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A Wearable Biosensor for Sweat Lactate as a Proxy for Sport Performance Monitoring

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In the last decade, sport performance assessment has significantly transformed due to the appearance of disruptive technologies. Subjective pen and paper notations have evolved into advanced wearable sensing systems that acquire performance-related data. The selection of adequate performance metric variables always causes a debate in sport physiology, and this becomes more relevant once new biochemical indicators are proposed, such as sweat lactate. Here, we analyze the correlation of real-time sweat lactate, obtained with a validated wearable biosensor, with the typical physiological parameters often recorded in sports laboratories (e.g., blood lactate, Borg scale for the rating of perceived exertion, heart rate, power output, blood glucose, and respiratory quotient). We found that the heart rate, power output, Borg scale, and blood lactate relate to sweat lactate in independent individuals during cycling activity. Hence, we demonstrate the potential to associate non-invasive, quantitative, and personalized analysis with sport practice.

Introduction

Among several bioindicators of sport performance, lactate is important because it belongs to a group of biomolecules involved in anaerobic metabolism.[1] In anaerobiosis, glycogen is consumed to produce energy, lactate, and other subproducts. Increased lactate levels can be detected in the bloodstream, and it can also accumulate in working muscles resulting in soreness, and possible pain and fatigue.[2] These symptoms have negative consequences on the athlete's performance,[3] and accordingly, determining blood lactate (or lactate thresholds) has been widely proposed to personalize exercise endurance measurements. Nonetheless, the measurement *per se* is invasive, unsafe, and easily affected by many factors; therefore, the field strives for alternative safe, convenient, and reliable tools. In addition, the blood lactate assessment appears to be sensitive to the collection site (earlobe, fingertip), method (venous, arterial, capillary), and laboratory analysis (lactate analyzer).[4]

In a radically different approach, non-invasive monitoring of sweat lactate in sports would allow easier and more practical solutions, given that sweat is actively excreted during sports. In terms of added value as an indicator for both sport performance and physiology, work is still pending in providing tangible and accurate evidence with massive (and validated) on-body data. Notably, most reports regarding sweat lactate are based on traditional sweat sampling methods, such as whole-body washdown, plastic bag, drape-based collection, and absorbent patches.[5–8] As highlighted in our recent perspective paper (Can Wearable Sweat Lactate Sensors Contribute to Sports Physiology?[9]), we found ten studies targeting sweat lactate sensing based on these sampling methods. Four out of the ten studies displayed an inverse relationship between sweat lactate and exercise intensity,[10–13] one study had inconclusive results,[14] and five studies suggested a positive relationship between sweat lactate and exercise.[15–19] These contradictory outcomes may be due to the different sweat collection methods and their intrinsic drawbacks and systematic errors. Moreover, we determined that all the studies showing a positive lactate-exercise relationship used different approaches to minimize the influence of sweat rate on the measurements.[15,17–19] In contrast, the other five studies (which showed a negative relationship) used the chosen sweat collection method (i.e., absorbent patch,[20] wound dressing,[7] sweat pouch,[21] or paper filter[22]) during very distinct periods (from 1 to 20 min), which caused large variation in the outcomes, but also the training protocols, and collection and transport of the sweat sample.

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Advanced microfluidic systems have been developed and applied to efficiently collect sweat samples, even at extremely low volumes, while avoiding the contamination and evaporation that may adversely affect the reliability of the results. Conveniently, microfluidic-based sweat sampling methods can be integrated with sensors for continuous, on-body analysis of sweat in real time without further laboratory-based analysis.[20,23–26] Some sweat lactate sensing technologies, for example, colorimetric, electrochemical, and electrochemiluminescence, have already been developed, and their potential in sweat lactate monitoring has been successfully proven.[23,27–29] Furthermore, several articles have used these new technologies to successfully investigate the correlations between sweat lactate and exercise intensity.[27,30–38] For example, the results of Seki et al. described monitoring of the anaerobic threshold in healthy subjects but also patients.[31] Overall, the integration of electrochemical sensors into a microfluidic system has shown great advantages in wearable sweat analysis with unique features, such as not disturbing the physical activity.

There are some already well-established parameters to trace sport performance, such as oxygen consumption (VO_2), work power, heart rate, the Borg scale for the rating of perceived exertion (RPE), blood glucose, blood lactate, and the respiratory quotient (RQ), which may have an association with sweat lactate levels.[39–46] Simoes et al. have reported that blood glucose and lactate are related during incremental exercise. In addition, the glucose threshold can be used as a prediction of lactate threshold for young, healthy individuals (Type 2 diabetes).[39–40] For exercise intensity, the heart rate has frequently been used as a physiological parameter to indicate training intensity, particularly in endurance sports.[42] It has also been reported that the ventilatory threshold (corresponding to VO_2) can be determined from the heart rate in well-trained subjects.[41] Indeed, VO_2 can be used to check how effectively the body combines the aerobic and anaerobic metabolisms to improve sport performance, reflecting the energy system at some point.[43] The RQ, i.e., the ratio between the metabolic production of CO_2 and the uptake of oxygen, has been used to indirectly identify the oxidative capacity of muscles to generate energy.[44] Well-trained subjects generally show lower RQ than untrained subjects, so RQ values can also be used to delay fatigue status during exercise. Finally, the perceived exertion ratings (the *Borg scale* is the most common) have been widely used in physiological studies and as a method to measure subjective exertion to quantify and monitor the intensity of physical activity.[45] This has been suggested as a primary tool in sports analysis because of the strong association with physiological parameters, such as blood lactate, VO_2, and respiratory rate.[46]

In this paper, a wearable lactate biosensor recently developed in our group, which demonstrated outstanding reliability and excellent sensing properties, is investigated as an on-body tool for analyzing sweat lactate.[23] Successfully, sweat lactate is continuously monitored in nine cyclists with other physiological parameters often accessible in sports laboratories (i.e., heart rate, power output, blood glucose, blood lactate, the Borg scale, and RQ). The correlations between all the outcomes are studied to understand the value of sweat lactate when measured at different body parts and in training programs. To the best of our knowledge, this is the first study to demonstrate the usefulness of sweat lactate reliably and unequivocally as an indicator of sport performance.

Results and Discussion

The device for on-body sweat lactate measurements

On-body sweat lactate measurements were performed using a wearable epidermal patch comprised of a lactate biosensor, which operates by means of amperometry. The patch was based on a microfluidic cell designed to preserve the natural flow of sweat from the skin to the surface of the biosensor, encompassing the perspiration of the subject. As illustrated in Figure 1a, the sweat inlet was connected to a microfluidic channel, in which the electrodes needed for the amperometry readout (working, reference, and counter) were placed, followed by the outlet. With such a design, once the inlet is full, the sweat flows through the channel until it reaches the outlet. The sweat is continuously replenished in the inlet, and thus in the channel due to active perspiration. The minimal dimensions provided for the fluidics allow the reproduction of a sweat flow velocity in the device that is similar as the perspiration rate in the subject.[23]

The working electrode is a lactate biosensor based on measuring the peroxide (H_2O_2) formed as a subproduct in the reaction of lactate with the lactate oxidase enzyme (LOx). The biosensor contains a series of layers (Figure 1b) that are prepared as detailed in the *Experimental Section*. Briefly, the end of a flat carbon path (prepared by manual screen-printing on a polyester substrate) was first modified with a Prussian Blue (PB) film. Then, the LOx enzyme trapped in a Nafion matrix was added to the top. Finally, an external layer based on a plasticized polymeric membrane was used to cover the electrode. As demonstrated in our recent paper,[23] the latter acts as a diffusion layer, limiting the lactate that reaches the enzyme, and thus, the linear range of the response of the biosensor is tuned to the lactate levels expected in sweat (1–25 mM). Once the sweat reaches the biosensor, the lactate is partitioned between the membrane and the sample and diffuses until it reaches the enzyme layer, where it is converted to pyruvate and H_2O_2. Meanwhile, the PB is in the form of Prussian White (PW) upon reduction due to the application of a constant potential (-50 mV) and the generated H_2O_2 is spontaneously reduced while the PW is oxidized to the original PB form. The PB is then reduced to PW by the applied potential, generating a current change proportional to the amount of PW generated and, thus, the H_2O_2 and the lactate involved in the overall reaction.

