1 <u>Article title</u>

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2 Intra- and Inter-session Reliability and Repeatability of an Infrared

3 Thermography Device Designed for Materials to Measure Skin

- 4 Temperature of the Triceps Surae Muscle Tissue of Athletes
- 5 <u>Runing title</u>
- 6 Reliability and Repeatability of Infrared Thermography
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32 33	Institutional Review Board Statement: This study was approved by the clinical intervention ethics committee of the Hospital Clínico San Carlos, Madrid (Spain), with internal code number 20/021-E on January 20, 2020.
34	Informed Consent Statement: Informed consent was obtained from all subjects involved in the study.
35	Data Availability Statement: Raw data are available in supplementary file 1.
36 37 38	Acknowledgments: Authors acknowledge the support from the Contract 83 between the Complutense University and PCE Ibérica S.L. entitle "Fiabilidad sobre el dispositivo de termografía Manual Infrared camera PCE-TC 30 en humanos" (Reference number: 6-2020), providing specific grant for the present research.
39 40 41	Conflicts of Interest: The authors declare conflict of interest due to the Contract 83 between the Complutense University and PCE Ibérica S.L. (Reference number: 6-2020) was specifically carried out to study the reliability of the Manual Infrared camera PCE-TC 30 thermography device.

42 43 | Article 44 Intra- and Inter-session Reliability and Repeatability of an 45 Infrared Thermography Device Designed for Materials to 46 Measure Skin Temperature of the Triceps Surae Muscle 47 Tissue of Athletes

48 Abstract

49	Background: Infrared thermography devices have been commonly applied to measure
50	superficial temperature in structural composites and walls. These tools were cheaper than
51	other thermographic devices used to measure superficial human muscle tissue temperature,
52	which could be of interest to athletes' triceps surae, cording to prior studies. Infrared
53	thermography devices have been commonly applied to measure superficial temperature in
54	structural composites and walls, being cheaper than other thermographic devices used to
55	measure superficial human muscle tissue temperature, which could be of interest in the triceps
56	surae of athletes according to prior studies.

57 Objective: The purpose was to determine the procedure's intra- and inter-session reliability 58 and repeatability to determine skin temperature within the Manual Infrared Camera PCE-TC 59 30 thermography device in the triceps surae muscle tissue of athletes, which was initially 60 designed to measure the superficial temperature of materials. The purpose was to determine 61 intra- and inter-session reliability and repeatability of the Manual Infrared Camera PCE-TC

62 30 thermography device designed initially to measure superficial temperature of materials in
63 the triceps surae muscle tissue of athletes.

64 Methods: A total of 34 triceps surae muscles were bilaterally assessed from 17 healthy 65 athletes using the Manual Infrared Camera PCE-TC 30 thermography device to determine intra- and inter-session reliability and repeatability of the skin temperature of the soleus, 66 medial and lateral gastrocnemius muscles. Statistical analyses comprised intraclass correlation 67 coefficient (ICC), standard error of measurement (SEM), minimum detectable change 68 (MCD), systematic error of measurement, correlation (r), and Bland-Altman plots completed 69 70 with linear regression models (*R*²). A total of 34 triceps surae muscles were bilaterally 71 assessed from 17 healthy athletes by the Manual Infrared Camera PCE-TC 30 thermography 72 device in order to determine intra- and inter-session reliability and repeatability of the skin 73 temperature of the soleus, medial and lateral gastrocnemius muscles. Intraclass correlation 74 coefficient (ICC), standard error of measurement (SEM), minimum detectable change 75 (MCD), systematic error of measurement, correlation (r) and Bland-Altman plots completed 76 with linear regression models (R^2) were analyzed.



85 between each pair of measurement moments was presented by the Bland-Altman plots 86 according to the limits of agreement and non-significant linear regression models (R^2 =0.000– 87 0.019; P>.05).

- 88 *Conclusions*: The proposed procedure to determine skin temperature within the Manual Infrared Camera PCE-TC 30 thermography device presented excellent intra- and inter-session 89 90 reliability and repeatability in athletes' triceps surae muscle tissue. Future studies should 91 consider the SEM and MDC of this procedure to measure the skin temperature of soleus, medial, and lateral gastrocnemius muscles to promote triceps surae muscle prevention and 92 93 recovery in athletes. The proposed Manual Infrared Camera PCE-TC 30 thermography device 94 designed initially to measure superficial temperature of materials presented excellent intraand inter-session reliability and repeatability in the triceps surae muscle tissue of athletes. 95 96 Future studies should consider the measurement errors of this tool to measure skin 97 temperature of soleus, medial and lateral gastrocnemius muscles in order to promote triceps surae muscle prevention and recovery in athletes. 98 *Keywords: Kewords:* Athletes, wer Extremity; Repeatability, producibility of Results; 99
- 100 Thermography.
- 101
- 102 *Highlights*:
- Prior high quality thermography devices were reliable in measuringto measure skin
 temperature
- The proposed device was initially designed for materials superficial temperature

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106	•	This device is reliable and repeatable <u>for measuring</u> to measure triceps surae ski
107		temperature

• This cheap tool could promote triceps surae prevention and recovery in athletes

109 1. Introduction

110	Infrared thermography was proposed as one of the most relevant non-ionizing radiation
111	tools to assess skin temperature related to muscle tissue conditions, such as compartmental
112	syndrome. Nevertheless, there is a lack of scientific evidence for other musculoskeletal
113	conditions (Sanchis-Sánchez et al., 2014). Among different application fields, the use of
114	inflammation and perfusion-based conditions may be evaluated by thermographic imaging in
115	<mark>sports medicine (Ramirez-Garcia Luna et al., 2022).</mark> This device was has been used to
116	determine the superficial temperature of muscle tissue of lower limbs from athletes for injury
117	prevention (Côrte et al., 2019), muscle activity assessment (Rodriguez-Sanz et al., 2019), and
118	<u>evaluations before and after treatments (Rodriguez et al., 2018; Benito-de-Pedro et al., 2019).</u>
119	In addition, assessments before and after running were performed in the triceps surae from
120	athletes as a main focus linked to musculoskeletal conditions (Rodríguez-Sanz et al., 2017;
121	Rodriguez-Sanz et al., 2018; Rodriguez et al., 2018; Benito-de-Pedro et al., 2019). Infrared
122	thermography was proposed as one of the most relevant non-ionizing radiation tools in order
123	to assess skin temperature related to muscle tissue conditions, such as compartmental
124	syndrome, but there is a lack of scientific evidence for other musculoskeletal conditions
125	(Sanchis-Sánchez et al., 2014). Among different application fields, inflammation and
126	perfusion-based conditions may be benefited by thermographic imaging use in the sport
127	medicine field (Ramirez-GarciaLuna et al., 2022). This device was used as a main outcome
128	measurement to determine superficial temperature in the human muscle tissue of athletes'

129	lower limbs for injury prevention (Côrte et al., 2019), muscle activity (Rodriguez-Sanz et al.,
130	2019), evaluation before and after treatments (Rodriguez et al., 2018; Benito-de-Pedro et al.,
131	2019) as well as assessments before and after running, being the triceps surae from athletes
132	considered as a main focus linked to musculoskeletal conditions (Rodríguez-Sanz et al., 2017;
133	Rodriguez-Sanz et al., 2018; Rodriguez et al., 2018; Benito-de-Pedro et al., 2019).
134	Different thermography methods to determine skin temperature in the calves' muscular
135	region showed good correlations among them ($R^2 = 0.885 - 0.924$) and between both sides (R^2
136	<u>= 0.754 – 0.881; r = 0.868 – 0.939) within adequate agreement by Bland-Altman plots</u>
137	(Ludwig et al., 2014). Thermography assessment was used to measure the cutaneous
138	temperature of triceps surae muscles in soccer players with equinus condition versus non-
139	equinus condition after running (Rodriguez-Sanz et al., 2018), as well as at rest (Rodríguez-
140	Sanz et al., 2017). In addition, this tool was applied to determine the treatment effects after
141	different physical therapy interventions, such as dry needling and ischemic compression, in
142	<u>triathletes (Benito-de-Pedro et al., 2019). In addition, infrared thermography was utilized to</u>
143	measure skin temperature changes after compressive versus standard stockings use in athletes
144	(Rodriguez et al., 2018). All these evaluations were carried out by the FLIR/SC3000/QWIP
145	Thermacan thermographic tool to measure the skin temperature of the triceps surae muscle
146	tissue. Thermography assessment was used to measure cutaneous temperature of triceps surae
147	muscles in soccer players with equinus condition after running (Rodriguez-Sanz et al., 2018),
148	as well as at rest (Rodríguez-Sanz et al., 2017). In addition, this tool was applied to determine
149	the treatment effects after different physical therapy interventions in triathletes (Benito-de-
150	Pedro et al., 2019). In addition, infrared thermography was utilize to measure skin

151 temperature changes after compressive stockings use in athletes (Rodriguez et al., 2018).

