kg with a respiratory rate of 15 breaths/min, positive end expiratory pressure (PEEP) of 5 cm H_2O, and an initial F_iO_2 (fraction of inspired oxygen) of 1.0. These settings were then titrated based on arterial blood gas results. A low tidal volume—high PEEP strategy was used to minimise ventilator-induced lung injury.

In order to obtain high-quality cardiorespiratory monitoring data, the instrumentation of the sheep was undertaken to acquire and derive the following physiological parameters using established standard methods at defined timepoints: core body temperature (T), pO_2, SpO_2, alveolar–arterial oxygen gradient $P(A\text{-}a)O_2$, PaO_2/FiO_2 ratio, end-tidal carbon dioxide concentration ($EtCO_2$), heart rate (HR), arterial blood pressure (BP) (systolic and diastolic), mean arterial BP (MAP), pulmonary artery pressure (PA) (systolic and diastolic), mean pulmonary artery pressure (MPAP), central venous pressure (CVP), pulmonary artery occlusion pressure (PAOP), mixed venous oxygen saturation (SvO_2), stroke volume (SV), continuous cardiac output (CCO), cardiac index (CI), systemic vascular resistance (SV), systemic vascular resistance index (SVRI), pulmonary vascular resistance index (PVRI), left ventricular stroke work index (LVSWI), right ventricular stroke work index (RVSWI), coronary perfusion pressure (CPP), arterial oxygen content (CaO_2), and oxygen delivery index (O_2EI). (These methods are detailed in data files that can be downloaded at doi:10.5061/dryad.3r2280gd5.)

Smoke inhalation injury

In the original study of the ECMO model [4], sheep inhaled standardised cotton smoke generated by a device that combusts material in an oxygen-deficient environment as previously described [11]. In brief, 8 g of cotton towelling was combusted in a chamber with transparent walls and 400 ml tidal volume. One tidal volume breath (approximately 10–12 ml/kg) of the smoke was delivered to the sheep via plastic tubing that was one m long connected to a tracheostomy tube. A fixed number (12) of breaths were given with each load of cotton over a period of approximately one minute. Serial arterial blood gas samples were taken to assess the effect of smoke inhalation, beginning at a predetermined time point after the smoke breath cycles.

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Physiological data management

155 Raw data were obtained from critical care monitoring of sheep undergoing treatment for
156 acute smoke-induced lung injury that involved several separate previous projects. Data analysed
157 were collected prior to 23 August 2013 and were obtained from two of the scientists (KD and SD –
158 see Acknowledgements) who developed the base model [4], as part of a research higher-degree
159 research project of the author at The University of Queensland [12,13]. All data files were stored in
160 Microsoft® Excel 97–2003 (Microsoft Corporation, Redmond, WA, USA) format and were grouped
161 per sheep and date of the experiment. Data comprised separate files of real-time physiological data
162 recorded in the hard drives of the monitoring devices (electronically acquired data) and parameters
163 manually recorded by those monitoring the sheep under anaesthesia (manually acquired data)—
164 which included data from the electronic monitoring equipment—as back-up if the electronic
165 monitors malfunctioned.

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Manually acquired physiological data workflow

168 A clone of the master manual data entry Excel spreadsheet was created by excluding the
169 formatting and formulas. Several members of the sheep ECMO research team repeatedly inspected
170 the data for errors to in order to ensure that all columns, rows, time points, and data points had been
171 copied correctly, including number formats. Redundant columns were omitted from the spreadsheet
172 and data were curated and aligned to predetermined experimental time points. While maintaining the
173 same experimental time point headers on the spreadsheet, data were grouped into the following
174 categories: ventilator settings, blood pressure and haemodynamics, fluids and urine output, arterial
175 blood gas values, activated clotting time, anaesthetics, anticoagulants, and ECLS circuit
176 observations.

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Electronically acquired physiological data workflow

Electronically acquired physiologic monitoring raw data were inspected for completeness. The data comprised 36 time points: ECLS pump time (min); time of day (h); electrocardiograph (heart rate); arterial blood pressure (mean, systolic, diastolic, heart rate); central venous pressure (mean); pulmonary artery pressure (mean, systolic, diastolic); oxygenator pressure (pre- and post-); capnography (end-tidal carbon dioxide ($etCO_2$), respiratory rate); pulse oximetry (SpO_2, heart rate); ECLS pump (flow rate, speed); ventilator (mode, frequency, oxygen, pressure control, inspiratory volume, expiratory volume, expiratory minute volume, pressure maximum, mean pressure, positive end-expiratory pressure, plateau pressure, inspiratory resistance, expiratory pressure, pulmonary compliance, inspiratory flow); mixed venous oxygen saturation (SvO_2); and continuous cardiac output (CCO). A baseline time point was established after instrumentation and an injury time point corresponding to the smoke inhalation time point was determined thereafter. It is important to note that there may or may not have been any data at any given point in time. The electronically acquired physiological monitoring data were inspected for errors and cleaned to provide data for downstream analysis.

Respiratory efficiency and haemodynamic monitoring

Manually acquired observations at specifically designated timepoints were recorded and extracted as detailed in the data filed at doi:10.5061/dryad.3r2280gd5. These timepoints were at baseline (soon after instrumentation of the sheep), smoke injury, five min post smoke injury, one hour post smoke injury. This was then followed by ECMO treatment, which was recorded in the following manner: 0, 0.25, 1, 1.5, 2, 4, 6, 6.5, 7, 8, 10, 12, 14, 16, 18, 20, 22 and 24 hours of ECMO.

Pre-data analysis checks

Thereafter, data were subjected to further integrity checks. An important step was to make a plot of data versus time together with descriptive statistics for all data points in the grouped data.

After artefact elimination and integrity checks, data for individual sheep were assigned to six categories: activated clotting time; anaesthetics + inotropes and anticoagulants; arterial blood gas values; blood pressure + ventilation and haemodynamic data; calculated respiratory + haemodynamic variables; and fluids and urine production. Using specially written macros, data were extracted from each experiment and grouped by parameters corresponding to experimental time points. All sheep treatment data were then filed according to parameter.

Data from the 19 sheep from groups E24H, SE24H, C24H and SC24H were processed further. Data integrity checks were performed again and repeated by several sheep ECMO research team members. The treatment timeline comprised 22 time points for all experiments in which sheep received acute smoke-induced lung injury (SE24H). A trend plot and descriptive statistics panel in Excel were used for data quality control processes for suitability for downstream data analysis and end-user applications.

Statistical methods

In order to meet the specific objective of the study, data from the groups, uninjured/treated and injured/treated groups were analysed. The means, medians and standard deviations of the weights of the sheep, where applicable, were tabulated and graphically compared. The physiological parameters of the groups were charted and compared with each other using one-way analysis of variance (ANOVA), where appropriate. Further, parameters between groups were compared using a paired two-tailed t-test. All p-values were two-sided and $p < 0.05$ was considered statistically significant. All statistical calculations were performed using GraphPad PRISM 6 software (GraphPad Software, La Jolla, CA, USA).

Results

The biodata of the sheep that were used in the current analysis are presented in Methods section (see Table 1). The weights of the uninjured/treated sheep, unlike the injured/treated group,

did not pass the D'Agostino–Pearson omnibus normality test; however, there was no significant difference in the weights of the sheep between the groups (Fig 1).

Fig 1. Body weights (Mean ± SD) of smoke and non-smoke injured sheep that received extracorporeal life support (ECLS) as compared to those of untreated controls.

Mechanical ventilation

A decrease in pulmonary compliance was found in all of the sheep during the course of the experiments, with the injured/treated (SE24H) animals having the most severe and drastic decrease followed by the uninjured/treated (E24H), injured/untreated (SC24), and placebo/untreated (C24) sheep in that order (Fig 2). There was a significant difference ($p = 0.0013$) in pulmonary compliance between uninjured/treated and injured/treated groups. The injured/treated sheep had consistently lower SpO_2 compared with the other groups, but there was no significant difference in SpO_2 readings between the groups (Fig 3). Further, there was an initial increase in $etCO_2$ followed by a rapid decrease that reduced 15 minutes after the treatment was began. The $etCO_2$ of the injured sheep continued to trend downward and plateaued in the uninjured groups (Fig 4). There was a significant difference ($p = 0.0147$) in the $etCO_2$ between the uninjured/treated and injured/treated groups.

Fig 2. Pulmonary compliance (Mean ± SD) in smoke and non-smoke injured sheep that received extracorporeal life support as compared to that in untreated controls. Dotted lines represent error bar margins.

Fig 3. Arterial oxyhaemoglobin saturation (SpO_2) (Mean ± SD) in smoke and non-smoke injured sheep that received extracorporeal life support as compared to that in untreated controls.

Fig 4. End-tidal carbon dioxide tension ($etCO_2$) (Mean ± SD) in smoke and non-smoke injured sheep that received extracorporeal life support as compared to that in untreated controls.

Arterial blood gas evaluation

Blood pH varied between the groups (Fig 5). The placebo/untreated sheep had the highest pH while the injured/treated group had the lowest. There was a significant difference in pH between the uninjured/treated and injured/treated groups ($p = 0.0343$).The pCO_2 in all but the uninjured/treated sheep increased initially before plummeting sharply, thereby forming a shallow trough corresponding to one hour after the treatment, followed by a slight increase before stabilising in all sheep (Fig 6). There was a gradual decrease in pO_2 in the treated groups of sheep from baseline before decreasing dramatically at the start of treatment with the injured sheep having the most profound decrease (Fig 7). However, there was no significant difference in pO_2 between the uninjured/treated and injured/treated groups.

Fig 5. Arterial blood pH (Mean ± SD) over a 24h period in smoke and non-smoke injured sheep that received extracorporeal life support as compared to that in untreated controls. The dots represent hourly time-points.

Fig 6. Mean arterial carbon dioxide partial pressure (pCO_2) (Mean ± SD) in smoke and non-smoke injured sheep that received extracorporeal life support as compared to that in untreated controls.

Fig 7. Arterial oxygen tension (pO_2) (Mean ± SD with no error bars shown) in smoke and non-smoke injured sheep that received extracorporeal life support as compared to that in untreated controls.

Haemoglobin dynamics

The concentration of haemoglobin [Hb] was found to decrease slightly from baseline before gradually increasing in the injured sheep and remained relatively constant over time in the uninjured sheep. There was a significant difference in [Hb] between the uninjured/treated and injured/treated ($p = 0.0131$) groups (Fig 8). The fraction of oxyhaemoglobin (FO_2Hb) decreased sharply with the lowest reading at five minutes post-injury before returning to near baseline levels within an hour of the treatment (Fig 9). The injured/treated sheep had a considerably deeper trough in FO_2Hb level and

there was a significant difference ($p = 0.046$) between troughs. There was no change in FO_2Hb for the uninjured sheep. Further, the fraction of carboxyhaemoglobin (FCOHb) increased sharply from baseline, peaking at approximately five minutes post-injury and decreased sharply thereafter to the beginning of treatment before gradually returning to near-baseline levels at approximately six hours after the treatment was begun in the injured sheep (Fig 10). The injured/treated sheep had a higher peak FCOHb than the injured/untreated sheep, although the difference was not significant. There was no change in FCOHb for the uninjured sheep. The fraction of methaemoglobin (MetHb) increased gradually from baseline, peaking at approximately five minutes post-injury and then gradually decreased when the treatment was begun (Fig 11). This was followed by a gradual return to near-baseline levels at approximately six hours after the treatment was begun in the injured/treated sheep. There was no change in MetHb for the uninjured sheep. There was an initial subtle decrease in calculated haematocrit (Hct) before a steady increase in the injured sheep and relatively flat slopes for the uninjured sheep (Fig 12).

Fig 8. Haemoglobin concentration [Hb] (Mean ± SD) in smoke and non-smoke injured sheep that received extracorporeal life support as compared to that in treated controls.

Fig 9. Fraction of oxyhaemoglobin (FO_2Hb) (Mean ± SD) in smoke and non-smoke injured sheep that received extracorporeal life support as compared to that in untreated controls

Fig 10. Fraction of carboxyhaemoglobin (FCOHb) (Mean ± SD) in smoke and non-smoke injured sheep that received extracorporeal life support as compared to that in untreated controls.

Fig 11. Fraction of methaemoglobin (FMetHb) (Mean ± SD) in smoke and non-smoke injured sheep that received extracorporeal life support as compared to that in untreated controls.

Fig 12. Calculated haematocrit (Hct) (%) (Mean ± SD) in smoke and non-smoke injured sheep that received extracorporeal life support as compared to that in untreated controls.

Electrolytes

The blood sodium concentration [Na^+] was relatively stable and there were no significant differences between groups (Fig 13). There was an initial decrease in the blood calcium [Ca^{2+}] level, with the lowest point at approximately one hour after the treatment was begun, before it levelled out thereafter in all groups (Fig 14). Further, there was a significant difference in [Ca^{2+}] between the uninjured/treated and the injured/treated groups ($p = 0.0001$). The placebo/untreated and injured/treated groups maintained the highest and lowest levels of [Ca^{2+}], respectively, throughout the experiments. Blood chloride [Cl^-] levels remained stable compared with baseline levels during the initial stages and then increased gradually thereafter (Fig 15). The blood potassium concentration [K^+] initially decreased as compared with baseline levels, reaching a minimum concentration one hour after the treatment was begun and then gradually increased with a peak at approximately 12 hours after treatment was begun in all experimental groups (Fig 16). Although the injured/untreated and injured/treated sheep had higher [K^+] than the uninjured sheep, the differences were not significant. Overall, the anion gap decreased gradually, achieving a relatively gentle slope at approximately six hours after the treatment was begun and did not change significantly, thereafter (Fig 17). There was a gradual decrease in anion gap from baseline in the course of the experiments and there was no significant difference in anion gap between the uninjured/treated and injured/treated groups.

Fig 13. Concentration of sodium ions in blood (Mean ± SD) in smoke and non-smoke injured sheep that received extracorporeal life support as compared to that in untreated controls.

Fig 14. Concentration of calcium ions in blood (Mean ± SD) in smoke and non-smoke injured sheep that received extracorporeal life support as compared to that in untreated controls.

Fig 15. Concentration of chloride ions in blood (Mean ± SD) in smoke and non-smoke injured sheep that received extracorporeal life support as compared to that in untreated controls.

Fig 16. Concentration of potassium ions in blood (Mean ± SD, error bars not shown) in smoke and non-smoke injured sheep that received extracorporeal life support as compared to that in

untreated controls.

Fig 17. Anion gap (Mean ± SD, error bars not shown) in smoke and non-smoke injured sheep that received extracorporeal life support as compared to that in untreated controls.

Metabolites

Although there was an increase in blood glucose level [Glu] for the injured/treated sheep after six hours of treatment, the change was not significant. There was an initial decrease in lactate levels [Lac] six hours after the treatment was begun, followed by a gradual increase for the injured sheep, particularly for the injured/treated group. There was no significant difference in [Lac] between the treated groups.

Fig 18. Blood glucose concentration (Mean ± SD) in smoke and non-smoke injured sheep that received extracorporeal life support as compared to that in untreated controls.

Fig 19. Blood lactate concentration (Mean ± SD) in smoke and non-smoke injured sheep that received extracorporeal life support as compared to that in untreated controls.

Acid-base balance

There was an increase in the blood base levels [Base (ecf)] that peaked one hour post-treatment, followed by a gradual decrease in the untreated group. [Base (ecf)] in the treated groups remained at baseline levels to one hour after the treatment begun, before decreasing markedly in the injured/treated sheep (Fig 20). There was a significant difference ($p = 0.0257$) in base (ecf) between the uninjured/treated and injured/treated groups. Further, blood bicarbonate concentrations [HCO_3^-] increased initially in the untreated groups before decreasing gradually; however, levels remained higher compared with the treated sheep (Fig 21).

Fig 20. Concentration of base (ecf) in blood (Mean ± SD) for smoke and non-smoke injured sheep receiving extracorporeal life support alongside untreated controls.

Fig 21. Blood bicarbonate concentration (Mean ± SD) for smoke and non-smoke injured sheep receiving extracorporeal life support alongside untreated controls.

Haemodynamics

There was a gradual decrease in heart rate (HR) of the sheep during the course of the experiments, with the placebo/untreated groups maintaining a higher HR compared with the injured/untreated, injured/treated, and uninjured/treated groups early in the experiments (Fig 22). There was no significant difference in HR between the uninjured/treated and injured/treated groups. The mean arterial blood pressure (MAP) decreased in the early stages of the experiments before subsequently increasing gradually, peaking at approximately the time that the treatment begun before gradually decreasing again in all but the placebo/untreated sheep (Fig 23). The injured/treated groups had a consistently lower MAP compared with the other groups and there was a significant difference in MAP ($p = 0.0058$) between the uninjured/treated and injured/treated groups. The mean pulmonary artery pressure (MPAP) increased gradually, with the injured/treated group having a consistently higher MPAP (Fig 24). There was no significant difference in MPAP between the uninjured/treated and injured/treated groups. There was an initial, subtle increase in the central venous pressure (CVP) that peaked at approximately one hour post-injury followed by a decrease that stabilised at approximately one hour after the treatment was begun. Further, CVP levels in the injured/treated and placebo/untreated sheep were consistently higher and lower, respectively, in the course of the experiments.

Fig 22. Composite heart rate (HR) (Mean ± SD) in smoke and non-smoke injured sheep that received extracorporeal life support as compared to that in untreated controls.

Fig 23. Mean arterial blood pressure (MAP) (Mean ± SD) in smoke and non-smoke injured sheep that received extracorporeal life support as compared to that in untreated controls. Dotted lines represent one-sided margins of error bars.

Fig 24. Pulmonary artery pressure (PAP) (Mean ± SD, no error bars shown) in smoke and non-smoke injured sheep that received extracorporeal life support as compared to that in untreated controls.

Fig 25. Mean central venous pressure (CVP) (Mean) in smoke and non-smoke injured sheep that received extracorporeal life support as compared to that in untreated controls.

Mixed venous oxygen saturation (SvO_2) had a lower baseline before eventually rising to a relatively stable and higher level for the treated sheep, and a slightly lower level for the untreated sheep (Fig 26). The injured/untreated sheep maintained a consistently lower SvO_2 compared with the other groups. Except for the placebo/untreated group, there was a decrease in continuous cardiac output (CCO) from baseline to approximately one hour after the treatment was begun (Fig 27). There was a significant difference (p = 0.0009) in CCO between the uninjured/treated and injured/treated groups with CCO in the treated groups increasing sharply before plateauing, particularly in the uninjured/treated group. There was also a subsequent gradual decrease in CCO in the injured/treated group. Stroke volume (SV) began to increase one hour after the treatment was begun for all groups, except for the injured/untreated group in which levels remained relatively constant (Fig 28). The SV in the injured/treated group began to decrease after 6 hours of treatment, while SV in the uninjured/treated and placebo/untreated sheep increased steadily before decreasing or levelling out after 12 hours or more of treatment. There were no significant differences in SV between the injured/untreated and placebo/untreated. The stroke volume index (SVI) began to increase one hour after the treatment was begun for all groups, except for the injured/untreated group, for which SVI remained relatively constant (Fig 29). The SVI in the injured/treated group began to decrease after six hours of treatment while SVI in the uninjured/treated and placebo/untreated groups increased before subsequently decreasing or levelling out after 12 hours or more of treatment. There were no significant differences in SVI between groups. While the cardiac index (CI) of the uninjured/treated and placebo/untreated groups remained relatively close to baseline levels (Fig 30), the CI of the injured/treated and injured/untreated groups declined gradually over the course of the experiments.

Fig 26. Mixed venous oxygen saturation (SvO_2) (Mean ± SD, error bars not shown) in smoke and non-smoke injured sheep that received extracorporeal life support as compared to that in

untreated controls.

Fig 27. Continuous cardiac output (CCO) (Mean ± SD, error bars not shown) in smoke and non-smoke injured sheep that received extracorporeal life support as compared to that in untreated controls.

Fig 28. Stroke volume (Mean ± SD error bars not shown) in smoke and non-smoke injured sheep that received extracorporeal life support as compared to that in untreated controls.

Fig 29. Stroke volume index (SVI) (Mean ± SD, error bars not shown) in smoke and non-smoke injured sheep that received extracorporeal life support as compared to that in untreated controls.

Fig 30. Cardiac index (Mean ± SD error bars not shown) in smoke and non-smoke injured sheep that received extracorporeal life support as compared to that in untreated controls.

After an initial increase in systemic vascular resistance index (SVRI) approximately one hour after treatment (Fig 31), SVRI began to decrease in all experimental groups before plateauing 12 hours after treatment, followed by a gentle increasing trend until the end of the experiments. The SVRI in the injured/treated group was consistently below that of the other groups during treatment while that of the injured/untreated group was correspondingly higher. There was no significant difference in SVRI between the groups. The pulmonary vascular resistance index (PVRI) remained close to baseline levels for all of the groups after one hour of treatment while that of the injured groups progressively increased and that of the uninjured groups remained lower with a subtle decrease after six hours of treatment (Fig 32). The PVRI in the placebo/untreated sheep remained close to baseline levels and the lowest throughout the course of the experiment. After a small peak attained at the beginning of the treatment, the right ventricular stroke work index (RVSWI) in the uninjured sheep gradually increased while that of the injured sheep decreased (Fig 33). There was a significant difference ($p = 0.0196$) in the RVSWI gap between the uninjured/treated and injured/treated groups. RVSWI in the placebo/untreated group remained high while that of the injured/treated group was consistently the lowest. The left ventricular stroke work index (LVSWI)

gradually increased in the uninjured/treated and placebo/untreated groups and plateaued 12 and 18 hours after treatment was begun, respectively, while LVSWI in the injured/untreated and injured/treated groups of sheep decreased and plateaued at 12 hours after treatment was begun and trended upward after 18 hours of treatment (Fig 34). LVSWI in the placebo/untreated group remained consistently higher than in the other groups while that of the injured/treated group was consistently the lowest.

Fig 31. Systemic vascular resistance index (SVRI) (Mean ± SD error bars not shown) in smoke and non-smoke injured sheep that received extracorporeal life support as compared to that in untreated controls.

Fig 32. Pulmonary vascular resistance index (PVRI) (Mean ± SD, error bars not shown) in smoke and non-smoke injured sheep that received extracorporeal life support as compared to that in controls.

Fig 33. Right ventricular stroke work index (RVWSI) (Mean ± SD, error bars not shown) in smoke and non-smoke injured sheep that received extracorporeal life support as compared to that in untreated controls.

Fig 34. Left ventricular stroke work index (Mean ± SD error bars not shown) in smoke and non-smoke injured sheep that received extracorporeal life support as compared to that in untreated controls.

Following a decrease in the coronary perfusion pressure (CPP) from baseline in the smoke-injured sheep, there was a subsequent increase in this parameter within five minutes prior to a sustained decrease up to 18 hours of treatment, followed by another increase for the subsequent six hours (Fig 35). There was a significant difference in CPP ($p = 0.0018$) between the uninjured/treated and injured/treated groups and CCP in the placebo/untreated sheep remained relatively stable after an initial, subtle increase.

Fig 35. Coronary perfusion pressure (CPP) (Mean ± SD, error bars not shown) in smoke and non-smoke injured sheep that received extracorporeal life support alongside as compared to

that in controls.

There was an initial subtle decrease in arterial oxygen content (C_aO_2) from baseline in all groups before a sustained increase in the injured/untreated group, a steady level in the placebo/untreated sheep, and a sharp trough in the injured/treated and uninjured/treated groups (Fig 36). Following the trough, the C_aO_2 of the injured/treated group gradually returned to baseline levels while that of the uninjured/treated group continued along a downward trend. There was a significant difference ($p < 0.0085$) in C_aO_2 between the uninjured/treated and injured/treated groups.

Fig 36. Arterial oxygen content (Mean ± SD error bars not shown) in smoke and non-smoke injured sheep that received extracorporeal life support as compared to that in untreated controls.

There was a slight decrease in the oxygen delivery index (DO_2I) in all groups one hour after treatment before a further marked decrease, except for the placebo/untreated sheep (Fig 37). There was a significant difference ($p = 0.0013$) in DO_2I between the uninjured/treated and injured/treated groups. The injured/treated group had the lowest DO_2I compared with the other groups while the placebo/untreated sheep maintained the highest DO_2I profile. The oxygen extraction index (O_2EI) decreased in all groups before plateauing after approximately six hours of treatment. Further, there was a significant difference ($p = 0.0247$) in O_2EI between the injured/treated and uninjured/treated groups (Fig 38). The O_2EI in the injured/treated and injured/untreated groups was consistently lower and higher, respectively, compared with those of the other groups.

Fig 37. Oxygen delivery index (DO_2I) (Mean ± SD error bars not shown) in smoke and non-smoke injured sheep that received extracorporeal life support as compared to that in untreated controls.

Fig 38. Oxygen extraction index (O_2EI) (Mean ± SD error bars not shown) in smoke and non-smoke injured sheep that received extracorporeal life support as compared to that in untreated

controls.

Fluid input and urine output

There was a variation in the volume of intravenous fluids administered to sheep in the different experimental groups. The injured/treated sheep had the highest fluid requirements, while the placebo/untreated sheep required the least (Fig 39). There was a significant difference ($p < 0.0001$) in fluid requirements between uninjured/treated and injured/treated sheep. The injured/untreated and injured/treated groups produced the least and most urine on average, respectively (Fig 40). There was no significant difference in urine output between the uninjured/treated and injured/treated groups.

Fig 39. Total running intravenous fluids per unit body weight (Mean ± SD) in smoke and non-smoke injured sheep that received extracorporeal life support as compared to those in untreated controls over a 24h period.

Fig 40. Urine output (Mean ± SD) in smoke and non-smoke injured sheep that received extracorporeal life support as compared to that in untreated controls over a 24h period. Lower and upper bar limits correspond to minimum and maximum urine output, respectively.

Anaesthetics

There was a significant difference ($p < 0.0001$) in the amount of alfaxalone required between the uninjured/treated and injured/treated groups (Fig 41). The uninjured/treated group required more alfaxalone on average and the injured/untreated group required the least amount on average. Ketamine requirements differed between groups, with the injured/untreated group requiring the highest amount on average and the injured/treated group requiring the least (Fig 42). There was no significant difference in the quantities of ketamine required between the uninjured/treated and injured/treated groups, but there were significant differences in midazolam requirements (Fig43), occurred between the uninjured/treated and injured/treated groups ($p = 0.0067$).

Fig 41. Alfaxalone infusion (Mean ± SD) indicating the titration range in smoke and non-smoke injured sheep that received extracorporeal life support as compared to that in untreated controls over a 24-hour period.

Fig 42. Ketamine IV infusion (Mean ± SD) indicating the titration range in smoke and non-smoke injured sheep that received extracorporeal life support as compared to that in untreated controls over a 24h period.

Fig 43. Midazolam IV infusion (Mean ± SD) indicating the titration range in smoke and non-smoke injured sheep receiving extracorporeal life support as compared to that in untreated controls over a 24-hour period.

Anticoagulation

There were no significant differences in heparin infusion doses between the uninjured/treated and injured/treated sheep. Heparin requirements for the placebo/untreated group were the lowest (Fig 44). Activated clotting time increased sharply from baseline during pre-treatment and peaked after one hour of treatment, before decreasing sharply and plateauing (Fig 45). There were no significant differences in activated clotting time between groups.

Fig 44. Heparin (10 U/mL) infusion rate (Mean ± SD, error bars not shown) in smoke and non-smoke injured sheep that received extracorporeal life support as compared to that in untreated controls.

Fig 45. Activated clotting time (ACT) (Mean ± SD, error bars not shown) in smoke and non-smoke injured sheep that received extracorporeal life support as compared to that in untreated controls.

ECLS circuit observations

There were significant differences in the ECLS pump speed (Fig 46), blood flow (Fig 47), and pressure differential (Fig 48) between the uninjured/treated and injured/treated groups. Further pump speed, blood flow, and pressure differential were significantly different ($p = 0.0022$), ($p =$

0.0095) and (p = 0.0041), respectively between the two groups that received ECLS. These parameters in the uninjured/treated group were consistently higher than those of the injured/treated group.

Fig 46. Extracorporeal life support pump speed (Mean ± SD) for smoke and non-smoke injured sheep that received treatment.

Fig 47. Blood flow (Mean ± SD) through an extracorporeal life support pump for smoke and non-smoke injured sheep that received treatment.

Fig 48. Pressure differential (Δ-p) (Mean ± SD) across the extracorporeal life support pump for smoke and non-smoke injured sheep that received treatment

Body temperature

Body temperature in the untreated groups gradually increased from baseline levels and plateaued at approximately after six hours of treatment, and remained higher than that for the treated groups (Fig 49). There was no significant difference in body temperature between the treated groups.

Fig 49. Body temperature (Mean ± SD) of smoke and non-smoke injured sheep that received extracorporeal life support as compared to that of untreated controls.