Figure 1c presents an image of the integration of the working electrode (biosensor) with the reference and counter electrodes in the microfluidic cell: the three electrodes are aligned on transparent adhesive tape and placed on top of a double-adhesive substrate, which is fixed in the microfluidic

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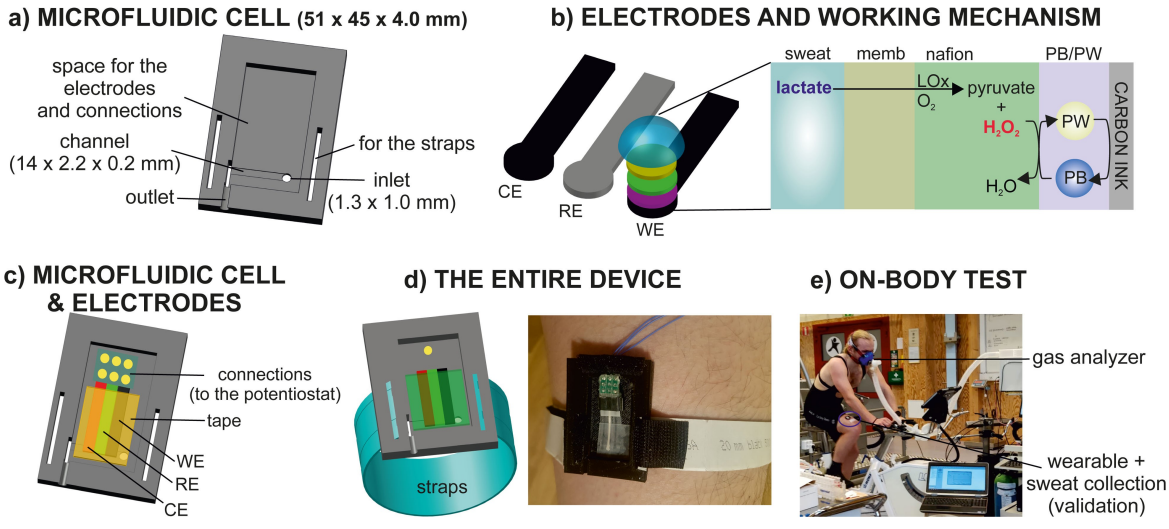

Figure 1. (a) 3D image of the microfluidic cell. **(b)** Schematic of the electrode and the working mechanism for lactate detection. **(c)** Schematic of the microfluidic cell, including the electrodes and the electronic connections. **(d)** 3D model and photograph of the entire wearable device. **(e)** Photograph of an on-body test monitoring a cyclist with the lactate wearable sensor and acquiring various physiological parameters.

channel as close as possible to the inlet by using another layer of the adhesive. This procedure ensures the generation of sweat flow over the entire three-electrode system. Figure 1d shows 3D images and photographs of the entire wearable device, which can be positioned on the forehead, back, arm, and thigh with appropriate straps. Figure 1e presents a photograph of an on-body test in which a cyclist is monitored using the wearable lactate sensor placed on his thigh while other parameters are recorded. For validation purposes, sweat samples were collected every 15 min using either special patches made from a cotton pad and Hydrofilm water-resistant tape or the Macroduct collector. For the patch, the cotton was squeezed in a syringe to extract the sweat immediately after it was collected from the skin.[47]

Monitoring sweat lactate in cyclists using the wearable lactate biosensor

Before starting an on-body test, the lactate biosensor was calibrated in flow mode (using a peristaltic pump at 5 $\mu L\,min^{-1}$) with solutions of 5-, 10-, 15-, and 20-mM lactate concentration in artificial sweat. Such a calibration curve is used to convert dynamic current profiles into lactate concentration, as illustrated in Figure 2. A typical calibration equation is $I(nA) = -10.3 \times c_{lactate}(nM) - 25.0$. Afterward, the patch is attached to the subject's skin and further secured with straps. A decrease in the current indicates an increase in the sweat lactate concentration and *vice versa*. Notably, there may be an initial time at which no lactate data is obtained, as a certain perspiration level is needed from the subject to create a sufficient sweat flow in the microfluidic channel and acquire the amperometric measurements.

The performance of nine well-trained and healthy cyclists was analyzed with the wearable lactate sensor positioned on

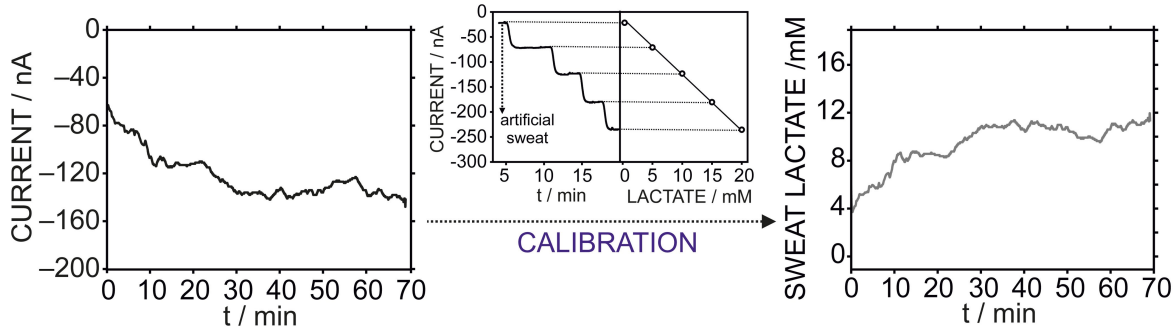

Figure 2. Illustration of the process to convert the current provided by the lactate biosensor in an on-body test into dynamic sweat lactate concentration using a previous calibration curve.

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either the forehead or the thigh, targeting active and passive muscles, respectively. Data was collected in the sports laboratory at Dalarna University (Sweden) under constant environmental conditions (20 °C and 40 % relative humidity). Volunteers were advised not to put rosin or similar products on the skin before the on-body tests to avoid the contamination of the sweat samples. The skin area where the sensor and the sweat collection patch were worn was cleaned with ethanol and water before the gadgets were attached. Also, the athletes were asked to arrive fully hydrated and rested before each training test to avoid any disruption to the test. The same validated cycle ergometer was used for all the subjects. Before data collection, the height and body weight of the subject were measured. A summary of the anthropometric characteristics, gender, and the place in which the wearable lactate sensor was attached is given in Table S1 in the *Supporting Information*.

Once the wearable sensor was attached, VO_2, power, heart rate, and RER measuring devices (that is, a commercial heart rate sensor, ergometer, and respiratory analyzer) were also placed on the subject. Then, the exercise by the cyclist was performed and monitored: 10 min of warmup (not included in the test results) and four rounds of 15 min of cycling at different intensities followed by ca. 2 min of rest. Blood samples were collected every 5 min from the finger of the subject, and sweat samples were collected every 15 min (while the subject was resting) from the cotton pads or the Macroduct collector. The glucose and lactate content in the blood samples were determined by a commercial glucose and lactate analyzer. The lactate content in the sweat samples was measured by ion chromatography (IC) (*Supporting Information*). In addition, every 5 min, the subject was asked to quantify their performance with a number (Borg scale or perceived exertion level). In essence, this number described the subject's feeling of exhaustion, where 6 represented "no exertion at all" and 20 denoted "maximal exertion".[45]

Figure 3 shows the dynamic sweat lactate concentration observed in the nine successful on-body tests: seven with the wearable sensor positioned on the forehead and two on the thigh. The figure also shows the sweat lactate averaged from the dynamic profile every 5 min, which coincides with the timing of the analyzed blood samples. Blood lactate is also presented. The gray squares indicate the resting periods, and the blue arrows indicate the moment the sweat flow in the microfluidic device was sufficient for the lactate biosensor to provide a proper readout. This lag time differs depending on

