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DietSee: An on-hand, portable, strip-type biosensor for lipolysis monitoring via real-time amperometric determination of glycerol in blood

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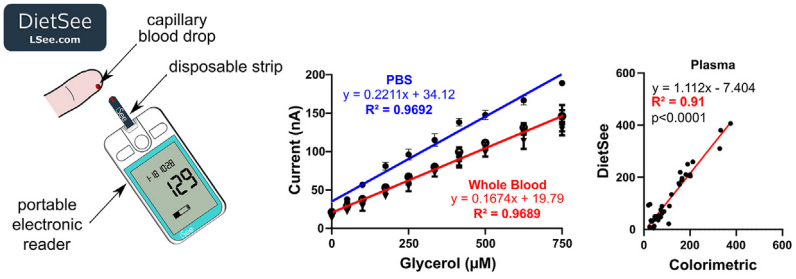
HIGHLIGHTS

- DietSee is a novel portable device for the detection of blood glycerol concentration.
- The glycerometer demonstrated very good linearity, selectivity, stability, and accuracy.
- The device showed comparable reliability in mice and humans.
- DietSee was successfully utilized for accurate lipolysis monitoring in real time.

GRAPHICAL ABSTRACT



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ABSTRACT

Glycerol is a clinical biomarker of lipolysis that is mainly produced by adipose tissues. Blood glycerol content increases in pathological conditions such as metabolic and cardiovascular diseases or cancer cachexia, but also in response to energetic stress such as physical exercise. Accurate glycerol monitoring is therefore important in a range of healthcare contexts. However, current methods available for the quantification of glycerol are expensive, time-consuming, and require the extraction of plasma from blood, from which blood glycerol content is then extrapolated. Here, we report the development of a new point-of-care glycerometer device, DietSee, based on a strip-type biosensor that enables the quantification of glycerol directly from whole blood in 6 s. The performance of the biosensor was first evaluated using buffer solutions and spiked human and mouse plasma samples, and its response was compared with that of the gold-standard colorimetric method. The results obtained using DietSee correlated strongly with those from the reference method and demonstrated a linear response to glycerol levels across a wide range of concentrations (40–750 µM) that were representative of those in the human body. Next, the biosensor was validated using spiked human blood samples over a range of 30–55% hematocrit; it also demonstrated a strong correlation with reference measurements under these conditions ($R^2 = 0.97$). In addition, the biosensor was only minimally affected by a variety of potential interferents (endogenous and exogenous) and was highly stable in storage (more than 2 years when strips were

Abbreviations: ATGL, Adipose triglyceride lipase; Hct, Hematocrit; HSL, hormone sensitive lipase; POCT, point-of-care testing.

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stored dry at 4 °C). Finally, we investigated the application of the biosensor to real-time monitoring of lipolysis and found that the DietSee is well adapted for this purpose in both human and mouse samples. To conclude, the novel DietSee glycerometer is a sensitive, selective, and rapid tool that enables characterization of the metabolic status of an individual by measuring the glycerol concentration from a single fingertip blood drop.

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1. Introduction

Glycerol is an abundant compound found in a variety of living tissues. It plays a key role in lipid metabolism through its involvement in the formation of neutral fats, fatty acid esters, and phosphoglycerides. In mammalian species, glycerol is obtained from dietary fat and by lipolysis of adipose tissues [1], a physiological process through which triglycerides stored in adipose tissue are mobilized to release fatty acids and glycerol, and thus supply energy to the whole body. The main enzymes responsible for this process are adipose triglyceride lipase (ATGL) and hormone sensitive lipase (HSL). Studies have suggested that dysfunction in lipolysis in adipose tissue could contribute to metabolic disorders through the accumulation of excess body fat [2,3].

In adult human plasma, the basal concentration of glycerol is in the range of 50–200 μM, but can vary depending on health status or lifestyle. Recently, glycerol has been reported to play a central role in the genesis of and/or in complications associated with metabolic syndrome anomalies such as obesity, adiposity, dyslipidemia, insulin resistance, and elevated plasma triacylglycerols [4,5]. Indeed, high levels of circulating glycerol have been identified in two distinct types of situations: (i) in cases of hyperglycemia, type 2 diabetes [6], and cardiovascular diseases, as well as in cases of cancer cachexia [7], and (ii) after physical exertion and/or dieting, when glycerol levels can increase significantly, potentially reaching more than 500 μM under certain conditions [8]. Thus, accurate monitoring of circulating glycerol concentration is crucial in a variety of therapeutic contexts as well as in the management of fat loss.