Inflammatory cells and cytokines

Data were initially available in abstract form and subsequently as a full publication on inflammatory cell infiltration into the lung tissue with a trend toward increased lung injury in sheep that inhaled smoke, revealing damage to the bronchiolar lining and infiltration of inflammatory cells [5,14-16].

Discussion

The results of this study agree with and confirm earlier preliminary observations that ECLS

causes a decrease in pulmonary compliance (Fig 2) over time [12]. It was expected that the injured sheep would have relatively lower SpO_2 readings (Fig 3) compared with the other groups because of episodes of hypotension with hypoxemia, which can affect pulse oximeter function [17]. The relatively low $etCO_2$ in the injured sheep suggested that the sheep may have hyperventilated (Fig 4), the causes of which were evaluated with respect to the reactive oxygen species or superoxide dismutase activity by a team from the source study [18,19].

The relatively low blood pH in the injured/treated sheep as depicted in Fig 5, suggested that the sheep tended to have metabolic acidosis, as the same group of animals also had low $etCO_2$. This also implies that there was no respiratory component that contributed to the observed acidosis. The low pCO_2 in the uninjured/treated sheep could be a result of hyperventilation and the high pCO_2 in the injured/untreated sheep suggested that CO_2 clearance was curtailed by injury (Fig 6).

The treatment of the sheep contributed to lung injury by causing deterioration of pO_2 (Fig 7). The low pO_2 translated to a low partial arterial oxygen pressure/inspired oxygen proportion (PaO_2/FiO_2 ratio), which was much worse in the injured sheep. This finding showed that ECLS contributed to the deterioration of the PaO_2/FiO_2 ratio in the injured/treated group of sheep, a novel finding that was also unexpected in the primary study (this has since been replicated in a more recent study[9]). It could also be argued from the data that perhaps VV ECMO was performed in a suboptimal manner, considering that the sheep oxygenation appeared to be less effective on ECMO than on the ventilator alone, but this remains to be explored further in future.

Further, the relatively higher levels of [Hb] in the injured sheep suggested that these animals could have been dehydrated as a secondary consequence of excessive fluid loss due to inflammation and increased vascular permeability [20] despite intravenous fluid replacement (Fig 8). However, blood total protein and albumin levels, which are better predictors of dehydration in sheep [21], were not measured.

The inverse decrease in FO_2Hb (Fig 9) relative to FCOHb (Fig 10) following smoke injury was expected and is a finding that is in agreement with other studies [20,22,23]. It has recently been

607 demonstrated that FCOHb is not correlated to the extent of lung injury [20]. The gradual decrease in
608 MetHb (Fig 11) was probably caused by the enzymatic activity of methaemoglobin reductase [24]
609 and the higher Hct observed in the injured sheep (Fig 12) could have been due to dehydration
610 because Hct was measured by an automated method.

611 As presented in Fig 14, the [Ca^{2+}] was lower than the published normal level of 2.4 mmol/L
612 [25]. Stress associated with yarding of the sheep and phosphorus imbalance in feed are the most
613 likely suggested causes of low [Ca^{2+}] [26]. Fasting the sheep for 24 hours prior to the experimental
614 procedures could also have contributed to the relatively low [Ca^{2+}].

615 The increase in Cl^- beyond the normal range of 105–110 mmol/L [25] during the experiments
616 (Fig 15) suggests that the sheep may have developed respiratory alkalosis. Hyperventilation or
617 metabolic acidosis resulting from sustained salivary loss of sodium bicarbonate that was more severe
618 in the injured/treated group may have played a role in hyperchloraemia, because Cl^- is known to
619 replace HCO_3^- when the latter is lost from the body [27,28]. Baseline [K^+] in all the sheep (Fig 16)
620 was below the published normal range of 4–5 mmol/L [25] and this relative hypokalaemia may have
621 been related to low K^+ in the diet [29-31]. The normal anion gap (Fig 17) with decreased HCO_3^- (Fig
622 21) confirmed the presence of hyperchloraemic acidosis in all but the placebo/untreated sheep. The
623 cause of the hyperchloraemia was likely the prolonged administration of 0.9% NaCl.

624 Although normal [Glu] in ruminants is usually lower than that for other species, its relative
625 progressive increase in the injured sheep (Fig 18) may have been related to stress and severe pain
626 associated with injury or the development of enterotoxaemia [32,33]. The relative increase in [Lac]
627 beyond the reported normal range of 1–2 mmol/L in the injured sheep (Fig 19) suggested
628 dehydration, trauma, and sepsis [34]. In particular, sepsis is a concern related to the sub-optimal
629 rumen function, leading to loss of its buffer effect and an increase in the number of anaerobic
630 bacteria with prolonged hypomotility, such as that which occurs during long-duration anaesthesia.
631 Therefore, the increases in both [Glu] and [Lac] are consistent with severe injury.

632 The elevated Base (ecf) above +2 mmol/L for most of the first 12 hours in the
633 placebo/untreated and injured/untreated sheep suggested that the sheep were metabolically alkalotic

[32] before returning to normal levels (Fig 20). The relatively low Base (ecf) (less than −2 mmol/L) was consistent with loss of HCO_3^- and the tendency of developing metabolic acidosis [32] in the injured/treated sheep. The marked decrease in [HCO_3^-] in the injured sheep was consistent with metabolic acidosis and was more severe in the injured/treated group, as illustrated in Fig 21, thereby suggesting that ECLS was a contributing factor.

The resting HR of sheep is 50–80 beats/min [25]. In a study that instrumented conscious sheep, the baseline heart rate was registered as 106 ± 9 beats/min [35]. In the present report, all of the sheep had a relatively high HR, thereby suggesting that stress and pain were contributing factors (Fig 22). The gradual decrease in HR during the course of the experiments was consistent with the effects of anaesthesia [25].

In sheep, a mean arterial pressure below 60 mmHg indicates inadequate tissue perfusion [25]. Although the MAP values in the injured sheep were lower than for the uninjured sheep (Fig 23), MAP values were still within the published normal value of 70 mmHg [25]; the magnitude of injury was again a predictor of how low the MAP was. Another predictor for the severity of the injury was the mean pulmonary artery pressure, which was highest for the injured/treated sheep as illustrated in Fig 24. The baseline values for MPAP were higher than the 17 ± 1 mmHg reported in another study that used sheep for experiments [35]. The baseline CVP in all of the sheep in the present report was > 10 mmHg (Fig 25), which was much higher than the 5.5 ± 1.2 mmHg reported elsewhere [35] in instrumented conscious sheep and a novel finding in this study. Thus in this study, the severity of injury and treatment contributed to the CVP elevations found among the sheep.

There was a benefit of ECLS treatment for SvO_2, as it remained high for both the injured/treated and uninjured treated groups (Fig 26). The consistently low SvO_2 in the injured/untreated group was expected because of the slightly reduced cardiac output in this group; however, this level of SvO_2 was still higher than that reported in other studies [35]. Smoke injury was associated with a sustained decrease in cardiac output in all the sheep that were exposed to smoke. As in CCO changes (Fig 27), the SV (Fig 28), SVI (Fig 29) and CI (Fig 30) all had similar profiles for different groups, with the injured sheep having lower values. The decrease in SVRI in all of the

sheep at a later stage in the experiments suggested that there was systemic vasodilation (Fig 31). In contrast, the increase in PVRI in the injured sheep suggested that vasoconstriction was caused by exposure to smoke injury (Fig 32). The exposure to smoke injury worsened both RVSWI (Fig 33) and LVSWI (Fig 34) while there was an increase in both parameters in the uninjured sheep. Reduced RVSWI is associated with poor functioning of the right ventricle [36,37] and LVSWI is a reliable parameter for left ventricular function [38].

The reduction in coronary perfusion pressure in the injured/treated—and to a certain extent the uninjured/ treated sheep—suggested that ECLS contributed to the decrease in CPP, in addition to smoke injury (Fig 35). The CPP is an indicator of myocardial perfusion and has been proposed as a drug target during resuscitation [39]. The observations in the present study support the suggestion that CPP could be used to predict the severity of injury in sheep.

Further, the apparent increase in CaO_2 in the injured sheep (Fig 36) could have been due to the relative increase in Hb secondary to dehydration as illustrated in Fig 8. The low DO_2I in the injured/treated and uninjured/treated groups suggested that ECLS also contributed to this, in addition to smoke, based on the relatively higher DO_2I in the injured/untreated sheep (Fig 37). Interestingly, the O_2EI (Fig 38) had a comparable profile to that of the PaO_2/FiO_2 ratio and could also be used to predict the contribution of ECLS to smoke-related injury.

The smoke-injured sheep required considerable amounts of intravenous fluids (Fig 39) to compensate for the losses from pulmonary exudation and inflammation [20,22]. The mean urine production in all groups (Fig 40) was marginally lower than the published normal of 1.2 mL/kg/h [25] but still considered to be within the acceptable range for this cohort of sheep. Moreover, the dosage of anaesthetic drugs used was considered adequate for the experiments (Figs 41, 42 and 43). In addition, heparin infusion (Fig 44) was indicated to prolong the activated clotting time (Fig 45) in order to minimise the risk of thrombosis during intravascular procedures [40].

The reduction in the ECLS pump speed (Fig 46), flow (Fig 47), and pressure differential (Fig 48) could have resulted from systemic hypotension contributing to low amounts of blood to the pump. The ECLS was configured such that the centrifugal pump pulled blood from the inferior vena

cava and returned it into the right atrium; therefore, if the circulating volume was low, the flow would decrease for a given pump speed and in this case, both rpm and flow would reduce. Centrifugal ECLS pumps are known to be preload dependent and afterload sensitive [41], thereby making rpm and flows directly proportional to each other. The reason for the systemic hypotension remains undetermined. It is possible that an unknown pulmonary component or product produced in the smoke-damaged lungs of the sheep played a role. It must be noted that the body temperature of the sheep was generally within the physiological range (Fig 49).

Certain observations regarding this study could affect the interpretation of red blood cell indices and their derivatives. For example, animals differ from humans in that estimated changes in plasma volume is preferably determined by changes in packed cell volume (PCV) or haemoglobin concentration and total plasma protein (TPP) [42-44]. Moreover, in animals, there is a wider range of normal PCV than TPP [45]. In critical care for domestic animals, the change in both PCV and TPP is most useful as a crude index of change in plasma volume [46]. A centrifuge that spins minute amounts of blood for rapid, cost-effective determination of PCV and TPP permits instant adjustments in an animal's fluid needs. However, measurements of PCV and TPP were not conducted in the primary study. As with all data that are collected with different objectives, it was considerably tedious to align certain time points with real-time observations made in the laboratory, particularly for data that was manually input. There was also no information regarding pre-anaesthetic blood tests.

An additional limitation of this study is related to the overall objective of providing useful information relevant to the sheep ECMO model in particular, and to the scientific community interested in large animal experimentation and veterinary medicine in general. Because the method of data collection method has not been validated across several laboratories or research groups, it is considered relatively preliminary and further validation studies are required. Moreover, the number of sheep (Fig 1) was low and this was particularly so in the injured/untreated and placebo/untreated groups, thereby preventing comparisons between the treated and untreated sheep. A further limitation is that cytokine levels, as predictors of lung injury, were not quantified. Using ELISA assays to

quantify cytokine levels proved difficult and the cost was prohibitive in the present study, although subsequent efforts were made by a few members of the original research group in this regard [5]. It is partially for this reason that pioneering studies [47] have been proposed for the development of proteogenomic assays as an alternative to ELISA—to learn from circulating markers of acute inflammation in injured sheep used as models of intensive care, to understand critical illness.

Nevertheless, although ECMO treatment has been demonstrated to contribute to, and exacerbate the deterioration of pulmonary pathology by reducing lung compliance and PaO_2/FiO_2 ratio in sheep studies [5,9,15], the understanding of ECMO in respiratory life-support in human medicine continues to grow in a positive direction overall; moreover, there is evidence that it is useful as a life-saving treatment. These novel observations from sheep could help in understanding similar pathology such as that which occurs in animal victims of smoke inhalation from house or bush fires, aspiration pneumonia secondary to tick paralysis, and in the management of COVID-19 in humans [48-50].

The World Health Organisation (WHO) has recently recognised and classified a novel coronavirus SARS-CoV-2 that causes COVID-19 as a global pandemic and public health emergency [51,52]. The National Institute of Allergy and Infectious Disease (NIAID) of the United States of America recognises that coronaviruses constitute a large group of viruses known to cause respiratory diseases, including the common cold [53]. However, in recent years, three novel members of this family of viruses have arisen from animals to cause severe and extensive infection and death in humans [54]. In addition to bats, a large number of coronaviruses are known to circulate in certain domestic animals like cats and occasionally spill over to humans and cause serious illnesses, such as the SARS coronavirus (SARS-CoV) that emerged in Southern China in 2002 [55]. As a future perspective, the outcomes of the present study could be used to guide additional studies to enable the mortality indicators and prognostic indicators associated with ECMO and allied technology to be further evaluated and well understood in sheep and other experimental animals.

Conclusions

The results of this study demonstrated that ECLS contributed to the worsening of pulmonary pathology by reducing lung compliance and PaO_2/FiO_2 ratio. The O_2EI changes mirrored those of the PaO_2/FiO_2 ratio and decreasing CPP was a predictor of a greater magnitude of cardiopulmonary injury in sheep. These novel observations could help in further understanding similar pathology in other patients; for example, in the resuscitation of animals injured from in house or bush fires. A similar data acquisition approach could be used in evaluating the effectiveness of a given experimental or clinical intervention to further the understanding of the clinical condition being studied and to aid in the formulation of treatments aimed at improving the survival of animal patients. In veterinary medicine, albeit now a considerably expensive and remote option, ECLS knowledge could complement the treatment of potentially reversible aspiration pneumonia, a secondary complication associated with both *Ixodes holocyclus* toxicity and laryngeal paralysis, in valuable companion animals and in critically ill humans who require respiratory support, like COVID-19 patients.

Competing interests

A previously undisclosed conflict of interest became apparent from a section of adjunct persons within the research group when important early findings of this paper were first presented at an academic milestone seminar at The University of Queensland in August 2013. Therefore, this report comprises work completed during studies for higher-degree research from 7th September 2012 to 23rd August 2013, only.

Author Contributions

The author (SC) was solely responsible for the study design, writing the manuscript, analysing and interpreting the data, and final approval of the manuscript. In addition, SC is fully accountable for the work.

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References

1. Chemonges S, Shekar K, Tung JP, Dunster KR, Diab S, et al. Optimal management of the critically ill: anaesthesia, monitoring, data capture, and point-of-care technological practices in ovine models of critical care. Biomed Res Int. 2014;468309.

2. Platts DG, Fraser JF, Hilton A, Diab S, McDonald C, et al. A novel echocardiographic imaging technique, intracatheter echocardiography, to guide veno-venous extracorporeal membrane oxygenation cannulae placement in a validated ovine model. Intensive Care Medicine Experimental. 2014;2:2.

3. Platts DG, Diab S, Dunster KR, Shekar K, Burstow DJ, et al. Feasibility of perflutren microsphere contrast transthoracic echocardiography in the visualization of ventricular endocardium during venovenous extracorporeal membrane oxygenation in a validated ovine model. Echocardiography. 2015;32:548-56.

4. Shekar K, Fung YL, Diab S, Mullany DV, McDonald CI, et al. Development of simulated and ovine models of extracorporeal life support to improve understanding of circuit-host interactions. Crit Care Resusc. 2012;14:105-111.

5. Passmore MR, Fung YL, Simonova G, Foley SR, Dunster KR, et al. Inflammation and lung injury in an ovine model of extracorporeal membrane oxygenation support. Am J Physiol Lung Cell Mol Physiol. 2016:311:L1202-L1212.

6. Rais-Bahrami K, Van Meurs KP. Venoarterial versus venovenous ECMO for neonatal respiratory failure. Seminars in Perinatology. 2014;38:71-77.

7. Brenner P, Zelger T, Kainzinger S, Sodian R, Überfuhr P, et al. Prolonged extracorporeal membrane oxygenation (ECMO) in 231 patients after different indications with low cardiac output—a single-center experience. The Thoracic and Cardiovascular Surgeon. 2012;60:V23.

8. Fraser JF, Platts DG, Mullany DV, Fung YL, Shekar K, et al. ECMO—the clinician's view. ISBT Science Series. 2012;7:82-88.

9. Millar JE, Bartnikowski N, Passmore MR, Obonyo NG, Malfertheiner MV, et al. Combined mesenchymal stromal cell therapy and ECMO in ARDS: a controlled experimental study in sheep. Am J Respir Crit Care Med. 2020

10. NHMRC. Australian code of practice for the care and use of animals for scientific purposes. Canberra, Australia: NHMRC; 2013.

11. Riedel T, Fraser JF, Dunster K, Fitzgibbon J, Schibler A. Effect of smoke inhalation on viscoelastic properties and ventilation distribution in sheep. Journal of Applied Physiology. 2006;101:763-70.

12. Chemonges S. Critical care management of sheep receiving extra-corporeal membrane oxygenation due

812 to smoke induced acute lung injury (ECMO S-ALI) and acute sepsis. Australian and New Zealand
813 College of Veterinary Scientists: College Science Week. Surfers Paradise, Australia: ANZCVS; 2013.
814 13. Chemonges S. Contemporary data capture, anaesthesia monitoring and point-of-care technology in critical
815 care research settings for animal models. Australian and New Zealand College of Veterinary
816 Scientists: College Science Week. Surfers Paradise, Australia: ANZCVS; 2013.
817 14. Margaret Passmore LF, Dunster K, Chemonges S, Diab S, Minchinton R, Shekar K, Fraser J. ECMO
818 contributes to lung injury in an ovine model of veno-venous ECMO. ANZICS/ACCCN Intensive Care
819 Annual Scientific Meeting (ASM). Hotel Grand Chancellor Hobart; 2013.
820 15. Margaret Passmore LF, Dunster K, Chemonges S, Diab S, Minchinton R, Shekar K, Fraser J. ECMO
821 contributes to lung injury in an ovine model of veno-venous ECMO; ; Beijing, China; 2013.
822 16. Margaret P, Yoke LF, Kimble RD, Sara D, Kiran S, et al. Lung inflammatory changes in an ovine model of
823 venovenous extracorporeal membrane oxygenation. B47 Acute Respiratory Distress Syndrome:
824 American Thoracic Society. 2014;A3064-A3064.
825 17. Quinn CT, Raisis AL, Musk GC. Evaluation of masimo signal extraction technology pulse oximetry in
826 anaesthetized pregnant sheep. Veterinary Anaesthesia and Analgesia. 2013; 40: 149-56.
827 18. McDonald CI, Fung YL, Fraser JF. Antioxidant trace element reduction in an in vitro cardiopulmonary
828 bypass circuit. ASAIO Journal. 2012;58:217-22.
829 19. McDonald CI, Fung YL, Shekar K, Diab SD, Dunster KR, et al. The impact of acute lung injury, ECMO and
830 transfusion on oxidative stress and plasma selenium levels in an ovine model. Journal of Trace
831 Elements in Medicine and Biology. 2015;30:4-10.
832 20. Lange M, Cox RA, Traber DL, Hamahata A, Nakano Y, et al. No correlation between initial arterial
833 carboxyhemoglobin level and degree of lung injury following ovine burn and smoke inhalation.
834 Experimental Lung Research. 2014;40:99.
835 21. Combs MDA, Rendell D, Reed KFM, Mace WJ, Quinn JC. Evidence of dehydration and electrolyte
836 disturbances in cases of perennial ryegrass toxicosis in Australian sheep. Australian Veterinary
837 Journal. 2014;92:107-13.
838 22. Westphal M, Morita N, Enkhbaatar P, Murakami K, Traber DL, et al. Carboxyhemoglobin formation
839 following smoke inhalation injury in sheep is interrelated with pulmonary shunt fraction. Biochemical
840 and Biophysical Research Communications. 2003;311:754-58.
841 23. Westphal M, Murakami K, Burke AS, Westphal-Varghese BB, Rudloff HE, et al. Combined burn and
842 smoke inhalation injury impairs ovine hypoxic pulmonary vasoconstriction. Critical Care Medicine.
843 2006;34:1428-36.
844 24. Bradberry S. Methaemoglobinaemia. Medicine. 2007;35:552-53.

845 25. Adams D, McKinley M, Colditz I, Dart C. The sheep. Australian and New Zealand Council for the Care of
846 Animals in Research & Teaching (ANZCCART) Humane Science. The University of Adelaide:
847 Australian and New Zealand Council for the Care of Animals in Research & Teaching; 2009.
848 26. McGrath SR, Lievaart JJ, Friend MA. Extent of utilisation of dual-purpose wheat for grazing by late-
849 pregnant and lambing ewes and producer-reported incidence of health issues in southern New South
850 Wales. Australian Veterinary Journal. 2013; 91:432-36.
851 27. Handy JM, Soni N Physiological effects of hyperchloraemia and acidosis. British Journal of Anaesthesia.
852 2008;101:141-50.
853 28. Peacock RE, Smart L. False hyperchloraemia in a dog secondary to ingestion of horse feed supplemented
854 with potassium bromide. Australian Veterinary Journal. 2013;91:320-22.
855 29. Duhm J, Göbel BO. Na+-K+ transport and volume of rat erythrocytes under dietary K+ deficiency. The
856 American Journal of Physiology. 1984;246:C20-29.
857 30. Mien TXN, Li EY, Nicholas KF, Donna HL, Hetal K, et al. Effects of K+-deficient diets with and without
858 NaCl supplementation on Na+, K+, and H2O transporters' abundance along the nephron. American
859 Journal of Physiology—Renal Physiology. 2012;303:92-104.
860 31. Oh YT, Kim J, Youn JH. Role of pituitary in K+ homeostasis: impaired renal responses to altered K+ intake
861 in hypophysectomized rats. American Journal of Physiology Regulatory, Integrative and Comparative
862 Physiology. 2013;304:R1166-74.
863 32. Russell KE, Roussel AJ. Evaluation of the ruminant serum chemistry profile. Veterinary Clinics of North
864 America: Food Animal Practice. 2007;23:403-26.
865 33. Sejrsen K, Hvelplund T, Nielsen MO. Ruminant physiology: digestion, metabolism and impact of nutrition
866 on gene expression, immunology and stress. Wageningen: Wageningen Academic Publishers; 2006.
867 34. Ewaschuk JB, Naylor JM, Zello GA D-lactate in human and ruminant metabolism. J Nutr. 2005;135:1619-
868 25.
869 35. Pohlmann JR, Akay B, Camboni D, Koch KL, Mervak BM, et al. A low mortality model of chronic
870 pulmonary hypertension in sheep. The Journal of Surgical Research. 2012;175:44-8.
871 36. Frea S, Bovolo V, Bergerone S, D'Ascenzo F, Antolini M, et al. Echocardiographic evaluation of right
872 ventricular stroke work index in advanced heart failure: a new index? Journal of Cardiac Failure.
873 2012;18:886-93.
874 37. Cameli M, Bernazzali S, Lisi M, Tsioulpas C, Croccia MG, et al. Right ventricular longitudinal strain and
875 right ventricular stroke work index in patients with severe heart failure: left ventricular assist device
876 suitability for transplant candidates. Transplantation Proceedings. 2012;44:2013.
877 38. Choi J-O, Park SW, Park PW, Lee S-C, Choi SH, et al. Noninvasive assessment of left ventricular stroke

work index in patients with severe mitral regurgitation: correlation with invasive measurement and exercise capacity. Echocardiography (Mount Kisco, NY). 2010;27:1161.

39. Raghunathan K, Barbeito A, MacLeod D. Goal-directed advanced cardiac life support: coronary perfusion pressure as a target during resuscitation. Critical Care Medicine. 2013;41:2817-18.

40. Oliver WC. Anticoagulation and coagulation management for ECMO. Seminars in Cardiothoracic and Vascular Anaesthesia. 2009;13:154-75.

41. Jayanthkumar HS, Murugesan C, Rajkumar J, Harish BR, Muralidhar K. Our experience with implantation of VentrAssist left ventricular assist device. Indian J Anaesth. 2013;57:56-61.

42. Falzon LC, Menzies PI, Shakya KP, Jones-Bitton A, Vanleeuwen J, et al. A longitudinal study on the effect of lambing season on the periparturient egg rise in Ontario sheep flocks. Preventive Veterinary Medicine. 2013;110:467-80.

43. Hayes GM, Mathews K, Floras A, Dewey C. Refractometric total plasma protein measurement as a cage-side indicator of hypoalbuminemia and hypoproteinemia in hospitalized dogs. Journal of Veterinary Emergency and Critical Care. 2011;21:356-62.

44. White A, Reyes A, Godoy A, Martínez R. Effects of transport and racing on ionic changes in thoroughbred race horses. Comparative Biochemistry and Physiology—Part A: Physiology. 1991;99:343-46.

45. Tang Z, Lee JH, Louie RF, Kost GJ. Effects of different hematocrit levels on glucose measurements with handheld meters for point-of-care testing. Archives of pathology & laboratory medicine. 2000;124: 1135-40.

46. Stafford KJ, Mellor DJ, Todd SE, Gregory NG, Bruce RA, et al. The physical state and plasma biochemical profile of young calves on arrival at a slaughter plant. New Zealand Veterinary Journal. 2001;49:142-42.

47. Chemonges S, Tung JP, Fraser JF. Proteogenomics of selective susceptibility to endotoxin using circulating acute phase biomarkers and bioassay development in sheep: a review. Proteome Sci. 2014;12:12.

48. Jacobs JP, Stammers AH, St Louis J, Hayanga JWA, Firstenberg MS, et al. Extracorporeal membrane oxygenation in the treatment of severe pulmonary and cardiac compromise in COVID-19: Experience with 32 patients. ASAIO J; 2020.

49. Ramanathan K, Antognini D, Combes A, Paden M, Zakhary B, et al. Planning and provision of ECMO services for severe ARDS during the COVID-19 pandemic and other outbreaks of emerging infectious diseases. Lancet Respir Med. 2020;8:518-26.

50. Rajagopal K, Keller SP, Akkanti B, Bime C, Loyalka P, et al. Advanced pulmonary and cardiac support of COVID-19 patients: Emerging recommendations from ASAIO-A "Living Working Document". ASAIO

J;2020.

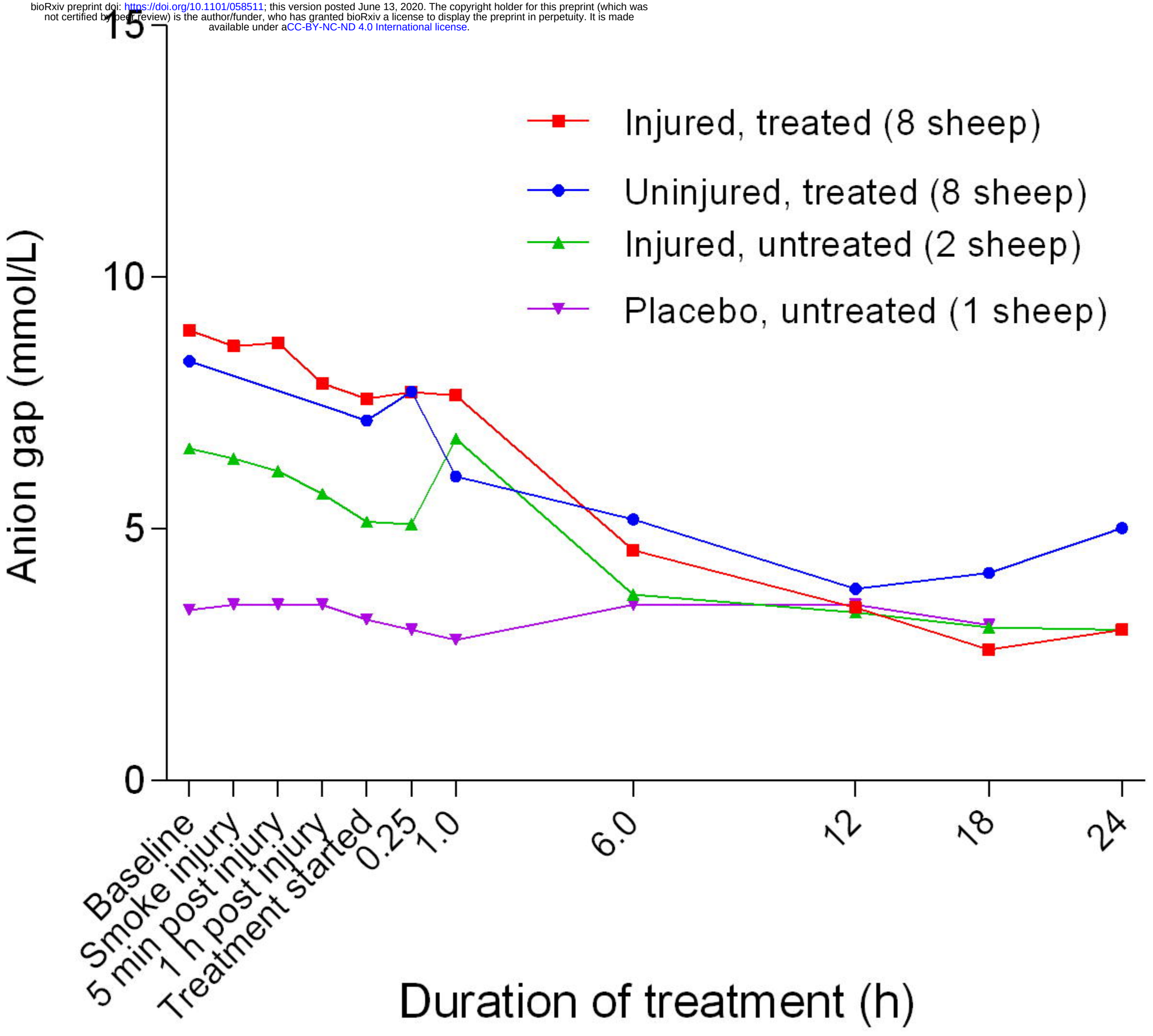
51. Sohrabi C, Alsafi Z, O'Neill N, Khan M, Kerwan A, et al. World Health Organization declares global emergency: A review of the 2019 novel coronavirus (COVID-19). Int J Surg. 2020;76:71-6.