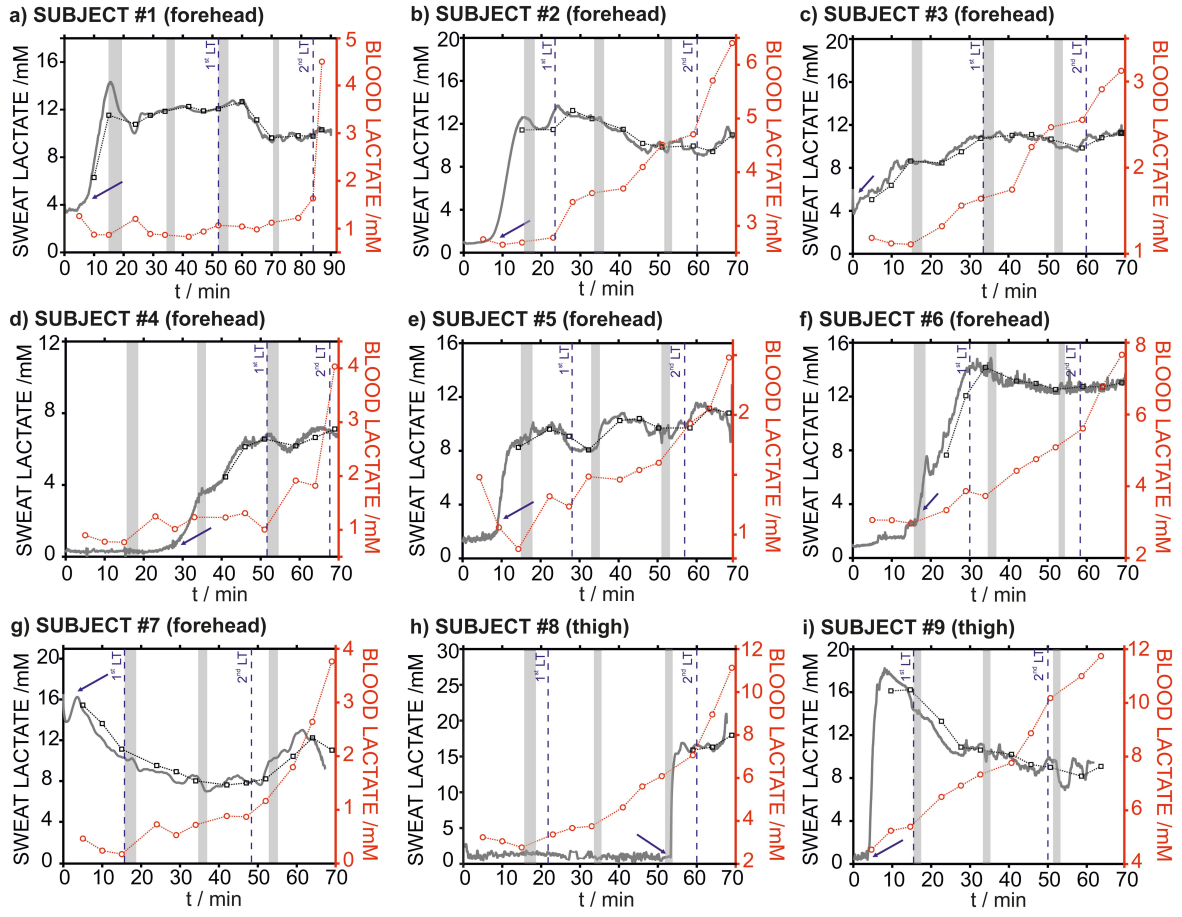

Figure 3. Dynamic sweat lactate profiles were observed in 9 athletes (a–i). Sweat lactate averaged from the dynamic profile every 5 min is represented as squares connected by a dotted line. Blood lactate analyzed every 5 min is also presented in red. Gray rectangles indicate the resting periods. Blue arrows indicate the moment the sweat flow in the microfluidic device was sufficient for the lactate biosensor to provide a proper readout. Blue lines indicate the first and second lactate thresholds as per the blood lactate dynamic profiles observed for each subject.

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the initial perspiration level of the subject (i.e., after the warmup period) and the body part to which the wearable sensor is attached. Thus, measurements from the forehead start before those from the thigh.

The profiles for sweat and blood lactate are next described for each subject. Notably, the blood lactate profile is expected to qualitatively follow the typical lactate performance curve with the first (aerobic) and second (anaerobic) lactate thresholds depending on whether exercise intensity was low, moderate/hard, or high (Figure 4a). Accordingly, the prediction of the first and second lactate thresholds are marked with blue lines in each subject profile and are herein utilized for the description of concentrations and physiological trends.

For **subject #1** (Figure 3a), sweat lactate was rather constant in the 20–60 min interval (average of 11.8 ± 0.6 mM), then it decreased and remained constant (averaged concentration of 9.9 ± 0.3 mM) until the end of the training period. This kind of decrease in sweat lactate while practicing a physical activity has previously been shown in the literature,[26–27] and was likely due to lactate dilution from increased perspiration rates, which occurred as physical activity continue:[48] The lactate concentration should, in principle, be constant or increase with sport intensity but an increase in the perspiration rate diluted it in the sweat. In contrast, the blood lactate remained constant (1.1 ± 0.2 mM) for 80 min, after which a slight increase corresponding to one of the lactate thresholds (probably the first one) was identified. Any correlation between sweat and blood levels is not evident beyond higher levels in sweat; for example, sweat levels were 12 times higher than blood in the 25–60 min period and 2.3 times higher at 90 min.

For **subject #2** (Figure 3b), a similar trend was observed for the sweat lactate in the first 60 min: a fairly constant concentration (12.4 ± 0.9 mM from 20 to 35 min), followed by a decrease and another constant period (10.0 ± 0.2 mM from 45 to 60 min) were observed. However, after 60 min, sweat lactate increased, coinciding with an increase in the blood lactate: from 9.0 mM to 11.0 mM in sweat (22.2% increase) and from 4.7 mM to 6.4 mM in blood (36.2% increase). Before that, the blood lactate was constant from 5 to 20 min and then gradually increased for 60 min, after which it increased more rapidly, as previously described. The later change in the speed of lactate increase traditionally reflects the transition between the aerobic and anaerobic threshold. The sweat lactate was around 4.1