152	<u>The FLIR/SC3000/QWIP Thermacan infrared thermal device presented an</u> 8–9 µm
153	spectral range, a temperature sensitivity of 0.02 K, a display of 320 × 240 pixels with 20° lens
154	and a spatial resolution of 1.1 mrad, being frequently used to evaluate the superficial
155	<u>temperature in the human tissue with adequate</u> reliability and repeatability The
156	FLIR/SC3000/QWIP Thermacan infrared thermal device presented a 8–9 µm spectral range, a
157	temperature sensitivity of 0.02 K, a display of 320 x 240 pixels with 20° lens and a spatial
158	resolution of 1.1 mrad, being frequently used to evaluate superficial temperature in the human
159	tissue with an adequate reliability and repeatability (Rodríguez-Sanz et al., 2017; Rodriguez-
160	Sanz et al., 2018; Rodriguez et al., 2018; Benito-de-Pedro et al., 2019), by both manual and
161	automatic thermographic software package measurement methods with an adequate
162	agreement and excellent intraclass correlation coefficient (ICC > 0.80) (Requena-Bueno et al.,
163	2020). Prior statistical procedures, such as ICC and Bland-Altman plots, including limits of
164	agreement (LoA), Prior statistical procedures, such as ICC and Bland-Altman plots including
165	limits of agreement (LoA), were used to compare infrared thermographic values in the lower
166	limbs showing that both manual and automatic definition devices presented an excellent ICC
167	from 0.92 to 0.99 with an adequate agreement by visual distribution and similar LoA by the
168	ThermoHuman [®] devices (Fernandez-Cuevas et al., 2017; Requena-Bueno et al., 2020),
169	excellent inter-session reproducibility with an ICC of 0.88 using the digital infrared camera
170	IRTIS-2000 [®] (Zaproudina et al., 2008), and almost perfect agreement in replication with an
171	ICC from 0.94 to 0.97 by the Thermofocus® thermal imaging device (Petrova et al., 2018) <mark>_</mark> .
172	Nevertheless, these devices showed a higher cost
173	designed to evaluate superficial temperature on materials. One of these thermographic devices
174	was the Manual Infrared Camera PCE-TC 30. This tool displayed a sensor resolution of
175	80x80, a measurement range from 0 to 250°C, a display of 320 × 240 pixels, a thermal

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176	<u>sensitivity of 80 mK, and an 8 mm lens (De Villoria et al., 2011; Pérez-Urrestarazu et al.,</u>
177	2014). Despite this, the thermal imaging system presented very low parameters concerning
178	geometrical resolution (80 × 80 pixels) and thermal sensitivity of 80 mK, while other thermal
179	imaging systems displayed high definition resolution (1280 \times 960 pixels) and higher thermal
180	sensitivity of 20 mK Nevertheless, these devices showed a higher cost compared with an
181	infrared thermography device to evaluate superficial temperature on materials, such as the
182	Manual Infrared Camera PCE-TC 30, which displayed a sensor resolution of 80x80, a
183	measurement range from 0 to 250°C, a display of 320 x 240 pixels, a thermal sensitivity of 80
184	mK and 8 mm lens (De Villoria et al., 2011; Pérez-Urrestarazu et al., 2014). Despite this
185	thermal imaging system presented very low parameters concerning geometrical resolution (80
186	x 80 pixels) and thermal sensitivity of 80 mK, while other thermal imaging systems displayed
187	high definition resolution (1280 x 960 pixels) and higher thermal sensitivity of 20 mK
188	(Fernandez-Cuevas et al., 2017; Requena-Bueno et al., 2020), the Manual Infrared Camera
189	PCE-TC 30 geometrical resolution and thermal sensitivity features could present adequate
190	reliability to measure triceps surae muscle tissue temperature variations (Rodríguez-Sanz et
191	al., 2017; Rodriguez-Sanz et al., 2018; Rodriguez et al., 2018; Benito-de-Pedro et al., 2019).
192	Indeed, the PCE-TC 30 thermal camera has already been employed to assess the thermal
193	behavior of fencing uniforms in athletes (Lamberti et al., 2020), but the reliability and
194	repeatability of this tool directly on the skin of the human muscle need to be has yet to be
195	determined.
196	This infrared thermography device was used to measure superficial temperature in
197	structural composites and walls according to quality inspections, such as reproducibility,
198	stability, reliability, and operating temperature (De Villoria et al., 2011; Pérez-Urrestarazu et
199	al., 2014). Although this tool is used to assess thermal temperature in the fencing uniforms of

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200	athletes (Lamberti et al., 2020), it has not been applied directly to measure superficial human
201	muscle tissue temperature, which could be of interest in the triceps surae of athletes
202	(Rodríguez-Sanz et al., 2017; Rodriguez-Sanz et al., 2018; Rodriguez et al., 2018; Benito-de-
203	Pedro et al., 2019; Requena-Bueno et al., 2020). This cheaper infrared thermography device
204	was applied to measure superficial temperature in structural composites and walls according
205	to quality inspections such as reproducibility, stability, reliability and operating temperature
206	(De Villoria et al., 2011; Pérez-Urrestarazu et al., 2014), but it has not been applied to
207	measure superficial human muscle tissue temperature, which could be of interest in the triceps
208	surae of athletes according to our prior studies (Rodríguez-Sanz et al., 2017; Rodriguez-Sanz
209	et al., 2018; Rodriguez et al., 2018; Benito-de-Pedro et al., 2019; Requena-Bueno et al.,
210	2020).
211	<u>We hypothesized that this device – developed initially for non-Vivo structures – could</u>
212	provide adequate reliability and repeatability to determine skin temperature in the triceps
213	<u>surae muscle human tissue of athletes, being cheaperless expensive than the infrared</u>
214	thermography device used in human studies. Thus, the purpose of the present study was to
215	determine the intra- and inter-session reliability and repeatability of the procedure to assess
216	skin temperature within the Manual Infrared Camera PCE-TC 30 thermography device in the
217	triceps surae muscle tissue of athletes, which was designed initially to measure the superficial
218	temperature of materials. We hypothesized that this device developed initially for non-vivo
219	structures could provide an adequate reliability and repeatability to determine skin
220	temperature in the triceps surae muscle human tissue of athletes, being cheaper with respect to
221	the infrared thermography device used in human studies. Thus, the purpose of the present
222	study was to determine the intra- and inter-session reliability and repeatability of the Manual

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223	Infrared Camera PCE-TC 30 thermography device designed initially to measure superficial
224	temperature of materials in the triceps surae muscle tissue of athletes.

225 2. Materials and Methods

226 2.1. Study design

227	The present study was carried out from January 2020 to May 2021 according to The
228	Strengthening the Reporting of Observational Studies in Epidemiology (STROBE) criteria
229	(Appendix 1). The Helsinki Declarations, as well as specific human experimentation ethical
230	rules, were taken into account. The ethics committee for clinical interventions of the Hospital
231	Clínico San Carlos, Madrid (Spain) approved this study with internal code number 20/021-E
232	on January 20, 2020. Before the study began, all participants signed the informed consent
233	form. In addition, the present study was supported by Contract 83 between Complutense
234	University and PCE Ibérica S.L. (Reference number: 6-2020), providing a specific grant for
235	this research project. The present study was carried out from January 2020 to May 2021
236	according to The Strengthening the Reporting of Observational Studies in Epidemiology
237	(STROBE) criteria (Vandenbroucke et al., 2014). Furthermore, Helsinki Declarations as well
238	as specific human experimentation ethical rules were taken into account.(Holt, 2014) The
239	ethic committee for clinical interventions of the Hospital Clínico San Carlos, Madrid (Spain)
240	approved this study with internal code number 20/021-E on January 20, 2020 on January 20,
241	2020. All study subjects signed the document of the informed consent form prior to beginning
242	this reliability study. In addition, the present study study was supported by a Contract 83
243	between the between the Complutense University and PCE Ibérica S.L. (Reference number:
244	6-2020), providing specific grant for this research project.