Glycerol is widely and routinely measured by colorimetric methods. Because of colorimetric interference in whole blood, glycerol quantification is performed in plasma. This process requires specialized laboratory equipment for blood collection and sample preparation, as well as skilled personnel; these constraints can hinder the widespread and rapid quantification of glycerol. There is a clear need for an easy-to-use, cost-effective technology for the determination of glycerol concentration in blood. Recent efforts to address this challenge have resulted in the development of electrochemical sensors for this purpose; however, this approach has thus far been confined to the detection of glycerol in food products [9] and biodiesel quality control [10], with medical applications limited to clinical laboratories using human plasma [11] or urine [12]. To date, no electrochemical sensor has been described in the literature for the detection of glycerol in blood without sample pretreatment. Thus, the challenge of developing a biosensor for real-time monitoring of glycerol (glycerometer) remains.

Here, we report the development of a novel, disposable, strip-type biosensor—DietSee—for the amperometric determination of glycerol. This device is able to determine blood glycerol concentrations using only a single fingertip blood drop. We first evaluated its sensitivity and selectivity compared to standard reference methods. We also assessed its suitability for applications in mice or humans in which real-time monitoring of lipolysis is necessary; here, the scenarios investigated were validation of a lipase-deficient mouse model and evaluation of fat-burning level in humans after physical exercise or a diet program.

2. Materials and methods

2.1. Materials

Acetaminophen (#A3035), ascorbic acid (#A92902), aspirin (#A2093), bilirubin (#B4126), dopamine (#H8502), cholesterol (#C8667), creatinine (#C4255), EDTA (#03620), galactose (#15522), gentisic acid (#G5129), glutathione (#PHR1359), hemoglobin (#H7379), heparin (#H0200000), ibuprofen (#I4883), L-dopa (#D9628), maltose (#M9171), salicylate (#S3007), tolbutamide (#T0891), triglyceride (#17810), uric acid (#U2625), and xylose (#X1500) were purchased from Sigma-Aldrich. All chemicals were analytical grade and were used in this study without further purification. The most frequently used buffer in these experiments was phosphate buffer solution (1X PBS, #P4417 Sigma-Aldrich). Buffers and other solutions were prepared with deionized water. For glycerol measurements, the DietSee glycerometer (#LS01LE01) and test strips (#LS01BA01) were supplied by LSee SAS (LSee.com). Free glycerol reagent (#F6428, Sigma-Aldrich) was used for comparison.

2.2. DietSee test strip design

The strip-style sensor is described in detail in French patent FR3069323A1, with update WO2019016451 [13]. Briefly, a three-electrode system was fabricated by screen-printing three layers—carbon ink, silver chloride ink, and an insulating layer—on an insulating substrate. On the exposed part of the working electrode, a mixture composed of 1.5 mM ferrocene-methanol, 1 U μL^{-1} glycerol kinase (GK), 0.33 U μL^{-1} glycerol phosphate oxidase (GPO), 1 U μL^{-1} peroxidase, 27 mM ATP, 27 mM $MgCl_2$, 0.25% hydroxypropyl methylcellulose, and 0.1% Triton X-100 in 1X PBS was dispensed and dried for 5 min at 40 °C. Then, the prepared electrode was covered with a protective layer of polyethylene terephthalate and affixed with a double-sided adhesive. Prepared strips were then stored in the dark at 4 °C.

2.3. Comparative accuracy of DietSee vs. gold-standard colorimetric assay

As a first step in evaluating the accuracy of the DietSee biosensor, we compared measurements generated by this novel device with those obtained from a commercially available colorimetric assay (*Free Glycerol Reagent,* Sigma-Aldrich). First, a standard curve was created using serial dilutions of glycerol in PBS buffer, from 0 to 1000 μM; this was conducted in triplicate for both methods. Using these data, we plotted a calibration curve for DietSee and determined the correlation between the two methods using linear regression and a correlation test. Next, both methods were used to quantify glycerol levels in samples of human and mouse plasma ($n = 36$), and the percentage of difference (bias) was then plotted using the Bland-Altman method.

2.4. Blood and hematocrit

Next, we evaluated the performance of the DietSee biosensor using samples of whole blood. A standard curve was obtained using serial dilutions of glycerol solution in PBS buffer and in human blood (n = 4), with concentrations from 0 to 750 μM. Data were collected in triplicate with the DietSee biosensor, and calibration curves were plotted using linear regression and a correlation test. To investigate the effect of Hct variability, a subset of blood samples was spiked with concentrated glycerol solution to achieve a concentration of 500 μM. By removing or adding plasma following centrifugation, hematocrit levels in samples were adjusted to be between 20% and 60%. Samples were tested using DietSee strips at room temperature (21–25 °C). The mean glycerol value determined at a hematocrit level of 40% (female blood) or 45% (male blood) was normalized to 100% in order to determine the potential bias (percentage deviation) occurring at other hematocrit levels.

