Label-free study of intracellular glycogen level in metformin and resveratrol-treated insulin-resistant HepG2 by live-cell FTIR spectroscopy

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ABSTRACT

Conventional in vitro study often involves the destruction of the cells followed by purification and dilution steps before applying enzymatic assay or metabolomic analysis. It is a costly and laborious process, and it cannot monitor changes as a function of time. Recently, we have developed a new label-free live-cell FTIR approach that can directly measure biochemical compositional changes within living cells in situ and the spectral changes are shown to be highly specific to the drug applied. In this work, we have demonstrated for the first time the effect of two anti-diabetic drugs, metformin and Resveratrol, on insulin-resistant liver cells (HepG2). Using live-cell FTIR with principal component analysis, we have shown the differences in the biochemical profiles between normal and insulin-resistant cells (p < 0.05), the lack of response/difference from the insulin-resistant cell to insulin (p > 0.05) and the restoration of the biochemical profile and sensitivity to insulin from the insulin-resistant cells after the drug treatment (p < 0.05). Particularly, a rise in the glycogen level, marked by three distinctive peaks at 1150, 1080 and 1020 cm⁻¹, within the living cells after the anti-diabetic drug treatments is observed. The live-cell FTIR results are confirmed by a parallel gold-standard biochemical assay, demonstrating the restoration of insulin sensitivity of the insulin-resistance cells. Live-cell FTIR can be a complementary tool for drug efficacy screening, especially for insulin sensitizers.

1. Introduction

Insulin resistance is a complex pathological condition with impaired cellular response to insulin in insulin-related cells such as hepatocytes, myocytes, adipocytes, and cardiomyocytes (Samuel and Shulman, 2016). This condition is considered a significant risk factor of metabolic syndrome, one of the most dangerous factors for increasing heart attack risks (Carr et al., 2004; Hu et al., 2004). It is estimated that a quarter of the world’s adults population has metabolic syndrome, which can increase the risk of developing type 2 diabetes by 5-fold (Stern et al., 2004). This can lead to an increase of up to 460 million people with diabetes, exacerbating the most common worldwide chronic disease - one of the four leading causes of non-communicable disease mortality in 2021 (World Health, 2021). The accumulation of cardiovascular disease (CVD) risk factors that classify metabolic syndrome is now appraised to be the root of a new CVD epidemic.

Regarding metabolic syndrome contributing to the global epidemic of type 2 diabetes and CVD, current drug treatments remain sub-optimum; therefore, better targeted therapies are urgently needed. This can be achieved through innovative research approaches at both pre-clinical and clinical stages. Advances in understanding the molecular dynamic inside cell systems are essential toward novel or targeted drug development.

Current in vitro diabetes assays typically focus on using metabolic profiling technologies, e.g., metabolomics (for example, by liquid chromatography-mass spectrometry), multi-target immunoassay and/or biochemical assay to obtain information about treatment response especially during the pre-clinical stage. The drug efficacy can also be monitored by a particular biomarker produced from the drug-cell interaction. However, these techniques are costly, laborious, and the biomarker needs to be identified first, which could also involve the time-consuming task of designing of a suitable dye. Recently, non-invasive, label-free, high-throughput screening techniques that can provide molecular information and detect cell-produced metabolites in pre-clinical research have attracted much attention (O’Farrell et al., 2013).

Fourier-Transform Infrared (FTIR) spectroscopy is a non-destructive, non-invasive, label-free, high sensitivity, high accuracy, and rapid technique. Recently, FTIR has shown to be applicable to study a wide

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range of cellular events and diseases, e.g., distinguishing the different stages of the cell cycle (Holman et al., 2000), cell death (Kuimova et al., 2009), drug-cell sensitivity and resistance (Falal et al., 2015; Rutter et al., 2014), diagnosis of diseases (Roy et al., 2017) including diabetes (Eikje, 2010; Scott et al., 2010; Severcan et al., 2010; Singh et al., 2018; Yoshida et al., 2012), and biochemical responses from the numerous type of cancers (Harvey et al., 2007; Kumar et al., 2014; Liu et al., 2001; Reich, 2005). However, it has yet to be applied in the study of diabetes treatments. Our preliminary work has shown that studying living cell culture in situ using FTIR (so called “live-cell FTIR”) can detect changes in glycogen and ATP levels in a label-free manner when living HepG2 cells were exposed in medium containing high glucose concentration (Poonprasartporn and Chan, 2021). In that simple study, we have demonstrated the potential of live-cell FTIR for studying metabolic changes in cells at the molecular level. The advantages of live-cell FTIR over FTIR study of dead cells are the reduced artifacts associated with the fixative procedure and the opportunity to measure dynamic events (Phelan et al., 2020). Importantly, FTIR is a quantitative method whereby absorbance of peaks are linearly related to sample concentration following the Beer-Lambert’s law (Parker, 1971). We have recently shown that live-cell FTIR method can quantify drug within living respiratory cells at micromolar level in situ when drug solution was added to the culture medium (Terakosolphan et al., 2021). Moreover, emerging low-cost, battery-operated and miniaturised (chip-based) infrared spectrometer with demonstrated ability to provide quantitative data is now available providing opportunities to apply the developed label-free spectroscopic method in automated high-throughput settings (Ng et al., 2021).