246	The sample size determination was calculated by bi <mark>-</mark> variate correlations statistical
247	procedures through G*Power 3.1.9.2 program (G*Power [©] , from Dusseldorf University, in
248	<u>Germany), considering a 0.4 correlation coefficient to achieve a moderate correlation (Lobo et</u>
249	<u>al., 2016)</u> between infrared thermography measurements <mark>, applying a</mark> 1-tailed hypothesis, a
250	0.05 α error and <mark>a</mark> 0.80 power. Lastly, <mark>the sample</mark> size was 34 triceps surae muscles to achieve
251	the required thermography measurements for a 0.801 actual power.
252	The determination for sample size determination was calculated by bi-variate correlations
253	statistical procedures through G*Power 3.1.9.2 programme (G*Power [®] , from Dusseldorf
254	University, in Germany), considering a 0.4 correlation coefficient to achieve a moderate
255	correlation(Lobo et al., 2016) between infrared thermography measurements, applying a 1-
256	tailed hypothesis, a .05 α error and a 0.80 power. Lastly, the considered sample size was 34
257	triceps surae muscles in order to achieve the required thermography measurements for a 0.801
258	actual power.

259 2.3. Participants

260	Thirty-four triceps surae muscles were bilaterally analyzed from 17 healthy athletes
261	considering a consecutive sampling recruitment procedure. Inclusion criteria comprised
262	specifically healthy athletes aged 18–65 years providing the consent information document
263	previously, carrying out sports activities and training for at least 2 h as well as one day per
264	week, with moderate (level-II) or vigorous (level-III) intensities for physical activity with
265	metabolic equivalent indexes greater than 600 METs/min/week, measured by the International
266	Questionnaire for Physical Activity (IPAQ) (Roman-Viñas et al., 2010).
267	Thirty-four triceps surae muscles were bilaterally analyzed from 17 healthy athletes
268	considering a consecutive sampling recruitment procedure. First, inclusion criteria comprised

269 specifically healthy athletes who aged 18-65 years providing previously the consent inform

270	document, carrying out sport activities and training for at least two hours as well as one day
271	per week, with moderate (level-II) or vigorous (level-III) intensities for physical activity with
272	metabolic equivalent indexes greater than 600 METs/min/week, measured by the International
273	Questionnaire for Physical Activity (IPAQ) (Roman-Viñas et al., 2010). Exclusion criteria
274	included muscle soreness, congenital dysfunctions, neuromuscular conditions, rheumatic
275	alterations, body mass index (BMI) greater than 31 kg/m ² , previous neurological conditions,
276	prior surgeries, skin pathologies, and some alterations in the lower limbs region (i.e., chronic
277	ankle instability presence, prior sprains or previous fractures) according to the thermographic
278	influence of compartmental, stress, inflammation, and perfusion-based conditions (Sanchis-
279	<u>Sánchez et al., 2014; Ramirez-GarciaLuna et al., 2022)</u> and, <u>lastly, difficulties or inability to</u>
279 280	Sánchez et al., 2014; Ramirez-GarciaLuna et al., 2022) and, lastly, difficulties or inability to carry out the procedure to complete the study course, explained below.
279 280 281	Sánchez et al., 2014; Ramirez-GarciaLuna et al., 2022) and, lastly, difficulties or inability to carry out the procedure to complete the study course, explained below.
279 280 281 282	Sánchez et al., 2014; Ramirez-GarciaLuna et al., 2022) and, lastly, difficulties or inability to carry out the procedure to complete the study course, explained below. Such, exclusion criteria included soreness, congenital dysfunctions, neuromuscular conditions, rheumatic alterations, body mass index (BMI) greater than 31 kg/m ² , previous neurological conditions,
279 280 281 282 283	Sánchez et al., 2014; Ramirez-GarciaLuna et al., 2022) and, lastly, difficulties or inability to carry out the procedure to complete the study course, explained below. Subd, exclusion criteria included soreness, congenital dysfunctions, neuromuscular conditions, rheumatic alterations, body mass index (BMI) greater than 31 kg/m ² , previous neurological conditions, prior surgeries, skin pathologies, and some alterations in the lower limbs region (i.e. chronic
279 280 281 282 283 283	Sánchez et al., 2014; Ramirez-GarciaLuna et al., 2022) and, lastly, difficulties or inability to carry out the procedure to complete the study course, explained below. Such, exclusion criteria included soreness, congenital dysfunctions, neuromuscular conditions, rheumatic alterations, body mass index (BMI) greater than 31 kg/m ² , previous neurological conditions, prior surgeries, skin pathologies, and some alterations in the lower limbs region (i.e. chronic ankle instability presence, prior sprains or previous fractures) according to the thermographic
279 280 281 282 283 283 284 285	Sánchez et al., 2014; Ramirez-GarciaLuna et al., 2022) and, lastly, difficulties or inability to carry out the procedure to complete the study course, explained below. Sound, exclusion criteria included soreness, congenital dysfunctions, neuromuscular conditions, rheumatic alterations, body mass index (BMI) greater than 31 kg/m ² , previous neurological conditions, prior surgeries, skin pathologies, and some alterations in the lower limbs region (i.e. chronic ankle instability presence, prior sprains or previous fractures) according to the thermographic influence of compartmental, stress, inflammation and perfusion-based conditions (Sanchis-
279 280 281 282 283 284 285 286	Sánchez et al., 2014; Ramirez-GarciaLuna et al., 2022) and, lastly, difficulties or inability to carry out the procedure to complete the study course, explained below. Sound, exclusion criteria included soreness, congenital dysfunctions, neuromuscular conditions, rheumatic alterations, body mass index (BMI) greater than 31 kg/m ² , previous neurological conditions, prior surgeries, skin pathologies, and some alterations in the lower limbs region (i.e. chronic ankle instability presence, prior sprains or previous fractures) according to the thermographic influence of compartmental, stress, inflammation and perfusion-based conditions (Sanchis-Sánchez et al., 2014; Ramirez-GarciaLuna et al., 2022) and, lastly, difficulties or inability in

2.4. Procedure

289	Intra- and inter-session reliability and repeatability of the skin temperature of the triceps
290	surae muscles were bilaterally assessed by the Manual Infrared Camera PCE-TC 30 to
291	determine measurement agreement and concordance at the same day separated by 1 hour
292	(considered as intra-day measurements) and alternate days separated by 48 hours (considered
293	as inter-day measurements), respectively. Indeed, participants were asked to continue with in

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294	their daily life and physical exercise routine (avoiding unusual efforts or activity changes)
295	between measurements and not taking drugs the prior week nor vasomotor substances
296	(i.e., caffeine) on the same measurement day, as well as heavy metals were not allowed. A
297	period of 5 minutes of acclimatization of the subjects to the room was applied (Figure 1). All
298	measurements were assessed with patients standing up in a relaxed position in the same room
299	within a 24.1 \pm 1 C° temperature and a 45 \pm 10% humidity, without direct ventilation flow
300	<mark>toward</mark> examiners or participants <u>(Rodríguez-Sanz et al., 2017)</u> . Intra- and inter-session
301	reliability and repeatability of the skin temperature of the triceps surae muscles were
302	bilaterally assessed by the Manual Infrared Camera PCE-TC 30 at the same day separated by
303	1 hour (considered as intra-day measurements) and alternate days separated by 48 hours
304	(considered as inter-day measurements), respectively (De Villoria et al., 2011; Pérez-
305	Urrestarazu et al., 2014). Indeed, participants were asked for continue within their daily life
306	and physical exercise routine (avoiding unusual efforts or activity changes) between
307	measurements and no taking drugs at the prior week nor vasomotor substances (i.e., caffeine)
308	at the same measurement day, as well as heavy metals were not allowed. All measurements
309	were assessed with patients standing up in relaxed position in the same room within a 24.1 ± 1
310	C° temperature and a 45 \pm 10% humidity, without direct ventilation flow towards examiners
311	or participants (Rodríguez-Sanz et al., 2017).
312	
313	
314	Please, include Figure 1 here or hereabouts
³¹⁵ 316 317	Figure 1. Infographics for evaluating and analyzing the infrared thermography images of the triceps surae complex.
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319 2.5. Descriptive data

320	Descriptive data including sex (categorized as male or female), age (measured in years),
321	height <mark>(measured in cm), weight (measured in kg), and BMI (expressed as kg/em² following)</mark>
322	the Quetelet's index) (Garrow, 1986), main sports category (divided into fitness considered as
323	bodybuilding exercise or soccer), side (categorized as right or left), and dominance (expressed
324	as yes or no <mark>)</mark> , and smoker <mark>(</mark> expressed as yes or no <mark>)</mark> were detailed (Calvo-Lobo et a <mark>l</mark> . 2019). As
325	a tool with adequate psychometric properties, metabolic equivalent index per minute per week
326	(METs/min/week) was evaluated by the IPAQ to determine physical activity and its
327	categorization as moderate (600–1500 METs/min/week) and vigorous (\geq 1500
328	METs/min/week) physical activity (Gauthier, Lariviere & Young, 2009). Descriptive data
329	including sex (categorized as male or female), age (measured in years), height (measured in
330	cm), weight (measured in kg), BMI (expressed as kg/cm ² following the Quetelet's index)
331	(Garrow, 1986). main sport category (divided into fitness considered as bodybuilding exercise
332	or soccer), side (categorized as right or left), and dominance (expressed as yes or no) and
333	smoker (expressed as yes or no) were detailed (Calvo-Lobo et al., 2019). As a tool with
334	adequate psychometric properties, metabolic equivalent index per minute per week
335	(METs/min/week) were evaluated by the IPAQ in order to determine physical activity and its
336	categorization as moderate (600 – 1500 METs/min/week) and vigorous (\geq 1500
337	METs/min/week) physical activity (Gauthier, Lariviere & Young, 2009).