2.5. Interference with DietSee test strips

The effect of potential interferents on the response of the biosensor was evaluated in whole blood with or without the addition of 500 μM glycerol. To these samples, we added different concentrations (up to 10 times the respective therapeutic or physiological concentration, Table 1) of 14 exogenous substances such as therapeutic agents or vitamins (acetaminophen, ascorbic acid, aspirin, dopamine, EDTA, gentisic acid, glutathione, heparin, ibuprofen, L-dopa, maltose, salicylate, tolbutamide, xylose) and 7 endogenous substances (bilirubin, cholesterol, creatinine, galactose, hemoglobin, triglyceride, uric acid). We then tested each sample with DietSee strips at room temperature (21–25 °C) to characterize the potential interfering effect of these substances.

2.6. Storage stability of DietSee test strips

The long-term stability of the biosensor in storage was analyzed through the course of regular use over a period of 600+ days at four storage temperatures (4 °C, 25 °C, 40 °C, and 45 °C). The loss in sensitivity in storage was calculated as the difference between values obtained at the starting point and at the end of storage, i.e. remaining sensitivity (%) = $\text{Slope}_{\text{storage time point}}/\text{Slope}_{\text{starting point}} \times 100\%$. The criterion for acceptance was a remaining sensitivity that was not less than 80% for two consecutive storage time points.

2.7. Biological applications of DietSee

The mice used in this study are described in Ref. [14]. All experimental procedures were approved by our institutional animal care and use committee, CEEA122 (#7796–2016112509262218) and conducted according to Inserm guidelines and European Directive 2010/63/UE for the care and use of laboratory animals. For the lipolysis test, mice were injected intraperitoneally with CL-316, 243 (1 mg/kg, Sigma #C5976). A drop of tail-vein blood was directly applied to the strip of the glycerometer device to quantify the glycerol in the sample.

Human fingertip blood samples were obtained from healthy adult volunteers after physical training and/or dieting. To determine circulating glycerol levels, a drop of blood was directly applied to the glycerometer strip. To investigate the effect of physical training, human blood was obtained from the CAMEX study. Blood from nine young, lean, healthy, and recreationally active male volunteers was sampled in a randomized fashion on three occasions: 1) at rest, 2) during endurance-type exercise of moderate intensity (1 h at 60% of maximal oxygen uptake), and 3) during sprint-interval-training (SIT)-type exercise (seven high-intensity 30-s intervals of cycling at 130% peak power output with 4.5-min recovery between intervals) on a bicycle ergometer. A blood sample was taken before and at the end of each exercise trial. The intensity of both trials was determined using the results of an incremental exercise test to exhaustion. The study was approved by the Ethics Committee of University College Dublin after written informed consent was obtained from all participants. To assess the effect of a diet program, human blood was obtained from two healthy adult volunteers who were following either a standard (20% protein, 30% fat, 50% carbohydrate) or a low-carb (30% protein, 55% fat, 15% carbohydrate) diet. A blood sample was taken every 5 min during a 45-min run.

Table 1
Effect of possible interfering substances on DietSee response.

Exogenous Interfering substance	Maximum tolerance	Therapeutic level (mg/dL)
Acetaminophen[a]	≦ 26.5 mM (400 mg/dL)	1.0–3.0
Ascorbic acid	≦ 42.6 μM (0.75 mg/dL)	0.4–1.5
Aspirin[a]	≦ 5.55 mM (100 mg/dL)	3–30
Dopamine	≦ 26.4 μM (0.5 mg/dL)	0,03
EDTA	≦ 5.37 mM (200 mg/dL)	
Gentisic acid	≦260 μM (4.0 mg/dL)	0.20–0.60
Glutathione[a]	≦ 32.5 μM (100 mg/dL)	
Heparin[a]	≦ 10.0 KU/dL	350–1000 U/L
Ibuprofen[a]	≦ 4.85 mM (100 mg/dL)	0.5–0.7
L-dopa	≦ 50.7 μM (1.0 mg/dL)	0.02–0.3
Maltose[a]	≦ 29.2 mM (1000 mg/dL)	
Salicylate	≦ 29.0 μM (400 mg/dL)	15–30
Tolbutamide	≦ 1.85 mM (50 mg/dL)	5.4–10.8
Xylose[a]	≦ 66.6 mM (1000 mg/dL)	
Endogenous Interfering substance	**Maximum tolerance**	**Physiological level (mg/dL)**
Bilirubin[a]	≦ 855 μM (50 mg/dL)	0–2.0
Cholesterol[a]	≦ 12.9 mM (500 mg/dL)	160–240
Creatinine[a]	≦ 4.42 mM (50 mg/dL)	0.2–1.5
Galactose[a]	≦ 55.5 mM (1000 mg/dL)	<5.0
Hemoglobin[a]	≦ 78.1 μM (500 mg/dL)	10–20
Triglyceride	≦ 2.26 mM (200 mg/dL)	<100
Uric acid[a]	≦ 2.97 mM (50 mg/dL)	2.5–8.0

[a] The tolerance of the maximum concentration might be higher.