We hypothesize that live-cell FTIR can directly monitor the changes in intracellular composition and glycogen level because of glucose uptake to reveal the effect of anti-diabetic drugs, which can reinstate the insulin sensitivity and metabolic profile of cells. We aim to illustrate that the live-cell FTIR approach can discriminate the biochemical alteration of HepG2 cells in four different culture conditions; normal glucose and high glucose conditions with/without insulin for 24 h (Adeva et al., 2016; Cho and Cho, 2019; Ding et al., 2019; Huang et al., 2015). We will be demonstrating the approach by studying the effect of metformin (a first line insulin sensitizer (American Diabetes Association Professional Practice, 2021)) and Resveratrol (a plant-derived polyphenol compound that has been reported for insulin sensitizer in diabetic HepG2 model (Teng et al., 2018b)). This is the first time that the efficacy of anti-diabetic drugs is directly studied by the label-free live-cell FTIR method.

2. Experimental section

2.1. Multi-reflection ATR FTIR

A temperature-controlled ZnS attenuated total reflectance (ATR) multi (10)-reflection accessory trough plate (HATR, Pike technologies) with a 45° ZnS ATR element (Crystar Ltd., UK) was used. The effective pathlength in the living cells produced from this accessory is ~ 20–30 μm, with a penetration depth of around 2–3 μm (Falal et al., 2015). The ATR trough plate has a measurement area of ~ 500 mm², where the live cells form a monolayer. The modest penetration depth of the measurement, which is smaller than the thickness of the living cell (~10 μm), ensures that the absorbances are produced mainly from the attached live cells rather than from the medium (Falal et al., 2015; Gaigneaux and Goormaghtigh, 2013; Kazarian and Chan, 2013; Welbe et al., 2012).

2.2. Live-cell FTIR preparation

Human hepatocyte carcinoma cell line (HepG2) was obtained from the maintained stock (passage Number 18) of cell lines from Professor Khuloud Al-Jamal at the School of Cancer and Pharmaceutical Science, King’s College London. Cells were maintained in T25 culture flasks using Dulbecco’s Modified Eagle’s high glucose Medium (4.5 g/L) (DMEM from Sigma Aldrich, UK) supplemented with 10% fetal bovine serum (FBS, from Sigma Aldrich, UK), 50 μM/L penicillin, 100 μg/mL streptomycin, 1% non-essential amino acid and 1% L-glutamine. Live-cell FTIR was prepared as described in our previous work (Poonprasartporn and Chan, 2021). In brief, cells were maintained in an incubator in a 5% CO2 humid environment at 37 °C. HepG2 cells were trypsinized and harvested when they reached ~ 90% confluence. The cells were then centrifuged into a pellet and resuspended in fresh DMEM CO2 independent medium (Thermofisher, UK) supplemented with 10% FBS, 1% non-essential amino acid, 50 μM/L penicillin, and 100 μg/mL streptomycin. DMEM CO2 independent medium was used during live-cell FTIR measurement to avoid the requirement of 5% CO2 at the spectrometer. The cell suspension was then diluted to a density of 1 × 10⁶ cell/mL when 2 mL were seeded in the muli-reflection, temperature-controlled (at 37 °C) ATR trough, where it was then sealed with a 37 °C lid to prevent evaporation (Fig. 1). After incubating in the ATR trough plate for 24 h, a 10 × reflective microscope with a digital camera (Optica, Italy) was used to confirm the cell layer has reached >80% confluence and were fully attached.