338 *2.6. Infrared thermography*

We used a Manual Infrared Camera PCE-TC 30 (PCE Instruments UK Ltd;
 Southampton, United Kingdom), which displayed a sensor resolution of 80 × 80, a
 measurement range from 0 to 250°C, a display of 230 × 240 pixels, a thermal sensitivity of 80

342	mK and an 8 <mark>-</mark> mm lens (De Villoria et al., 2011; Pérez-Urrestarazu et al., 2014). The infrared
343	thermography imaging process was performed with the participant standing up in a relaxed
344	position 1 m from the camera. Bilaterally, the triceps surae complex, including lateral (Figure
345	1A and 1B) and medial (Figure 1C and 1D) gastrocnemius, as well as soleus (Figure 1E and
346	1F) muscles, was measured by a region of interest (ROI) through five infrared thermography
347	images for each muscle. Removing the highest and lowest values, the mean of the three
348	measurements was used for data analysis. Firstly, the Manual Infrared Camera PCE-TC 30
349	(PCE Instruments UK Ltd; Southampton, United Kingdom), which displayed a sensor
350	resolution of 80x80, a measurement range from 0 to 250°C, a display of 230 x 240 pixels, a
351	thermal sensitivity of 80 mK and 8 mm lens (De Villoria et al., 2011; Pérez-Urrestarazu et al.,
352	2014). The infrared thermography imaging process was performed with the participant placed
353	at 1 m of distance from the camera and standing up in a relaxed position. Bilaterally, the
354	triceps surae complex, including lateral (Figure 1A and 1B) and medial (Figure 1C and 1D)
355	gastrocnemius as well as soleus (Figure 1E and 1F) muscles, was measured by a region of
356	interest (ROI) through 5 infrared thermography images for each muscle. Removing the
357	highest and lowest values, the mean of the 3 measurements were used for data analysis.
358	Infrared images and data were analyzed by a blinded and experienced evaluator using the
359	<u>Guide TM</u> Report Express (PCE Instruments UK Ltd; Southampton, United Kingdom)
360	(Rodríguez-Sanz et al., 2017). This software provided the mean thermal value (°C) of the
361	selected ROI of 1 cm ² coinciding with the center of a landmark for each muscle (Figure 2).
362	Infrared images and data were analyzed by a blinded and experienced evaluator using the

363 Guide[™] Report Express (PCE Instruments UK Ltd; Southampton, United Kingdom)
364 (Rodríguez-Sanz et al., 2017). This software provided the mean thermal value (°C) of the

365 selected ROI of 1 cm² coinciding with the center of a landmark for each muscle (Figure 1).

366	These landmarks were used to determine superficial skin temperature and placed superior to	
367	the Achilles tendon for the soleus muscle and in the thickest part of the medial and lateral	
368	gastrocnemius muscles according to prior similar studies (Benito-de-Pedro et al., 2019; Rojas-	
369	Valverde et al., 2021).	
370		
371	<u>Please, include <mark>Figure 2</mark> here or hereabouts</u>	
372 373 374 375 376	Figure 2. Infrared thermography images of the triceps surae complex, including left (A) and right (B) lateral gastrocnemius, left (C) and right (D) medial gastrocnemius, and left (E) and right (F) soleus muscles, including the thermal values of the region of interest (ROI) of 1 cm ² .	
377	Please, include Figure 1 here or hereabouts	
378 379 380 381	Figure 1. Infrared thermography images of the triceps surae complex, including left (A) and right (B) lateral gastrocnemius, left (C) and right (D) medial gastrocnemius, and left (E) and right (F) soleus muscles, including the thermal values of the region of interest (ROI) of 1 cm ² .	
382	2.7. Statistical Analyses	
383	<u>The 24.0 Statistical Package <mark>Program</mark> for Social Science (<mark>named</mark>-SPSS<mark>;</mark> from IBM-Corp,</u>	
384	<u>in Armonk, NY) <mark>was used</mark> for data analyses. <mark>The α error was</mark> set at 0.05, and thus a <i>P</i>-value</u>	
385	lower than 0.05 was considered statistical significance. The Kolmogorov-Smirnov statistical	
386	<u>test and visual <mark>inspection of</mark> histograms were considered <mark>to detail normality distribution.</mark> The</u>	
387	24.0 Statistical Package programme for Social Science (named SPSS; from IBM-Corp, in	
388	Armonk, NY) was considered as the package for data analyses. The α error as set at .05 and	
389	thus a <i>P</i> -value lower than .05 was considered as statistical significance. Kolmogorov-Smirnov	
390	statistical test and visual inspection of histograms were considered to detail the distribution of	
391	normality. Data adjusted to normal distribution were detailed through means \pm standard	
392	deviations (SD) in conjunction with the upper and lower limits of 95% confidence interval	
393	(CI). Data adjusted to non-normal distribution were detailed through medians ± interquartile	
394	ranges (IR). <u>). Infrared thermography measurements</u> for intra- and inter-session evaluations	

395	were compared through <mark>paired-sample</mark> Student t-tests considering parametric tests and
396	Wilcoxon tests regarding non-parametric tests. ICC analyzed the reliability and repeatability
397	between each pair of measurements for bidirectional absolute agreement and Pearson (r) or
398	Spearman (ρ) correlation coefficients as parametric or non-parametric tests, respectively.
399	Furthermore, ICC $_{(2,1)}$ values were specifically interpreted as poor for ≤ 0.40 ICC $_{(2,1)}$, weak for
400	0.40–0.59 ICC _(2.1) , good for 0.60–0.74 ICC _(2.1) , and excellent for 0.75-1.00 ICC _(2.1) (Calvo-
401	Lobo et al., 2019). Infrared thermography measurements for intra- as well as inter-session
402	evaluations were compared through paired-samples Student t tests considering parametric
403	tests as well as Wilcoxon tests regarding non-parametric tests. The reliability and repeatability
404	between each pair of measurements were analyzed by ICC_for bidirectional absolute
405	agreement as well as Pearson (r) or Spearman (ρ) correlations coefficients as parametric or
406	non-parametric tests, respectively. Furthermore, ICC (2,1) values were specifically interpreted
407	as poor for < 0.40 ICC $_{(2,1)}$, weak for 0.40-0.59 ICC $_{(2,1)}$, good for 0.60-0.74 ICC $_{(2,1)}$, as well as
408	excellent for 0.75-1.00 ICC _(2,1) (Calvo-Lobo et al., 2019).
409	Next, correlation coefficients were specifically interpreted as weak for 0.00–0.40 r or ρ ,
410	moderate for 0.41–0.69 r or ρ , and strong for 0.70–1.00 r or ρ (Lobo et al., 2016). Standard
411	errors for measurements (SEM) values were detailed through SD $\times \sqrt{\Box}$. Next, correlation
412	coefficients were specifically interpreted as week for 0.00-0.40 r or ρ , moderate for 0.41-0.69
413	<i>r</i> or ρ , as well as strong for 0.70-1.00 <i>r</i> or ρ (Lobo et al., 2016). Standard errors for
414	measurements (SEM) values were detailed through SD $\times \sqrt{\Box}$. After, minimum detectable
415	changes (MDC) values were detailed through $\sqrt{2} \times 1.96 \times SEM$ for 95% CI. Both MDC and
416	SEM were detailed through Bland and Altman recommendations (Calvo-Lobo et al., 2019).
417	Limits for agreement (LoA) for each pair of measurements were detailed through differences

418 means ± 1.96 × *SD* for 95% CI in line with Bland and <u>Altman (Bland & Altman, 2010; Calvo-</u>
419 Lobo et al., 2019).