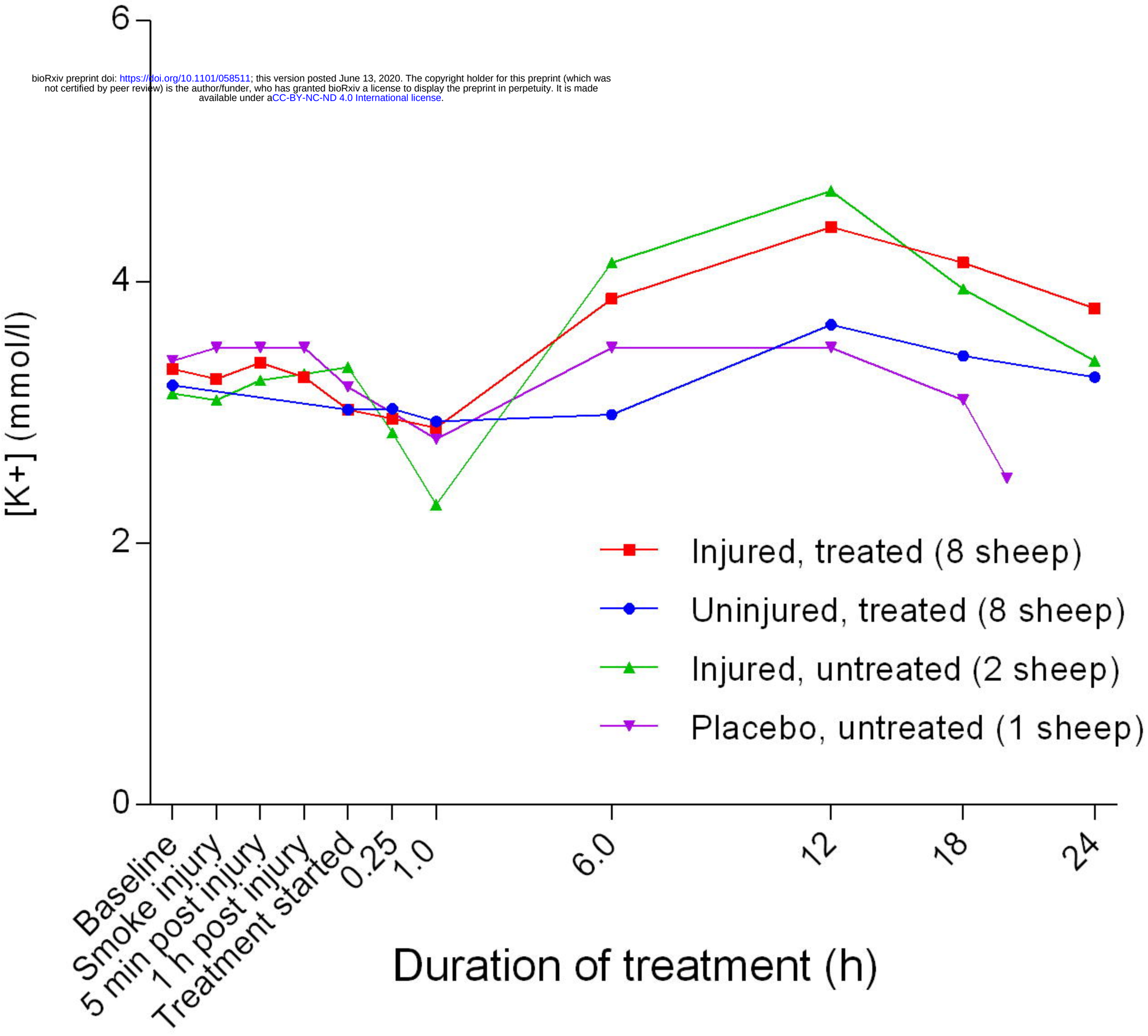
52. Rothan HA, Byrareddy SN. The epidemiology and pathogenesis of coronavirus disease (COVID-19) outbreak. J Autoimmun. 2020;109:26.

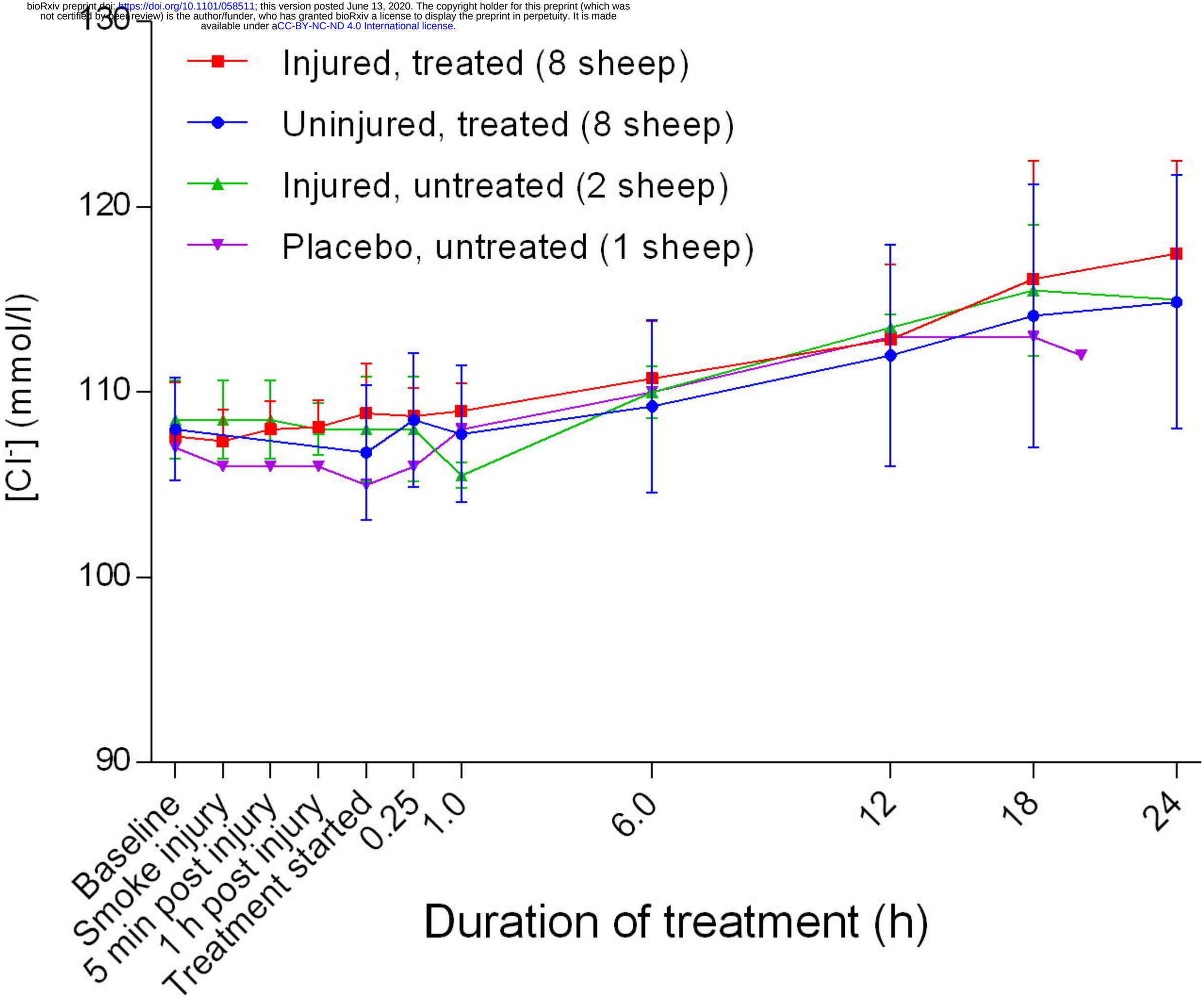
53. NIAID Coronaviruses; 2020.

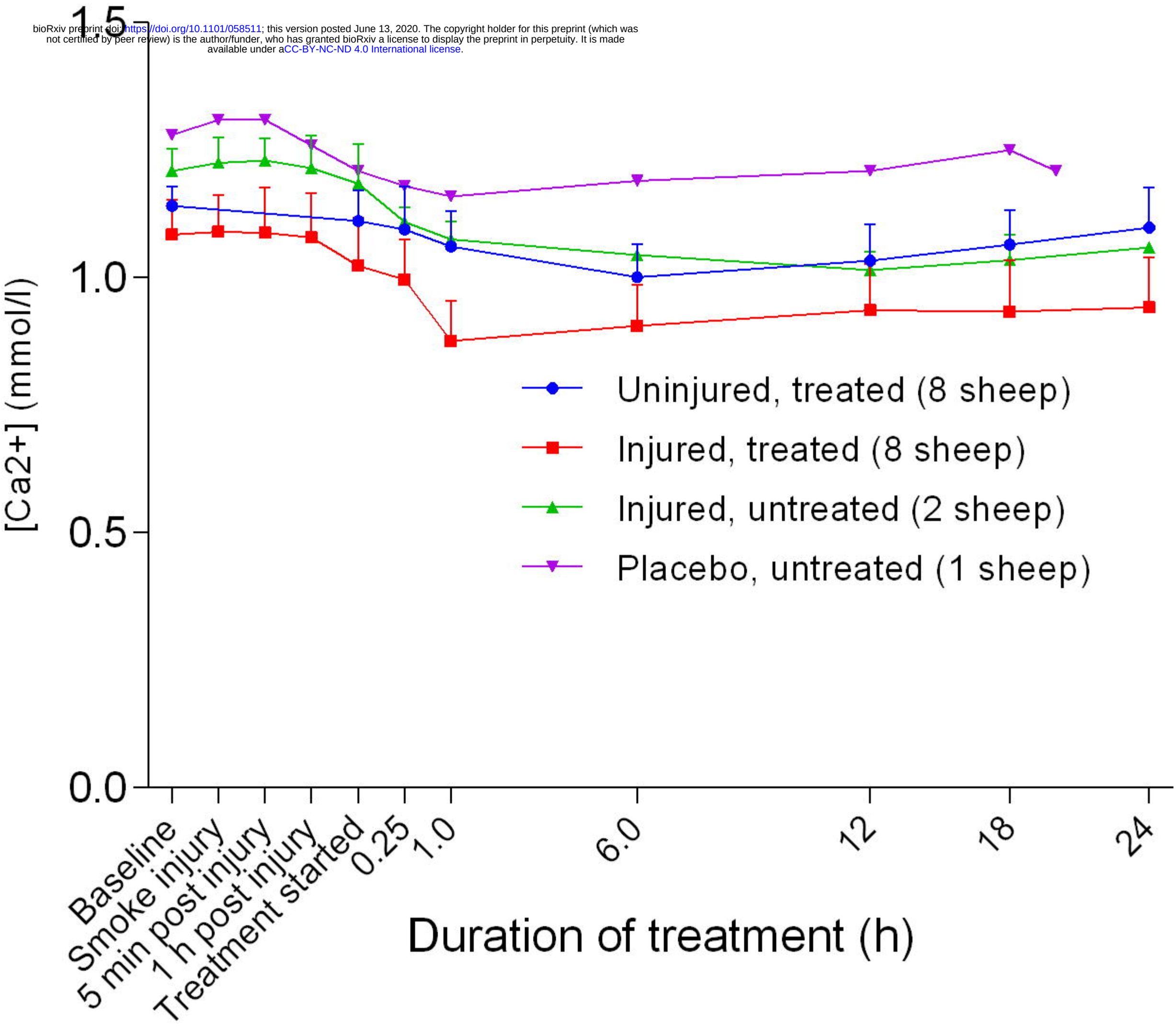
54. Chen Y, Liu Q, Guo D. Emerging coronaviruses: genome structure, replication, and pathogenesis. J Med Virol. 2020;92:418-423.

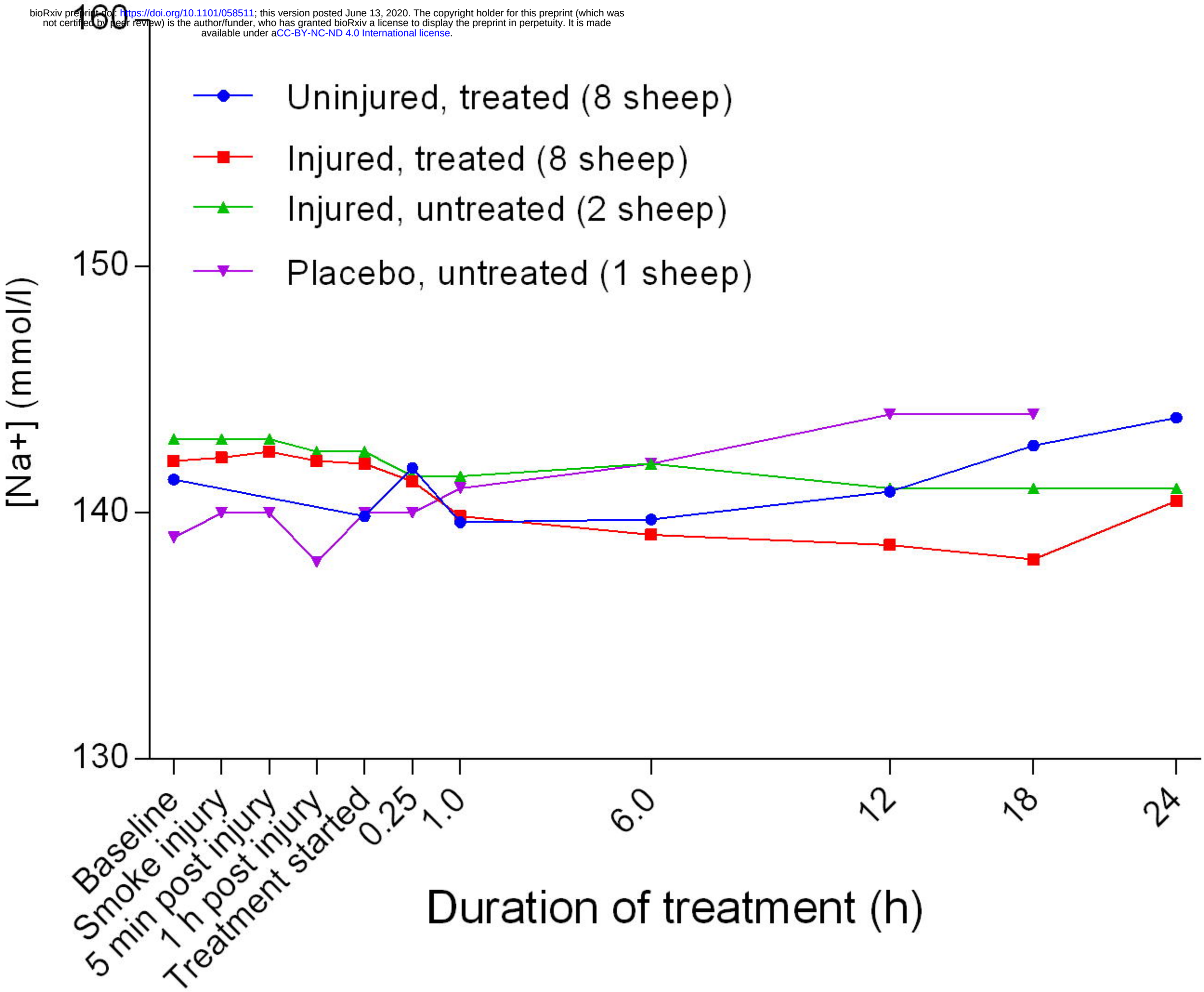
55. Chan JF, To KK, Tse H, Jin DY, Yuen KY. Interspecies transmission and emergence of novel viruses: lessons from bats and birds. Trends Microbiol. 2013;21:544-55.