2.8. Calculation of glycerol concentration from DietSee reading

The electronic reader gives a measurement result that corresponds to the intensity of the electrical current (in 10^{-8} A) generated by the biochemical reaction between glycerol and the strip reagents. This measurement can be correlated to blood glycerol level using the following formula: blood glycerol concentration (μmol/L) = A × Measurement-B, where A and B are two calibration parameters printed on the label of the strip vial (their values depend on the test strip batch).

3. Results and discussion

3.1. DietSee device

The purpose of the DietSee glycerometer is to measure, in real-time, the glycerol concentration in fingertip blood from an individual (Fig. 1). The biochemical reagents included in the reactive part of the strip react specifically with the glycerol in the blood drop to generate a small electrical current (Fig. 2). Briefly, glycerol is first phosphorylated by ATP to glycerol-3-phosphate (G3P) and ADP in a reaction that is catalyzed by glycerol kinase (GK). Then, G3P is converted to dihydroxyacetone phosphate (DAP) and hydrogen peroxide (H_2O_2) by glycerophosphate oxidase (GPO). Finally, in the presence of a peroxidase, H_2O_2 is reduced to water and, simultaneously, a redox mediator (M) is oxidized (M(red) → M(ox)). The resulting electrical current, which is directly proportional to the glycerol concentration, is measured in 6 s by the electronic reader and is displayed to the user.

3.2. Validation of DietSee biosensor as a quantitative method

To assess the reliability of the novel biosensor compared to the standard colorimetric approach, we used both methods to quantify glycerol levels in PBS buffer and in plasma (the colorimetric method cannot be applied to whole blood) (Fig. 3). We first created a standard curve by using the DietSee device to determine the concentration of glycerol in serially diluted solutions of PBS buffer, from 0 to 1000 μM (Fig. 3A). Maximum linearity ($R^2 = 0.99$) of the response was obtained between glycerol concentrations of 0 and 500 μM. For comparison, we also measured the concentration of these glycerol solutions using commercially available *Free Glycerol Reagent* (Fig. 3B, Fig. S1). An excellent correlation ($R^2 = 0.99$) was observed between the novel biosensor and the colorimetric method. Based on these results, we determined that the maximum accuracy of the biosensor was obtained between glycerol concentrations of 40 and 750 μM. For concentrations outside this range, the device cannot be considered reliable.

We next compared glycerol measurements in 36 human plasma samples using the DietSee biosensor and colorimetric method (Fig. 3C). A strong correlation ($R^2 = 0.91$) was obtained between the two methods. To investigate differences between the two sets of results, we constructed a Bland-Altman plot based on the paired datapoints from each sample (n = 36; Fig. 3D). For 32 of the 36 samples (89%), measurements from the two methods were within the 95% limit of agreement. Furthermore, similar results were obtained from mouse plasma (Fig. S2). The high degree of concordance between the results of the two methods serves as strong evidence for the suitability of the DietSee biosensor for glycerol determination in human and mouse plasma.

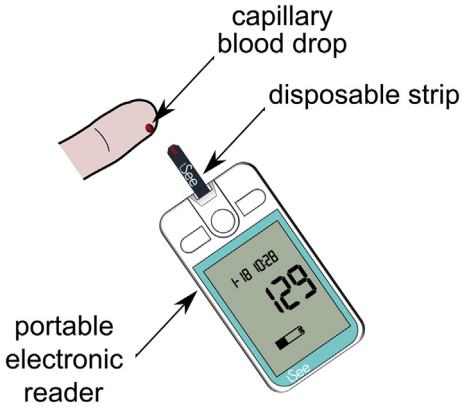

Fig. 1. Schematic illustration of the DietSee biosensor device.