420 Altman.(Bland & Altman, 2010; Calvo-Lobo et al., 2019)

421 In addition, Bland-Altman plots were shown to detail visual agreements for each pair of measurements showing systematic measurement errors of the differences in means 422 423 distributions for each pair of measurements located at the Y-axis with regards to the means 424 for each pair of measurements located at the X-axis. These Bland-Altman plots were shown in conjunction with linear regression models. R^2 coefficients were calculated to detail the 425 426 adjustment quality. The mean values for each pair of measurements were considered 427 independent variables. Lastly, the differences for each pair of measurements were considered 428 dependent variables (Bland & Altman, 2010). In addition, Bland-Altman plots were shown to 429 detail visual agreements for each pair of measurements showing systematic measurement 430 errors of the differences means distributions for each pair of measurements located at Y axis 431 with regards to the means for each pair of measurements located at X axis. These Bland-432 Altman plots were shown in conjunction with linear regression models. R² coefficients were 433 calculated to detail the adjustment quality. The means values for each pair of measurements 434 were considered as independent variables. Lastly, the differences for each pair of 435 measurements were considered as dependent variables (Bland & Altman, 2010).

436

437 3. Results

438 3.1. Descriptive data

439 The final sample comprised 34 triceps surae muscles bilaterally from 17 healthy athletes,
440 nine (52.9%) males and eight (47.1%) females, with mean ± SD (95% CI) age of 41.76 ±

39 ⁴⁰

441	<u>14.42 (36.73–</u> 46.79) years, the weight of 68.57 ± 14.57 (63.40 <mark>–</mark> 73.64) kg, the height of 1.69 ±
442	0.09 (1.66–1.73) m, and BMI of 23.45 ± 3.47 (22.24–24.66) kg/em ² . Regarding the main
443	<mark>sports</mark> category <mark>, these</mark> athletes performed <mark>in</mark> fitness (n = 14; 82.40%) and soccer (n = 3;
444	<u>17.6%). All athletes presented <mark>the dominant right side</mark> (n = 17; <mark>100%</mark>); <mark>most were</mark> non-</u>
445	smokers (n = 12; 70.60%). Considering the IPAQ, the mean ± SD (95% CI) of metabolic
446	<u>equivalents index per minute per week was 3276.08 ± 1876.49 (2621.34 <mark>–</mark> 3930.82)</u>
447	METs/min/week, including eight (47.10%) athletes who performed vigorous physical activity
448	and <mark>nine</mark> (52.90%) athletes who performed moderate physical activity. Table 1 shows the
449	normality statistics and significance according to the Kolmogorov-Smirnov test. The final
450	sample comprised 34 triceps surae muscles bilaterally from 17 healthy athletes, 9 (52.9%)
451	males and 8 (47.1%) females, with mean ± SD (95% CI) age of 41.76 ± 14.42 (36.73 – 46.79)
452	years, weight of 68.57 ± 14.57 (63.40 – 73.64) kg, height of 1.69 ±0.09 (1.66 – 1.73) m, and
453	BMI of 23.45 ± 3.47 (22.24 – 24.66) kg/cm ² . Regarding the main sport category, these
454	athletes performed fitness (n = 14; 82.40%) and soccer (n = 3; 17.6%). All athletes presented
455	right dominant side (n = 17; 100%) and most of them were non-smokers (n = 12; 70.60%).
456	Considering the IPAQ, mean ± SD (95% CI) of metabolic equivalents index per minute per
457	week was 3276.08 ± 1876.49 (2621.34 – 3930.82) METs/min/week, including 8 (47.10%)
458	athletes who performed vigorous physical activity and 9 (52.90%) athlete who performed
459	moderate physical activity. Table 1 showed the normality statistics and significance according
460	to Kolmogorov-Smirnov test.
461	
462	Please, include Table 1 here or hereabouts
463 464	Table 1. Descriptive data and normality statistics and significance according to Kolmogorov-Smirnov test.



466	According to Table 2, intra-session measurements of the infrared thermography device
467	designed for materials (Manual Infrared Camera PCE-TC 30) in the triceps surae muscle
468	tissue of athletes showed excellent reliability (ICC _(1,2) = $0.969 - 0.977$), measurement errors
469	(SEM = $0.186-0.212^{\circ}C$; MDC = $0.515-0.587^{\circ}C$) and did not present any statistically
470	significant systematic error of measurements ($P > 0.05$). According to Table 2, intra-session
471	measurements of the infrared thermography device designed for materials (Manual Infrared
472	Camera PCE-TC 30) in the triceps surae muscle tissue of athletes showed excellent reliability
473	(ICC _(1,2) = 0.969 − 0.977), measurement errors (SEM = 0.186 − 0.212 °C; MDC = 0.515 −
474	0.587 °C) and did not present any statistically significant systematic error of measurements (P
475	<u>≻.05).</u>
476	
477	<u>Please, include Table 2 here or hereabouts</u>
478 479 480	Table 2. Intra-session reliability and repeatability of an infrared thermography device designed for materials (Manual Infrared Camera PCE-TC 30) in athletes' triceps surae muscle tissue.
481	Please, include Table 2 here or hereabouts
482 483 484	Table 2. Intra-session reliability and repeatability of an infrared thermography device designed for materials (Manual Infrared Camera PCE-TC 30) in the triceps surae muscle tissue of athletes.
485	Regarding intra-session measurements, bivariate correlations were excellent for the
486	soleus ($r = 0.953$; $P < 0.001$), medial gastrocnemius ($\rho = 0.885$; $P < 0.001$), and lateral
487	gastrocnemius ($r = 0.939$; $P < 0.001$). Regarding intra-session measurements, bivariate
488	correlations were excellent for the soleus ($r = 0.953$; $P < .001$), medial gastrocnemius ($\rho =$
489	0.885; $P < .001$) and lateral gastrocnemius ($r = 0.939$; $P < .001$).
490	In addition, Bland-Altman plots presented an adequate agreement for intra-session
491	
	measurements of the infrared thermography device designed for materials (Manual Infrared

493	gastrocnemius (Figure 5) muscles of athletes, due to visual distributions of the difference
494	means for each pair of measurements at Y axis concerning the mean for each pair of
495	measurements at X-axis did not present any systematic measurement error and most
496	thermographic measurements were between the upper and lower LoA.In addition, Bland-
497	Altman plots presented an adequate agreement for intra-session measurements of the infrared
498	thermography device designed for materials (Manual Infrared Camera PCE-TC 30) in the
499	soleus (Figure 2), medial gastrocnemius (Figure 3) and lateral gastrocnemius (Figure 4)
500	muscles of athletes, due to visual distributions of the difference means for each pair of
501	measurements at Y axis with respect to the mean for each pair of measurements at X axis did
502	not present any systematic measurement error and most thermographic measurements were
503	between the upper and lower LoA. In addition to Bland-Altman plots, linear regression
504	models did not show any statistical significance for soleus ($R^2 = 0.000$; $\beta = 0.003$; $F_{1,32} =$
505	0.003; $P = 0.954$), medial gastrocnemius ($R^2 = 0.002$; $\beta = -0.017$; $F_{1,32} = 0.065$; $P = 0.800$).
506	and lateral gastrocnemius ($R^2 = 0.019$; $\beta = -0.050$; $F_{1,32} = 0.626$; $P = 0.435$) intra-session
507	measurements.
508	
509	<u>Please, include</u> Figure 3 here or hereabouts
510 511 512 513	Figure 3. Bland-Altman plots agreement for intra-session measurements of the infrared thermography device designed for materials (Manual Infrared Camera PCE-TC 30) in the soleus muscle tissue of athletes, completed with the upper and lower limits of agreement (LoA).
514	Please, include Figure 2 here or hereabouts
515 516 517 518 519	Figure 2. Bland-Altman plots agreement for intra-session measurements of the infrared thermography device designed for materials (Manual Infrared Camera PCE-TC 30) in the soleus muscle tissue of athletes, completed with the upper and lower limits of agreement (LoA).
520	<u>Please, include <mark>Figure 4</mark> here or hereabouts</u>