2.2.1. Live-cell FTIR measurements

For the insulin response study, the attached HepG2 cells on the ATR element were first incubated in the high glucose (25 mM, Gibco, UK) or normal glucose (4 mM) medium for 24 h, respectively, to generate the diabetic model and the normal model. To avoid the interference from cell proliferation and the risk of bacterial infection, a reduced FBS supplement (2% v/v instead of 10% v/v) and 50 U/mL of penicillin and 100 μg/mL of streptomycin in the DMEM CO2 independent medium was used in all treatments (Baker et al., 2014; Poonprasartporn and Chan, 2021). After the incubation, 100 nM of insulin (Sigma, UK) or 67 μL of PBS 1× (pH 7.4) (the control) were added in the cultured medium and the cells were measured for another 24 h. For the anti-diabetic drug study, 2 mM of metformin (Sigma, UK) or 50 μL of Resveratrol (Fluorochek, UK, 98% purity) in high glucose with 100 nM insulin (Zhu et al., 2018) were added and the cells were measured for 24 h where the results were compared to the same treatment but without drug (the control) using an FTIR spectrometer (Tensor II, Bruker Optics). In all cases, measurements were taken at every 10 min at 8 cm⁻¹ spectral resolution with 9 min scanning time (1024 scans) and a spectral range of 4000 to 900 cm⁻¹. The OPUS software (Bruker Optics, vers.7.8) was used for all data processing. All experiments were performed in triplicate based on 3 independent cultures with a passage number ranged between 33 and 43. Full spectra of the cells were acquired using the plain medium as the background. However, in the insulin response and anti-diabetic drug studies, difference spectra were obtained by using the attached cell spectrum (immediately after the treatment was applied, i.e., (time 0 h) as the background and then further subtracting the spectrum of the treated cells from the spectrum of the same cells before the treatment, using the amide II band as a reference. The same strategies were successfully used in previous live-cell FTIR studies on drug-cell interactions (Altharawi et al., 2019; Poonprasartporn and Chan, 2021).

2.3. Data processing and statistical analysis

The water vapor compensation algorithm (OPUS 7.8, Bruker Optics) was applied to all FTIR spectra followed by subtracting the cell spectrum before treatment using the amide II peak as a reference to account for the difference due to cell proliferation between experiments. The spectra were cut to the wavelength range between 1500 and 950 cm⁻¹ followed by concave rubber band baseline correction (1 iteration with 16 baseline points) (Poonprasartporn and Chan, 2021; Terakosolphan et al., 2021) and vector normalization (Baker et al., 2014; Chiriboga et al., 1998).

The spectral range between 1500 and 950 cm⁻¹ was selected to focus the analysis on the carbohydrate and phosphate absorbance bands and to
avoid noise from the residual water vapor absorbance, especially around the amide I band at 1650 cm\(^{-1}\) (Chiriboga et al., 1998). Pairwise principal component analysis (PCA) was performed using the PyChem\textsuperscript{®} software (available from http://pychem.sourceforge.net/) for correlating changes from the different treatment settings. T-test was applied to determine the statistical significance between the treatment groups and the control. The difference was considered significance for p-value <0.05, calculated using Microsoft Excel Malek et al., 2014.

2.4. Cell viability assay

To confirm that HepG2 cells remain viable in all treatments investigated during the live-cell FTIR experiment, the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay was performed. HepG2 cells were tested in the four treatment groups, with a CO\(_2\) independent medium containing 25 mM or 4 mM of glucose supplemented with 2% FBS for 24 h, then added with 100 nM of insulin or 1 \(\times\) PBS for another 24 h to mimic the live-cell FTIR experiments. Cells concentration of 2.0 \(\times\) 10\(^4\)–200 \(\mu\)L were cultured in 96-well plates with 3 replicates. Then, 100 \(\mu\)L of the MTT reagent (0.5 mg/mL) (VWR International) was added to each well. After 4 h of incubation, 100 \(\mu\)L of DMSO was added for the detection of formazan. The absorbance at 570 nm was measured using a spectrophotometer (The Spark\textsuperscript® multimode microplate reader). The absorbance values measured from each well plate were plotted, and the percentage cell viability with standard deviation was calculated over each concentration compared to the control by the GraphPad prism version 9.3.1 program.