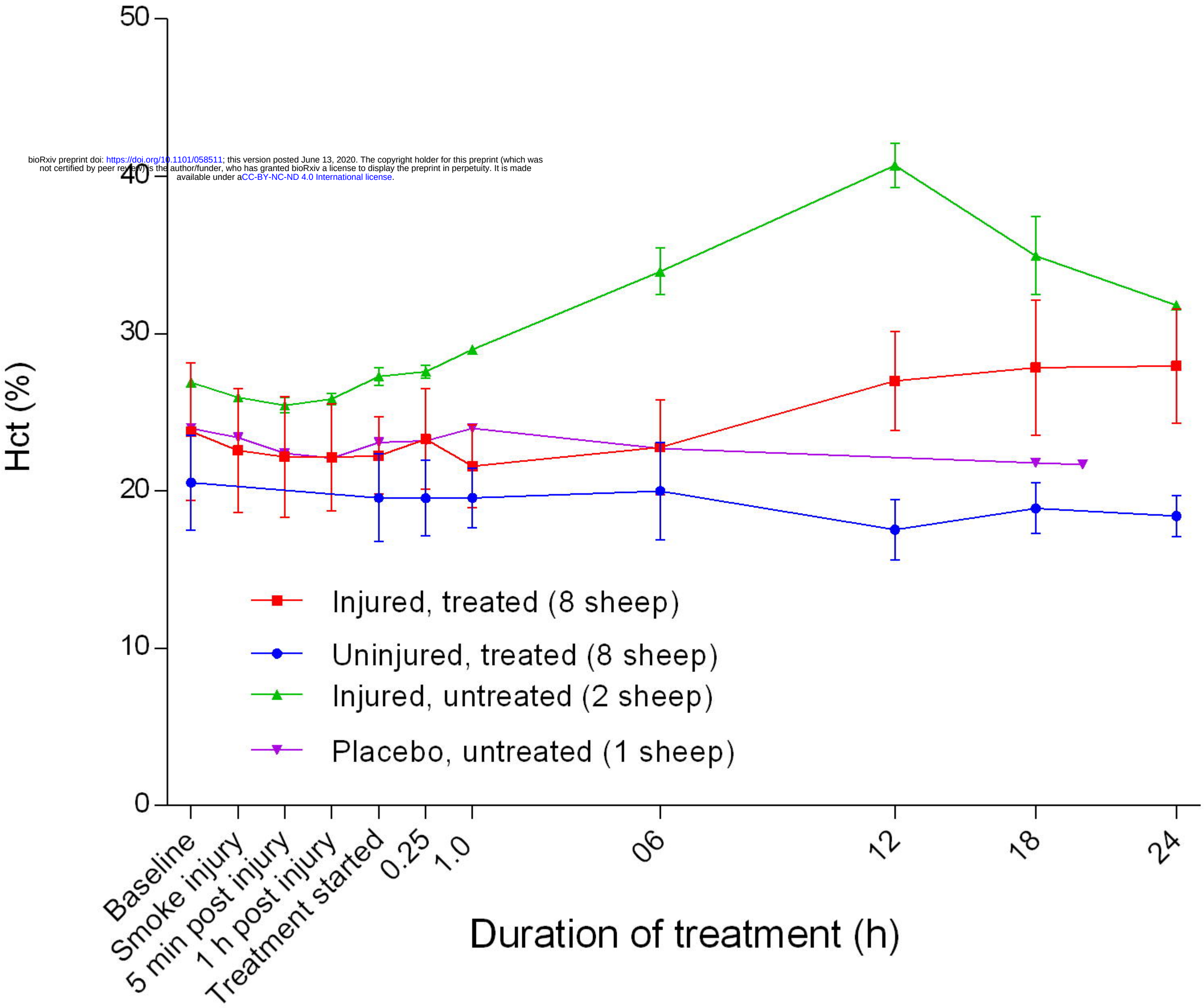


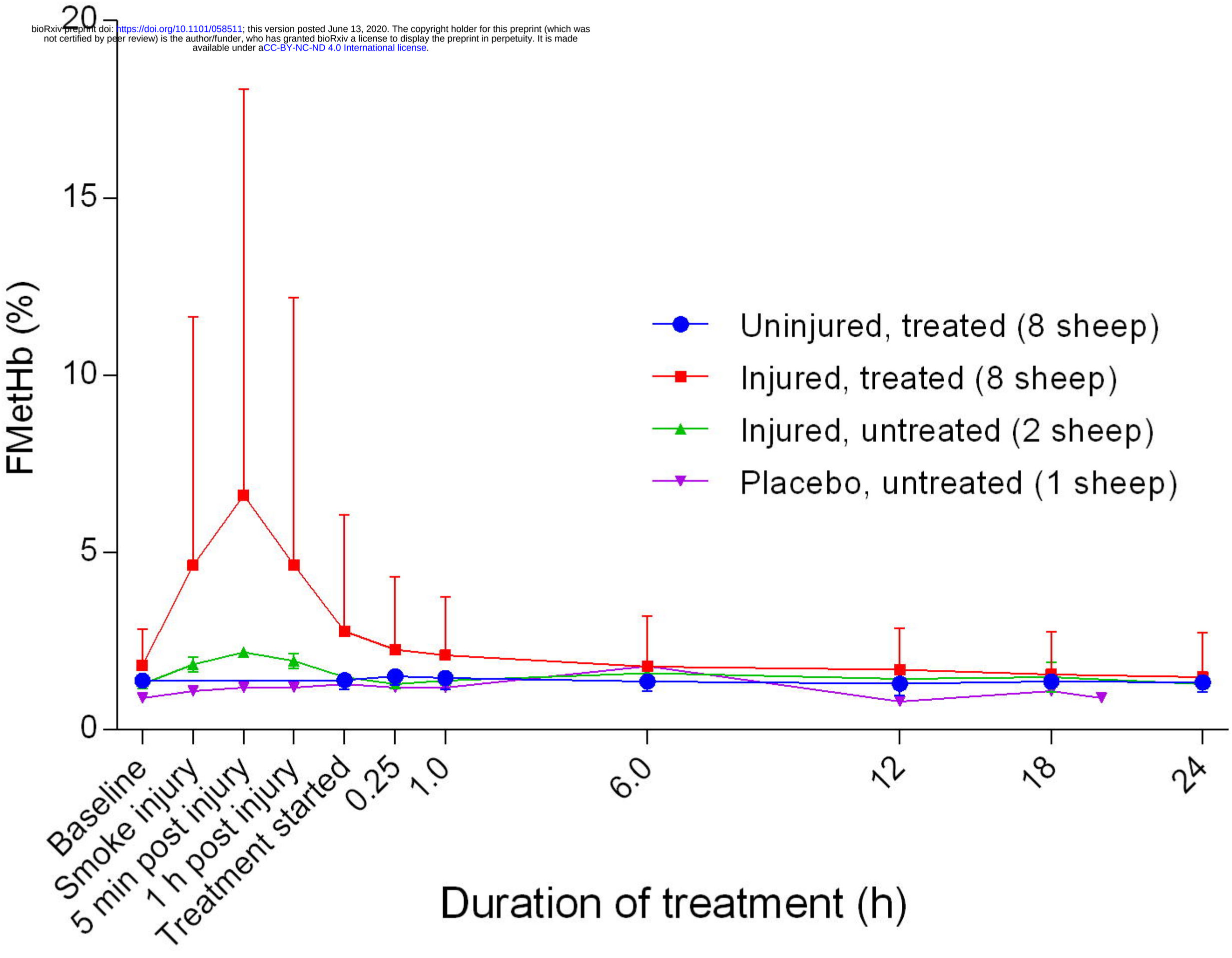


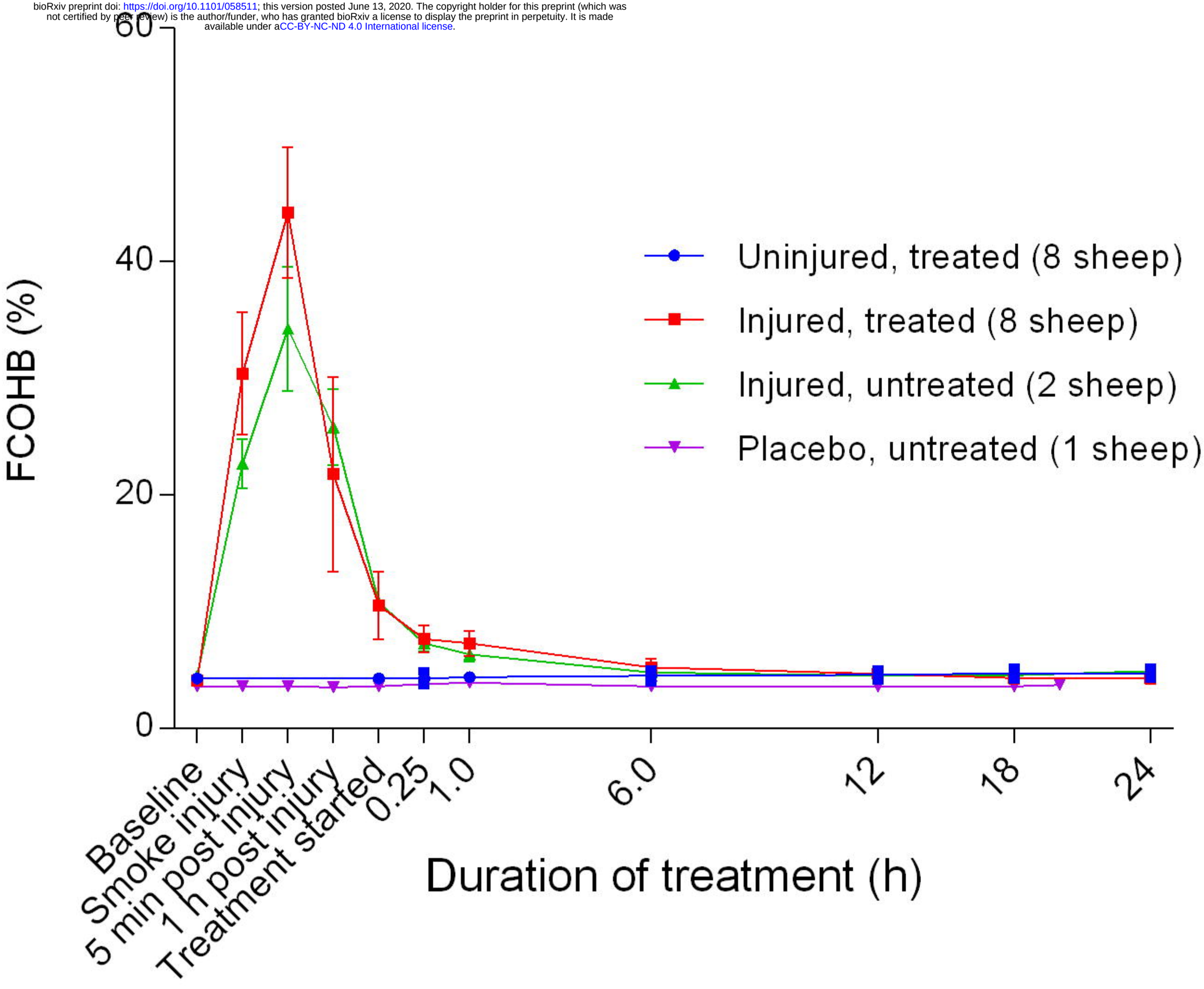


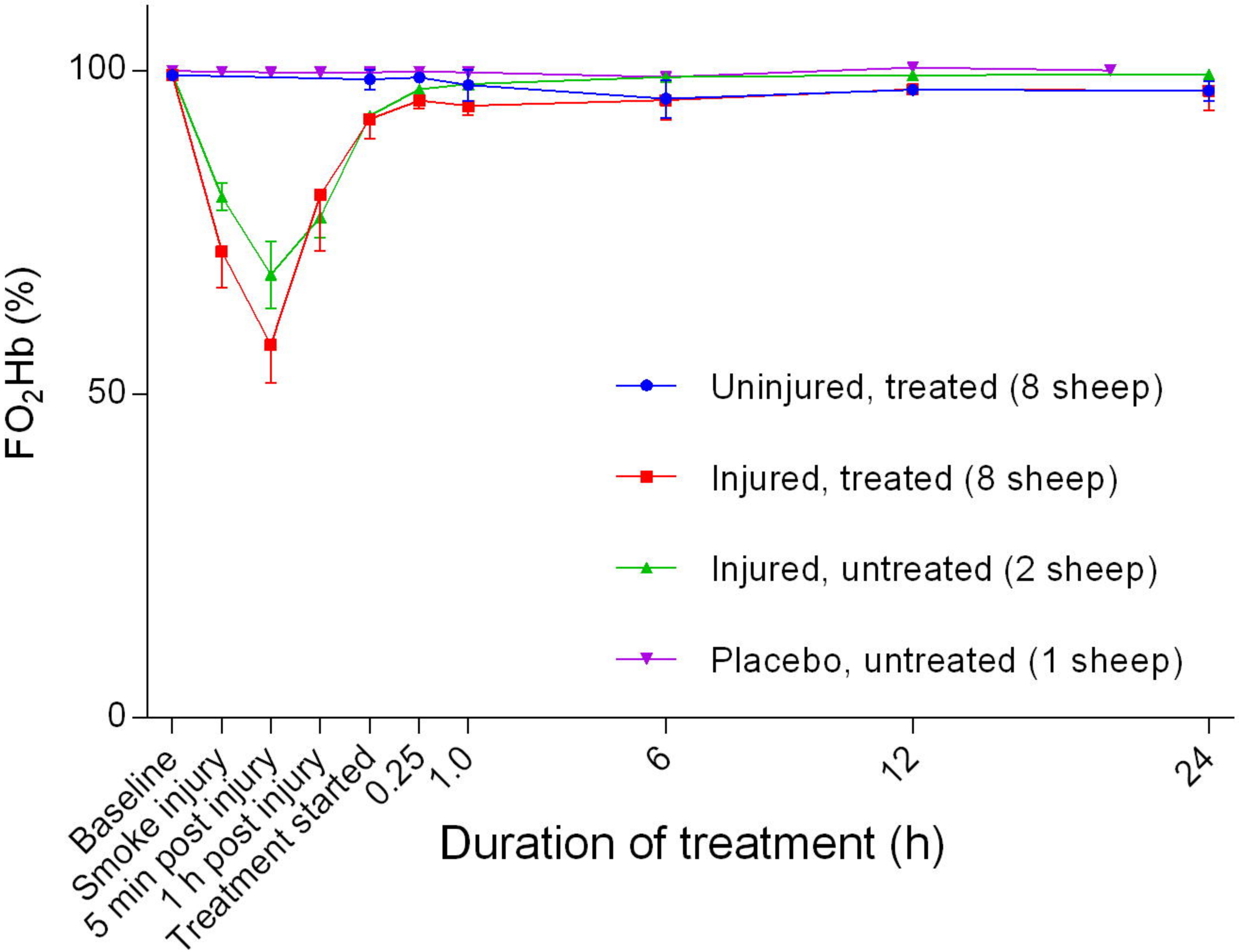


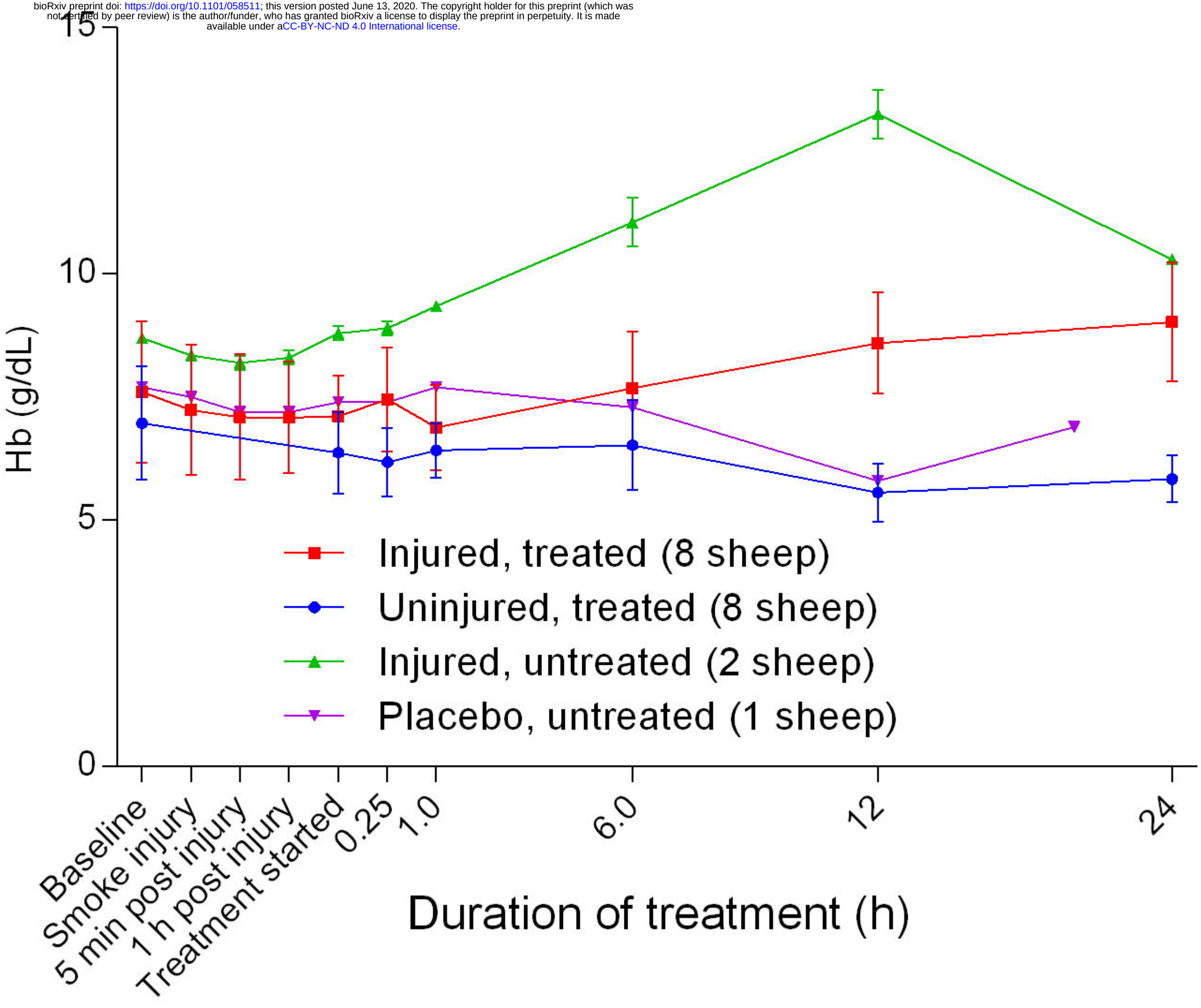


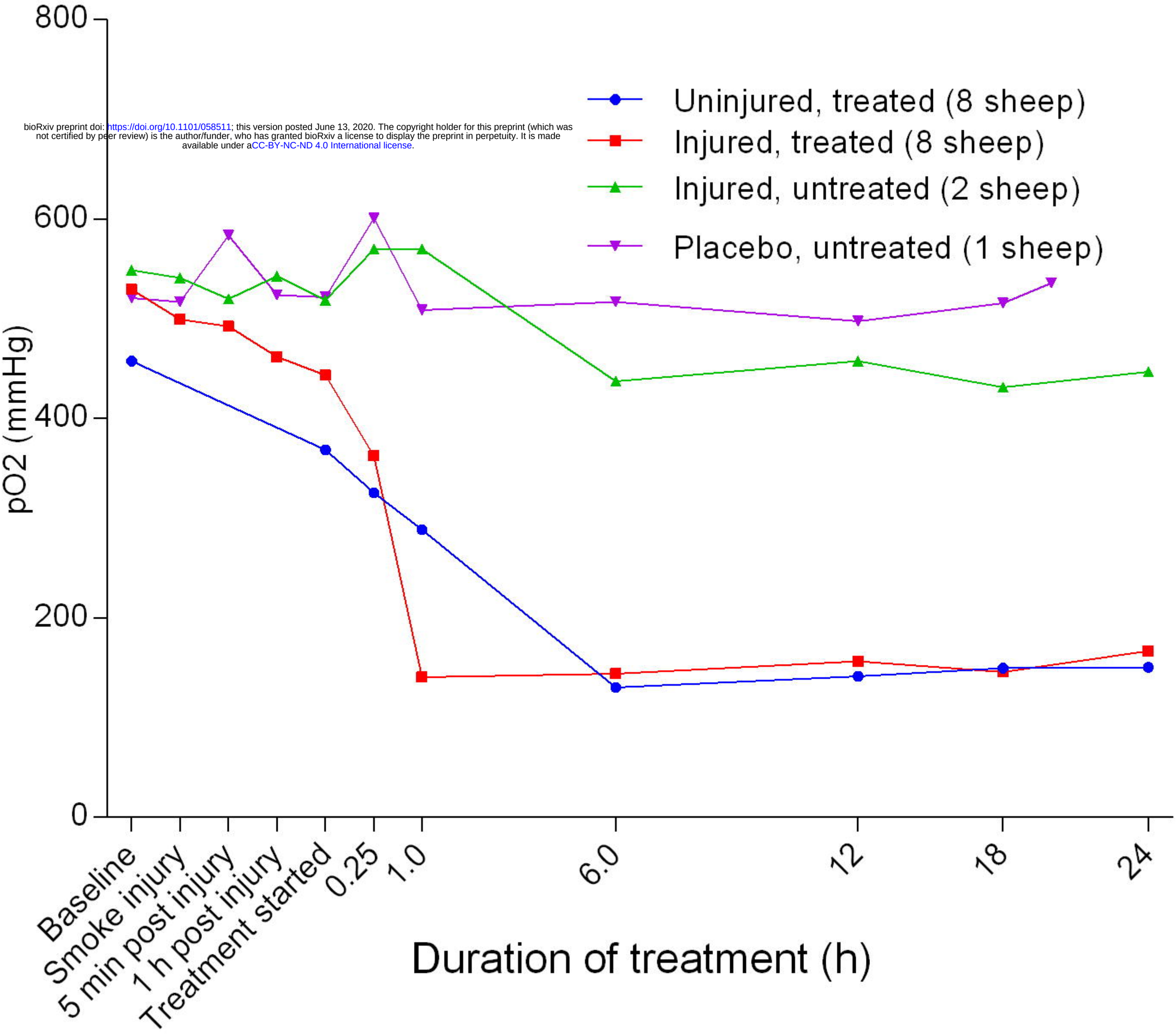


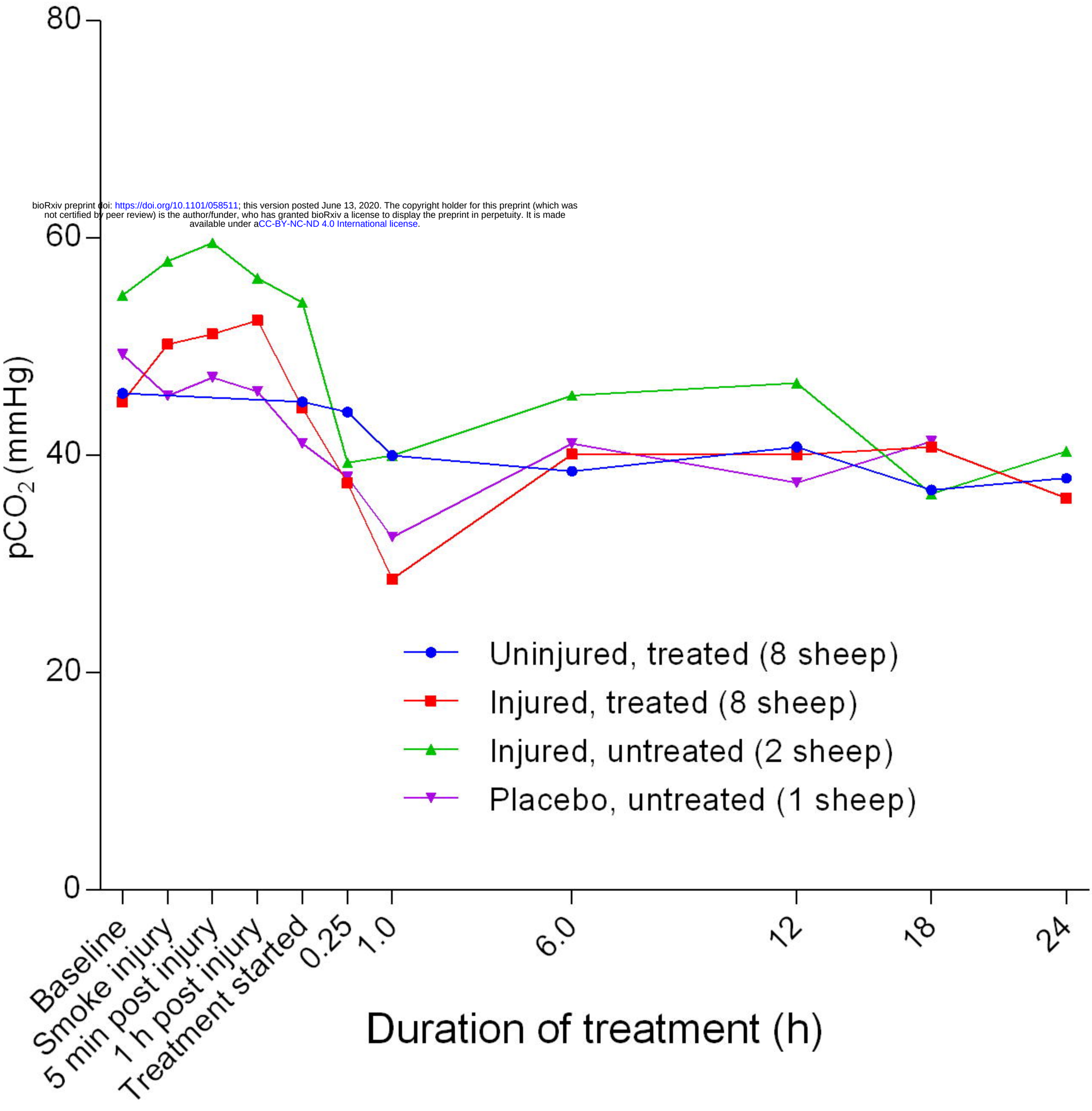


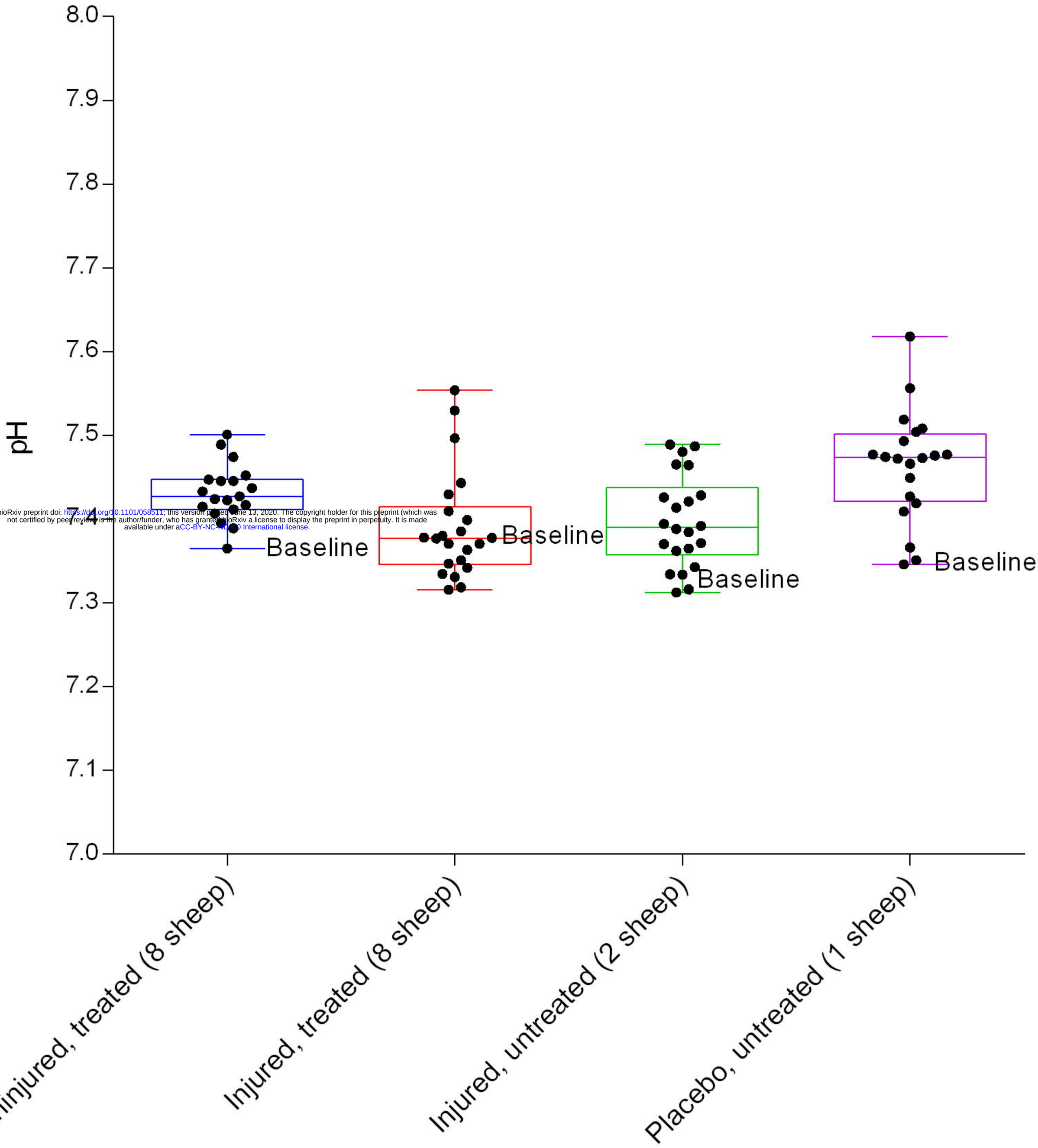


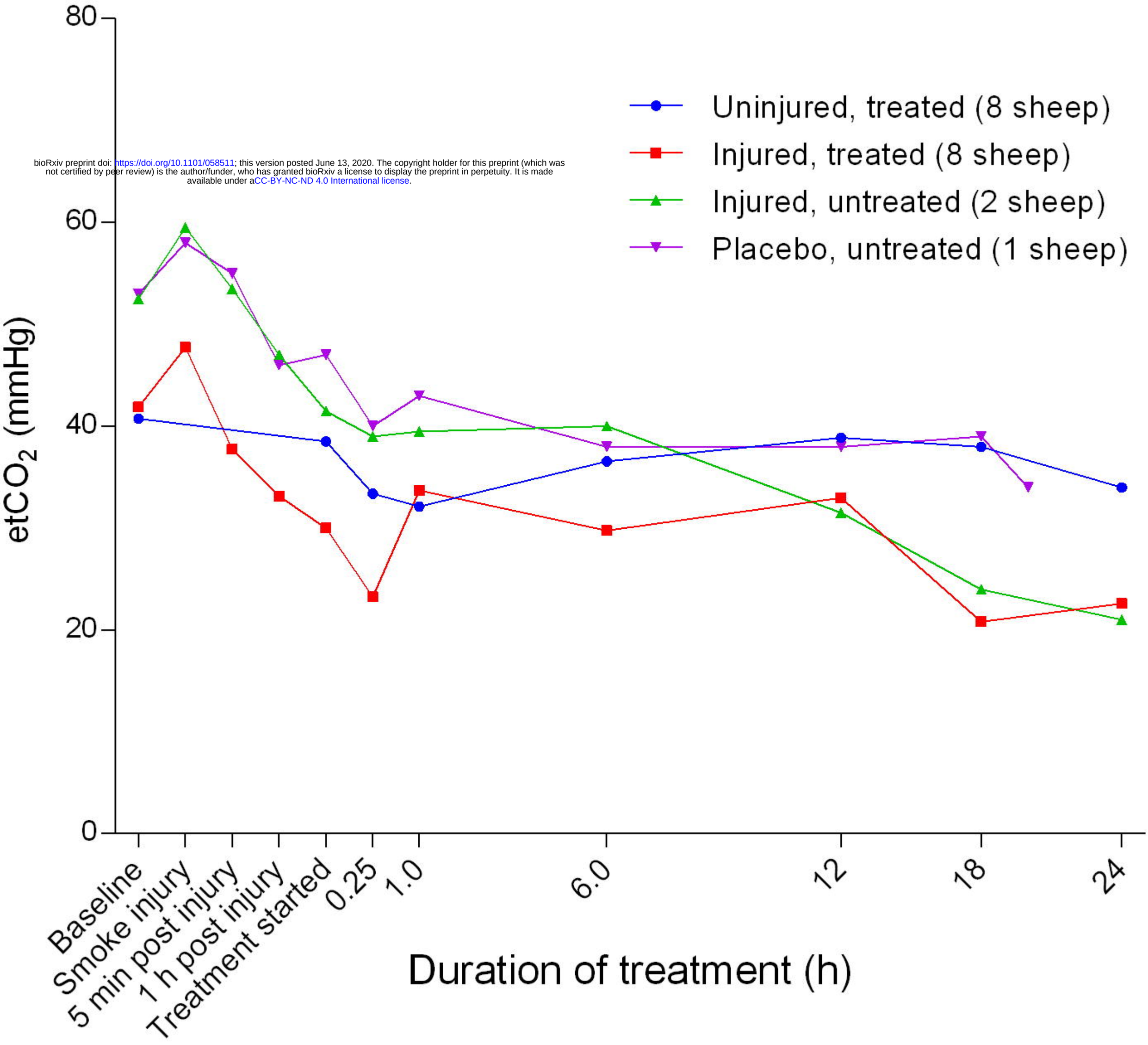


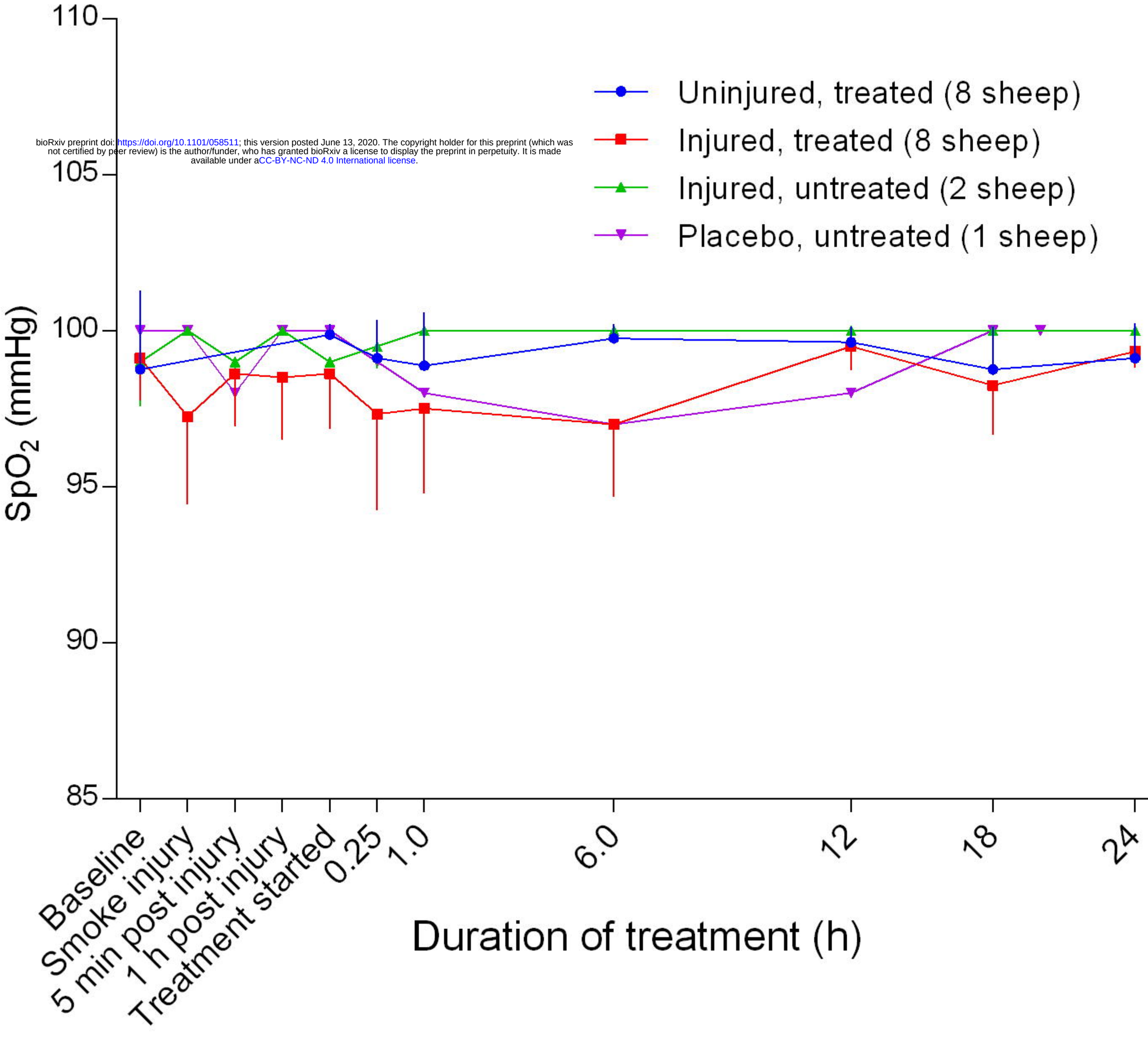


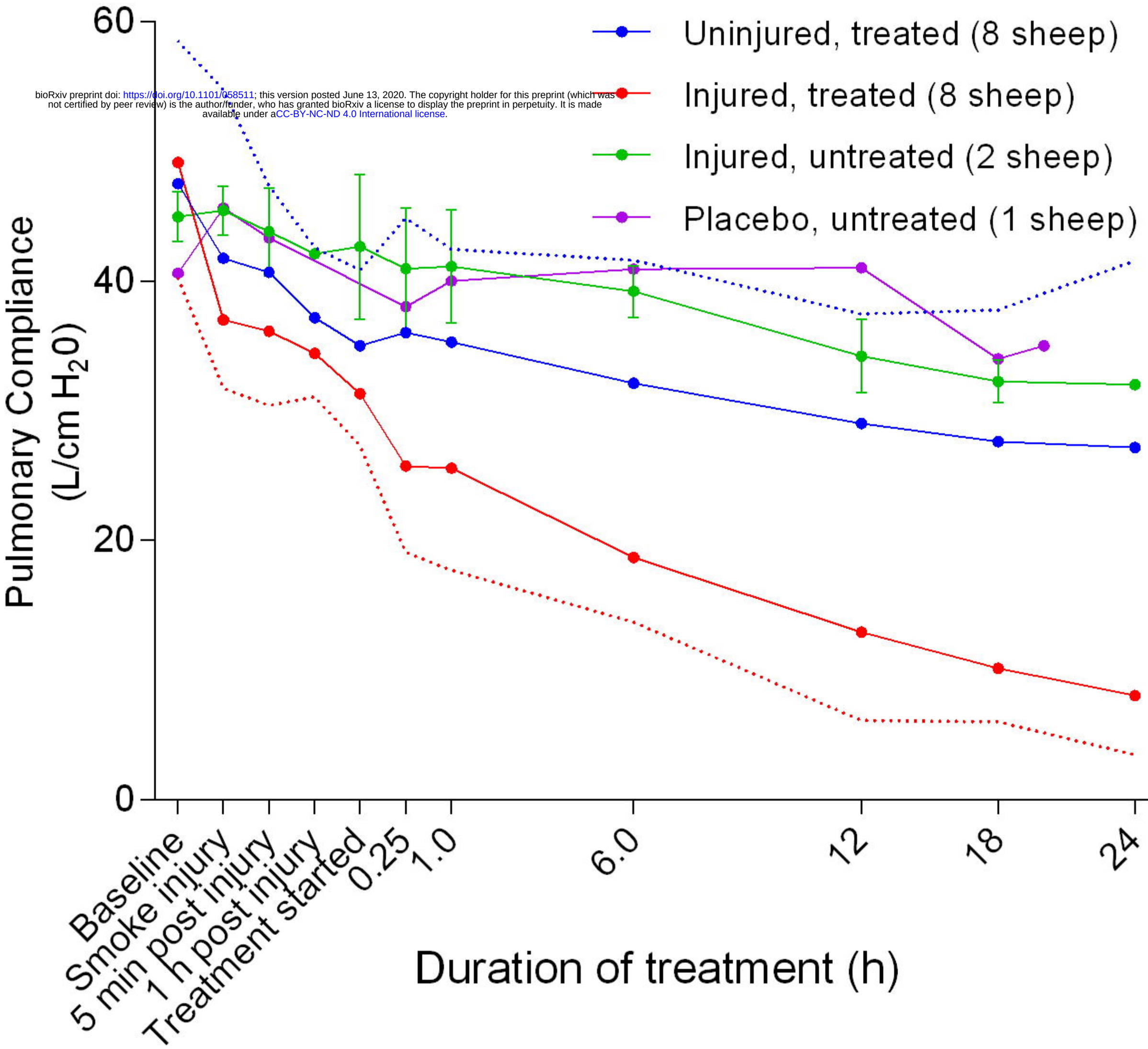


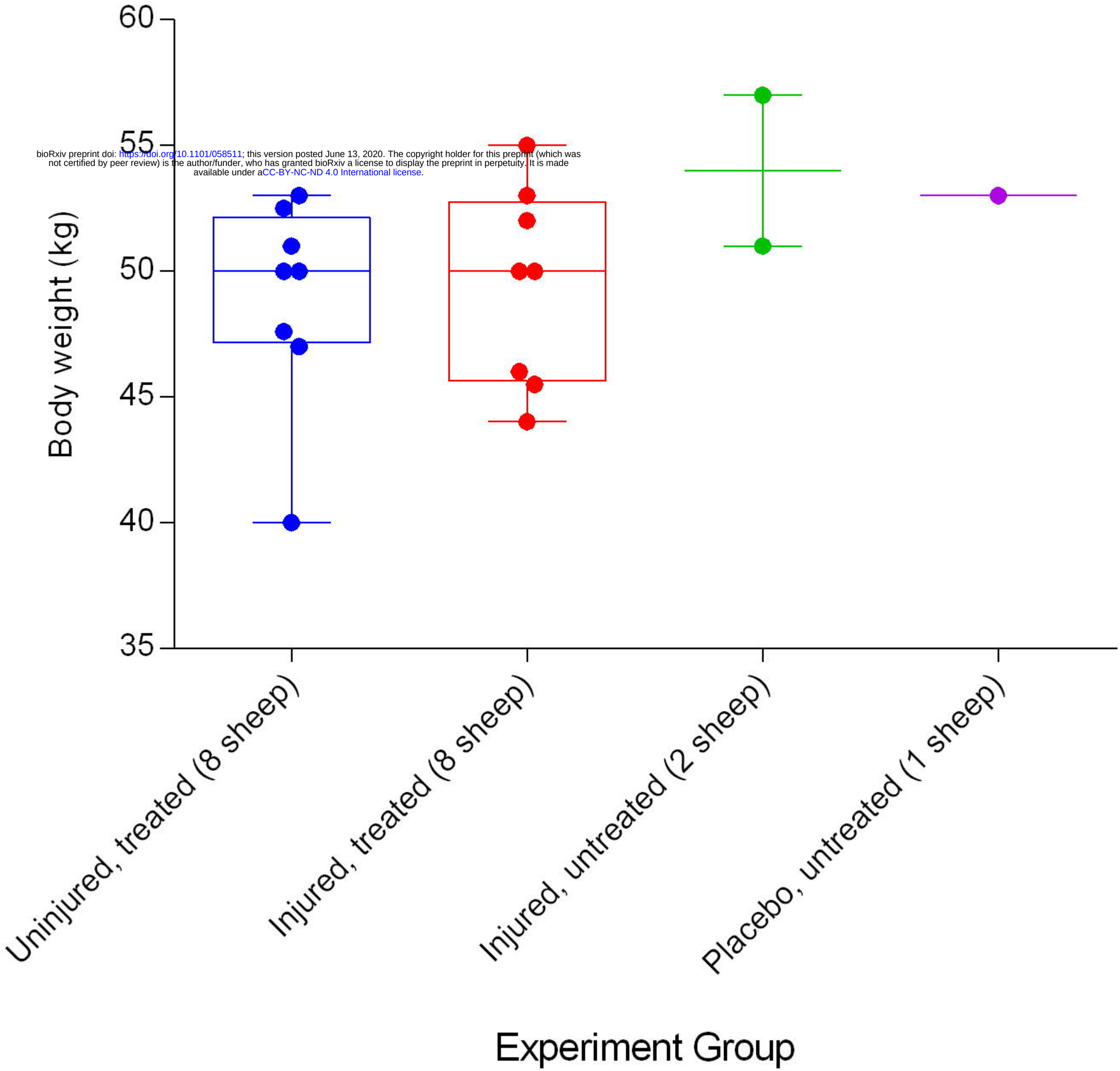


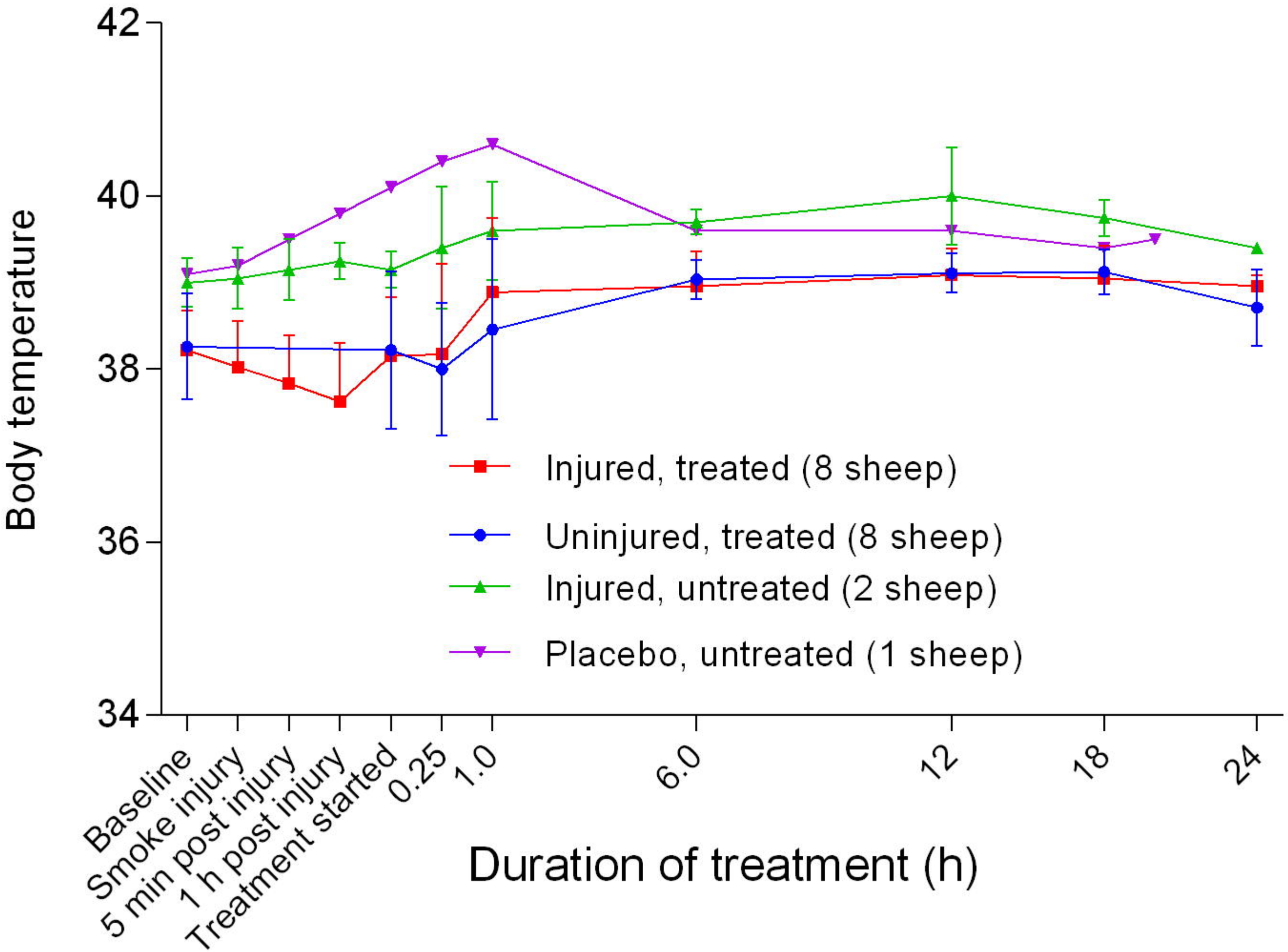


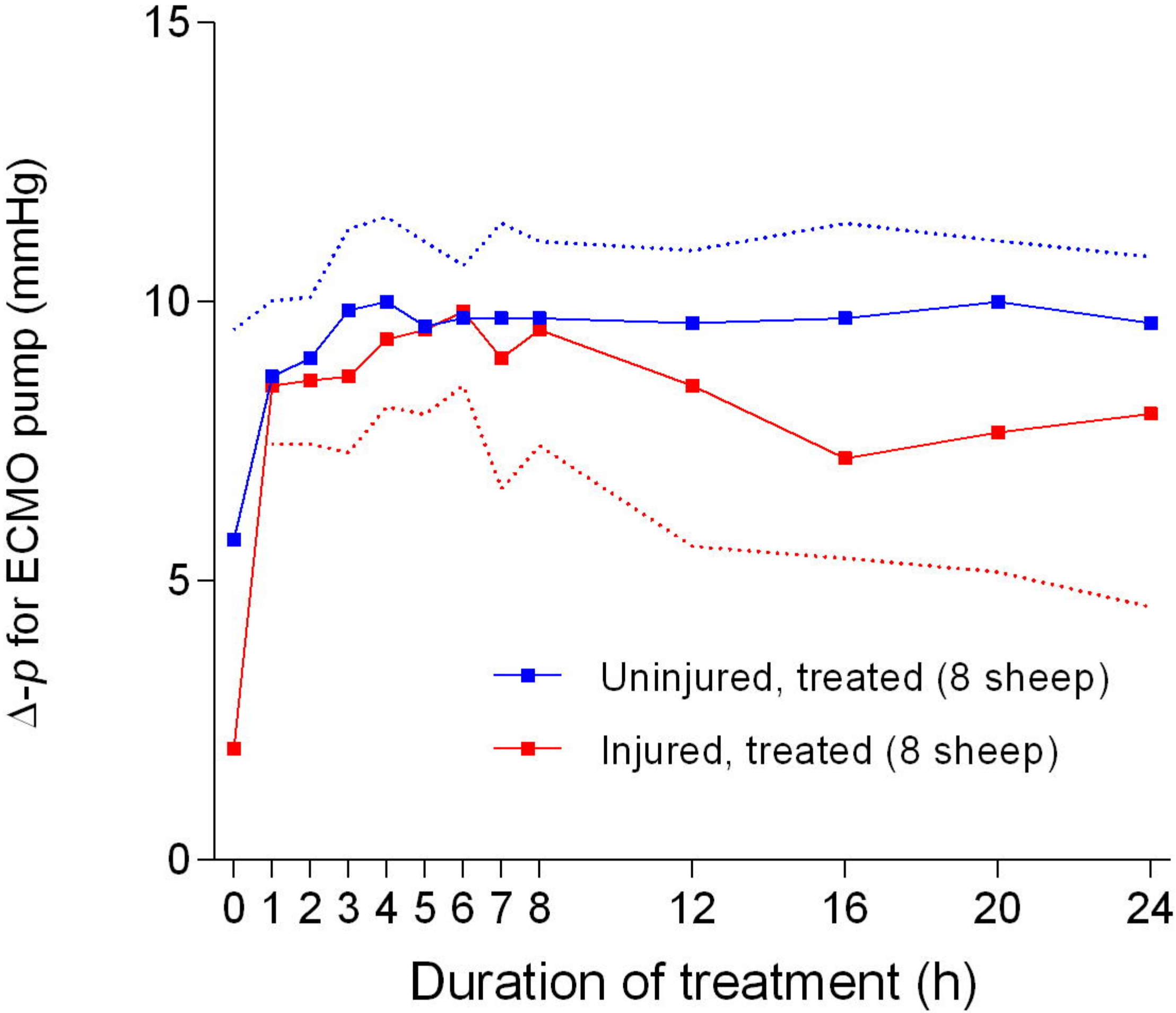


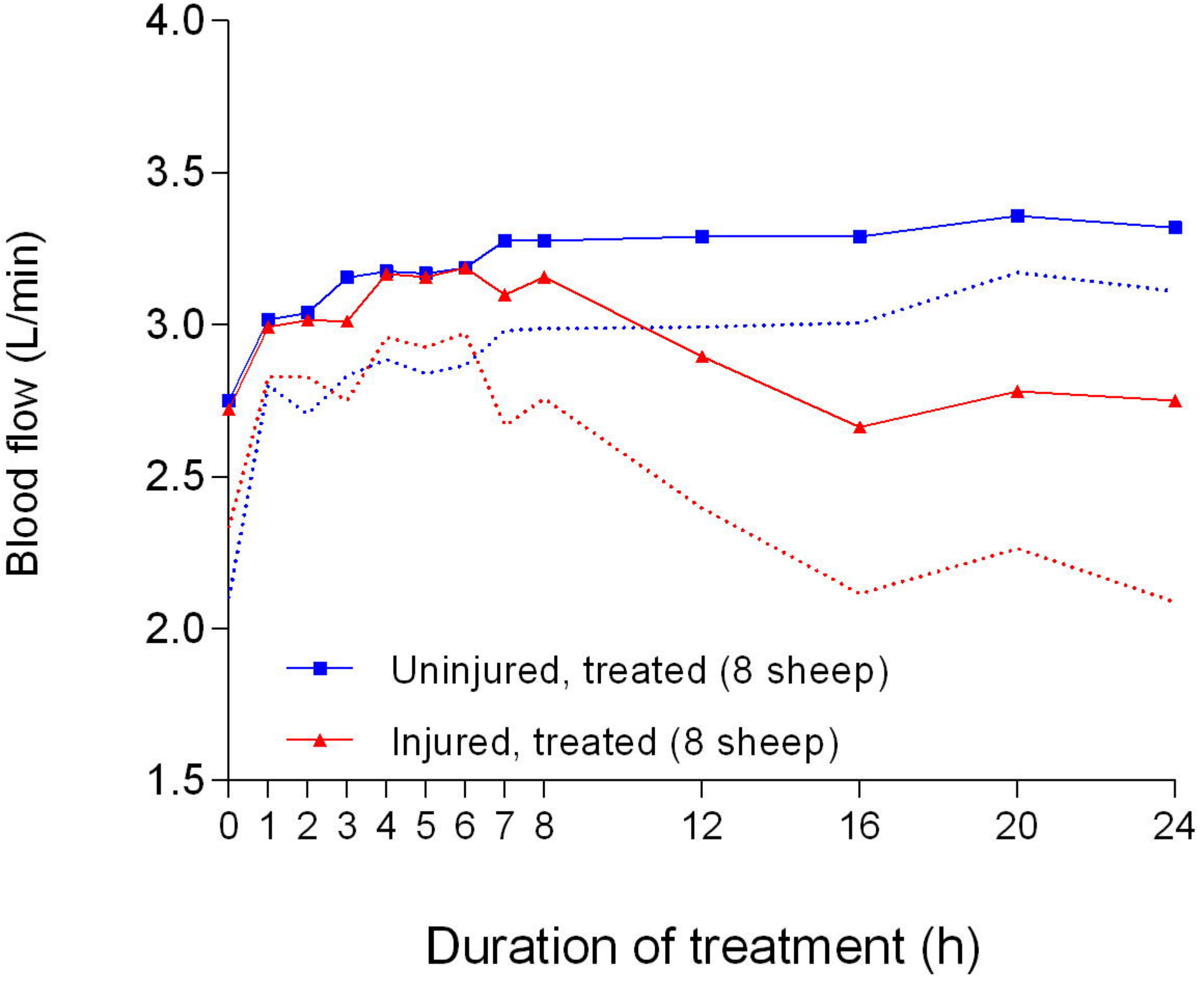


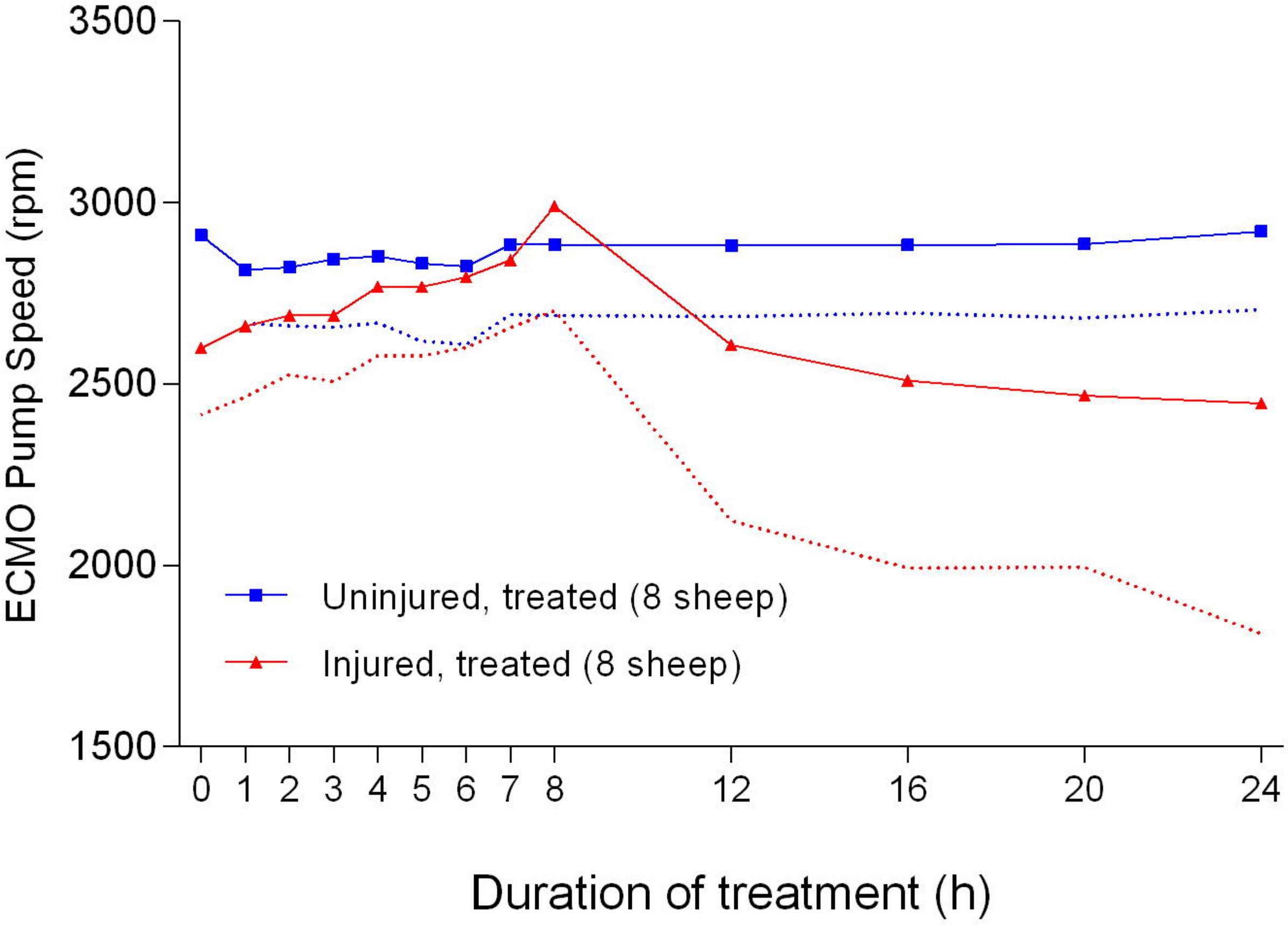