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521 522 523	Figure 4. Bland-Altman plots agreement for intra-session measurements of the infrared thermography device designed for materials (Manual Infrared Camera PCE-TC 30) in the medial gastrocnemius muscle tissue of athletes, completed with the upper and lower limits of agreement (LoA).
524	
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526	Please, include Figure 3 here or hereabouts
527 528 529	Figure 3. Bland-Altman plots agreement for intra-session measurements of the infrared thermography device designed for materials (Manual Infrared Camera PCE-TC 30) in the medial gastrocnemius muscle tissue of athletes, completed with the upper and lower limits of agreement (LoA).
530	
531	<u>Please, include <mark>Figure 5</mark> here or hereabouts</u>
532 533 534	Figure 5. Bland-Altman plots agreement for intra-session measurements of the infrared thermography device designed for materials (Manual Infrared Camera PCE-TC 30) in athletes' lateral gastrocnemius muscle tissue, completed with the upper and lower limits of agreement (LoA).
535	
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537	Please, include Figure 4 here or hereabouts
537 538 539 540	<i>Please, include Figure 4 here or hereabouts</i> Figure 4. Bland-Altman plots agreement for intra-session measurements of the infrared thermography device designed for materials (Manual Infrared Camera PCE-TC 30) in the lateral gastrocnemius muscle tissue of athletes, completed with the upper and lower limits of agreement (LoA).
537 538 539 540 541	<i>Please, include Figure 4 here or hereabouts</i> Figure 4. Bland-Altman plots agreement for intra-session measurements of the infrared thermography device designed for materials (Manual Infrared Camera PCE-TC 30) in the lateral gastrocnemius muscle tissue of athletes, completed with the upper and lower limits of agreement (LoA).
537 538 539 540 541	<i>Please, include Figure 4 here or hereabouts</i> Figure 4. Bland-Altman plots agreement for intra-session measurements of the infrared thermography device designed for materials (Manual Infrared Camera PCE-TC 30) in the lateral gastrocnemius muscle tissue of athletes, completed with the upper and lower limits of agreement (LoA).
537 ⁵³⁸ ⁵³⁹ ⁵⁴⁰ 541 542	Please, include Figure 4 here or hereabouts Figure 4. Bland-Altman plots agreement for intra-session measurements of the infrared thermography device designed for materials (Manual Infrared Camera PCE-TC 30) in the lateral gastrocnemius muscle tissue of athletes, completed with the upper and lower limits of agreement (LoA). 3.3. Inter-session reliability and repeatability
537 538 539 540 541 542 543	Please, include Figure 4 here or hereabouts Figure 4. Bland-Altman plots agreement for intra-session measurements of the infrared thermography device designed for materials (Manual Infrared Camera PCE-TC 30) in the lateral gastrocnemius muscle tissue of athletes, completed with the upper and lower limits of agreement (LoA). 3.3. Inter-session reliability and repeatability According to Table 3, inter-session measurements of the infrared thermography device
537 538 539 541 542 542 543 544	Please, include Figure 4 here or hereabouts Figure 4. Bland Altman plots agreement for intra-session measurements of the infrared thermography device designed for materials (Manual Infrared Camera PCE-TC 30) in the lateral gastrocnemius muscle tissue of athletes, completed with the upper and lower limits of agreement (LoA). 3.3. Inter-session reliability and repeatability According to Table 3, inter-session measurements of the infrared thermography device designed for materials (Manual Infrared Camera PCE-TC 30) in the triceps surae muscle
537 538 540 541 542 543 544 545	Please, include Figure 4 here or hereabouts Figure 4. Bland-Altman plots agreement for intra-session measurements of the infrared thermography device designed for materials (Manual Infrared Camera PCE-TC 30) in the lateral gastrocnemius muscle tissue of athletes, completed with the upper and lower limits of agreement (LoA). 3.3. Inter-session reliability and repeatability According to Table 3, inter-session measurements of the infrared thermography device designed for materials (Manual Infrared Camera PCE-TC 30) in the triceps surae muscle tissue of athletes presented excellent reliability (ICC _(1,2) = 0.956–0.974), measurement errors
537 538 540 541 542 543 543 544 545 546	Please, include Figure 4 here or hereabouts Figure 4. Bland-Altman plots agreement for intra-session measurements of the infrared thermography device designed for materials (Manual Infrared Camera PCE-TC 30) in the lateral gastrocnemius muscle tissue of athletes, completed with the upper and lower limits of agreement (LoA). 3.3. Inter-session reliability and repeatability According to Table 3, inter-session measurements of the infrared thermography device designed for materials (Manual Infrared Camera PCE-TC 30) in the triceps surae muscle tissue of athletes presented excellent reliability (ICC _(1,2) = 0.956_0.974), measurement errors (SEM = 0.187_0.232 °C; MDC = 0.518_0.643 °C), and did not present any statistically
537 538 540 541 542 543 544 545 546 547	Please, include Figure 4 here or hereaboutsFigure 4. Bland-Altman plots agreement for intra-session measurements of the infrared thermography device designed for materials (Manual Infrared Camera PCE-TC 30) in the lateral gastrocnemius muscle tissue of athletes, completed with the upper and lower limits of agreement (LoA).3.3. Inter-session reliability and repeatabilityAccording to Table 3, inter-session measurements of the infrared thermography device designed for materials (Manual Infrared Camera PCE-TC 30) in the triceps surae muscle tissue of athletes presented excellent reliability (ICC _(1,2) = 0.956–0.974), measurement errors (SEM = 0.187–0.232 °C; MDC = 0.518–0.643 °C), and did not present any statistically significant systematic error of measurements ($P > 0.05$).

- 549 designed for materials (Manual Infrared Camera PCE-TC 30) in the triceps surae muscle
- 550 tissue of athletes presented excellent reliability (ICC_(1,2) = 0.956 0.974), measurement errors

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551	(SEM = 0.187 – 0.232 °C; MDC = 0.518 – 0.643 °C) and did not present any statistically
552	significant systematic error of measurements ($P > .05$).
553	
554	Please, include Table 3 here or hereabouts
555 556	Table 3. Inter-session reliability and repeatability of an infrared thermography device designed for materials (Manual Infrared Camera PCE-TC 30) in the triceps surae muscle tissue of athletes
557 558 559	Considering inter-session measurements, bivariate correlations were also excellent for the
560	soleus ($r = 0.949$; $P < 0.001$), medial gastrocnemius ($r = 0.914$; $P < 0.001$), and lateral
561	gastrocnemius ($r = 0.938$; $P < 0.001$). Considering inter-session measurements, bivariate
562	correlations were also excellent for the soleus ($r = 0.949$; $P < .001$), medial gastrocnemius (r
563	= 0.914; $P < .001$) and lateral gastrocnemius ($r = 0.938$; $P < .001$).
564	Lastly, Bland-Altman plots showed an adequate agreement for inter-session
565	measurements of the infrared thermography device designed for materials (Manual Infrared
566	<u>Camera PCE-TC 30) in the soleus (Figure 6), medial gastrocnemius (Figure 7), and lateral</u>
567	gastrocnemius (Figure 8) muscles of athletes, since visual distributions of the difference
568	means for each pair of measurements at the Y axis concerning the mean for each pair of
569	measurements at the X axis did not present any systematic measurement error, and most
570	thermographic measurements were between the upper and lower LoA. In conjunction with the
571	Bland-Altman plots, linear regression models did not show any statistical significance for
572	soleus (R^2 = 0.014; β = 0.039; $F_{1,32}$ = 0.463; P = 0.501), medial gastrocnemius (R^2 = 0.000; β =
573	<u>-0.009; $F_{1,32} = 0.014$; P = 0.907</u> , and lateral gastrocnemius ($R^2 = 0.019$; $\beta = -0.050$; $F_{1,32} =$
574	<u>0.621; <i>P</i> =0.436) inter-session measurements.</u>
575	Lastly, Bland-Altman plots showed an adequate agreement for inter-session
576	measurements of the infrared thermography device designed for materials (Manual Infrared
577	Camera PCE-TC 30) in the soleus (Figure 4), medial gastrocnemius (Figure 5) and lateral

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578	gastrocnemius (Figure 6) muscles of athletes, since visual distributions of the difference
579	means for each pair of measurements at Y axis with respect to the mean for each pair of
580	measurements at X axis did not present any systematic measurement error and most
581	thermographic measurements were between the upper and lower LoA. In conjunction with
582	Bland-Altman plots, linear regression models did not show any statistical significance for
583	soleus ($R^2 = 0.014$; $\beta = 0.039$; $F_{1,32} = 0.463$; $P = 0.501$), medial gastrocnemius ($R^2 = 0.000$; $\beta = 0.0000$; $\beta = 0.0000$;
584	-0.009; $F_{1,32} = 0.014$; <i>P</i> =0.907) and lateral gastrocnemius (<i>R</i> ² = 0.019; β = -0.050; $F_{1,32} =$
585	0.621; <i>P</i> =0.436) inter-session measurements.
586	
587	<u>Please, include <mark>Figure 6</mark> here or hereabouts</u>
588 589 590	Figure 6. Bland-Altman plots agreement for inter-session measurements of the infrared thermography device designed for materials (Manual Infrared Camera PCE-TC 30) in the soleus muscle tissue of athletes, completed with the upper and lower limits of agreement (LoA).
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592	
593	Please, include Figure 5 here or hereabouts