2.5. Glycogen assay

Cellular glycogen content was determined using the glycogen assay kit (MAK-016, Sigma, UK). The experiment was repeated 3 times on 3 separate cell culture flasks. 1 million HepG2 cells were seeded in 6-well plates using 10% FBS-supplemented DMEM high glucose medium. After incubating overnight, all treatments - high glucose (25 mM) and insulin 100 nM with or without 2 mM metformin/50 \(\mu\)M Resveratrol in 2% DMEM CO\(_2\) independent medium - were introduced for 18 h. The medium was then removed followed by washing in cold 1 \(\times\) PBS, 5 min in 1 \(\times\) trypsin, counted to 1 million and then centrifuged at 1000 rpm to a cell pellet. The packed cells were added with 100 \(\mu\)L of water for homogenization, where the intracellular glycogen was extracted. A triplicate measurement was performed following the assay kit protocol, using the colorimetric method, with the absorbance value measured at 570 nm. The glycogen concentration in the treated cells was subtracted from the glycogen content of the blank to obtain the amount of glycogen production.

3. Results

3.1. A schematic of FTIR setting

MTT assay was used to verify that HepG2 cells remain viable in the insulin-resistant model in the live-cell FTIR experiment conducted in the CO\(_2\) independent medium. The results show that both high glucose (25 mM) + high insulin (100 nM) and normal glucose (4 mM) + high insulin (100 nM) conditions were well-tolerated by cells with up to 100% viability compared to the control (see supplementary data, Fig. S1), which is in good agreement with the literatures (Ding et al., 2019; Huang et al., 2015; Nagarajan et al., 2019; Sefried et al.). Although diabetic HepG2 cells containing either 2 mM metformin or 50 \(\mu\)M Resveratrol have shown lower viability to 80 and 78%, respectively, the viability is still high and cells were showing healthy morphology (supplementary data, Fig. S2–4). The results demonstrated that the conditions used (based on anti-diabetic study literatures (Li et al., 2019; Norouzzadeh et al., 2016; Teng et al., 2018b)) were suitable.

3.2. Viability assay

3.3. Live-cell ATR FTIR experiment

To perform the live-cell FTIR experiment, HepG2 cells were first established on the measuring surface of the ATR trough plate (Poonprasartporn and Chan, 2021). The reproducibility of the establishment of the living cells in the ATR trough plate was confirmed by the small standard deviation of the absorbance 24 h after seeding between 3 independent cultures (see supplementary data, Fig. S5). The HepG2 cell layer on the ATR trough plate was then pre-treated in high glucose medium (25 mM) for 24 h to develop the insulin-resistant model (Ding et al., 2019; Huang et al., 2015; Nagarajan et al., 2019; Sefried et al.). A study was carried out to demonstrate the lack of insulin response from these insulin-resistant cells. The insulin-resistant cells with and without treatment of 100 nM of insulin were compared and the results, including all three replicates at the 1\(^{st}\) and 18\(^{th}\) h after treatment, are shown in Fig. 2A and B. The analysis was focused in the spectral range of 1500–950 cm\(^{-1}\) where the influence from the background water absorbance is minimal whilst many spectral features of metabolites are found.

Fig. 1. A schematic representation of the live-cell ATR FTIR experiment setting.
plot from the pairwise PCA have shown an increase in the absorbance between 1000-1150 and 1150-1200 cm\(^{-1}\) regions for the insulin-treated group for all time points after the treatment. Interestingly, this resembles the spectral changes observed when cells were treated in high glucose (25 mM) condition. The score plot has also shown that PC1 has shown significant difference between with and without insulin (\(p < 0.001\)) for all time points (Fig. 2C and D).

Next, we explored if the live-cell FTIR approach can be used to study the effect of anti-diabetic drugs. Metformin (2 mM), an insulin sensitizer, and 100 nM insulin were added to the insulin-resistant cell. The spectral changes were compared to the same experiment but without the addition of metformin. The pairwise PCA results have shown a significant difference between with and without metformin treatments for both time points (\(p < 0.05\)) (Fig. 3A and B). Likewise, in the Resveratrol treatment study, the PCA of the FTIR spectra show significant differences at both time points (\(p < 0.05\)) (Fig. 3C and D). Notably, the PCA loadings plot for the 1st metformin treatment has shown three characteristic peaks at 1150, 1080, and 1020 cm\(^{-1}\), which closely resemble the peaks of glycogen (Chiriboga et al., 1998; Lasch et al., 2002; Wiercigroch et al., 2017). Fig. 3A3, therefore, suggests an increase in glycogen level in the cells, while no peaks associated with metformin were found. At 18th h of the treatment, the loading plots (Fig. 3B) show peaks at around 1080, 1235, 1400 and 1450 cm\(^{-1}\), resembling the peaks observed when the cells were treated in normal glucose condition (Fig. 2C and D).