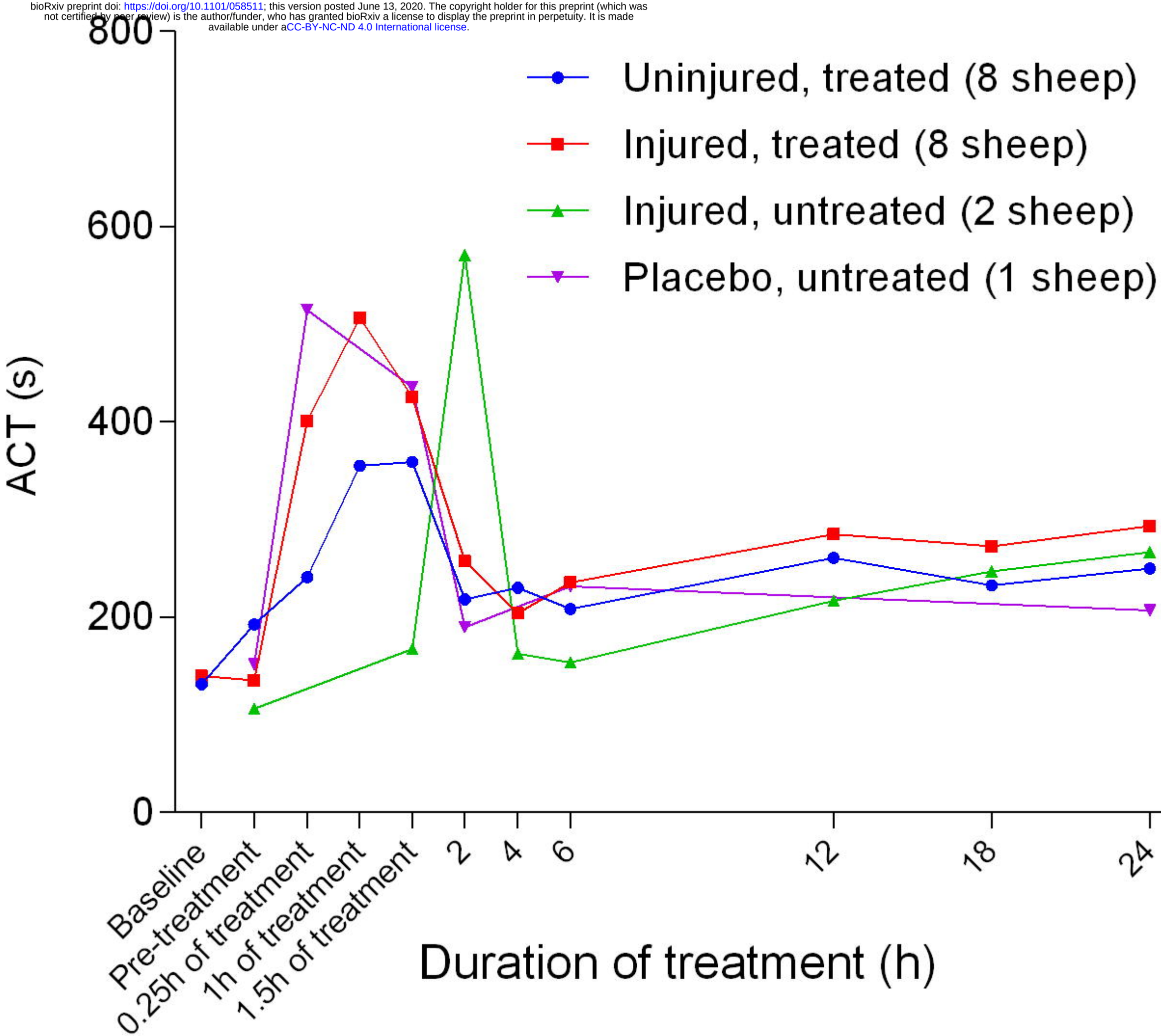





15
10
5
0
Δ-p for ECMO pump (mmHg)
0 1 2 3 4 5 6 7 8 12 16 20 24
Duration of treatment (h)
Uninjured, treated (8 sheep)
Injured, treated (8 sheep)


4.0
3.5
3.0
2.5
2.0
1.5
Blood flow (L/min)
Uninjured, treated (8 sheep)
Injured, treated (8 sheep)
0 1 2 3 4 5 6 7 8 12 16 20 24
Duration of treatment (h)


ECMO Pump Speed (rpm)
3500
3000
2500
2000
1500
0
1
2
3
4
5
6
7
8
12
16
20
24
Uninjured, treated (8 sheep)
Injured, treated (8 sheep)
Duration of treatment (h)



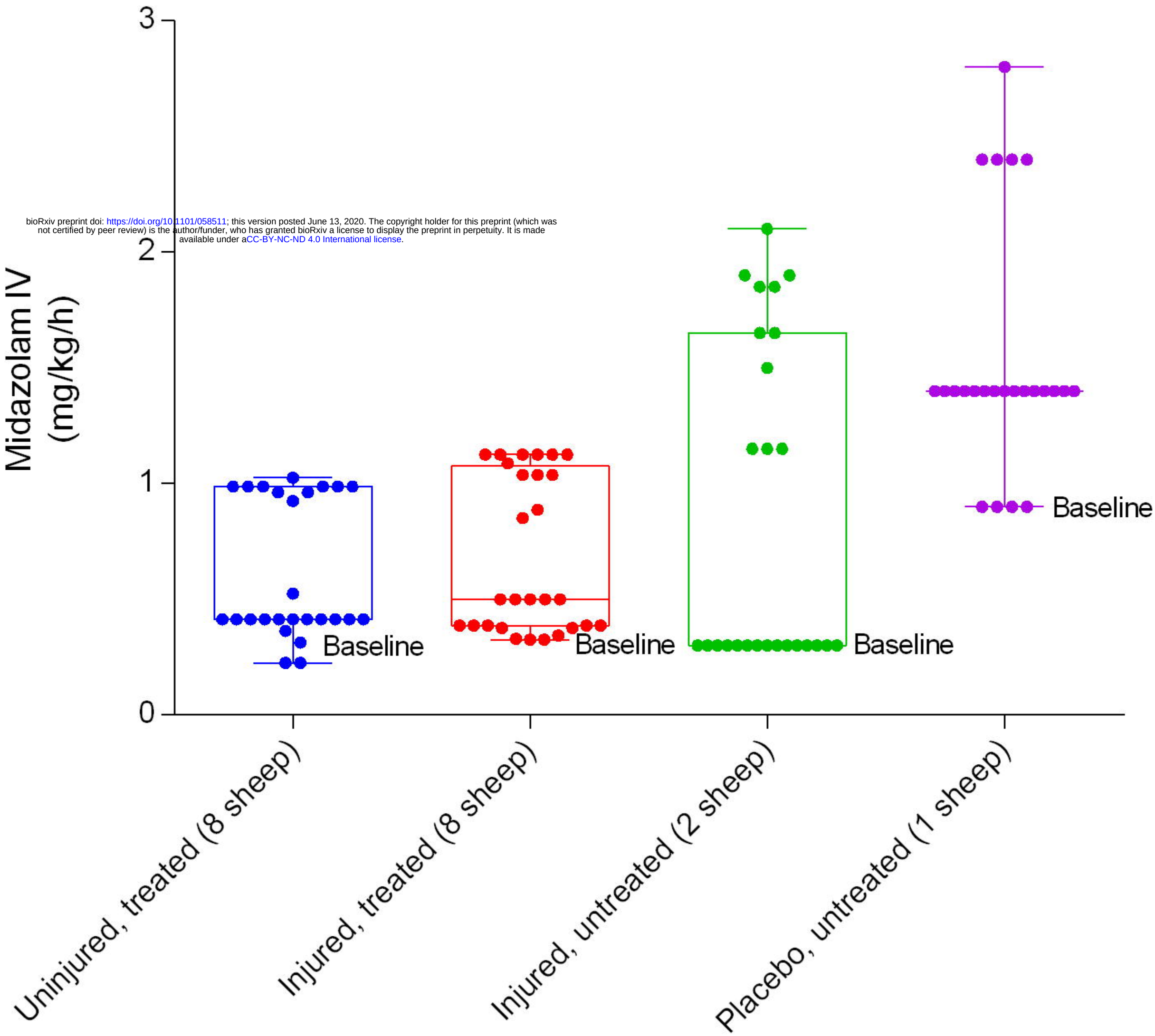
Uninjured, treated (8 sheep)
Injured, treated (8 sheep)
Injured, untreated (2 sheep)
Placebo, untreated (1 sheep)