594 595 596	Figure 5. Bland-Altman plots agreement for inter-session measurements of the infrared thermography device designed for materials (Manual Infrared Camera PCE-TC 30) in the soleus muscle tissue of athletes, completed with the upper and lower limits of agreement (LoA).
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598	<u>Please, include <mark>Figure 7</mark> here or hereabouts</u>
599 600 601	Figure 7. Bland-Altman plots agreement for inter-session measurements of the infrared thermography device designed for materials (Manual Infrared Camera PCE-TC 30) in the medial gastrocnemius muscle tissue of athletes, completed with the upper and lower limits of agreement (LoA).
602	
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604	Please, include Figure 6 here or hereabouts
605 606 607	Figure 6. Bland-Altman plots agreement for inter-session measurements of the infrared thermography device designed for materials (Manual Infrared Camera PCE-TC 30) in the medial gastrocnemius muscle tissue of athletes, completed with the upper and lower limits of agreement (LoA).
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610	<u>Please, include <mark>Figure 8</mark> here or hereabouts</u>
611 612 613	Figure 8. Bland-Altman plots agreement for inter-session measurements of the infrared thermography device designed for materials (Manual Infrared Camera PCE-TC 30) in athletes' lateral gastrocnemius muscle tissue, completed with the upper and lower limits of agreement (LoA).
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616	Please, include Figure 7 here or hereabouts
617 618 619	Figure 7. Bland-Altman plots agreement for inter-session measurements of the infrared thermography device designed for materials (Manual Infrared Camera PCE-TC 30) in the lateral gastrocnemius muscle tissue of athletes, completed with the upper and lower limits of agreement (LoA).
620	
621	4. Discussion
622	The proposed procedure within the Manual Infrared Camera PCE-TC 30 thermography
623	device presented excellent intra- and inter-session reliability and repeatability with an
624	adequate agreement avoiding systematic errors of measurement to measure skin temperature
625	of soleus, medial and lateral gastrocnemius muscles. Although this tool was initially designed
626	<u>to assess the superficial temperature of materials (De Villoria et al., 2011; Pérez-Urrestarazu</u>
627	<u>et al., 2014), it could be a cheap toolless expensive device to promote triceps surae muscle</u>
628	<u>prevention and recovery in athletes (Rodríguez-Sanz et al., 2017; Rodriguez-Sanz et al., 2018;</u>
629	<u>Rodriguez et al., 2018; Benito-de-Pedro et al., 2019).</u> In addition, the PCE-TC 30 thermal
630	camera may be employed to assess the thermal behavior of fencing uniforms in athletes
631	(Lamberti et al., 2020). Our study supports that this device may be directly used to determine
632	superficial human muscle tissue temperature in the triceps surae of athletes with adequate

- 633 <u>reliability and repeatability (Rodríguez-Sanz et al., 2017; Rodriguez-Sanz et al., 2018;</u>
- 634 Rodriguez et al., 2018; Benito-de-Pedro et al., 2019; Requena-Bueno et al., 2020). The
- 635 Manual Infrared Camera PCE-TC 30 thermography device, which was initially designed to

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636	assess superficial temperature of materials (De Villoria et al., 2011; Pérez-Urrestarazu et al.,
637	2014), presented excellent intra- and inter-session reliability and repeatability with an
638	adequate agreement avoiding systematic errors of measurement to measure skin temperature
639	of soleus, medial and lateral gastrocnemius muscles, which may be a cheap tool to promote
640	triceps surae muscle prevention and recovery in athletes (Rodríguez-Sanz et al., 2017;
641	Rodriguez-Sanz et al., 2018; Rodriguez et al., 2018; Benito-de-Pedro et al., 2019).
642	The PCE-TC 30 thermography device showed similar excellent reliability (ICC > 0.8)
643	compared to the infrared ThermoHuman® device (Fernandez-Cuevas et al., 2017; Requena-
644	Bueno et al., 2020), the digital infrared camera IRTIS-2000® (Zaproudina et al., 2008), and
645	Thermofocus® thermal imaging device (Petrova et al., 2018), which were specifically
646	<mark>designed for human tissue temperature measurements in lower limbs.</mark> Indeed, different
647	<u>ThermoHuman® devices showed an excellent intra-session (ICC = 0.99; LoA = $0.0 \pm 0.4 - 0.1$</u>
648	\pm 0.4 °C) and inter-session (ICC = 0.92; LoA = $0.1 \pm 0.4 - 0.1 \pm 0.5$ °C) reliability before and
649	after running, respectively, detailing small differences effect sizes (Cohen's $d < 0.4$) for foot
650	<u>skin temperature (Requena-Bueno et al., 2020).</u>
651	This tool showed similar excellent reliability (ICC > 0.8) compared to the infrared
652	ThermoHuman® device (Fernandez-Cuevas et al., 2017; Requena-Bueno et al., 2020), the
653	digital infrared camera IRTIS-2000®(Zaproudina et al., 2008) and Thermofocus® thermal
654	imaging device (Petrova et al., 2018), which were specifically designed for human tissue
655	temperature measurements in lower limbs. Indeed, different ThermoHuman® devices showed
656	an excellent intra-session (ICC = 0.99; LoA = 0.0±0.4 – 0.1±0.4 °C) and inter-session (ICC =
657	0.92 ; LoA = $0.1\pm0.4 - 0.1\pm0.5$ °C) reliability before and after running, respectively, detailing
658	small differences effect sizes (Cohen's $d < 0.4$) for foot skin temperature (Requena-Bueno et

659 al., 2020).

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660	Specifically, the digital infrared camera IRTIS-2000® applied in the calf region
661	showed adequate intra-session (ICC = 0.84) and inter-session (ICC = 0.66) reliability
662	with similar temperature mean \pm SD values for anterior (31.2 \pm 0.6 °C), posterior (30.8 \pm
663	<mark>0.6°C)</mark> , and lateral (31.3 ± <mark>0.6°C</mark>) calf regions <u>(Zaproudina et al., 2008)</u> . Finally, the
664	Thermofocus® thermal imaging device presented almost perfect intra-session agreement in
665	<u>different foot regions (ICC = 0.94–0.97)</u> , showing non-significant replication interactions (<i>P</i> -
666	values = 0.23_0.84)posterior (30.8 ± 0.6 °C) and lateral (31.3 ± 0.6 °C) calf regions
667	(Zaproudina et al., 2008). Finally, the Thermofocus® thermal imaging device presented
668	almost perfect intra-session agreement in different foot regions (ICC = 0.94 - 0.97) showing
669	non-significant replication interactions (<i>P</i> -values = 0.23 – 0.84) (Petrova et al., 2018). Despite
670	the use of such modern thermal imaging systems with better definition resolutions could
671	provide more reliable measurements and improve the quality of the thermograms obtained,
672	the reliability and measurement errors provided by the Manual Infrared Camera PCE-TC 30
673	thermography may be enough to detect temperature differences linked to clinical
674	musculoskeletal changes (Côrte et al., 2019).
675	Athletes may be exposed to physical stress under training loads and competitions with
676	overload reactions which could cause blood flow changes affecting skin temperature (Merla et
677	al., 2010). Infrared thermography may not display anatomical abnormalities, although
678	<u>functional changes may be shown and linked to skin temperature control (Merla et al., 2010;</u>
679	Ring & Ammer, 2012). Infrared thermography use was proposed as a complementary tool to
680	apply preventive measures, such as cryotherapy, physiotherapy, training load reduction, and
681	massage or recovery boot use, to avoid muscle conditions in professional soccer players. The
682	asymmetry reference values range from 0.5°C to 1°C between both right and left lower limbs
683	was proposed to initiate this preventive protocol, which reduced up to 63% muscle injuries in

684	a professional soccer season by thermographic monitoring <u>(Côrte et al., 2019)</u> . Thus, the
685	Manual Infrared Camera PCE-TC 30 thermography device could be used to determine these
686	cut-off values due to the MDC values varied from 0.515 to 0.587 °C and from 0.518 to
687	0.643°C for intra- and inter-session evaluations, respectively.
688	Our research group carried out a prior thermographic study addressing the thermal skin
689	evaluation of the triceps surae muscles. This study showed that skin temperature after running
690	was deeply linked to electromyography, which may indirectly reflect triceps surae muscle
691	activity. Although the Manual Infrared Camera PCE-TC 30 thermography device has not yet
692	been correlated with electromyography values, thermal values could be related to muscle
693	activity in the triceps surae muscles of athletes using this tool according to a similar study
694	using the FLIR/SC3000/QWIP Thermacan-infrared thermal deviceA prior thermographic
695	study carried out by our research group showed that skin thermal evaluation of the triceps
696	surae muscles showed that skin temperature after running was deeply linked to
697	electromyography, which may indirectly reflect triceps surae muscle activity. Despite the
698	Manual Infrared Camera PCE-TC 30 thermography device have not been yet correlated with
699	electromyography values, thermal values could be related with muscle activity in the triceps
700	surae muscles of athletes using this tool according to a similar study using the
701	FLIR/SC3000/QWIP Thermacan-infrared thermal devi.ce (Rodriguez-Sanz et al., 2019)
702	Athletes may be exposed to physical stress under training loads and competitions with
703	overload reactions which could cause blood flow changes affecting skin temperature (Merla et
704	al., 2010). Infrared thermography may not display anatomical abnormalities, although
705	functional changes may be shown and linked to skin temperature control (Merla et al., 2010;
706	Ring & Ammer, 2012). Infrared thermography use was proposed as a complementary tool to
707	apply preventive measures, such as cryotherapy, physiotherapy, training load reduction, and