The increase of intracellular glycogen is an indicator of an increased

Fig. 2. The comparison of three replicate HepG2 FTIR spectra between high glucose (blue) and high glucose + insulin (red) with PCA score at time 1 h (A) and 18 h (B) and the comparison of three replicate HepG2 FTIR spectra between normal glucose (light green) and normal glucose + insulin (magenta) with PCA score and loadings at time 1 h (C), and 18 h (D) with error bars representing the standard deviation between the three independent repeated experiments.
glucose uptake when an insulin sensitizer was added. The mean integrated absorbance of the 1020 cm\(^{-1}\) peak between 1060 and 1000 cm\(^{-1}\) of the treatment and control has been plotted to semi-quantitatively approximate and compare the change in the glycogen level in cells over 24 h of treatment. The results have clearly shown that both metformin and Resveratrol treated cells have more glycogen compared to the control as presented in Fig. 4 A. This integrated glycogen absorbance supports the PCA results that glycogen is being generated after the insulin sensitizer treatment (Fig. 3 A3).

3.4. Glycogen assay

To confirm the live-cell FTIR findings, the same experiment was repeated with the intracellular glycogen level measured using the glycogen enzymatic assay kit. The results (Fig. 4B) have shown that after the introduction of metformin and Resveratrol, cells have produced significantly higher intracellular glycogen at time 1\(^{st}\) and 18\(^{th}\) h compared to diabetic cells without treatment (the control), which is in good agreement with the FTIR results that show an accumulation of glycogen after adding insulin sensitizer overtime (Fig. 4A). It is important to highlight that live-cell FTIR measurement does not require expensive reagents and provides an opportunity to obtain time profile of glycogen level of the same batch of living cells, which is impossible with the glycogen biochemical assay.
In brief, the capital cost of a FTIR instrument is similar to a fluo-
slow-cost, highly reproducible, and non-destructive.

4. Discussion

In diabetes studies and development of a new drug for diabetic
treatments, in vitro models are often used to understand cellular meta-
bolism. Current in vitro insulin-resistance analytical methods include

glucose uptake assay, glycogen assay, western blot, and qPCR analysis
(Lo et al., 2013; Zhang et al., 2008). These conventional biochemical

assays provide precision and accuracy in both quantitative and qual-
itative studies. However, multiple biochemical assays are often required,
which is time-consuming, expensive, and laborious (Esber, 1989). The

live-cell FTIR approach combined with PCA is a promising tool for
studying cellular response to drug treatments because it is label-free,
low-cost, high throughput, highly reproducible, and non-destructive.
In brief, the capital cost of a FTIR instrument is similar to a fluo-

rescence/UV–vis plate reader, but the live-cell FTIR approach does not
require the reagents used in the glycogen assay, which is ~£500 per set
of experiment. This is the first time that live-cell FTIR is applied for

testing the effect of two anti-diabetic compounds on a diabetic HepG2
model.

The cells pre-treated with 25 mM of glucose for 24 h did not respond
to the 100 nM insulin treatment is expected because the cells were
already diabetic. Both the insulin-treated and the non-treated diabetic
cells have shown similar spectral bands at 1100 and 1250 cm$^{-1}$, which
could be used as the spectral marker for diabetic cells. When healthy
cells (cultured in normal glucose) were treated in 100 nM of insulin, a
similar spectral profile to the diabetic cells showing both the 1100 and

the 1250 cm$^{-1}$ bands, was emerged (Fig. 2 C and D). This is in contrast to

the non-treated healthy cells, which has shown a different spectral
profile with peaks at 1080, 1235, 1400 and 1450 cm$^{-1}$. The high insulin
treatment can induce diabetes in HepG2 cells (Allister et al., 2008;
Huang et al., 2015; Nagarajan et al., 2019; Sefried et al., 2018) and the
results have demonstrated that FTIR can capture this change. Moreover,
when cells were simultaneously treated in 25 mM of glucose and 100 nM
of insulin, which is another method to generate diabetic models (Bri-
cambert et al., 2010; Cho and Cho, 2019; Hao et al., 2018; Zhu et al.,
2018), the characteristic diabetic cells spectral profile was again
observed as shown in Fig. 3A–D. The results have shown that live-cell
FTIR can provide information regarding insulin sensitivity and insulin
resistance. The introduction of metformin to the diabetes HepG2 model
has produced a significant biochemical response and is clearly shown by
the spectral changes. Concomitant with an apparent increase in
glycogen peaks at ~1020, 1080, and 1150 cm$^{-1}$ 1 h after the intro-
duction of metformin (Fig. 3A3) provided a clear evidence of the
increased glycogen level in the living HepG2 cell. At the longer drug
treatment time (18 h), the loading plot, which highlights the differences
between the spectrum of cells with and without the presence of drug, has
shown a return of a spectral profile that is similar to the cells treated in
normal glucose condition. These findings suggested that metformin has
improved insulin sensitivity and restored the metabolic profile of the
cells (Huang et al., 2015; Petersen and Shulman, 2018).