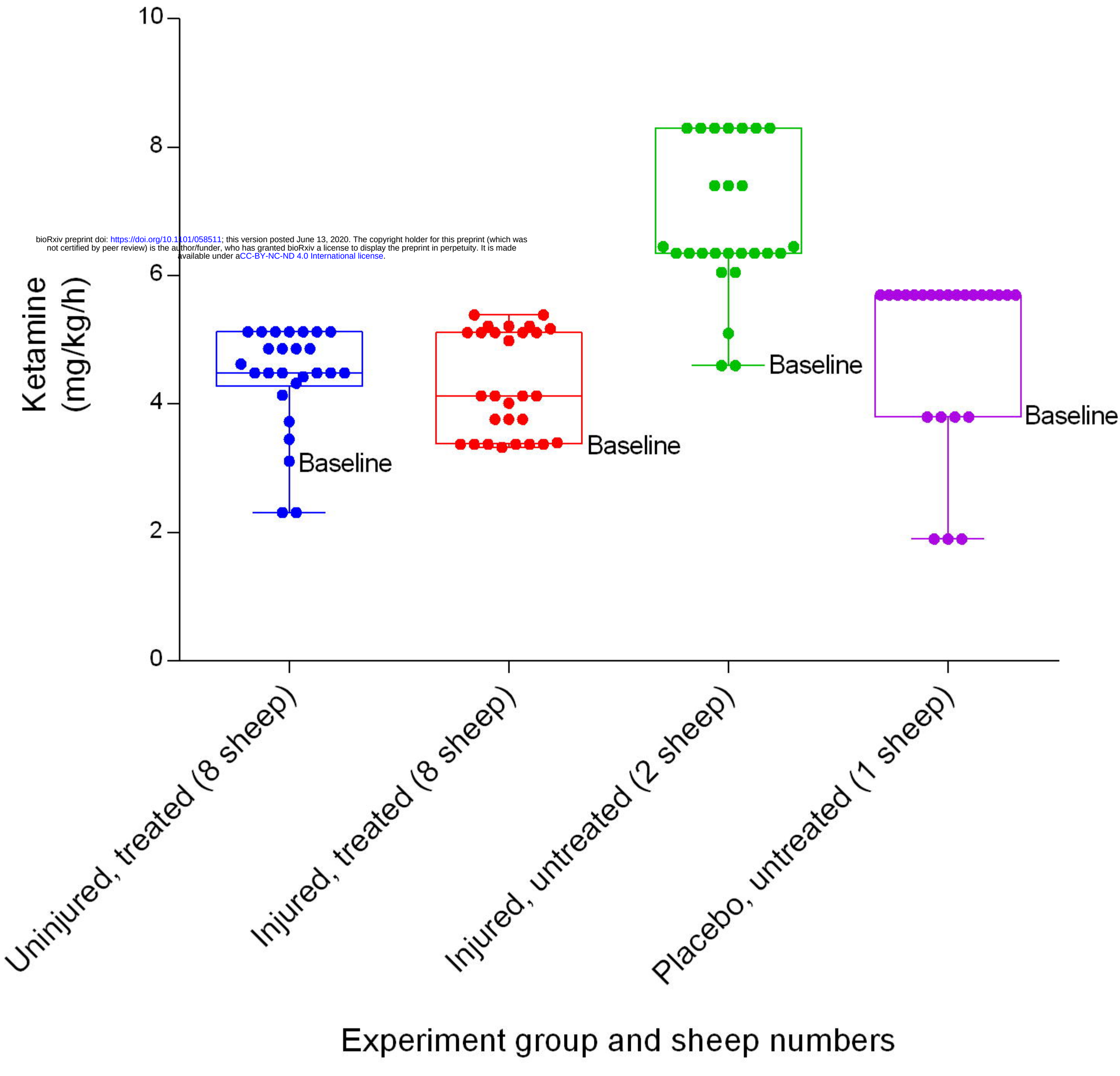
Heparin -10 U/mL infusion (mL/h)

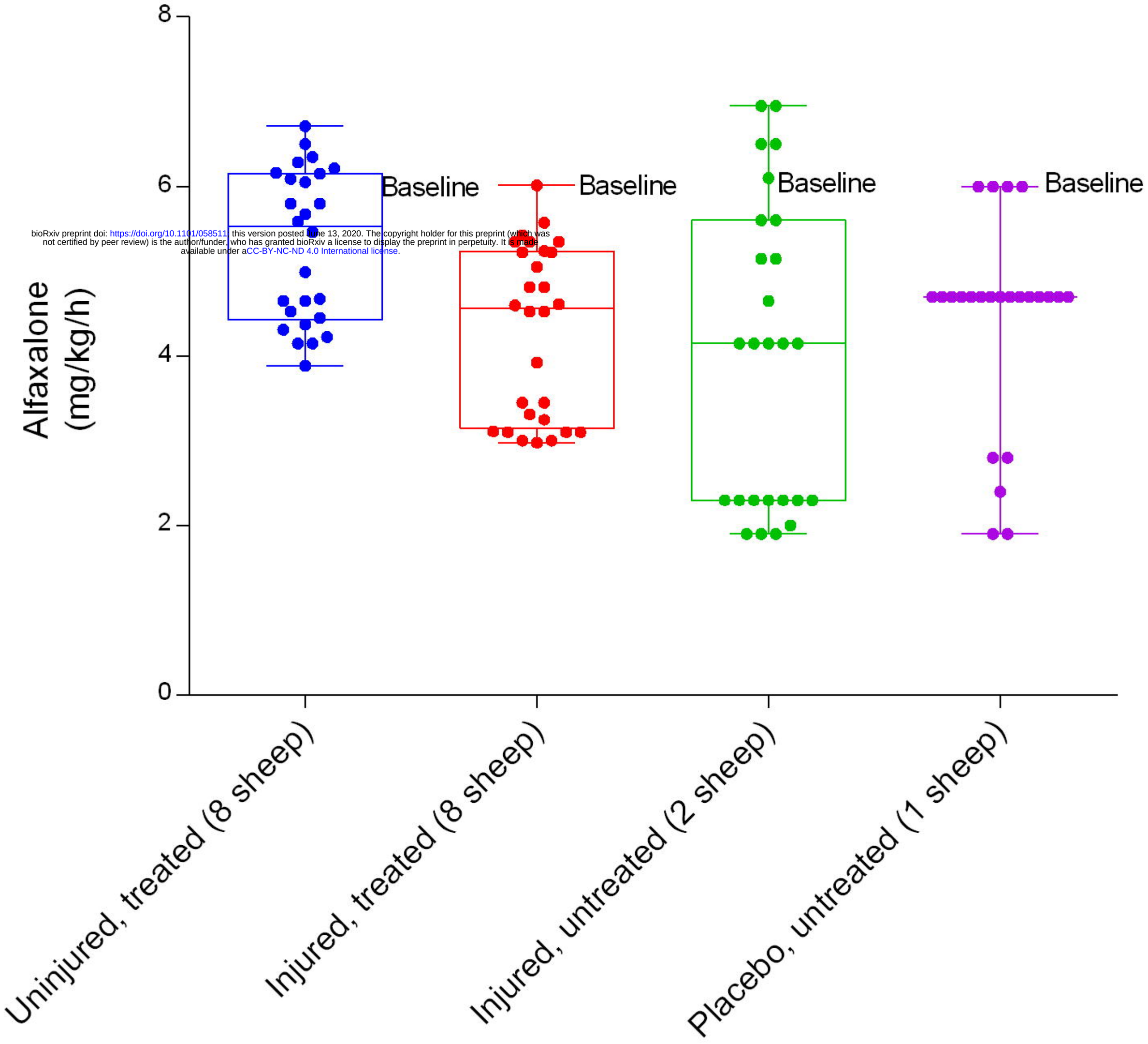
100
80
60
40
20
0

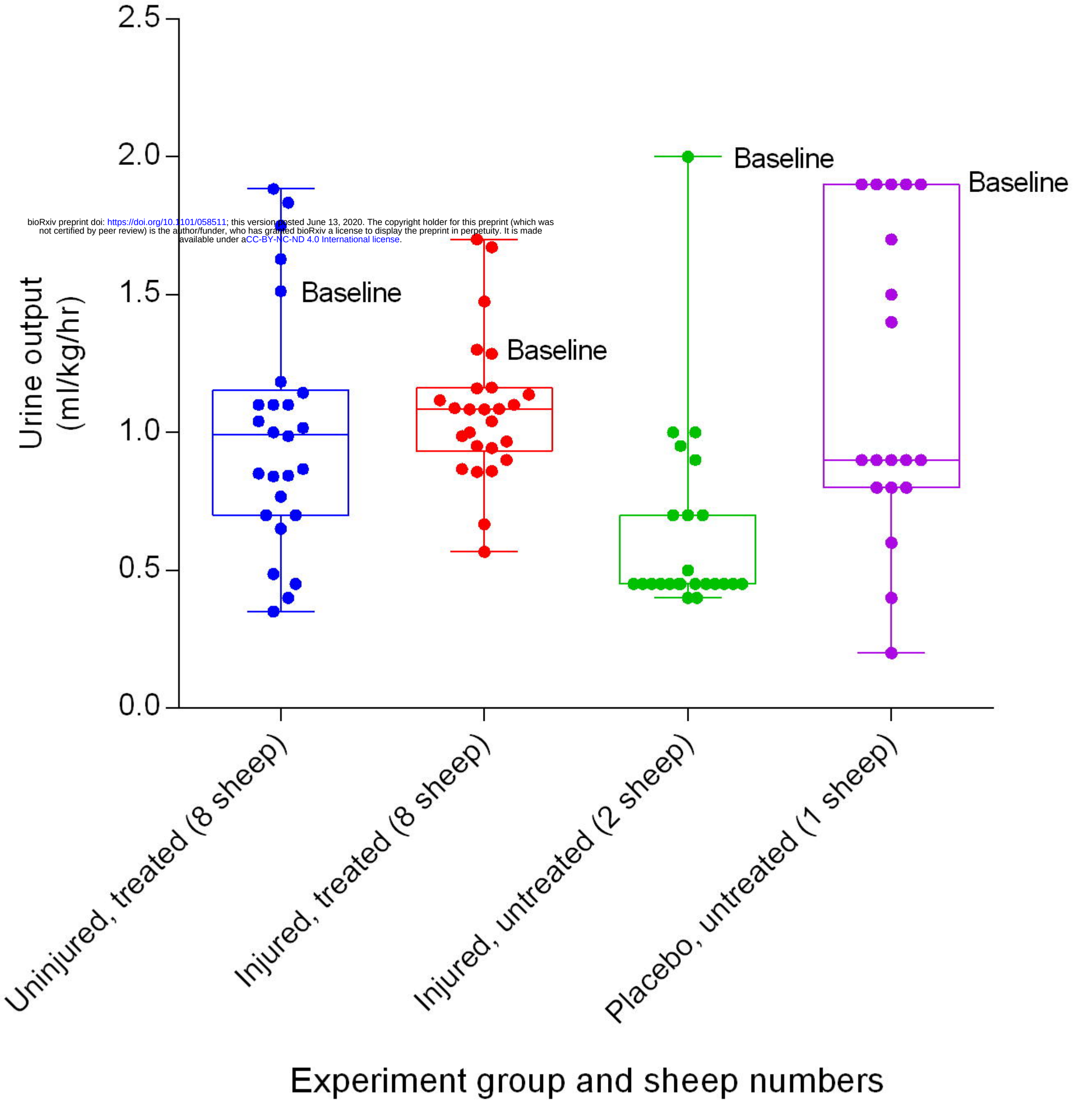
4
6
8
10
12
18
24

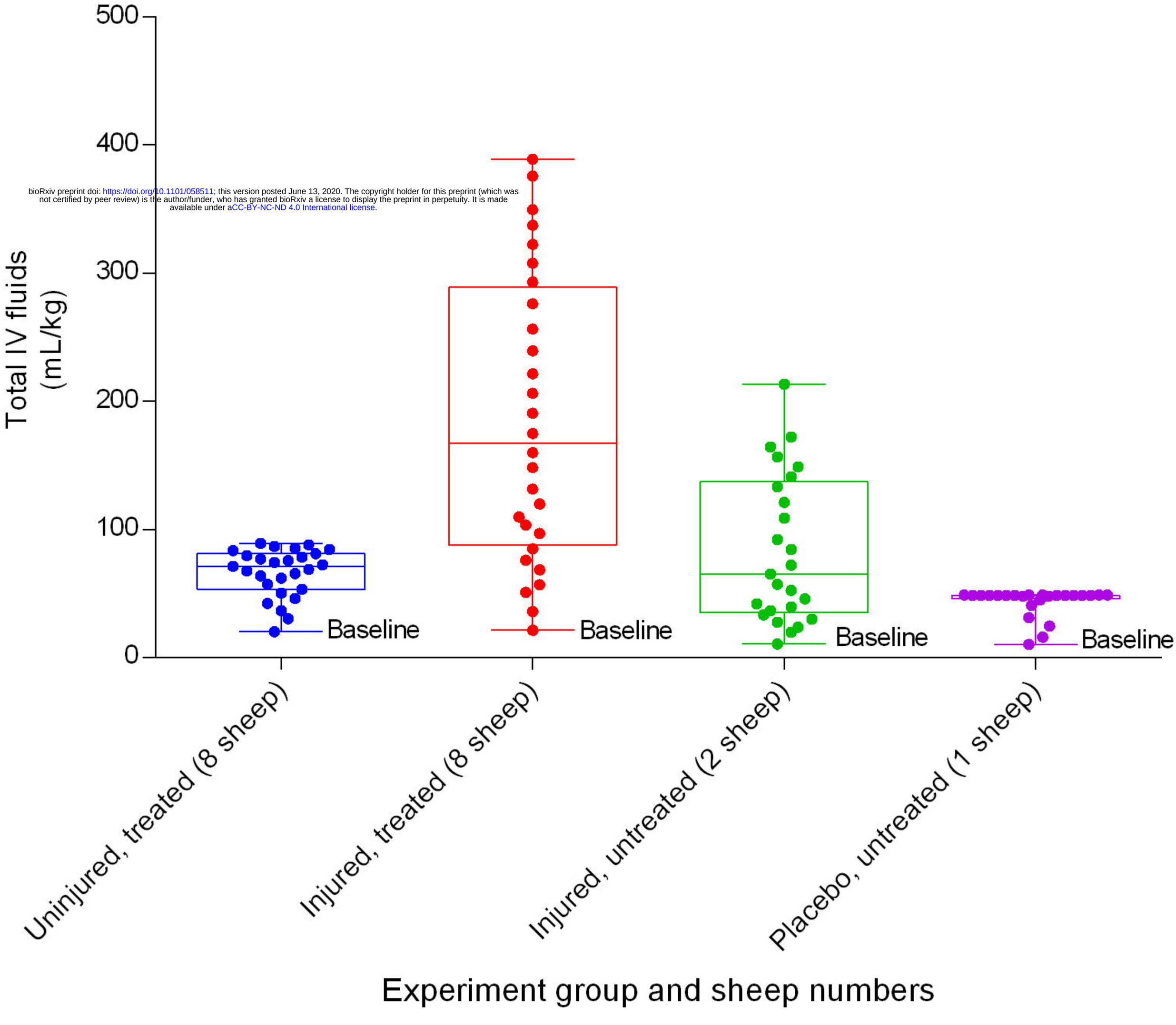
Duration of treatment (h)