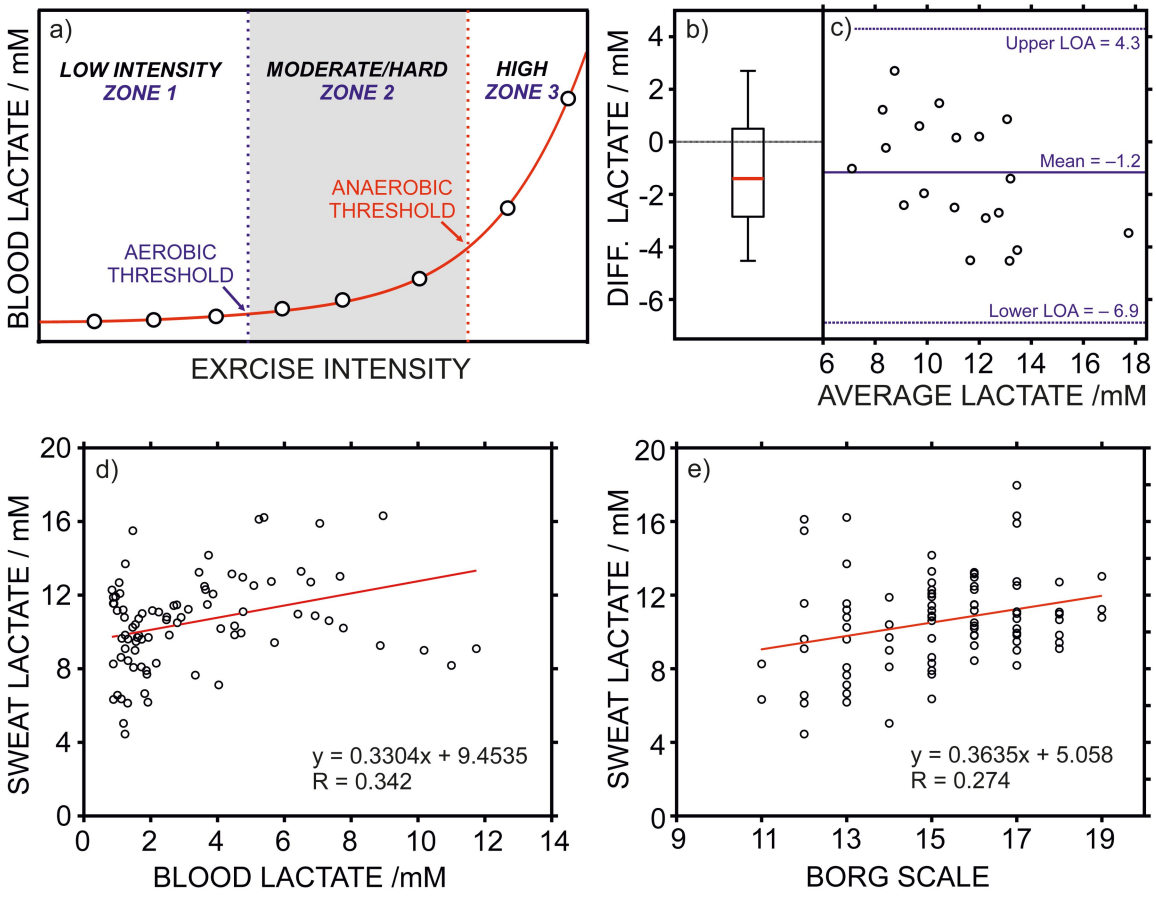

Figure 4. **(a)** Blood lactate performance profile showing the first (aerobic) and second (anaerobic) lactate thresholds depending on low, moderate/hard, and high exercise intensity. **(b)** Boxplot for differences in lactate concentration observed with the wearable biosensor and IC. **(c)** Bland-Altman plot represents the differences between the measurements provided by the wearable biosensor and IC against the average lactate concentration. A solid horizontal line represents the mean value. Dashed horizontal lines indicate the upper and lower limit of agreement at a 99% confidence level. **(d)** Plot of the sweat lactate *vs.* blood lactate observed in the samples collected from all the subjects (number of samples = 85). **(e)** Plot of the sweat lactate *vs.* Borg scale for all the subjects (number of samples = 85).

times higher than blood lactate before the aerobic threshold was reached, 3.1–3.8 times higher between the two thresholds, and 1.6–2.5 times higher after the anaerobic threshold.

For **subject #3** (Figure 3c), overall, the sweat lactate increased (from 3.9 mM to 11.4 mM), with a later period (from 35 to 50 min) during which a constant concentration was observed (10.9 ± 0.2 mM). The blood lactate was constant from 5–25 min and then increased, showing the transition between the aerobic and anaerobic threshold at ca. 45 min. The sweat lactate was 7.8 to 3.6 times higher than the blood lactate.

For **subject #4** (Figure 3d), the sweat lactate measurements were not observed during the first 25 min of training, and the dynamic profile showed a globally increasing concentration (from 4.1 to 7.2 mM, a 76% increase). The decrease at around 55 min could have been caused by the resting stage of this period. The blood lactate was rather constant until 50 min, and then it started to increase, seemingly presenting the aerobic threshold and an unclear anaerobic threshold at 65–70 min. At that time, the sweat lactate was 1.8 times higher than the blood lactate, while before the first threshold and between the first and second thresholds, it was 3.6–6.5 and 3.2–3.6 times higher, respectively.

For **subject #5** (Figure 3e), sweat lactate increased once measurements were obtained from ca. 10 min (from 8.3 to 11.3 mM), except on two occasions: (i) at ca. 28–35 min, which the dilution effect may explain; and (ii) at ca. 50 min, coinciding with one resting period. Regarding the blood lactate, the profile showed an initial decrease until 15 min had passed, which was not expected unless the subject performed a warmup stage with higher intensity than the training *per se*. Then, the blood lactate increased and probably showed the second threshold from ca. 55 min. In the period from 15 to 70 min, the sweat lactate was between 9.4 and 4.4 times higher than the blood lactate.

For **subject #6** (Figure 3f), sweat lactate increased from 8.1 to 14.4 mM between ca. 20 and 35 min, followed by a slight decrease and then remaining constant until the end of the test (12.85 ± 0.2 mM). The blood lactate was constant for the first 25 min, with the transition between the aerobic and anaerobic threshold occurring between 25 and 60 min. The sweat lactate was 2.3 to 3.8 times higher than the blood lactate before the second threshold and 1.7 to 1.9 times higher after that.

The sweat lactate profile observed for **subject #7** (Figure 3g) differed slightly from the other subjects: the lactate concentration decreased for the first 35 min, remained constant (8.0 ± 0.2 mM) until 52 min had passed, then increased from 52 to 60 min, and finally decreased again. The first decrease coincided with the constant blood lactate (9.6–11.1 times lower than the sweat lactate). The constant region corresponded to the transition between the first and second thresholds in the blood (concentrations 4.2–5.9 times lower than that of the sweat lactate), and the final increase and decrease related to the anaerobic threshold in the blood (concentrations between 2.3–3.8 times lower in blood than in sweat). Notably, this subject perspired much more than others, and therefore, the last decrease in the sweat lactate likely comes from lactate dilution, as described above.

Subject #8 (Figure 3h) was monitored at the thigh, and due to the lower perspiration rate, lactate sweat data were not obtained for the first 50 min of training. At that point, the subject was in the final part of the transition between the first and second threshold, and thus, the sweat lactate was found to be constant (16.1 ± 0.3 mM) and increased from 16.3 mM to 20.9 mM, or 1.6–2.3 times higher than the corresponding blood lactate concentration. In contrast, for **subject #9** (Figure 3i), also monitored at the thigh, the sweat lactate decreased during the first 30 min of exercise and remained constant (9.6 ± 1.0 mM). The blood lactate increased over the entire training period, with the second lactate threshold appearing after 55 min. The sweat lactate was 1.4–3.1 times higher than the blood lactate before 40 min had passed and 0.7–1.3 times higher after that. Both sweat and blood lactate levels were generally higher in measurements related to the active muscle (thigh) than the passive one (forehead). We did not identify any significant difference between the male and female subjects.

Validation of sweat measurements observed with the wearable lactate biosensor

To confirm the analytical accuracy of the on-body sweat measurements made with the wearable biosensor, these were averaged every 15 min to coincide with the collection of sweat samples with either a cotton pad (forehead) or the Macroduct collector (thigh), which were analyzed by IC. Accordingly, Table S2 *(Supporting Information)* shows the lactate concentration of 19 sweat samples provided by the wearable biosensor and the IC results together with the difference (in %). When a collection period for a subject does not appear in the table, it is because the volume of sweat collected was insufficient to perform IC measurements. In most cases, a difference of less than 20% was observed, which is an appropriate cutoff value to confirm the reliability of the results obtained with the wearable lactate biosensor.

A dependent sample *t*-test, which estimates whether the average difference between the results provided by two analytical techniques (in this case, the wearable biosensor and IC) on the measurement of multiple samples is zero, was performed. Considering a 99% confidence level, the calculated *t*-score was lower than the critical value (2.59 and 2.88, respectively), which means no statistically meaningful differences were found. These results are graphically illustrated in Figure 4b with a boxplot of the differences in lactate concentration provided by the wearable biosensor and IC. As observed, most of the samples had an absolute value close to zero and deviated in the case that the values determined by IC were higher than the wearable biosensor, as inferred by a median value of −1.4 mM and a first quartile further from zero than the third quartile (−2.9 and 0.5 mM, respectively).

A closer evaluation of the individual agreement between samples can be accomplished with the Bland-Altman plot presented in Figure 4c, which represents the difference between both analytical techniques versus their average values allowing the identification of trends and inconsistencies in

variability. A homogeneous error distribution was found in the lactate concentration ranging from 7.1 to 17.7 mM, indicating that the variability and discrepancy are independent of the lactate concentration. The lower (-6.9 mM) and upper limits (4.3 mM) of agreement were also calculated at a confidence level of 99%. The broad range of concentration observed may be due to two reasons: (i) some measurements could be considered outliers (e.g., the third rounds of subject #5 and subject #9), and their omission would improve the results of the validation study; and (ii) the different frequency of the measurements provided by each technique, i.e., the comparison is between averaged continuous measurements and samples collected at discrete intervals. Overall, the results confirmed the adequate accuracy of the sweat lactate measurements provided by the wearable lactate biosensor.