3.3. Complex matrices and hematocrit effects

We further evaluated the suitability, accuracy, and precision of the novel biosensor by analyzing spiked samples of human and mouse whole blood and comparing the results to those obtained from standards in PBS (Fig. 4A, Fig. S3). First, we created a calibration curve for glycerol (from 0 to 750 μM) in human blood (n = 4). The equation for the standard curve in PBS was Current (nA) = 0.2211×glycerol concentration (μM) + 34.12, whereas the equation for the spiked blood calibration curve was Current (nA) = 0.1775 × Glycerol concentration (μM) + 17.97. Although a slight difference in slope was observed between the standard (0.2211) and calibration (0.1775) curves, the fact that both presented the same degree of linearity ($R^2 = 0.97$) indicated a high level of agreement.

We next assessed the impact of different hematocrit levels on the performance of the biosensor. Venous blood samples were manipulated to have hematocrit levels between 20% and 60% and either low or high glycerol concentrations (0 μM and 500 μM, respectively). The results from the biosensor for these samples were then plotted as bias with respect to hematocrit level (Fig. 4B). The values generated by the biosensor were within acceptable limits (i.e. bias less than 10%) when hematocrit levels were between 30 and 55%.

Based on these operating ranges (40–750 μM for glycerol detection, 30–55% for hematocrit level), the DietSee biosensor appears to be highly suitable for the detection of glycerol concentration in whole blood under most circumstances. Depending on the physiological or health status of an individual, circulating glycerol levels in humans are generally in the range of 50–750 μM [6–8], while hematocrit levels in typical patients tend to be more constant, around a value of 40–45% (41–50% for men, 36–44% for women). In the case of hematocrit, deviation from these reference values may occur in individuals with severe diseases or under certain conditions, such as in pregnancy, during strenuous exercise, or under other specific lifestyle or environmental conditions [15–17].

3.4. Interference of endogenous and exogenous substances with DietSee performance

To investigate the possibility that certain compounds present in blood samples might interfere with the response of the biosensor, we tested the potential for interference from both exogenous (originating outside the body, such as therapeutic agents or vitamins) and endogenous (produced or originating inside the body) substances. The concentration at which each substance was tested was based on the guidelines provided in the Clinical and Laboratory Standards Institute's (CLSI) document EP7-A2, section 5.5: "Interferent Test Concentrations". These recommended concentrations vary among substances, but all substances were tested at a

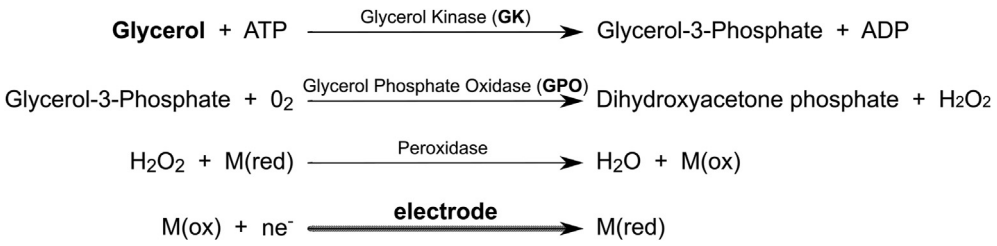

Fig. 2. Glycerol response mechanism on the DietSee test strip.