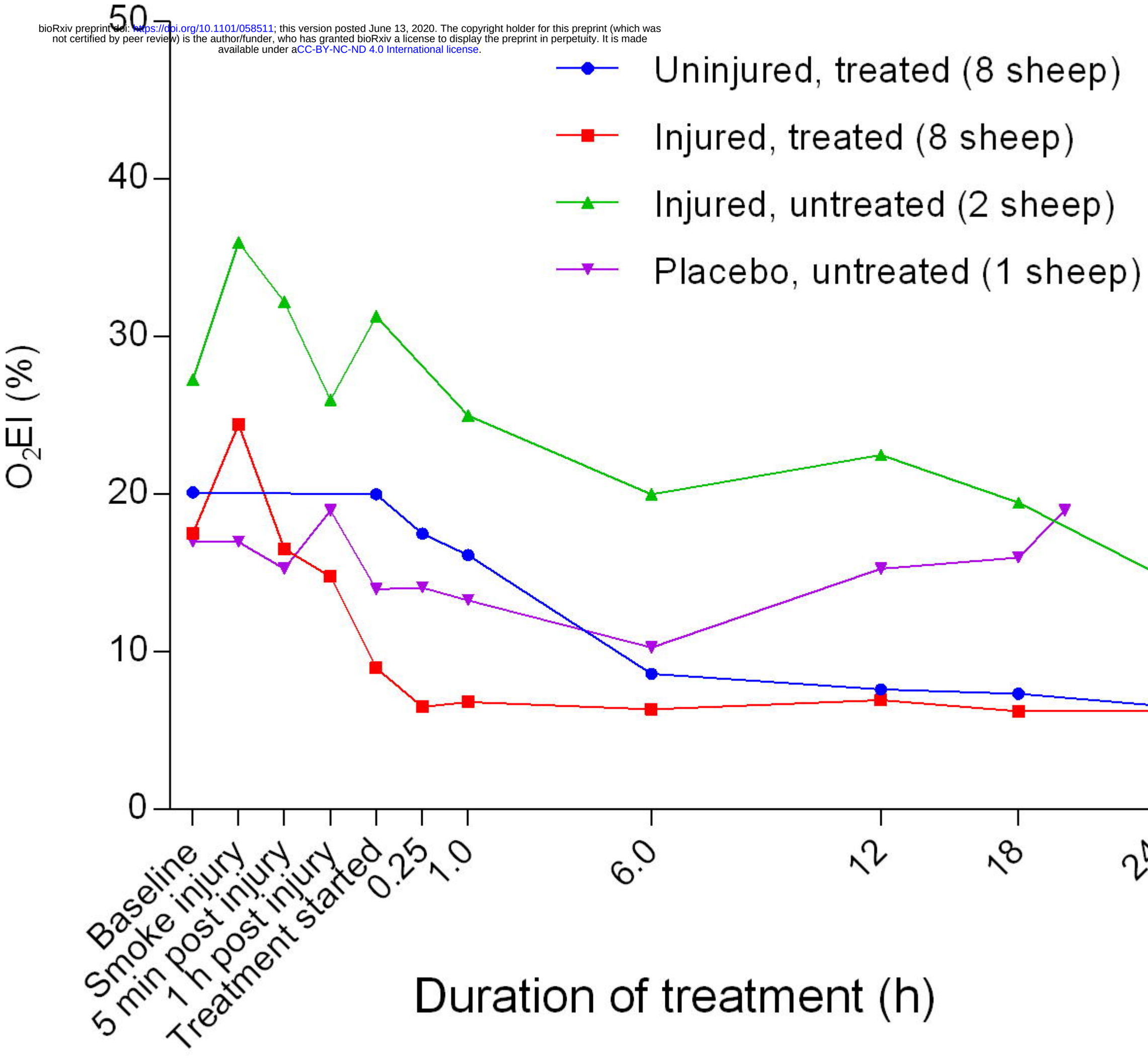


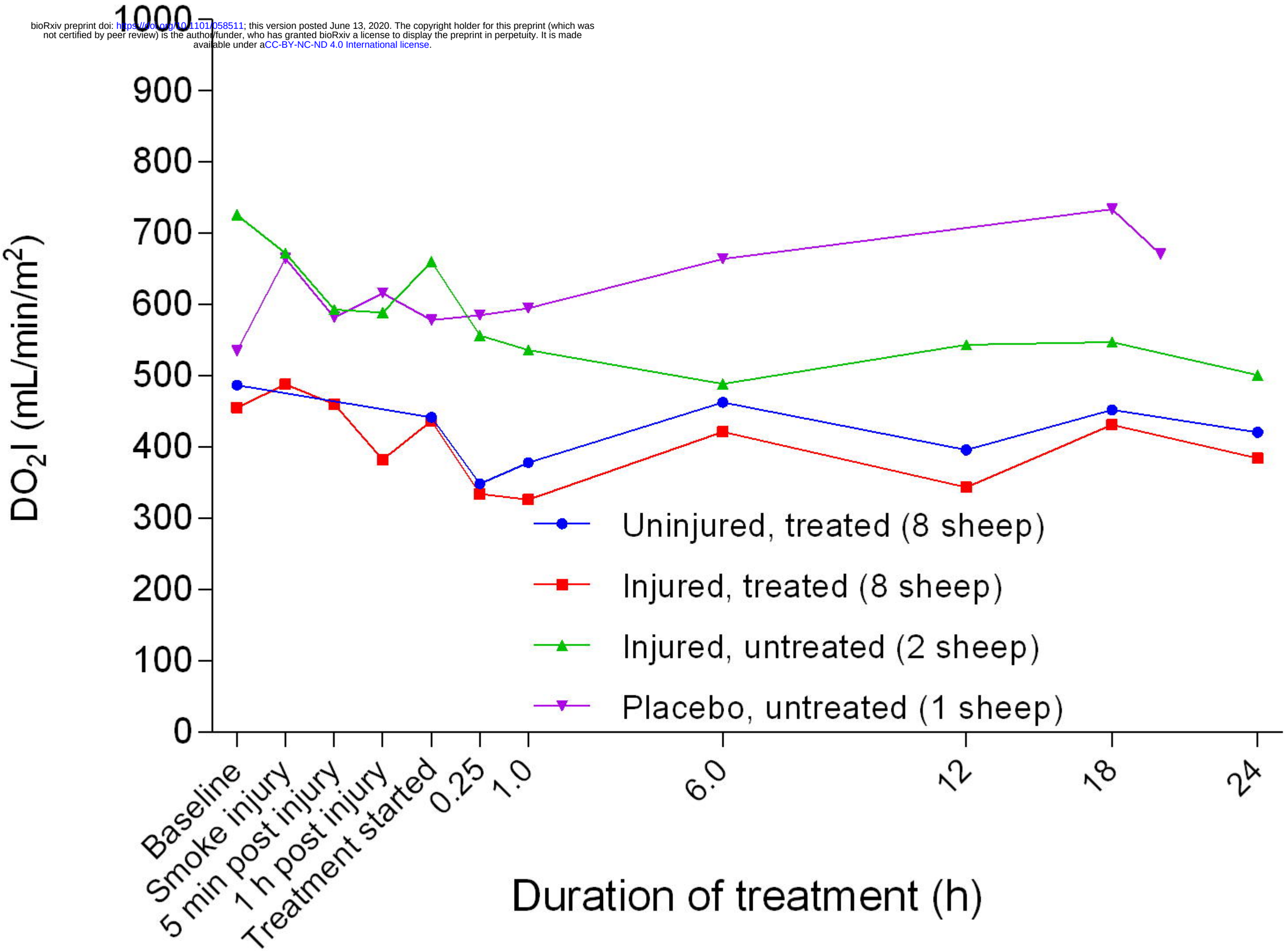


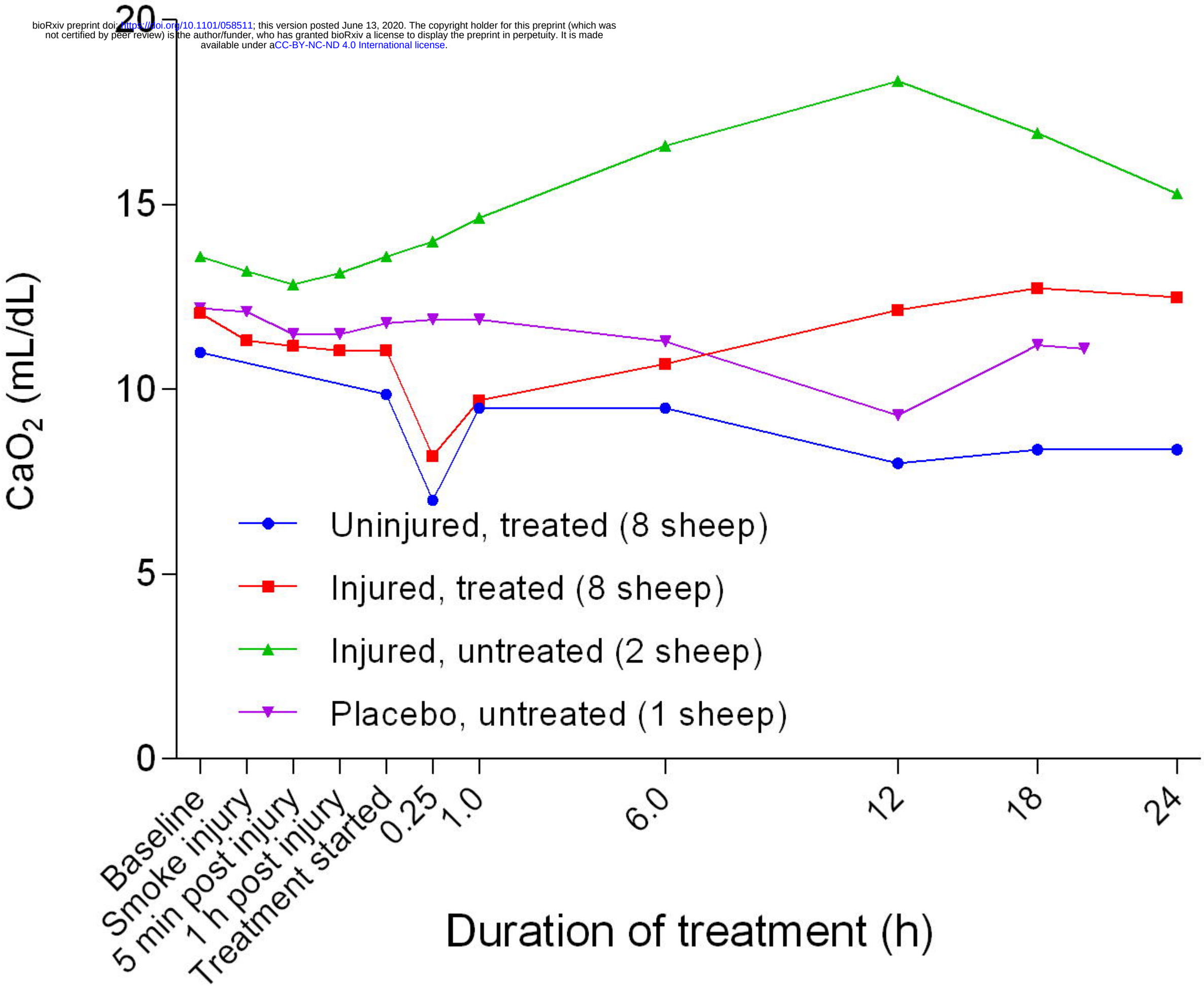


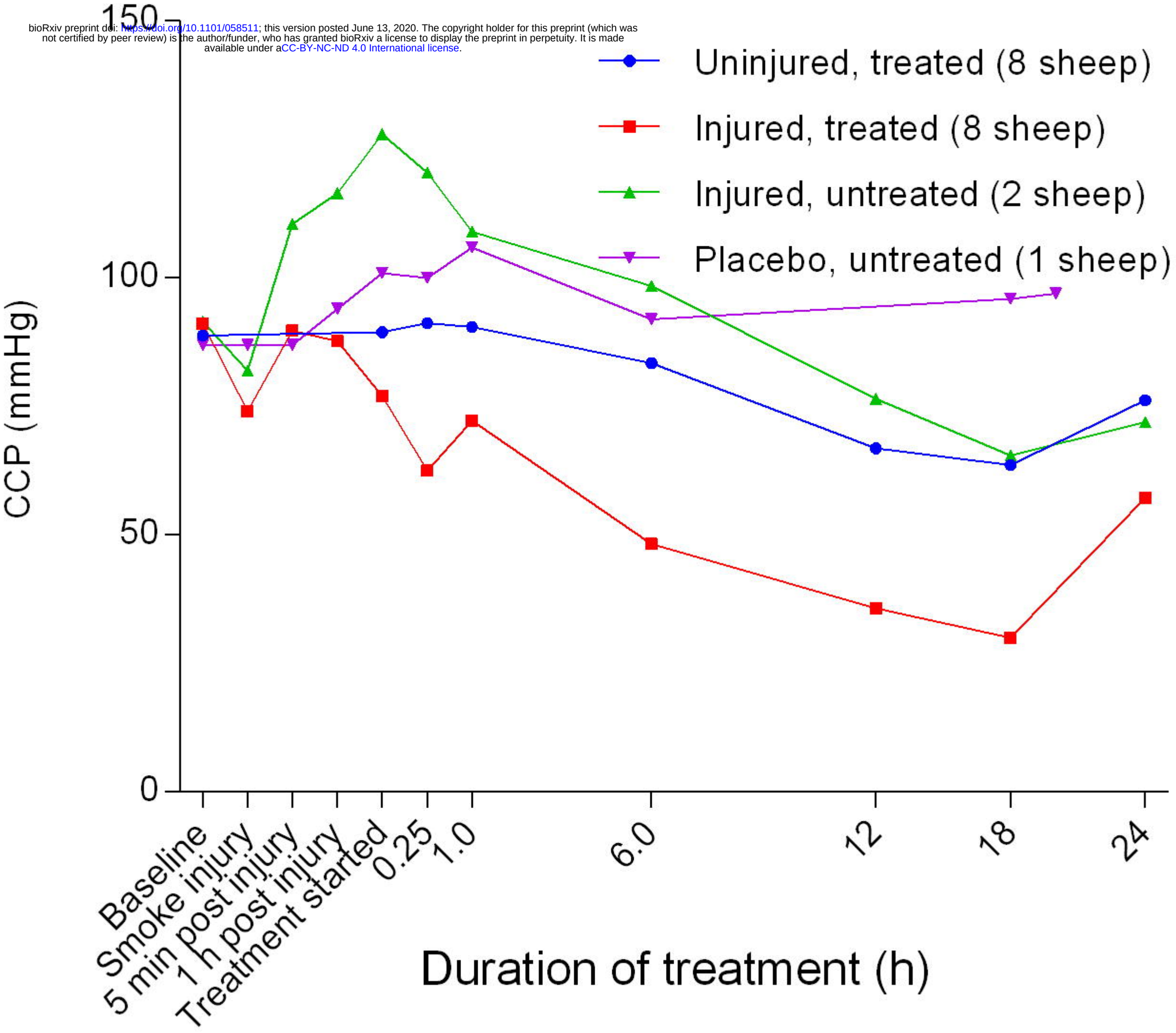


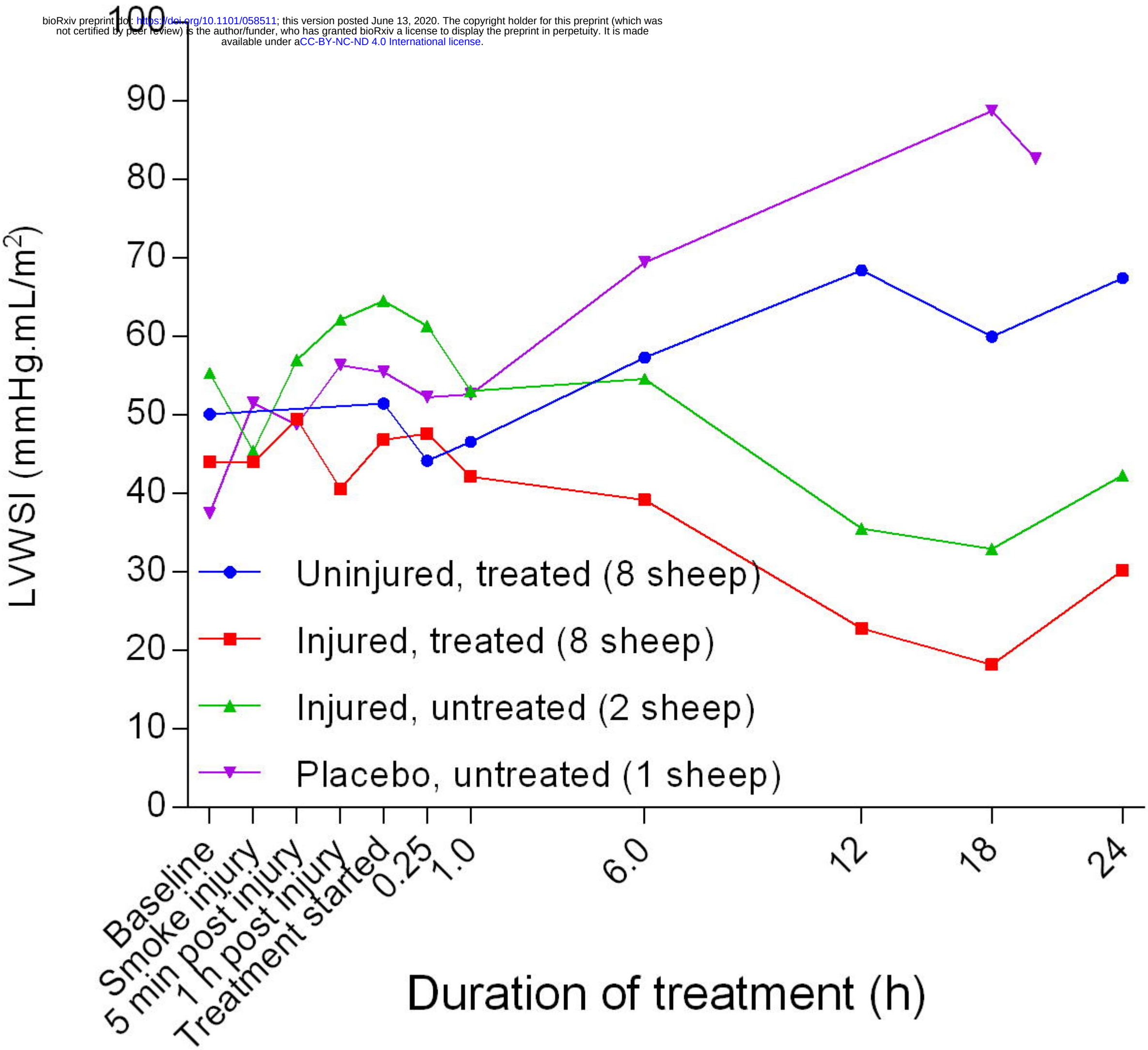


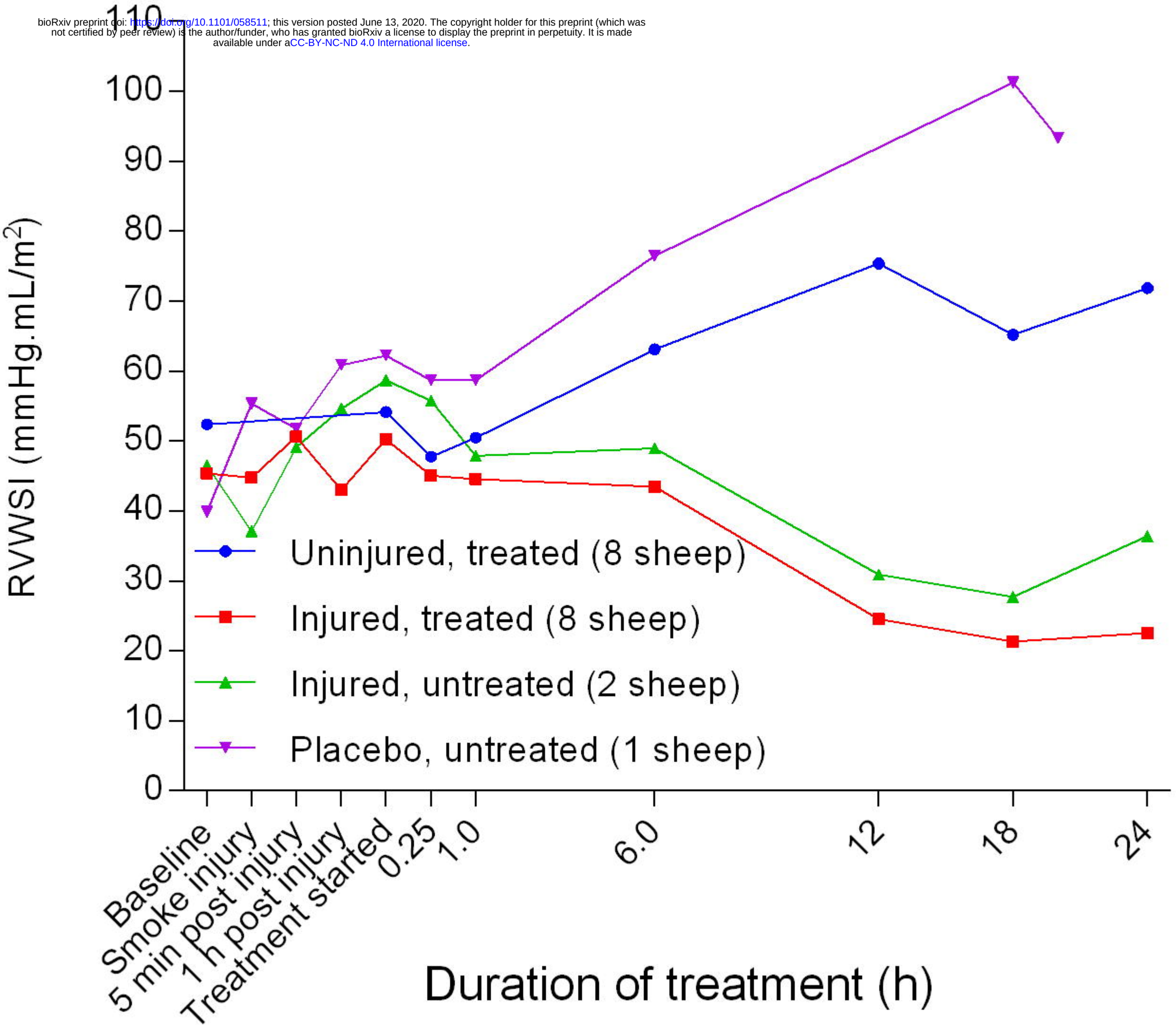


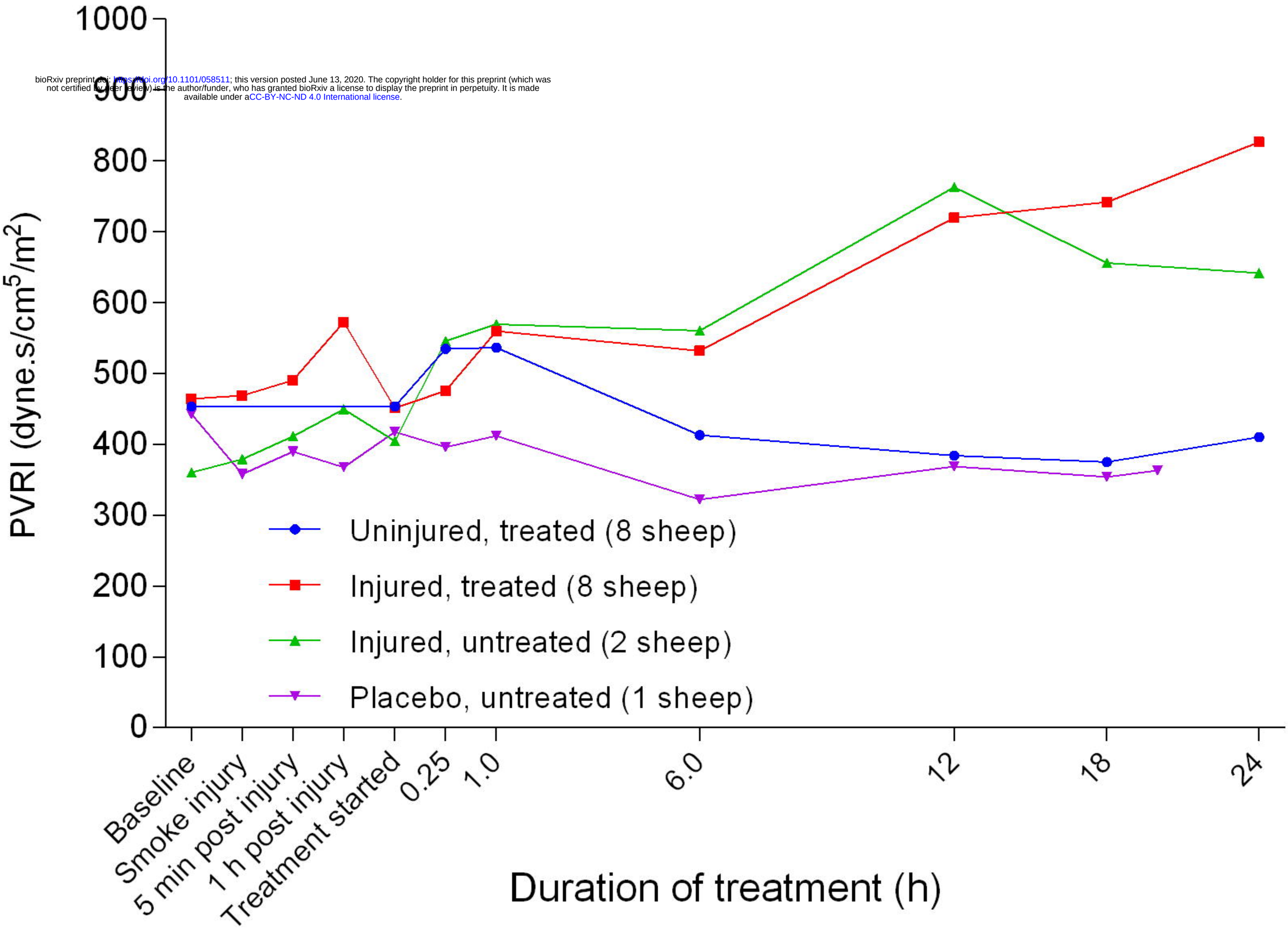


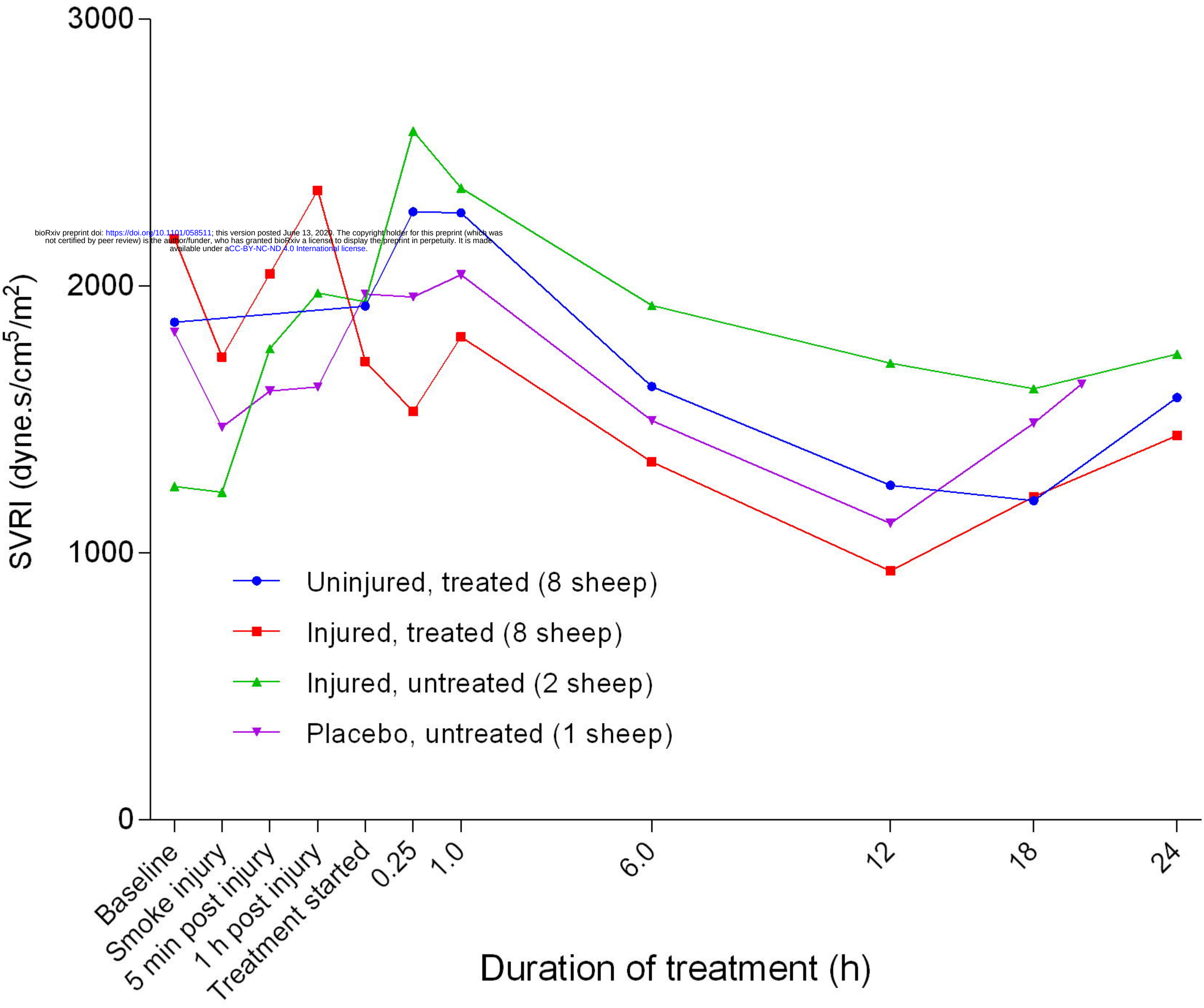


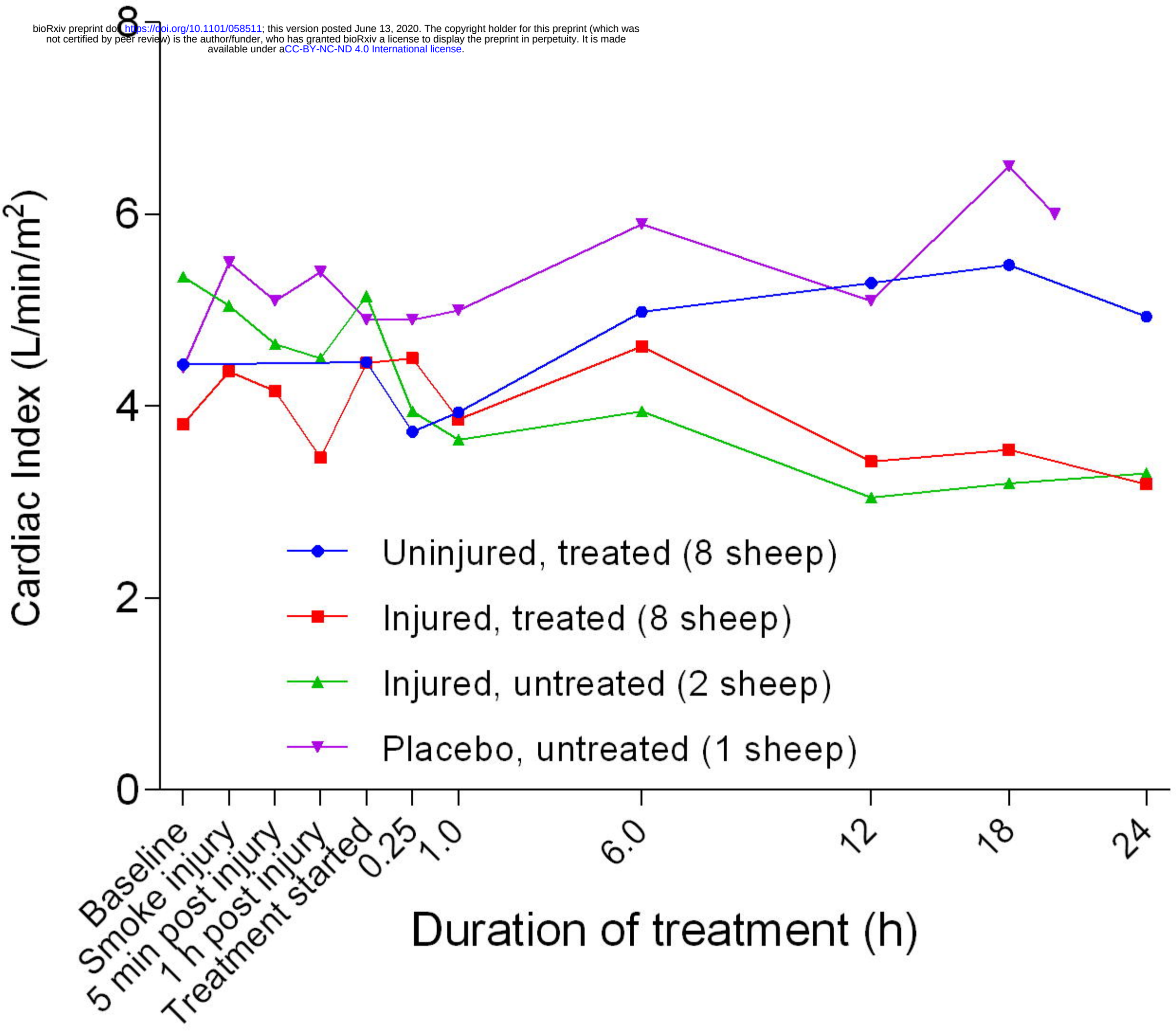


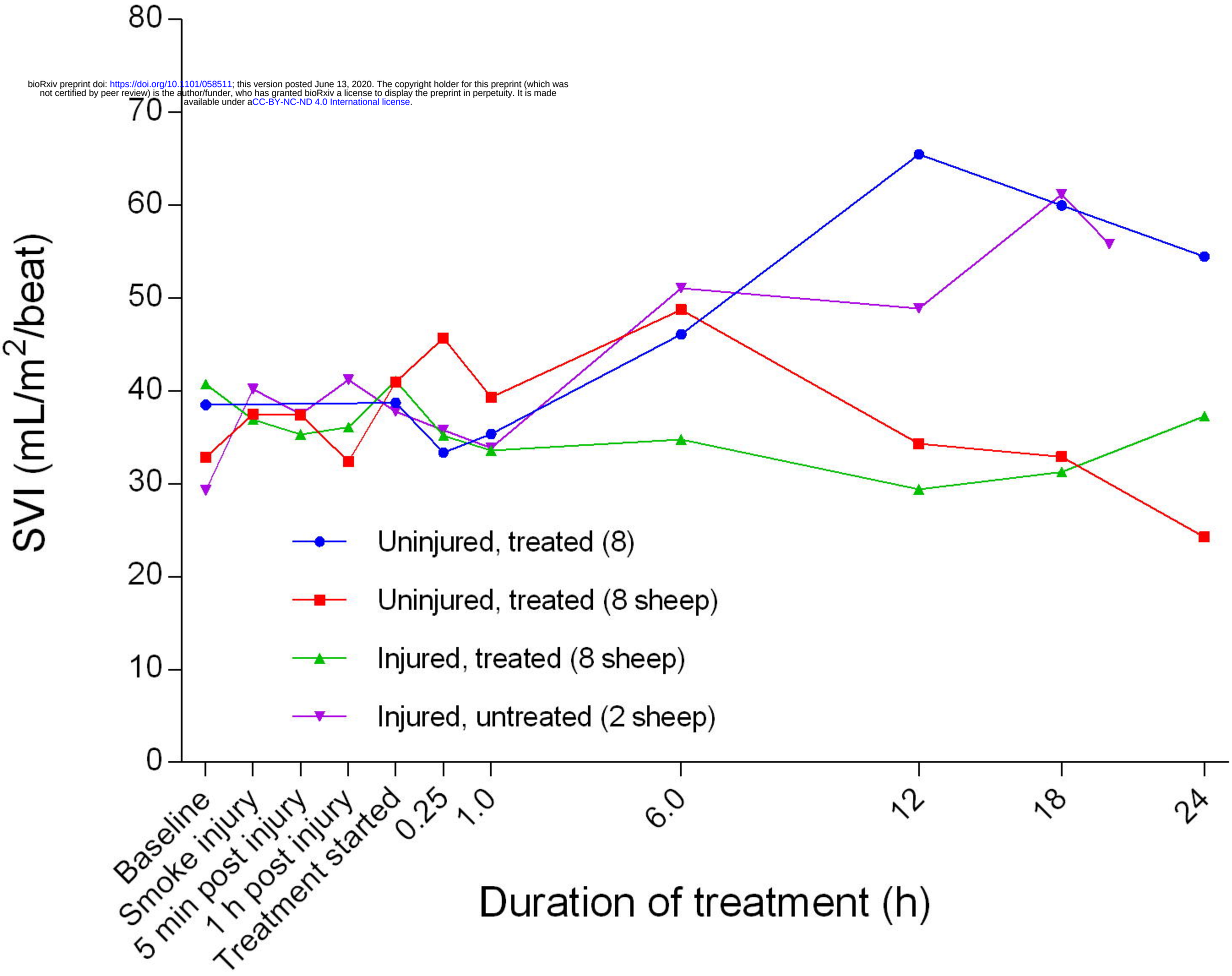


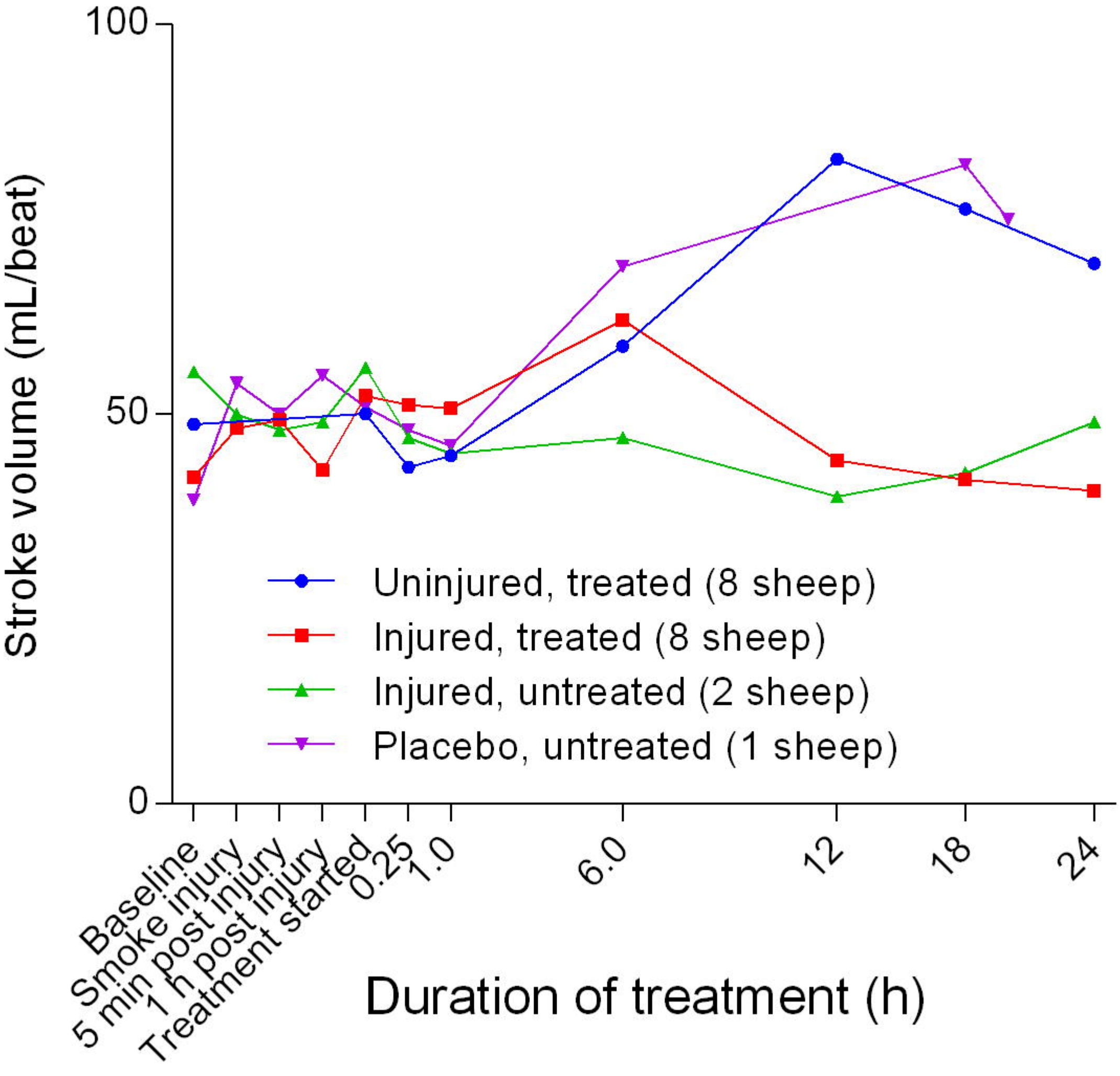


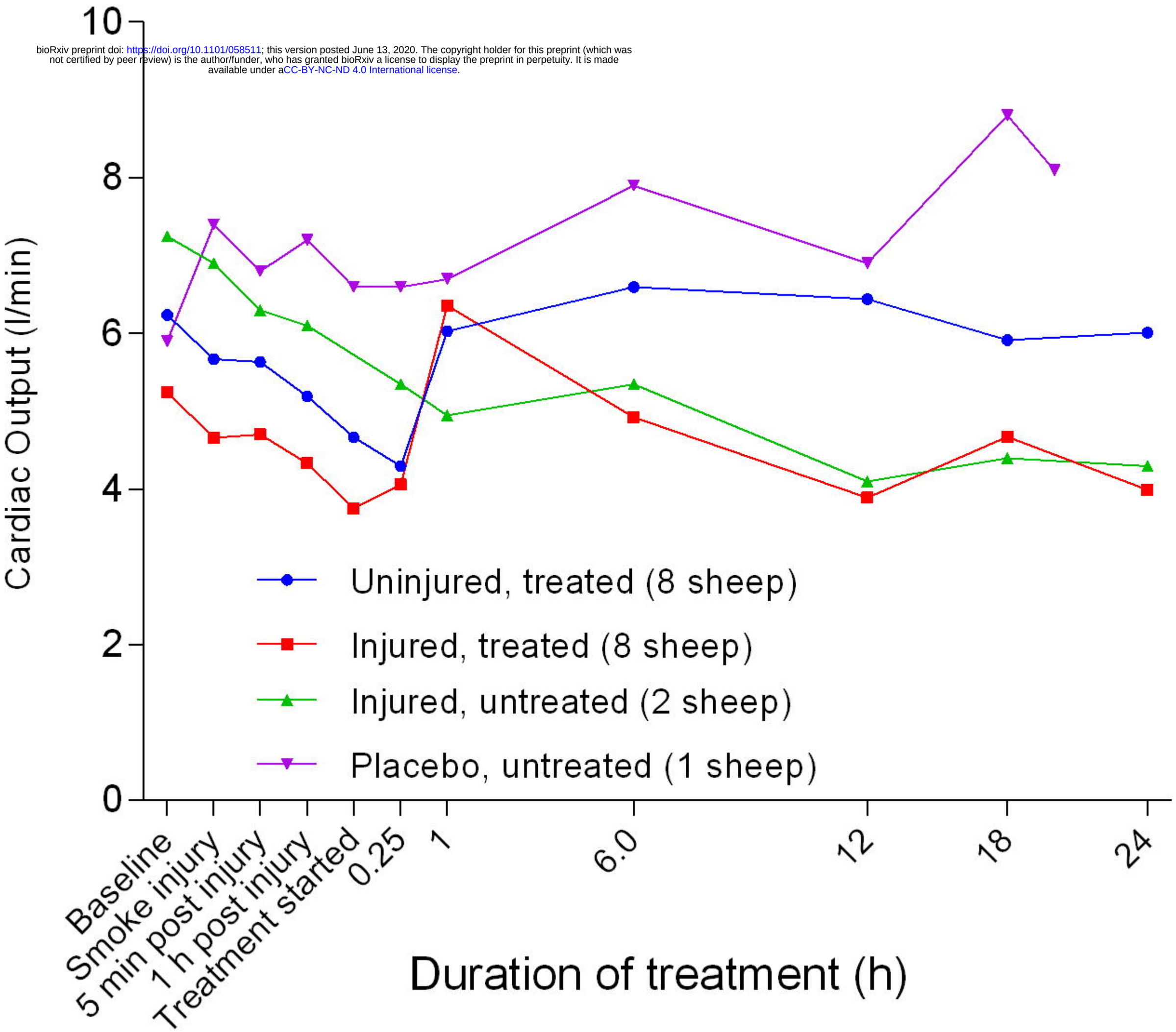


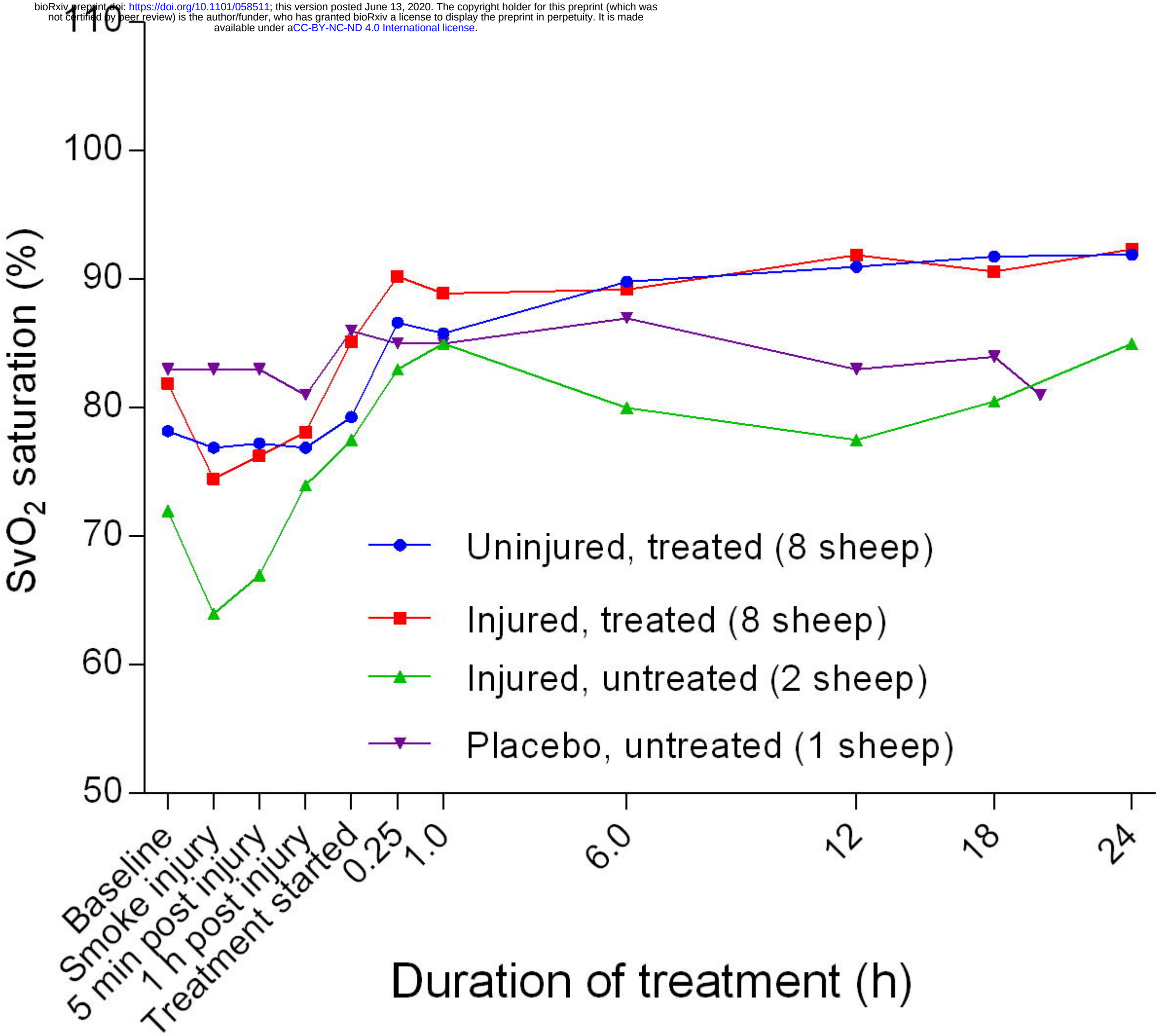


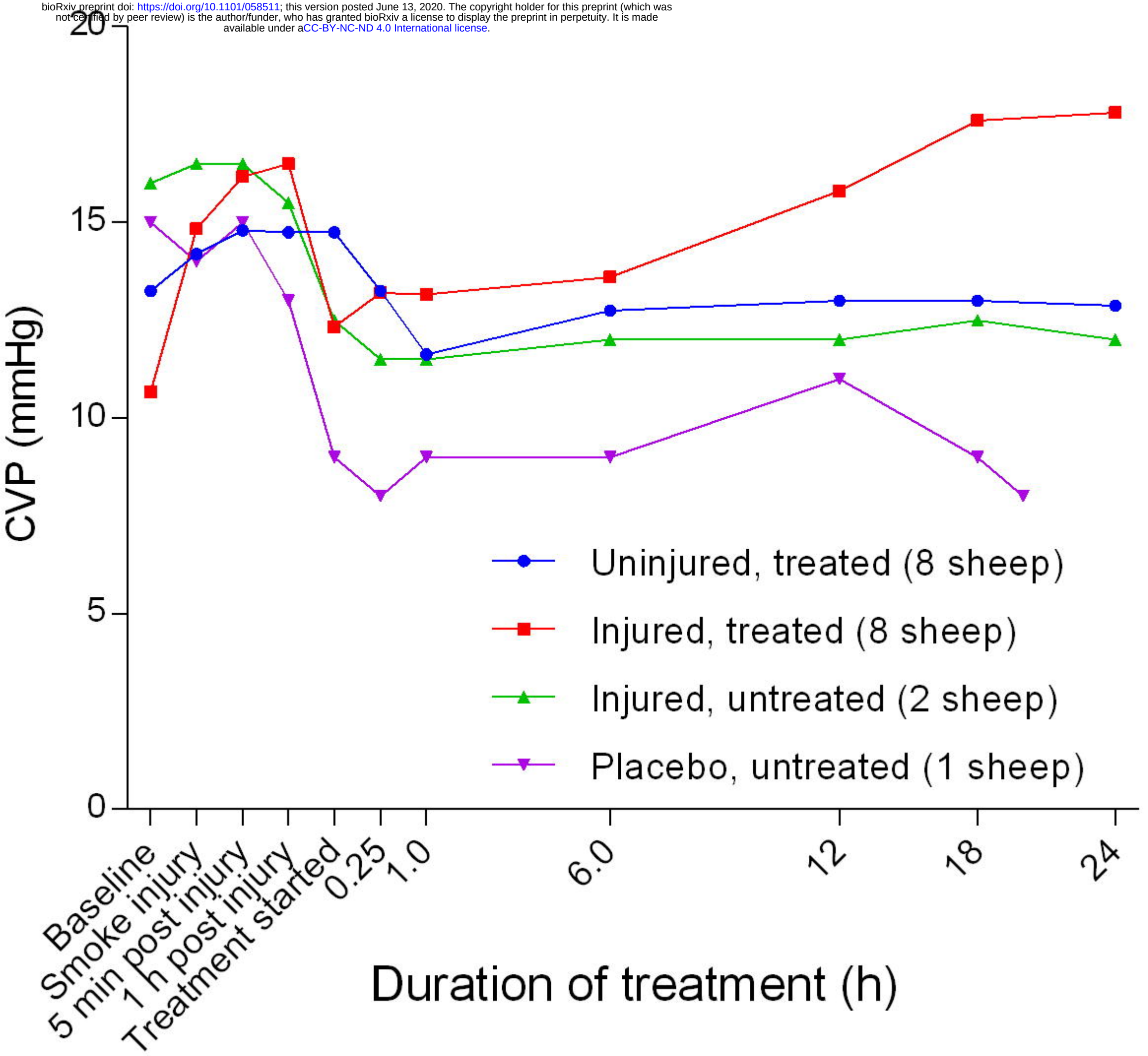


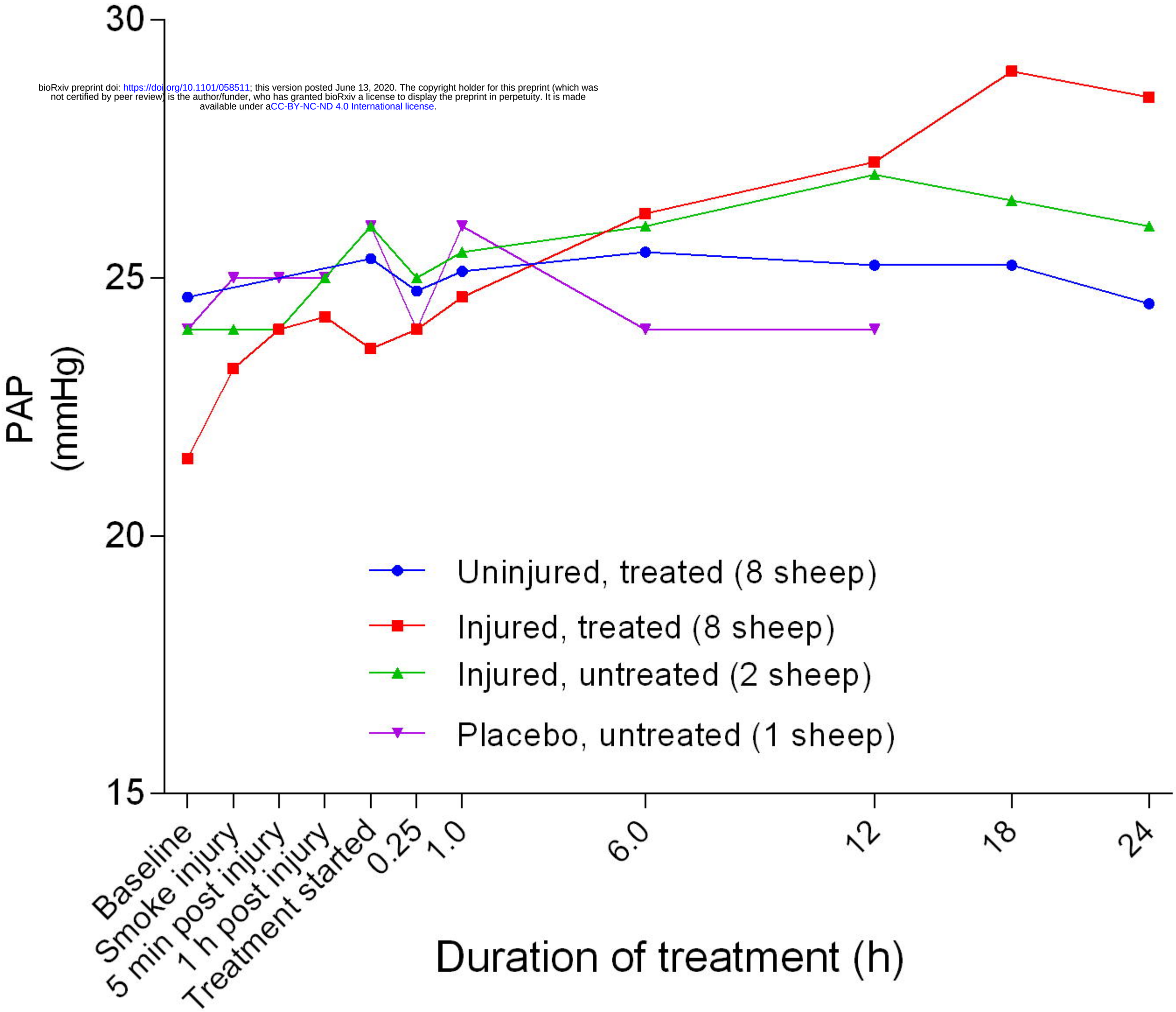


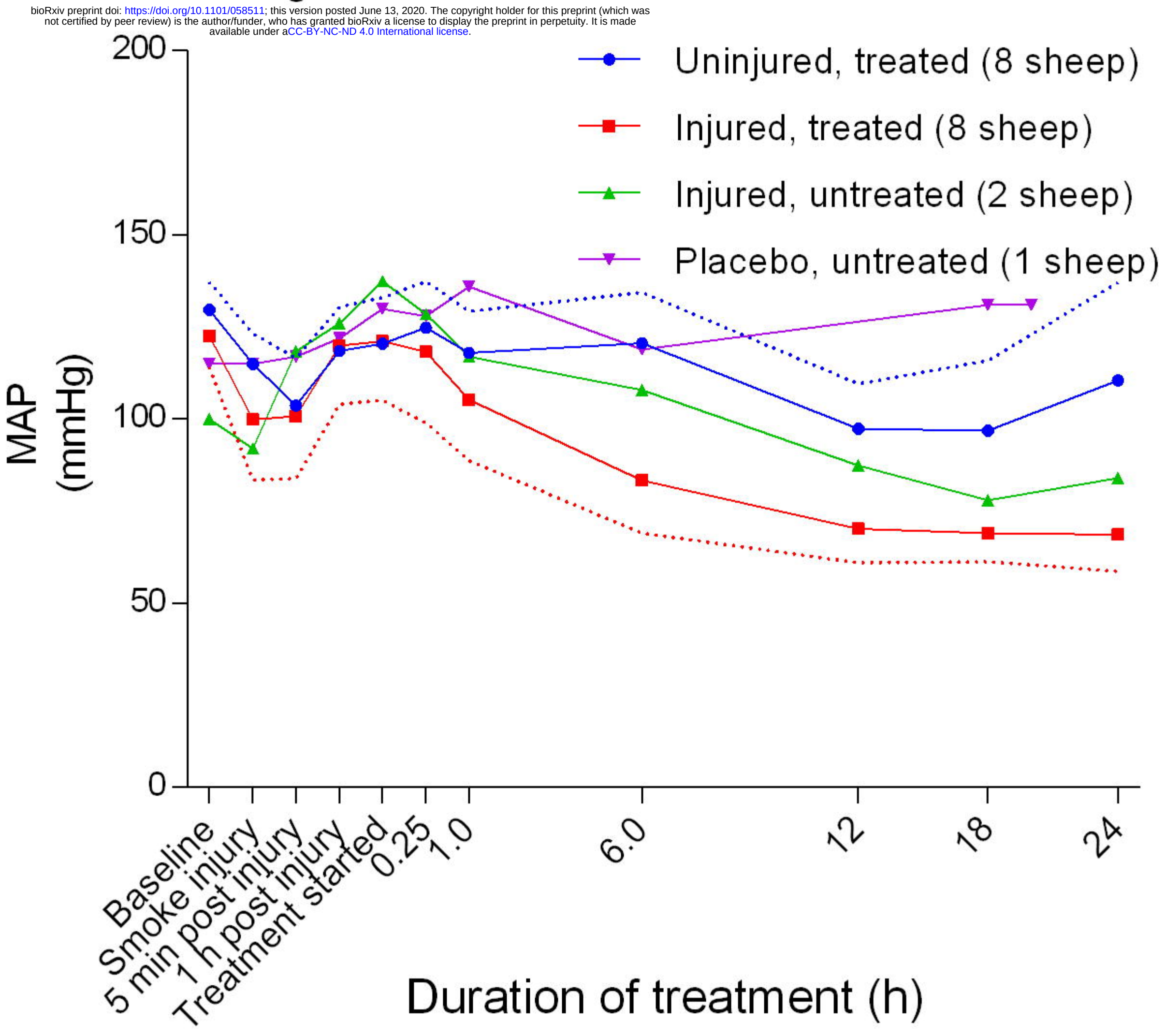


