Treating metabolic syndrome's metaflammation with low level light therapy: preliminary results

Tania Mateus Yoshimura[a], Ilka Tiemy Kato[a], Alessandro Melo Deana[b], Martha Simões Ribeiro[a]
[a]Center for Laser and Applications, Nuclear and Energy Research Institute, Av. Professor Lineu Prestes, 2242, Cidade Universitária, São Paulo, SP, Brazil, 05508-000; [b]Post-graduate program in Biophotonics applied to Health Sciences, Universidade Nove de Julho, Av. Dr. Adolpho Pinto, 109, Barra Funda, São Paulo, SP, Brazil, 01156-050

ABSTRACT

Metabolic syndrome comprises a constellation of morbidities such as insulin resistance, hyperinsulinemia, atherogenic dyslipidemia, dysglycemia and obesity (especially abdominal). Metabolic alterations are observed in major insulin target organs, increasing the risk of cardiovascular diseases, type-2 diabetes and therefore mortality. Tissue alterations are characterized by immune cells infiltrates (especially activated macrophages). Released inflammatory mediators such as TNF-α induce chronic inflammation in subjects with metabolic syndrome, since inflammatory pathways are activated in the neighboring cells. The intra-abdominal adipose tissue appears to be of particular importance in the onset of the inflammatory state, and strategies contributing to modulate the inflammatory process within this adipose tissue can mitigate the metabolic syndrome consequences. Considering the low level light therapy (LLLT) recognized benefits in inflammatory conditions, we hypothesized this therapeutic approach could promote positive effects in modulating the inflammatory state of metabolic syndrome. That being the scope of this study, male C57BL/6 mice were submitted to a high-fat/high-fructose diet among 8 weeks to induce metabolic syndrome. Animals were then irradiated on the abdominal region during 21 days using an 850 nm LED (6 sessions, 300 seconds per session, 60 mW output power, ~6 J/cm² fluence, ~19 mW/cm² fluence rate). Before and during treatment, blood was sampled either from the retro-orbital plexus or from tail puncture for glucose, total cholesterol and triglycerides analysis. So far our results indicate no alterations on these metabolic parameters after LLLT. For further investigations, blood was collected for plasma inflammatory cytokine quantification and fresh *ex vivo* samples of liver and intra-abdominal adipose tissue were harvested for immunohistochemistry purposes.

Keywords: meta-inflammation, phototherapy, diabetes, cholesterol, triglycerides, obesity

INTRODUCTION

According to the World Health Organization [1], in 2008, 36 million people died due to noncommunicable diseases (NCDs). This number corresponds to 63 % of all deaths in that year. Infirmities like diabetes, cardiovascular diseases (CVD), cancers and respiratory diseases account for almost 80 % of all NCD deaths.

Responsible for nearly half of all NCDs deaths, CVDs and diabetes are preventable in 80% of the cases [2]. Their socioeconomic burden affects not only mortality and treatment costs, but also decreases quality of life and productivity. For an example, in 2005 Brazil have lost USD 2.7 billion in the national income by reason of diabetes and CVDs, a financial loss that can triplicate by 2015, as reported by WHO [3].

The Type-2 diabetes mellitus (T2DM) and CVD major risk factors are comprised in a medical condition known as metabolic syndrome (MetS). Dysglycemia, hypertension, atherogenic dyslipidemia and obesity (especially abdominal obesity) are strong predictors for the development and progression of the aforementioned diseases. It is yet to be determined which of these risk factors are MetS causes or consequences, but there is consensus throughout many experts

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societies that impaired insulin sensitivity and central adiposity are common denominators among individuals with MetS [4]. By some means, these two conditions contribute to metabolic alterations leading to the onset of T2DM and CVDs.

Metaflammation [5], the characteristic inflammation observed in obese individuals, may play an important role in MetS. Hostamisligil et al [6] were the firsts reporting an increased tumor necrosis factor alpha (TNF-α) expression in the adipose tissue of obese individuals showing that their findings in murine models of obesity [7, 8, 9] also occurred in humans. Since 1995, studies have been portraying the obese white adipose tissue as an organ richly permeated by leukocytes, having not only increased TNF-α levels, but also other cytokines and chemokines [10, 11, 12, 13, 14]. This inflammatory state is closely related to the development of insulin resistance once Toll-like receptors (TLRs) pathways activation leads to intracellular inhibition of insulin signaling [10, 11].