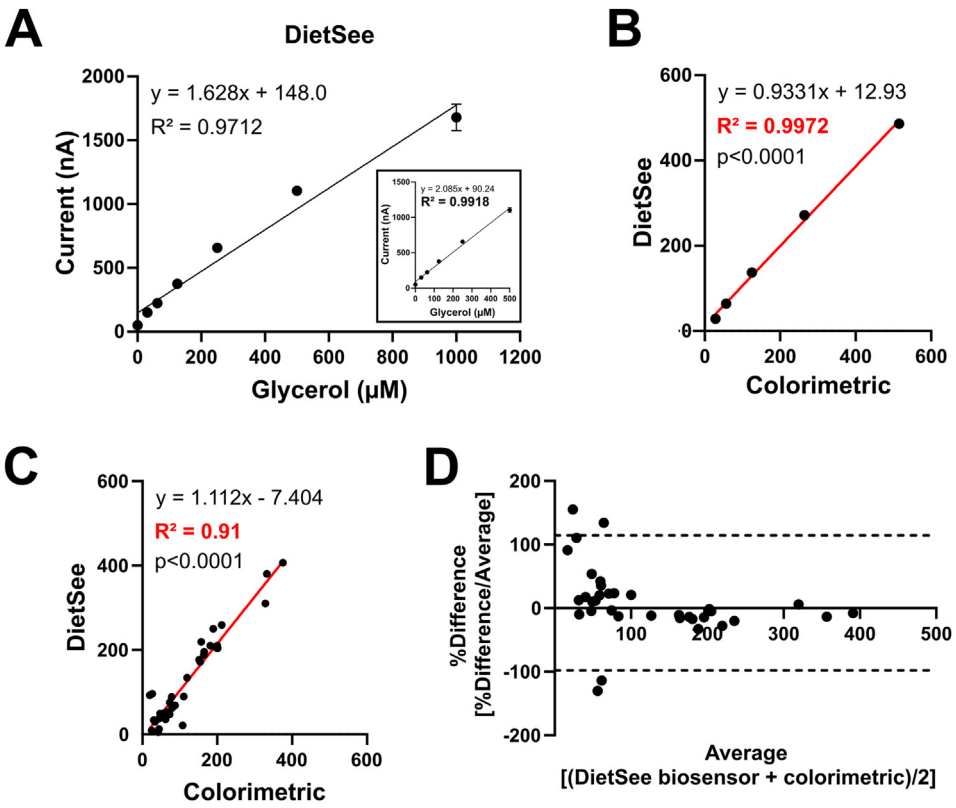

Fig. 3. Comparison of measurements from DietSee and colorimetric method. (A) Calibration curve for glycerol in PBS as determined by the DietSee device. The inset shows the best fit of the linear range of the calibration curve. Each measurement was performed in triplicate. Values are represented as mean ± SD. (B) Correlation between measurements of glycerol concentrations in PBS obtained using DietSee and standard colorimetry. Each measurement was performed in triplicate. Mean values are represented. (C) Correlation between measurements of glycerol concentrations in human plasma obtained using DietSee and colorimetry (n = 36). (D) Bland-Altman difference plot (n = 36) showing the correlation between results obtained using the biosensor and a commercial colorimetric assay. The difference is plotted against mean values, and the 95% limits of agreement (dashed lines) of the difference between the two methods of measurement are shown.

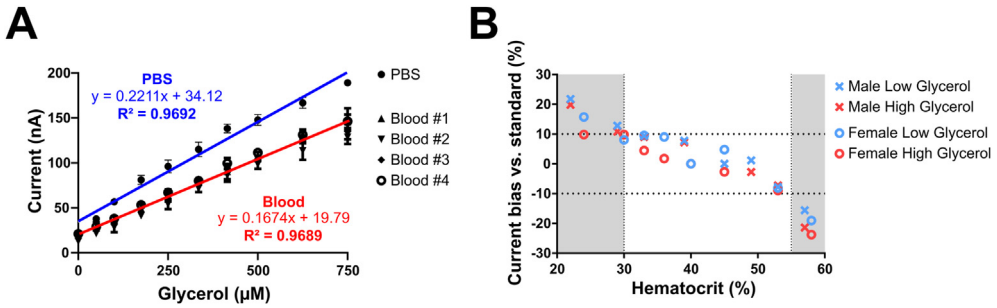

Fig. 4. Effect of complex matrices on glycerol determination using the DietSee device. (A) Calibration curve of glycerol in PBS or in blood (n = 4 individuals). Each measurement was performed in triplicate. Values are represented as mean ± SD. (B) Bias of blood glycerol measurement over a hematocrit range of 20–60% for DietSee strips. (n = 4 individuals, 2 males and 2 females). Each measurement was performed in triplicate. Values are represented as mean of bias vs. standard (40% for female and 45% for male). Hct limits of DietSee biosensor (<30% and >55%) are highlighted in gray and ±10% bias limits are shown (dashed lines).

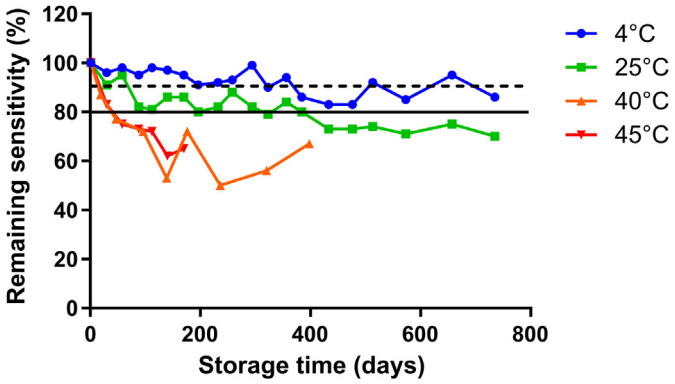

Fig. 5. Stability of sealed DietSee test strips stored at different temperatures. Each measurement was performed in triplicate. Mean values are represented. Remaining sensitivity of 90% (dashed line) and 80% (solid line) are shown.