Resveratrol is a plant-derived polyphenol reported for insulin resis-
tance improvement in diabetic animals and humans, and there are evi-
dences of the drug being effective in treating diabetic HepG2 cells
(Norouzzadeh et al., 2016; Teng et al., 2018b; Zhao et al., 2019). Teng
W. et al. (Teng et al., 2018a) have demonstrated the mechanism of ac-
tion of Resveratrol metabolism in insulin resistance HepG2 cells; the re-

sults have shown that Resveratrol, similar to metformin, can enhance

cellular glucose uptake, glycogen synthesis and the restoration of the
metabolic profile of cells. The live-cell FTIR study have demonstrated
that both drugs have produced similar results, which is expected as
Resveratrol also regulates insulin signalling and ameliorate insulin
resistance by modulating the IRS-1/AMPK signalling pathway, similar to
metformin (Teng et al., 2018b).

Traditional glycogen assays require extracting the intracellular
glycogen from the sample and removing the insoluble materials.
Through this process, intracellular glycogen is significantly diluted and
some glycogen could be lost even when samples are protected in cold
environment (Huijing, 1970). In contrast, the method presented mea-

sures glycogen level inside cells directly without the need of extraction.
The plot of the glycogen absorbance peak shown in Fig. 4 is indicative of
the relative increase of glycogen level in cells as a function of treatment
time and the increasing trend is in good agreement with the results
obtained from the standard glycogen bioassay kit. However, the precise
amount of glycogen produced cannot be determined using the simple
single peak integration method as shown in Fig. 4A because of the
complexity of cell spectrum with near-by overlapping bands. A multi-

variate calibration model, such as partial least square, could be devel-

oped by correlating the live cell spectra to the actual glycogen level
measured at many different conditions, so that a more accurate pre-
diction can be made should the quantity of glycogen in cells is of in-

terest. The current method is suitable for the semi-quantitatively
determination of glycogen increase (or decrease) in living cells. To
illustrate that this is possible, the spectra of 2 and 4 mg/mL glycogen
standard solution are shown in supplementary Fig. S5, highlighting the
absorbance of the 4 mg/mL glycogen solution is approximately double of
the 2 mg/mL solution. When comparing the glycogen spectra to
Resveratrol treated cell spectra at the 6th h time point, the glycogen
peaks can be clearly observed confirming that the method can detect and
semi-quantify the increase in glycogen level in cells. Further improve-
ments to the presented method include the development of multi-well
ATR plates or the use of chip-based infrared spectrometers (Ng et al.,
2021) for measuring live-cells exposed in different conditions at the
same time to increase the measurement throughput and better control of
the experiment so that a multivariate predictive model can be

developed.

Fig. 4. The comparison of the integrated absorbance of glycogen peak (1060-1000 cm$^{-1}$) by FTIR approach among metformin (purple), Resveratrol (orange), and control (green) over 24 h treatment (A) and the comparison of glycogen amount in diabetic HepG2 model by glucose assay among diabetic HepG2 (control) with metformin or resveratrol treatment at time 1, and 18 h (B) with error bars representing the standard deviation.
5. Conclusions

Live-cell FTIR spectroscopy was demonstrated to be suitable for studying the effect of drugs on insulin-resistance HepG2 diabetes model. It can be a complementary tool for drug efficacy screening, especially for insulin sensitizers. The measurement requires no extraction or dilution highlighting the advantages of the label-free method to detect the changes in intracellular metabolic profile and glycogen level. Metformin and Resveratrol were shown to produce an improvement in insulin sensitivity by the direct FTIR measurement of changes in intracellular glycogen level in a semi-quantitative manner and the restoration of the spectral profile, which represent the molecular composition of the cell. Accurate quantification of glycogen in cells will require the acquisition of a comprehensive calibration data set followed by the development of a multivariate model, which will be explored in the future.

CRediT authorship contribution statement

Anchisa Poonprasartporn: Methodology, Validation, Formal analysis, Investigation, Data curation, Writing – original draft, Visualization, Funding acquisition.
K.L. Andrew Chan: Conceptualization, Methodology, Resources, Data curation, Writing – review & editing, Supervision, Project administration.

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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