Investigation of the relationship between sweat and blood lactate

Next, the possible correlation between sweat and blood lactate was investigated after describing the sweat and blood lactate trends and confirming the reliability of the sweat measurements. The plot of the sweat lactate *vs.* the blood lactate (Figure 4d), from time-coincident, averaged measurements using the wearable biosensor and collected blood samples, revealed no correlation. While the general trend was that the higher the blood lactate, the higher the sweat lactate, such a positive correlation was not statistically significant (Pearson coefficient of $R = 0.342$). This lack of correlation, considering an absolute cutoff value for R of 0.80,[49] could be due to the mixture of different subjects and body zones within the data matrix. Therefore, individual correlations were considered (Table S3). Positive and negative correlations were randomly found, in agreement with previous results in the literature.[23] Moreover, only in the case of subjects #8 and #9, the R values were higher than the typical cutoff of 0.80 to confirm an existing correlation between sweat and blood lactate, indeed at the thigh part. However, this result was obtained from only a few samples (a total of eleven points obtained from the two subjects).

We then investigated any possible relationship between the sweat/blood lactate ratio and the lactate thresholds (defined as per the results in blood lactate) as the physical activity advanced for each subject. Notably, it is a common practice in the clinical field to establish ratios between the concentration of the same (bio)marker in two different biological fluids, one of them being the blood as it is the most analyzed one. For example, urine/blood ratios for proteins, creatinine, and chloride, as well as interstitial fluid/blood ratios for electrolytes and glucose, to mention but a few.[50–52] Interestingly, these ratios have been proposed as proxies of the physiological status of animals and humans. Nevertheless, ratios considering sweat are not really exploited in the literature for physiological purposes, and we strongly believe that this lack is caused by the absence of studies providing a relatively large group of data whose interpretation could be physiologically meaningful.

Figure 5 shows the individual temporal profiles for the sweat/blood ratios, indicating the lactate thresholds (LT) obtained from the blood measurements. In every case, the sweat/blood lactate ratio remained initially constant and then gradually decreased over the transition between the two LT to again remain almost constant after the second (anaerobic) threshold. This trend is much more evident and better described in subjects for which a higher number of samples were analyzed (i.e., all subjects except **#4** and **#8**). Some deviations from the described trends coincided with the measurements in which sample dilutions were identified; thus, a lower lactate concentration than expected was measured with the wearable biosensor. For example, the last point in the data from **subject #1**, the initial 3 points in **#5**, and the last point in **#7**. Thus, determining the perspiration rate while exploring the correlation between sweat lactate and sport performance may be recommended.[53] In addition, the initial points of some subjects (**#1, #4, #5**, and **#6)** slightly deviated from the averaged constant value expected before the first lactate threshold. This effect was likely due to the uncertainty associated with the very first measurements provided by the wearable biosensor before appropriate sweat flow was established in the microfluidic system, encompassing the natural perspiration of the subject.

In conclusion, a direct correlation between sweat and blood lactate was not found. However, a relationship between the sweat/blood lactate ratio and the LT for each subject was determined, which could be useful for further physiological investigations in personalized training strategies and with a larger number of on-body data. On the other hand, it is essential to investigate the relationship of sweat lactate with other performance indicators aside from blood lactate to unequivocally address whether sweat lactate can be used as an indicator for personalized training strategies and other sports trends.

Investigation of the relationship of sweat lactate with the Borg scale and other sport performance parameters

We present a thorough investigation of the relationship of sweat lactate with cycling intensity, with a special focus on the RPE (expressed using the Borg scale). The Borg scale is an athlete-subjective parameter that can be easily measured and considered with the sweat lactate measurements to monitor sports training daily. On the contrary, the acquisition of other parameters (blood lactate and glucose, heart rate, power output, and RQ) may cause discomfort in the subject or disturb the physical practice. Thus, the data collected from each subject (every 5 min) were grouped according to the measured Borg scale values. Figure 6 illustrates such a grouping for the case of **subject #1**, whereas the entire data distribution is presented in Table S4. It is worth mentioning that the number of Borg scale commonly differs among participants facing

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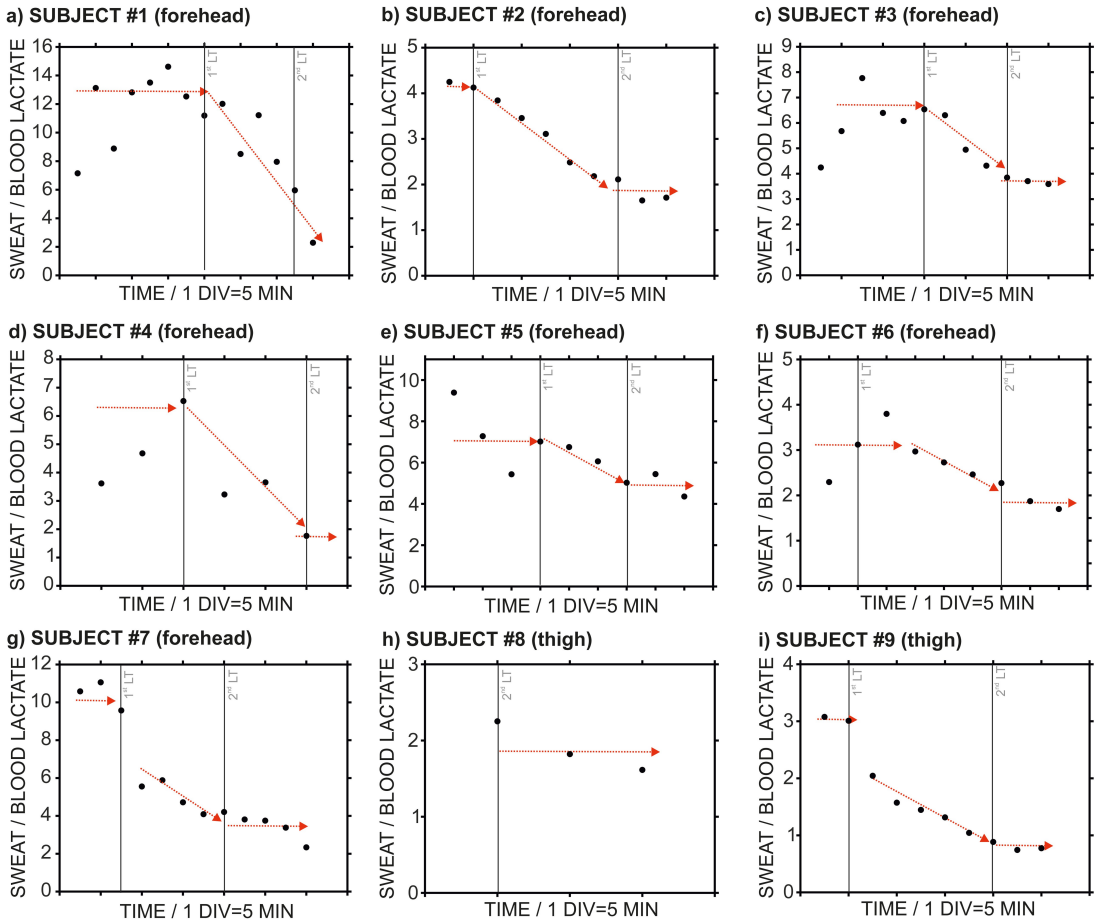

Figure 5. For subjects #1–#9 (a–i), individual temporal profiles of the sweat/blood lactate ratio and correspondence with the LT are identified by the blood measurements. Red arrows indicate the trends of the sweat/blood lactate ratio with time and hence, with increasing workout intensity. Gray lines indicate the first and second lactate thresholds (first LT and second LT, respectively).

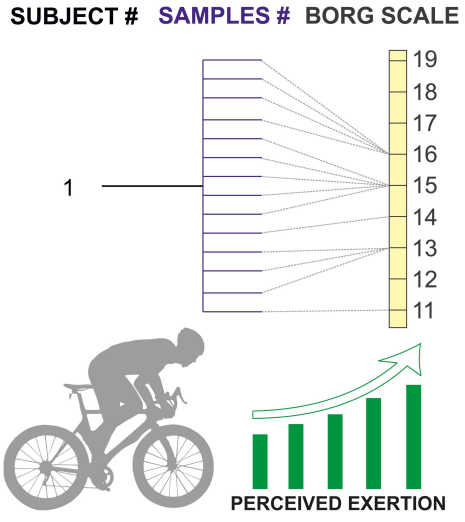

Figure 6. Illustration of the data analysis for on-body tests. The level of perceived exertion was recorded via the Borg Scale during cycling, establishing it as a reference to analyze and study any relationship between sweat lactate and other physiological parameters.

analogous physical activity due to the different body conditions.