concentration that was greater than the highest plasma concentration expected in the intended patient population [18]. In total, we tested a total of 14 exogenous substances (acetaminophen, ascorbic acid, aspirin, dopamine, EDTA, gentisic acid, glutathione, heparin, ibuprofen, L-dopa, maltose, salicylate, tolbutamide, xylose) and 7 endogenous substances (bilirubin, cholesterol, creatinine, galactose, hemoglobin, triglyceride, uric acid). To explore the effect of these interferents, we recorded the response current of the biosensor to low and high levels of glycerol (0 μM and 500 μM, respectively) over a range of concentrations for each interferent (up to 10 times the therapeutic or physiological concentration) in human blood. For each substance, the concentration that was determined to represent the maximum tolerance for the system is shown in Table 1. At the specified test concentrations, none of these substances resulted in noticeable interference, with the sole exception of ascorbic acid. Thus, if an ascorbic acid concentration greater than 42.6 μM is suspected, blood samples should be analyzed with care.

3.5. DietSee strip stability assessment

We evaluated the stability of DietSee strips in storage by measuring the response to a range of glycerol concentrations (0–750 μM) in PBS over the course of 735 days (4 °C and 25 °C), 545 days (40 °C), and 170 days (45 °C) (Fig. 5). The study was performed in accordance with the guidelines of ISO 23640:2011. The criterion for acceptance was a remaining sensitivity that was not lower than 80% for two consecutive time points. The biosensor presented a stability of one month at 40 °C and 45 °C, one year at 25 °C, and over two years at 4 °C; these values were much higher than those reported for another recently developed biosensor [11].

3.6. Biological applications of DietSee

To explore the potential applications of the DietSee device in practical analysis, we evaluated its performance in measuring the glycerol content in blood samples from three different biological scenarios which require real-time monitoring of lipolysis: validation of a lipase-deficient mouse model (Fig. 6A–B) and assessment of fat-burning level after physical exercise (Fig. 6C) or dieting (Fig. 6D).