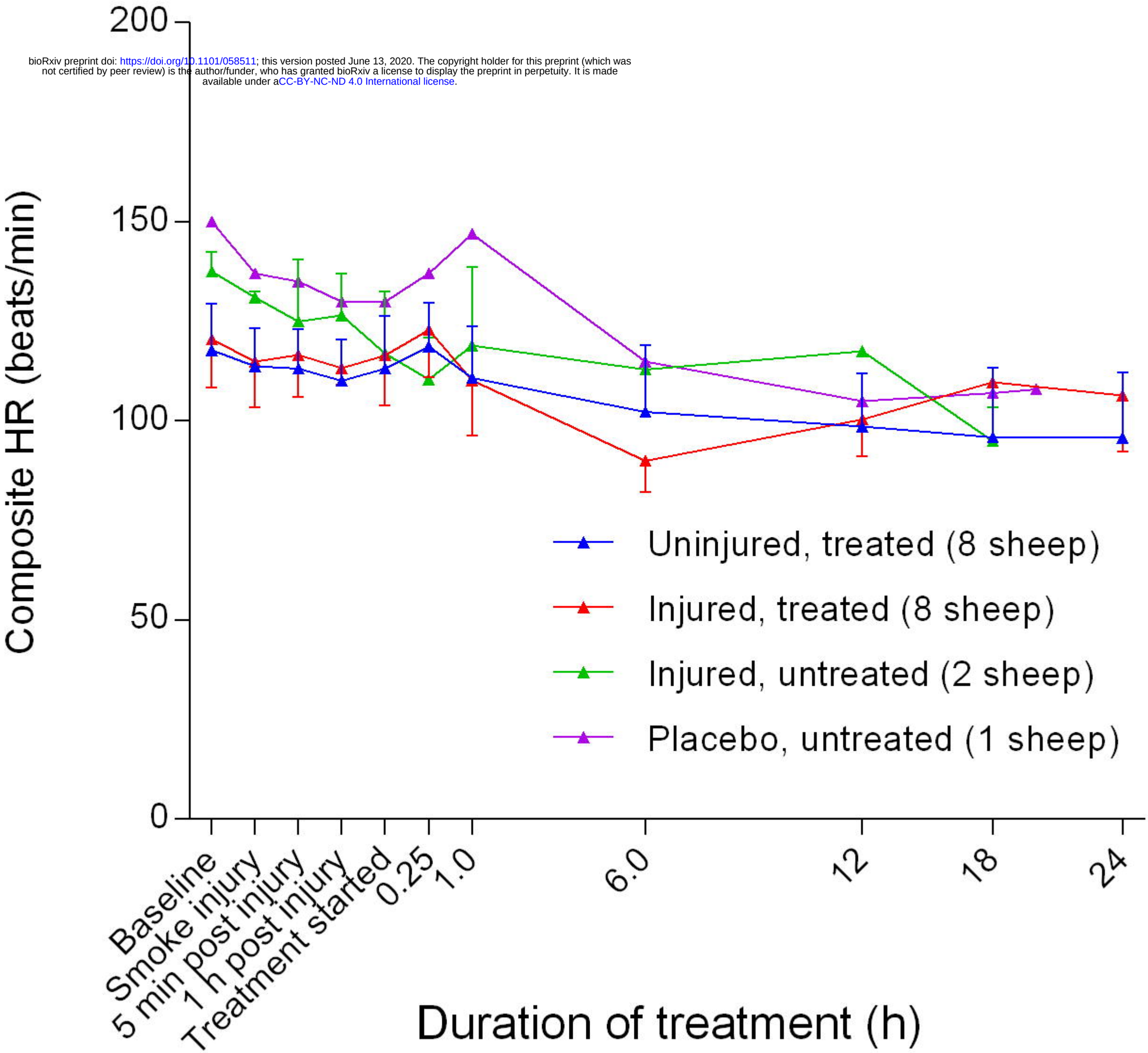


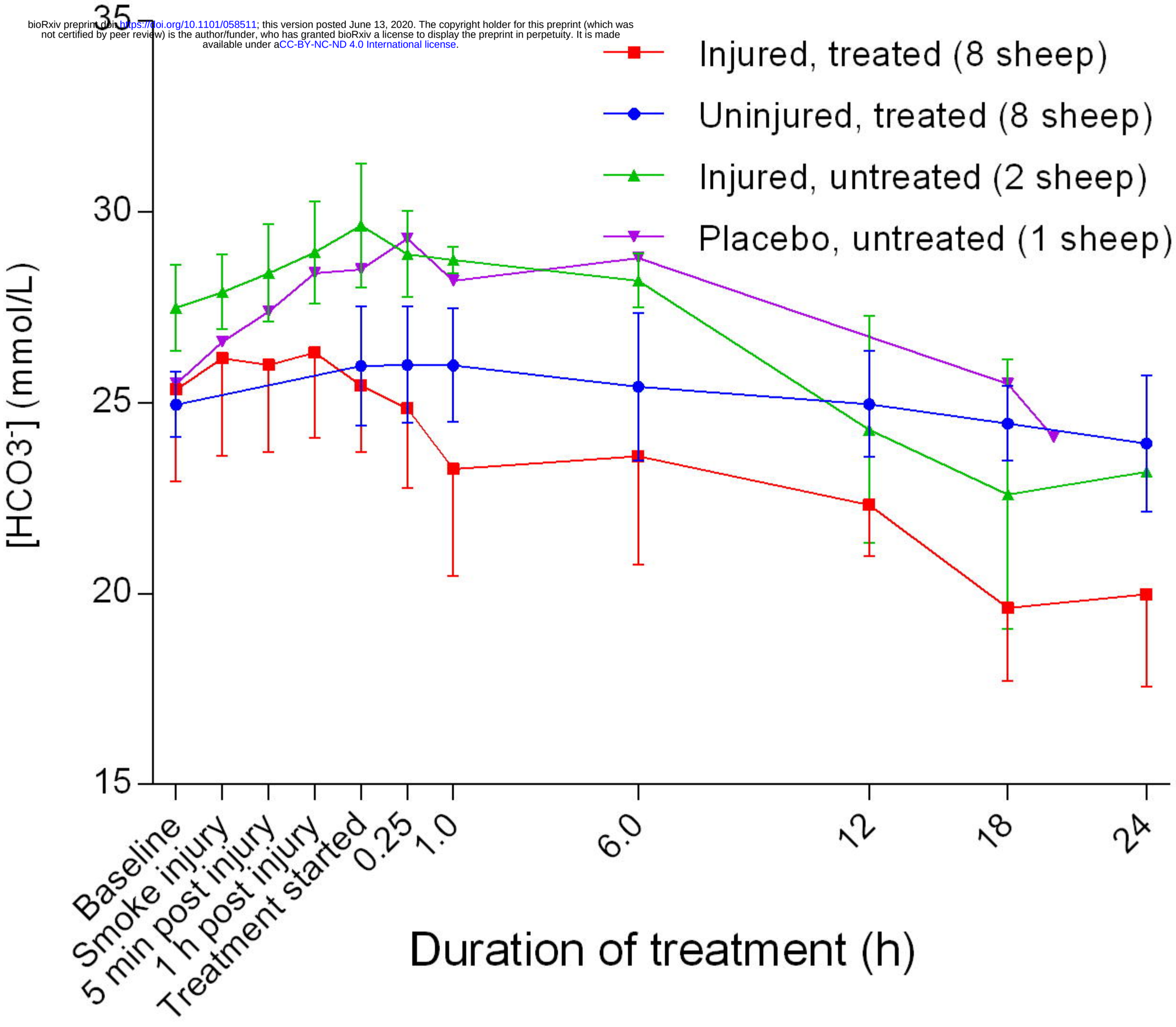


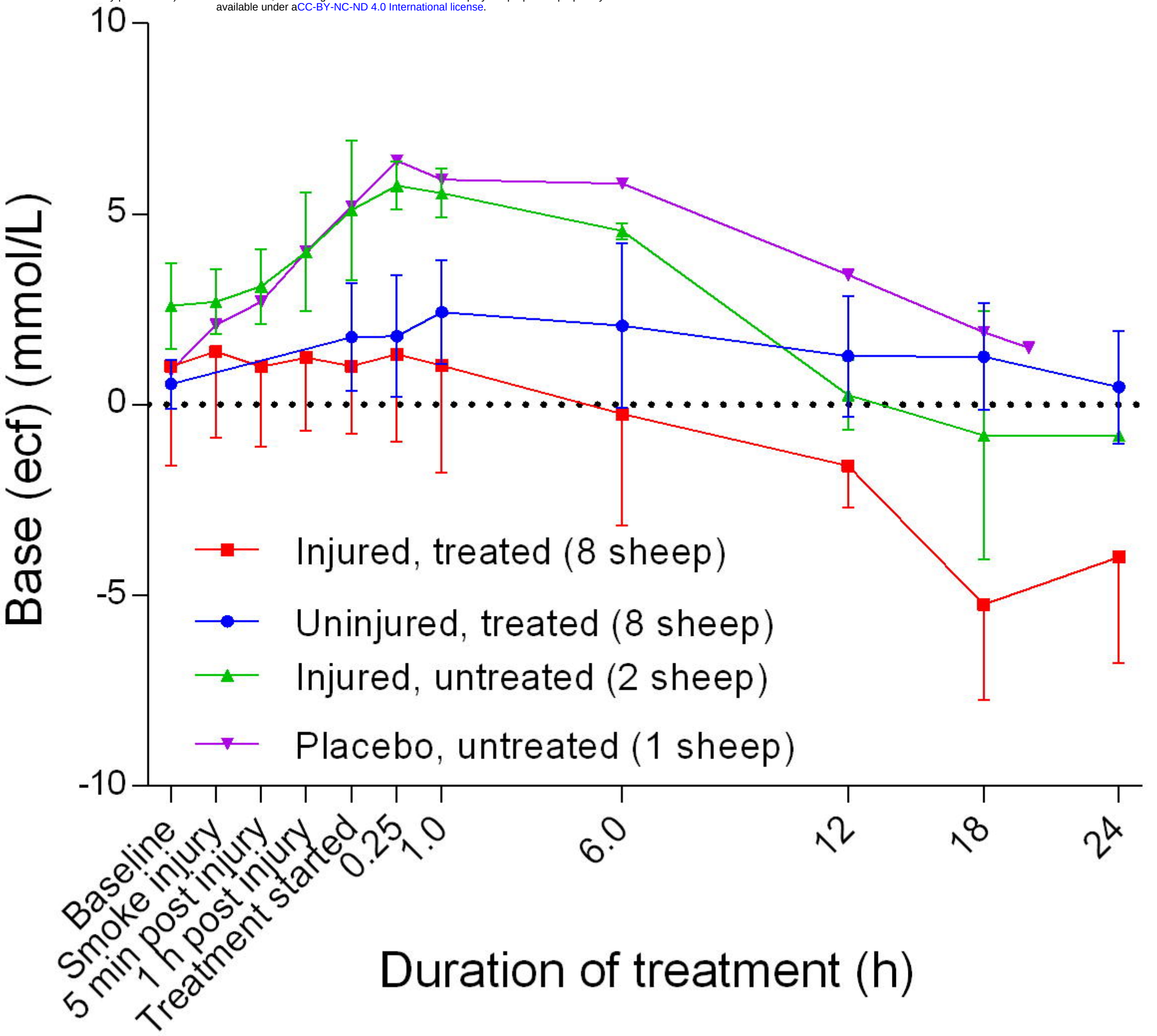












Injured, treated (8 sheep)
Uninjured, treated (8 sheep)
Injured, untreated (2 sheep)
Placebo, untreated (1 sheep)

[Lactate] (mmol/L)

0
1
2
3
4
5

Baseline
Smoke injury
5 min post injury
1 h post injury
Treatment started
0.25
1.0
6.0
12
18
24

Duration of treatment (h)

Injured, treated (8 sheep)
Uninjured, treated (8 sheep)
Injured, untreated (2 sheep)
Placebo, untreated (1 sheep)

[Glucose] (mmol/L)

10
8
6
4
2
0

Baseline
Smoke injury
5 min post injury
1 h post injury
Treatment started
0.25
1.0
6.0
12
18
24

Duration of treatment (h)