Figure 4e presents the sweat lactate concentrations averaged every 5 min with the corresponding Borg scale values. As in the case of blood lactate, no correlation was observed. However, inspecting the individual plots of the sweat lactate against the Borg scale, some interesting trends can be found (Figure 7). The first and second lactate thresholds observed in blood were marked to explore possible relationships with the Borg scale. It is noticeable that the same number of Borg scale contained several data points of blood lactate, which distributed between the 1st and 2nd LT or even above the 2nd LT for the same subject. Overall, the relationship between the lactate LTs and the Borg scale differed for each individual case, and the drawn of deeper conclusions is not foreseen. The other performance parameters (blood lactate, power, blood glucose, heart rate, VO_2, and RQ) were also analyzed *versus* the Borg scale values to facilitate interpretation (RQ is separately provided in Figure S1 in the *Supporting Information*).

Investigating the Borg scale values with respect to the sweat lactate, blood lactate, power, heart rate, and VO_2, the data from **subject #1** revealed that, despite increased Borg scale values due to increased power output during cycling,

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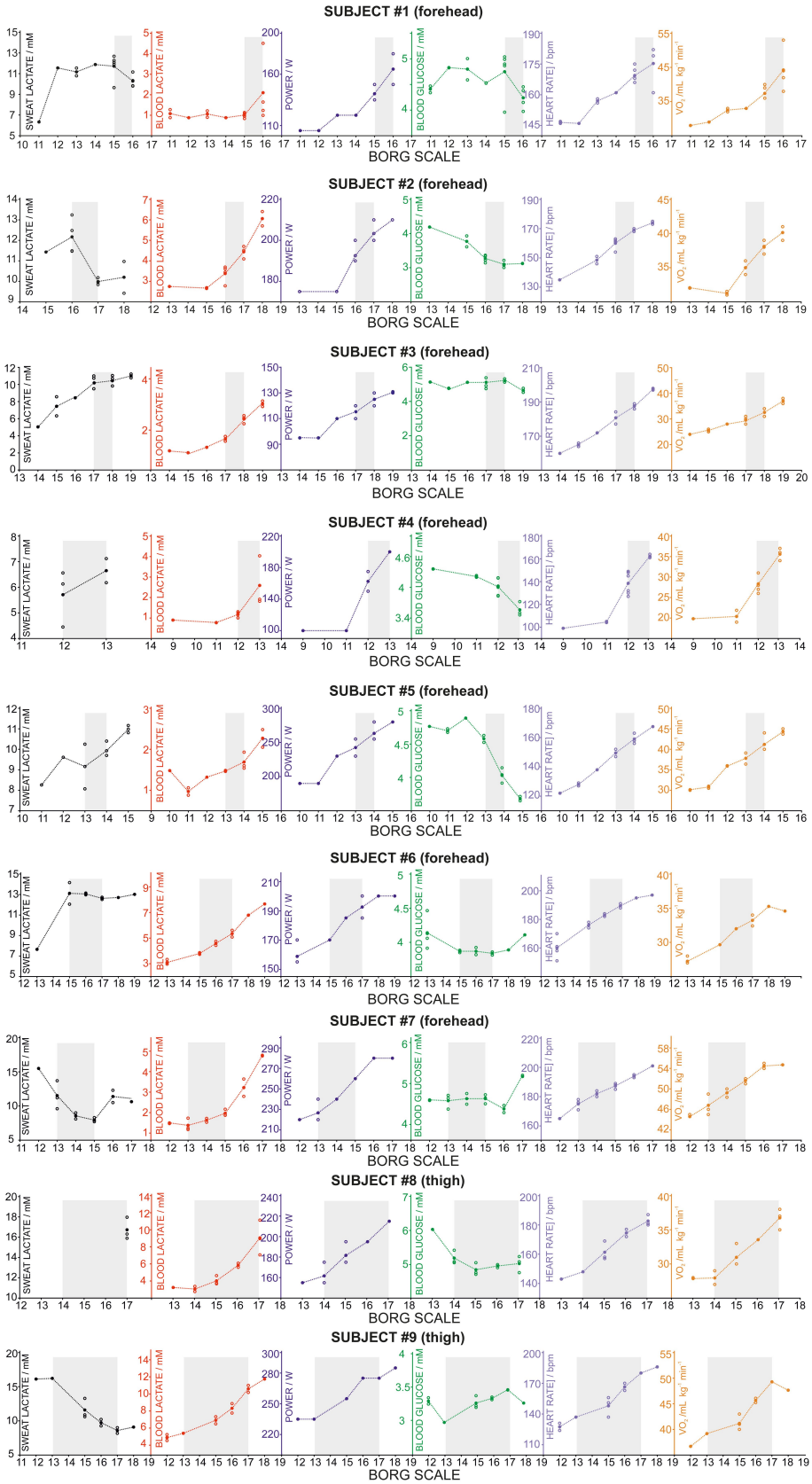

Figure 7. For subjects #1–#9, plots of the sweat lactate, blood lactate, power, blood glucose, heart rate, and VO_2 *vs.* the rating of perceived exertion expressed in the Borg scale. Filled dots present averaged data to guide the eye of the reader together with the dashed line in the overall trend in each plot. Gray areas indicate the transition between the 1st and 2nd lactate thresholds in blood.

which is also reflected in increased heart rate and VO_2, both blood lactate and sweat lactate on the forehead remained constant. Hence, the amount of energy that the anaerobic system produced (or was trained to produce) to reach a significant physical effort was minimal. In essence, the subject reached a maximum of 16 on the Borg scale, corresponding to the initiation of the first lactate threshold in blood, which did not manifest in the sweat lactate in the forehead. In contrast, for **subject #2**, the situation changed; it was possible to visualize the two LT and higher values on the Borg scale. In this case, the increase in the performance parameters coincided with an increase in the blood lactate and a decrease in the sweat lactate, caused by dilution as the sweat rate increased in the forehead.

In **subject #3**, the exertion reached a value of 19 on the Borg scale, indicating maximal effort in the cyclist's performance. Interestingly, all the parameters increased, including the sweat glucose in the forehead. A similar tendency was found for **subjects #4** and **#5** despite reaching a lower maximal value on the Borg scale (13 and 15, respectively) than **subject #3** and a less marked increase in the blood lactate. In the case of **subject #6**, sweat lactate in the forehead remained fairly constant despite reaching an exertion level of 19, whereas **subject #7** presented an uncommon decrease followed by an increase in the lactate level.

Regarding sweat measurements from the thigh, **subjects #8** and **#9** presented the maximum lactate levels, probably because active muscles (such as the quadriceps) were targeted, and these two subjects showed higher levels of and changes in blood lactate. Unfortunately, in **subject #8**, the sweat measurements that could be achieved with the wearable were all at the maximum exertion (17 on the Borg scale); thus, the results were inconclusive. For **subject #9**, the sweat lactate decreased over the training program because of the increased perspiration rate, while the rest of the performance parameters increased.

For each subject, a positive correlation of the Borg scale with the power output, heart rate, and VO_2 can be seen in Figure 7. When statistically significant correlations were explored for the power output and the heart rate (Figure S1 in the *Supporting Information*), considering the averaged values observed along the exertion scale, positive correlations were observed in all cases, except for **subject #8**, for which there were an insufficient number of points. Once again, by considering an absolute threshold value of 0.80 for the Pearson coefficient to confirm the correlations,[49] the investigation of the grouped data did not reveal significant relationships, unlike the individualized data (Figure S2). This result is logical since muscle (or physical) efficiency to reach comparable workouts evidently varied between subjects.

Another way to study the data is using a box plot, which presents the upper and lower quartiles, medians, potential outliers, and discrepancies in data distribution (Figure S3 in the *Supporting Information*). The sweat lactate values started at 6.7 mM at a Borg scale value of 11, which is considered low-level exertion, then rose to approximately 9.5 mM at the middle level of exertion (Borg scale of 12–14), and finally increased to 10.5 mM once the exertion level reached values above 15 on the Borg scale. This trend likely indicates that sweat lactate concentration shows some degree of correlation with sport performance expressed on the Borg scale; hence, it may be used as an indicator (alternative to blood tests) to identify the rate of exhaustion in long-term cycling tests.