- massage or recovery boot use, to avoid muscle conditions in professional soccer players. The
 asymmetry reference values range from 0.5 °C to 1 °C between both right and left lower limbs
 was proposed to initiate this preventive protocol, which reduced up to 63% muscle injuries in
 a professional soccer season by thermographic monitoring (Côrte et al., 2019). Thus, the
 Manual Infrared Camera PCE-TC 30 thermography device could be used to determine these
 cut-off values due to the MDC values varied from 0.5°C to 0.6°C.
- 714 <u>Although some less expensive commercially-available thermal cameras could be suitable</u>
- 715 for skin temperature assessment, employing the PCE-TC 30 camera to assess triceps surae
- 716 <u>muscle temperature providesd reliable and repeatable measures with MDC cut-off values</u>
- 717 <u>useful to determine preventive protocols for muscle injuries (Côrte et al., 2019).</u>
- 718 4.1. Future studies
- 719 Further studies should be designed as randomized clinical trials to determine if these 720 asymmetries reference cut-off values could prevent triceps surae muscle injuries (Côrte et al., 721 2019). According to prior studies (Hiemstra et al., 2007; Chung et al., 2015), the uninvolved normal side after injury may often be not normal, *i.e.*, presenting temperature values different 722 723 from healthy subjects, and cut-off values should also be detailed in the future muscle recovery 724 studies. In addition, thermographic measurement of the triceps surae with this device should 725 be analyzed by intra- and inter-rater reliability determining SEM and MDC values and 726 correlated with the other high-end infrared devices as possible gold standards such as <u>ThermoHuman® (Fernandez-Cuevas et al., 2017; Requena-Bueno et al., 2020), IRTIS-</u> 727 728 2000®(Zaproudina et al., 2008) and Thermofocus® (Petrova et al., 2018) tools. Furthermore, correlations with electromyography measurements of the triceps surae muscle activity should 729 be carried out (Rodriguez-Sanz et al., 2019). Lastly, the intramuscular temperature should 730

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731 <u>also be correlated with this device to determine concurrent validity concerning a gold</u>
732 <u>standard (Burnham, McKinley & Vincent, 2006).</u>

733 Further studies should be designed as randomized clinical trials in order to determine if 734 these asymmetry reference cut-off values could prevent triceps surae muscle injuries.(Côrte et 735 al., 2019) According to prior studies (Hiemstra et al., 2007; Chung et al., 2015), the 736 uninvolved normal side after injury may often be not normal, i.e. presenting temperature 737 values different from healthy subjects, and cut-off values should be also detailed in future 738 muscle recovery studies. In addition, thermographic measurement of the triceps surae with 739 this device should be correlated with the other high-end infrared devices as possible gold 740 standards such as ThermoHuman® (Fernandez-Cuevas et al., 2017; Requena-Bueno et al., 741 2020), IRTIS-2000®(Zaproudina et al., 2008) and Thermofocus®(Petrova et al., 2018) tools, 742 as well as correlations with electromyography measurements of the triceps surae muscle 743 activity should be carried out (Rodriguez-Sanz et al., 2019). Lastly, intramuscular temperature 744 should also be correlated with this device in order to determine concurrent validity with 745 respect to a gold standard (Burnham, McKinley & Vincent, 2006).

746 | 4.2. Limitations

747 Various limitations should be considered for the use of this thermographic device. First,
748 the MDC was superior to the lower limbs asymmetry reference range from 0.3°C to 0.4°C
749 proposed as a cut-off for following-up before a preventive protocol (Côrte et al., 2019) and
750 therefore, this device should not be used for values lower than 0.5°C–0.6 °C.
751 Second, the concurrent validity of this device has not been performed for human tissue
752 temperature, and this validity should be assessed in the future (Burnham, McKinley &
753 Vincent, 2006).

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754	Third, our sample only comprised healthy athletes, and further studies should analyze
755	skin temperature with this tool over injured muscle tissue (Alburquerque Santana et al., 2022).
756	Fourth, our sample size calculation was accurately detailed to determine a moderate
757	bivariate correlation between measurements, but our sample size was low to achieve the
758	actual power to perform comparisons classifying groups depending on sport category, BMI,
759	and other valued characteristics. In addition, despite statistical analyses were carried out
760	according to our prior sample size calculation model to detail bivariate comparisons for intra-
761	and inter-session measurements, future nested type studies should be designed as nested
762	statistical models such as analyses of variance (ANOVA) to determine more accurate
763	temperature comparisons.
764	Finally, the fact that the PCE-TC 30 thermography device was not calibrated for skin
765	temperature assessment could affect the repeatability of the measurement to a lesser extent
766	than its accuracy. Nevertheless, a comparison between the temperature assessed by a
767	validated thermal camera and the PCE-TC 30 was not reported. Future studies should evaluate
768	its accuracy due to a possible wrong estimation of the absolute skin temperature. Procedures
769	for thermographic assessment in sports and exercise sciences have been reviewed by Moreira
770	et al. (Moreira et al., 2017) in a consensus statement of the experts in the field. However, our
771	reliability study aimed to standardize the proposed procedure and contributed to improving
772	the methods behind measures. Various limitations should be considered for the use of this
773	thermographic device. First, the MDC was superior to the lower limbs asymmetry reference
774	range from 0.3 °C to 0.4°C proposed as a cut-off for following-up before a preventive protocol
775	(Côrte et al., 2019) and therefore this device should not be used for values lower than 0.5 °C –
775 776	(Côrte et al., 2019) and therefore this device should not be used for values lower than 0.5 °C – 0.6 °C. Second, concurrent validity of this device has not been performed for human tissue

- 778 Vincent, 2006). Third, our sample only comprised healthy athletes and further studies should 779 analyze skin temperature with this tool over injured muscle tissue (Alburguergue Santana et 780 al., 2022). Lastly, our sample size calculation was accurately detailed to determine a bivariate 781 moderate correlation between measurements, but our sample size was low to achieve the 782 actual power in order to perform comparisons classifying groups depending on sport category, 783 BMI, and other valued characteristics. In addition, despite statistical analyses were carried out 784 according to our prior sample size calculation model in order to detail bivariate comparisons 785 for intra- and inter-session measurements, future nested type studies should be designed as 786 nested statistical models such as analyses of variance (ANOVA) to determine more accurate 787 temperature comparisons.
- 788

789 5. Conclusion

790	The proposed procedure within the Manual Infrared Camera PCE-TC 30 thermography
791	device designed initially to measure the superficial temperature of materials presented
792	excellent intra- and inter-session reliability and repeatability to measure skin temperature in
793	the triceps surae muscle tissue of athletes. Future studies should consider the measurement
794	errors of this procedure to measure the skin temperature of soleus, medial, and lateral
795	gastrocnemius muscles to promote triceps surae muscle prevention and recovery in athletes.
796	The proposed Manual Infrared Camera PCE-TC 30 thermography device designed
707	
/9/	initially to measure superficial temperature of materials presented excellent intra- and inter-
797	initially to measure superficial temperature of materials presented excellent intra- and inter- session reliability and repeatability to measure skin temperature in the triceps surae muscle
797 798 799	initially to measure superficial temperature of materials presented excellent intra- and inter- session reliability and repeatability to measure skin temperature in the triceps surae muscle tissue of athletes. Future studies should consider the measurement errors of this tool to
797 798 799 800	initially to measure superficial temperature of materials presented excellent intra- and inter- session reliability and repeatability to measure skin temperature in the triceps surae muscle tissue of athletes. Future studies should consider the measurement errors of this tool to measure skin temperature of soleus, medial and lateral gastrocnemius muscles in order to

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813 University and PCE Ibérica S.L. (Reference number: 6-2020) was specifically carried out to
814 study the reliability of the Manual Infrared camera PCE-TC 30 thermography device.

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