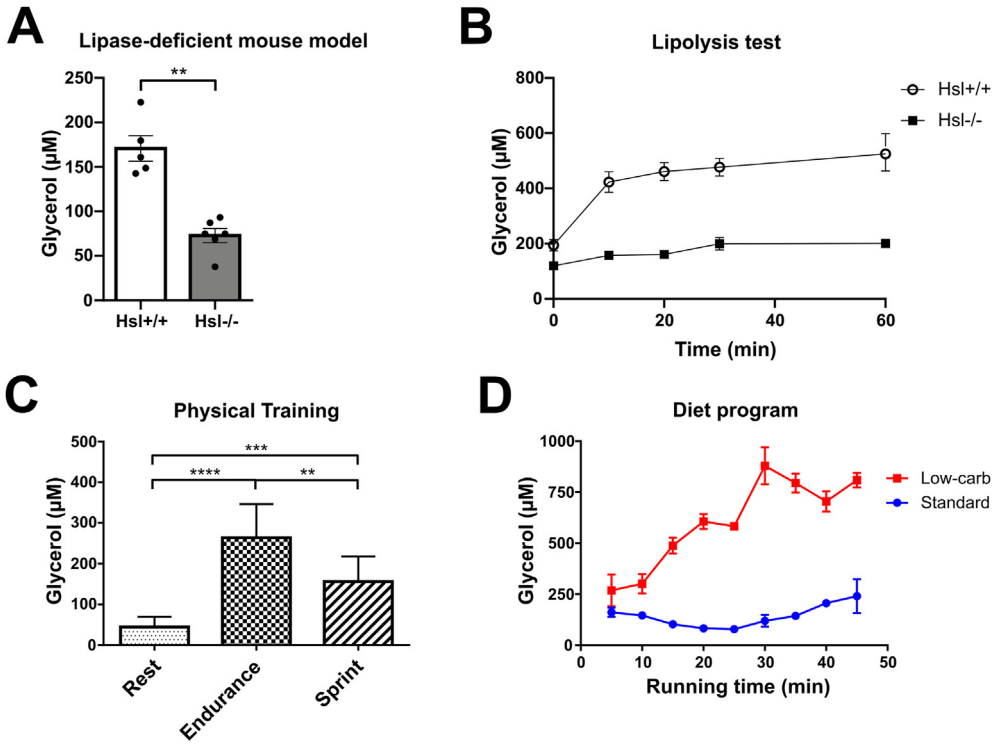

Fig. 6. Application of DietSee to real samples. (A) Measurements of blood glycerol of male $Hsl^{+/+}$ and $Hsl^{-/-}$ mice taken with the glycerometer device (n = 5–6). (B) Lipolysis test on $Hsl^{+/+}$ and $Hsl^{-/-}$ mice (n = 5–6). Blood glycerol was determined at four time points (10, 20, 30 and 60 min) after CL-316,243 injection (1 mg/kg). Values are represented as mean ± SEM. (C) Effect of physical training on lipolysis/glycerol concentration. Lean healthy human males (n = 18) were investigated at rest and after 1 h of exercise at 60% VO_2max, either endurance training (n = 9) or sprint interval training (SIT) (n = 9). Values are represented as mean ± SD. (D) Effect of diet on lipolysis during running. Blood glycerol concentrations were investigated during exercise in two healthy adult volunteers, one following a standard diet and the other following a low-carb diet program. Samples were taken every 5 min during a 45-min run. Statistical analysis was performed using paired *t*-tests to compare both types of training to resting levels and to each other, and an unpaired *t*-test to compare $Hsl^{-/-}$ to $Hsl^{+/+}$ mice (control). **P < 0.01, ***P < 0.001, ****P < 0.0001.

First, we tested the device on mice in which the hormone-sensitive lipase enzyme has been knocked out (Hsl $^{-/-}$ mice). HSL is one of the major players in triglyceride hydrolysis, which contributes to the release of fatty acids and glycerol in blood. As expected, the glycerometer revealed that Hsl$^{-/-}$ mice had blood glycerol levels that were 50% lower than those of their Hsl$^{+/+}$ counterparts (Fig. 6A). Approximately the same results were obtained using a mouse model in which Hsl was knocked out specifically in adipose tissue (Hsl^{adipoKO} mice, Fig. S4A). These results were consistent with previous results obtained in plasma from HSL-deficient mice [19,20]. To determine the efficacy of the glycerometer in a context that requires rapid blood glycerol measurements, we tested the device during an *in vivo* lipolysis test, in which blood glycerol was measured after an intraperitoneal injection of a beta 3−agonist (CL-316,243) that rapidly stimulates lipolysis. In the control mice, we observed that blood glycerol doubled within 10 min of injection, with a maximum at 20 min, while glycerol levels in HSL-deficient mice increased only marginally (Fig. 6B). The results obtained from adipose-knockout mice (Hsl^{adipoKO} mice) were similar to HSL-deficient mice (Fig. S4B). It is important to note that, in contrast to the standard method which requires the extraction of plasma from a much larger sample of blood, the DietSee glycerometer requires only a drop of blood. Moreover, it avoids the necessity of performing various treatment steps that can induce bias (such as hemolysis, time, thawing).

Next, we compared the level of fat burning reached using two different types of physical exercise, endurance and sprint interval training (SIT). Using the DietSee device, blood glycerol concentrations were measured in 18 lean healthy males (endurance, $n = 9$ and SIT, $n = 9$) (Fig. 6C). As expected, a significantly higher concentration of glycerol was detected after both types of physical exercise (endurance and SIT) compared to base levels before training (264 ± 83 μM and 156 ± 62 μM, respectively, vs. 44 ± 25 μM, $P < 0.0001$, $P < 0.001$, respectively) and a significantly higher concentration of glycerol was detected after endurance training compared to SIT (264 ± 83 μM vs. 156 ± 62 μM, $P < 0.0001$).

Finally, we used the glycerometer to determine the effect of a diet program on fat burning during a run (Fig. 6D). After a 45-min run, the subject on a standard diet presented lower and more-stable blood glycerol levels than the subject on a low-carb diet, whose blood glycerol level was high and increased during the course of the run. These results highlighted a metabolic difference between two subjects on different diets, with one person clearly using more lipid resources than the other.

4. Conclusions

In the present work, we evaluated the performance of the DietSee glycerometer, a new point-of-care amperometric device we developed to enhance the accuracy of blood glycerol monitoring. The DietSee device demonstrates a detection range that is suitable for both humans and mice, high substrate selectivity, and a rapid response time. It is easily operated, inexpensive, and highly stable in storage. Furthermore, its portability facilitates the real-time analysis of samples with a minimized risk of contamination. The versatility of this glycerometer lends it to a variety of uses, including applications in healthcare as well as in research focused on lipolysis and metabolic disorders.

Author contributions

The manuscript was written with contributions from all authors. All authors have given their approval to the final version of the manuscript.

CRediT authorship contribution statement

Séverine A. Degrelle: Visualization, Writing − review & editing. **Sébastien Delile:** Investigation, Methodology, Project administration. **Sophie Moog:** Investigation. **Etienne Mouisel:** Investigation. **Donal O'Gorman:** Resources. **Cédric Moro:** Investigation, Formal analysis, Writing − review & editing. **Pierre-Damien Denechaud:** Investigation, Formal analysis, Writing − review & editing. **Cyril Torre:** Conceptualization, Resources, Supervision, Project administration, Funding acquisition.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

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