The power level obtained at each Borg scale value displayed large variation, yielding large box plots with wide whiskers in each exhaustive stage, which is attributed to the variation in the training level of the subjects. Since it is hard to establish a potential trend from randomly distributed values from nine subjects, we suggest that participants in future studies have similar training levels. In the case of the heart rates, there was a clear increment as the Borg scale increased. Effectively, the narrow boxes and whiskers (with only one point in the outlier zone) indicated a strong association with the level of perceived exertion. This result indicates that a potential relationship between sweat lactate and heart rate could be investigated in a further study, which would be further evidence of a strong correlation between the lactate concentration in sweat and the level of exhaustion.

The levels of VO_2 can be used to assess how effectively the body combines aerobic and anaerobic metabolism, reflecting the production of the energy required for the sustenance of muscles during physical activity. From our results, the value of oxygen consumption increased up to level 13 of the Borg scale. From levels 13 to 19, the VO_2 remains nearly constant, likely indicating that the participants reach a maximum oxygen consumption. We also noted large variabilities of $\pm 14\ mL\,kg^{-1}\,min^{-1}$ that could have been caused by the different training levels of the subjects.

Next, considering the blood glucose, it tended to remain constant or decrease in some subjects when certain blood lactate levels were reached. Certainly, the variations found were not dramatic or physiologically relevant, while other authors have claimed that blood glucose tends to decrease with high concentrations of blood lactate, mirroring the second threshold.[39–40] The RQ (Figure S4) was calculated as the ratio between the metabolic production of CO_2 and the uptake of O_2 and can be used to indirectly identify how muscles efficiently get energy while doing physical activity. For example, lower RQ at a similar physical intensity is expected in well-trained over untrained subjects (e.g., close to 0.8 *vs.* close to 1.0), which means that maintaining a low RQ value is important to delay the time to becoming fatigued.[44] Then, RQ values close to or over 1.0 may cause muscle injury due to overexercise.[44]

In our studies, **subject #1** maintained a constant RQ value of 0.83 until a Borg scale of 14, then increased to 0.89 for the highest Borg scale value because of the increasing intensity of the physical activity. In contrast, the RQ value of **subjects #2 and #3** started from high values of 0.92 and 0.89, respectively, probably because the warmup step was not sufficiently effective. Then a slight decrease in RQ over the training period was observed, which may represent how the subject performed at their maximum capacity. Then, **subjects #4–#7** showed an overall gradual increase in RQ (and intensity) over the training period, never reaching values

higher than 0.96, and **subject #7** showed lower (and healthier) values. **Subjects #8** and **#9** also showed increasing values with increasing exertion, with values even higher than 1.0, which are not good for the healthy training of the individual. Overall, RQ was the parameter that showed not only the poorest relationship with the Borg scale values (Figure S4) but also the lowest correlation with sweat lactate measured from either the thigh or the forehead (Figure S5 in the *Supporting Information*).

Some hints for the appropriate utilization of sweat lactate in sport performance monitoring

From all the descriptions and discussions proposed until now, it is evident that: (i) outcomes should be interpreted for each individual and not for groups; (ii) there is a trend relating the sweat/blood lactate ratio with the LT and thus with the sport intensity; (iii) the Borg scale for exertion is a performance parameter that positively correlates with intensity measured as either power output, heart rate, or VO_2; (iv) the exertion (and thus the power output, heart rate, and VO_2) presents individualized tendencies with the sweat lactate, as the muscle efficiency to reach comparable workouts varied between subjects; (v) blood glucose and RQ seem to be unrelated to sweat lactate; (vi) it seems important to investigate a possible correction of the calculated sweat lactate according to changes in the perspiration rate of the subject; (vii) in addition to the Borg scale, the heart rate seems to be another parameter that can be co-monitored with the sweat lactate to further explore the correlation with performance; and (viii) sweat lactate levels in an area of skin close to active muscles (e.g., on the thigh) are higher than those in passive muscles (e.g., on the forehead),[54] although trends during training were similar for both. Regarding this latter, differences in sweat composition attending to the body part were already pointed out in previous scientific reports. In the case of lactate, it has been hypothesized that higher lactate concentration is related to a lactate removal process from blood and/or tissues in close vicinity to active muscles during the sport practice.[54–55]

Bearing in mind the final goal of a sports device providing a continuous reading of sweat lactate in real-time, the appropriate interpretation of such data requires further studies and ideally considering the Borg scale or heart rate as subjective or objective performance traces. It is important to initially "learn by doing" for each subject to create a personalized training strategy depending on their sweat lactate levels and their exertion related to the physical work. Our results indicated that no general conclusions regarding sweat lactate levels can be drawn but that evidences must be assigned to each individual. In addition, further efforts directed to maximize the reliability of the results provided by the lactate wearable sensor are recommended. These include the implementation of a sensor that monitors perspiration rate and the investigation of a strategy for data correction by compensation of changes in the perspiration rate of the subject. Furthermore, this dual sensor for lactate and perspiration rate may serve as a proxy to study the so-called "removal of lactate" via sweat during exercise.[56–57]

Conclusions

We have demonstrated continuous, real-time, on-body analysis of sweat lactate using a wearable lactate biosensor with an amperometric readout. The tendencies in levels, together with correlations with well-established physiological parameters (blood lactate, blood glucose, Borg scale, power output, heart rate, VO_2, and RQ), have been investigated using individualized and grouped data. Individualized contextualization is more appropriate since physical efficiency for reaching comparable workouts varies between subjects. From the data obtained from a cycling workout program involving nine experienced (healthy) subjects, continuous traces of sweat lactate revealed increased sweat lactate with increased training intensity expressed on the Borg scale. However, in some cases, when significantly high perspiration was identified, a decrease in sweat lactate was observed. Notably, the sweat/blood lactate ratio strongly correlates with the LT. Moreover, this correlation occurs independently of the targeted muscle, whether passive (forehead) or active (thigh). Overall, and bearing in mind the need to scale up the number of subjects in the study, as well as the positioning of the wearable biosensor on other body parts, the results in this work demonstrate the potential to associate non-invasive, quantitative, and personalized analysis of sweat lactate with sport practice labels.

Experimental Section

Chemicals

Potassium hexacyanoferrate(III) (CAS-13746-66-2, $>98\%$ purity), iron (III) chloride (CAS-7705-08-0, $>97\%$ purity), sodium l-lactate (CAS-867-56-1), chitosan (CAS-9012-76-4), Nafion® perfluorinated resin solution 5% (CAS-31175-20-9), bis(2-ethylhexyl)sebacate (DOS) (CAS-122-62-3, $\leq 97\%$ purity), polyurethane Tecoflex SG80A (PU) (CAS-68400-67-9), high molecular weight poly(vinyl chloride) (PVC), tetradodecylammonium tetrakis(4-chlorophenyl)borate (ETH 500), and tetrahydrofuran (THF) (CAS-109-99-9) were purchased from Sigma-Aldrich. Analytical-grade chloride salts of ammonium (CAS-12125-02-9), magnesium (CAS-7786-30-3), potassium (CAS-7447-40-7), sodium (CAS-7647-14-5), as well as sodium carbonate (CAS-497-19-8) and sodium phosphate (CAS-7558-79-4) were also purchased from Sigma-Aldrich. Lactate oxidase (LOx) was purchased from Sorachim SA, Switzerland (LAX-18C). Silver/silver chloride (Ag/AgCl) and carbon inks were purchased from Henkel, Germany. Polyurethane (PU) filament for the 3D printing of the sampling cell, TPU 95 A, Ultimaker Material 1756, was purchased from Ultimaker B.V., Netherlands. All solutions were prepared in 18.2 $M\Omega^{-1}$ doubly deionized water (Milli-Q water systems, Merck Millipore). Polyester sheets (thickness of 100 μm) to fabricate screen-printed carbon and Ag/AgCl electrode paths (for further modification to provide the lactate biosensor) were purchased from RS Components (Sweden). The Macroduct device for sweat collection was purchased from ELITechGroup (The Netherlands).

Preparation of the epidermal patch for lactate biosensing

The lactate biosensing patch consisted of an enzyme-based electrochemical lactate sensor integrated into a microfluidic system for sweat sampling. The lactate is measured using a three-electrode system (the working electrode, counter electrode, and reference electrode) individually fabricated from a polyester sheet (4×23 mm in dimensions). First, either carbon (for the working and counter electrodes) or Ag/AgCl (for the reference electrode) inks were manually screen-printed onto the polyester substrate and dried in the oven at 100 °C for 1 hour to prepare conducting paths for further modification. The paths contained circular (diameter of 1.5 mm, sensing area) and rectangular (width = 1 mm, length = 20 mm, for the electrical connection) parts (designed and cut using a Silhouette Cameo cutter, Silhouette Inc, The Netherlands).

The modification of the working electrode was carried out using a layer-by-layer deposition method, based on PB as the mediator layer, LOx enzyme entrapped in Nafion as the reaction layer, and a PVC-based external diffusion-limiting membrane. In more detail, 3 μL of 0.1 M potassium ferricyanide and 3 μL 0.1 M iron (III) chloride were drop-casted on the circle of the carbon path, mixed in situ, and left to react for 20 min at room temperature. Afterward, excess solution was removed with 0.01 M HCl, and the resulting PB layer was annealed in the oven at 100 °C for 1 hour. The LOx enzyme layer was constructed by drop-casting 2.5 μL of a solution containing 15 mg/mL LOx, 5 mg/mL BSA, and 0.5 wt% Nafion. This layer was allowed to dry for 20 min at room temperature before the outer polymeric membrane was deposited. For the latter, 1.5 μL of a solution containing 3 mg/mL ETH 500, 33 mg/mL PVC, and 66 mg/mL DOS in THF was drop-casted and dried at room temperature for 20 min. The reference and counter electrodes were prepared as reported elsewhere.[23] Finally, the three electrodes were conditioned overnight in artificial sweat containing 60 mM NaCl, 6 mM KCl, 5 mM NH_4Cl, 0.08 mM $MgCl_2$, 2.6 mM $NaHCO_3$, and 0.04 mM Na_2HPO_4 (pH 7.6) before further use.

The microfluidic cell for sweat sampling was designed and printed using AutoCAD and a 3D printer (Ultimaker B.V., The Netherlands). The electrodes were fixed, with the sensing area coinciding with the microfluidic channel and the working electrode as close to the inlet as possible (see Figure 1), with double adhesive tape (3 M VHB, RS components) forming the sweat channel (2.0×25.0×0.2 mm). Then, the rectangular parts of the electrodes were connected to three 150-centimeter-length aluminum wires (24 AWG, Jonard tools, USA), which were connected to the electrochemical instrument (Autolab, Metrohm Nordics AB, Sweden) using commercial 3-pin connectors (5-520315-3-ND, Digi-Key).

Participants in the on-body tests

A total of thirteen elite (national level) cyclists or triathletes were recruited to participate in this study in December 2020. However, sweat lactate was successfully monitored in nine athletes due to some technical aspects. All the participants provided written informed consent prior to the study. Ethical approval for the study in this paper was granted by the Swedish Ethical Review Authority (registration number: #2020-04206). All the research was conducted in accordance with the Code of Ethics of the World Medical Association (Declaration of Helsinki). Participants were asked not to use rosin or similar products before data collection to avoid contamination of the sweat samples.

To avoid any disruption of data collection, all athletes were informed to arrive in a fully hydrated and rested state before each training test. The same validated cycle ergometer was used for all the subjects, and individual handcycles were mounted before exercise. Before data collection, the height and mass of all the participants were measured to ensure subjects were physically fit and had no medical disorders, which may either affect the experimental results or prevent subjects from exercising safely.

Protocol of the on-body tests

All the data were collected in a sports laboratory (Dalarna University) at an ambient temperature of 20 °C and 40% relative humidity. The heart rate and VO_2 and carbon dioxide output (VCO_2) were monitored through a metabolic chart in mixing chamber mode (Jaeger Oxycon Pro, Erich Jaeger GmbH, Hoechberg, Germany). The cycling intensity and power output were recorded using a cycle ergometer (LC7, Monark Exercise AB, Vansbro, Sweden). Before data collection, each participant performed a 9 min warmup on the bike ergometer. The step consisted of three different cycling intensities (1.2, 1.4, and 1.6 W kg^{-1}) for 3 min each.

Based on the relationship between the power and the heart rate during the different intensities in the warmup, four submaximal, incremental cycling levels were established for the on-body tests (to be monitored with the lactate wearable biosensor). Each participant cycled for 15 min on each submaximal level, with around two min of rest between each submaximal level. Every 5 min, the participants were asked to rate their exertion using the 6–20 RPE (Borg scale). At the same time, 20 μL of capillary blood was extracted from a fingertip and immediately placed in a vial containing a commercial standard solution from EKF-diagnostic GmbH (Barleben, Germany). Before measuring the concentrations of blood lactate and glucose through BIOSEN C-line (EKF-diagnostic GmbH, Barleben, Germany), convection conditions were applied to the mixtures for around 20 min at a low speed via an automatic wiggle sample shaker (MiniMix, Molek AB, Årsta, Sweden).

For sweat lactate measurements, the influence of the sweat rate (mimicked by the flow rate of an automatic peristaltic pump) on the biosensor response was evaluated in our previous work.[23] The range for the tested flow rates corresponded to a sweat rate range from 0 to ca.13.8 μL cm^{-2} min^{-1}, which largely covered the typical human sweat rate (from 2 to 7 μL cm^{-2} min^{-1} depending on the subject and body part.) Overall, it was found a negligible influence of the flow rate in the amperometric response of the biosensor. Moreover, any influence of pH and T on the biosensor response was also discharged.[23]

The wearable biosensor was pre-calibrated prior to being attached to the body of the participant (forehead or thigh). The corresponding skin area was shaved and then cleaned with ethanol and water. The epidermal biosensor was attached and secured with straps. The sweat collector (cotton pad or Macroduct collector) was attached close to the sensing patch and replaced by a new collector during every rest period. The readout of the biosensor was obtained through a commercial potentiostat (Autolab, Metrohm Nordics AB, Sweden).

Analysis of lactate in the collected sweat samples

5-mL syringes (Henke Sass Wolf, Germany) were used to squeeze the cotton pads to extract the sweat samples into an Eppendorf vial, as reported for the modified regional absorbent patch method

elsewhere.[47] When the Macroduct sweat collector was used, sweat samples were collected to an Eppendorf tube using a 1-mL syringe with the corresponding needle (Henke Sass Wolf, Germany). The samples were packaged inside an ice bag, which can maintain the temperature under 10 °C to avoid any damage of the samples during transportation from Dalarna to KTH University (5 h). The samples were then stored in a refrigerator at 4 °C until analysis. The lactate contents in the collected sweat samples were analyzed in the laboratory using the IC (850 Professional IC, Metrohom, Switzerland): MetrosepA Supp 5–150/4.0 (ref. 6.1006.520) column, 1 mM $NaHCO_3$/3.2 mM Na_2CO_3 buffer as the mobile phase, flow rate of 0.8 mL min^{-1} and 100 mM H_2SO_4 as the solution in the suppressor.

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Conflict of Interest

The authors declare no conflict of interest.

Data Availability Statement

The data that support the findings of this study are available from the corresponding author upon reasonable request.

Keywords: Blood Lactate Correlation · Exertion Correlation · Sweat Lactate Analysis · Sports Performance · Wearable Biosensor

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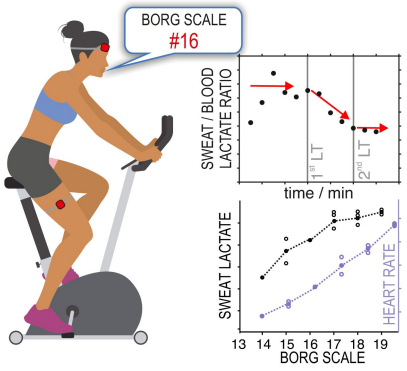
RESEARCH ARTICLE

Sweat lactate as a performance metric variable in sport: Accurate real-time sweat lactate assessment is possible by means of a wearable biosensor. Correlations with different well-established performance parameters are evaluated. The Borg scale for the rating of perceived exertion is a suitable parameter together with sweat lactate measurements to create individual training strategies.



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A Wearable Biosensor for Sweat Lactate as a Proxy for Sport Performance Monitoring