Several authors have highlighted the importance of inhibiting the inflammatory pathways in the MetS therapeutic management. Studies focusing on modulating the inflammation without impairing the subject's immune responses are considered of particular importance [10, 11, 15]. The World Health Organization on its latest global strategy for the prevention and control of NCDs [16] emphasizes the need to promote and support multisectoral and multidimensional investigations that generate or strengthen strategies to prevent and control these diseases. In this sense, we propose a non-invasive treatment without side effects, aiming to reduce the MetS characteristic inflammation using low level light therapy (LLLT).

According to Chung et al [17] the therapeutic use of LLLT in the red and near infrared wavelengths can be divided in three main areas: 1) reducing inflammation and edema; 2) wound healing and tissue repair and 3) pain relief and treatment from neurological disorders. Some examples from murine models include edema, inflammatory infiltrate and proinflammatory cytokines expression reduction in joint disorders [18, 19, 20, 21]; renal function improvement, decreased blood pressure and lowered expression of cytokines in renal disease [22, 23]; decreased fibrosis in diabetic rats with muscle injury [24]; and pain reduction after induced neuropathic pain [25]. *In vitro* assays have shown LLLT's immunomodulatory potential as demonstrated by lower expression and synthesis of inflammatory cytokines by murine monocytes/macrophages stimulated by proinflammatory agents like lipopolysaccharides or interferon-γ [26, 27]. Notwithstanding its recognized benefits on inflammatory conditions, in regard to the adipose tissue LLLT is more focused on aesthetic aspects [28]. Reported cases of spot fat reduction [29, 30] and "fat liquefaction" [31] are a few examples.

To our knowledge, there are no other studies using LLLT to treat MetS, a chronic, systemic and persistent inflammatory condition. Therefore, our main objective is to investigate this new application to phototherapy.

METHODOLOGY

2.1 Metabolic syndrome animal model

Male adult C57BL/6 mice were fed *ad libitum* a hypercaloric, high-fat, and fructose enriched chow during 8 weeks in order to induce MetS. The diet was modified from the standard chow to obtain at least 40 % of calories from fat, since high-fat diets are known to induce obesity and insulin resistance in rodents [32, 33, 34]. The diet's nutritional composition can be seen in

Table 1. Throughout the experiments, animals were individually housed in appropriate cages under a 12 h daylight cycle. All the procedures were in accordance with the Guide for Care and Use of Laboratory Animals [35] and were approved by the institutional animal ethics committee (number 95/11/CEUA-IPEN/SP).

Table 1: Hypercaloric, high-fat and fructose enriched diet nutritional composition.
* percent of total caloric value

NUTRITIONAL COMPOSITION		
Amount per 100 g		**%TCV***
Energy	434 kcal = 1816 kJ	100,0%
Carbohydrates	46 g	42,4%
Proteins	17 g	16,0%
Total fat	20 g	41,6%

2.2 LLLT

Following the eight weeks diet stage, animals were transcutaneously irradiated on the depilated abdominal surface with a one-spot light emitting diode (LED), under anesthesia (ketamine:xylazine: 80 mg:10 mg per kilogram of body mass). All irradiation parameters and LED specifications are shown in

Table 2: LED specifications and irradiation parameters

and Figure 1. Irradiations were performed at the days 1, 3, 7, 10, 14 and 21 after the eight weeks period. Animals from the control group (C) were handled in the same manner as the LED group, but they were placed under the powered off apparatus. Both groups continued receiving the modified diet throughout the experimental procedures.

Table 2: LED specifications and irradiation parameters

Irradiation Parameters	
Center wavelength [nm]	843.02
Spectral bandwidth (FWHM) [nm]	38.33
Operating mode	Continuous wave
Average radiant power [mW]	60
Polarization	Random
Aperture diameter [cm]	0.900
Irradiance at aperture [mW/cm^2]	94
Beam profile	Multimode
Beam spot area at target [cm^2]	3.14
Irradiance at target [mW/cm^2]	19
Exposure duration [s]	300
Radiant exposure [J/cm^2]	5.7
Radiant energy [J]	18
Number of points irradiated	1
Area irradiated [cm^2]	3.14
Application technique	90° to the abdominal surface, distanced 1.5 cm
Number of treatment sessions	6
Frequency of treatment sessions	days 1, 3, 7, 10, 14 and 21
Total radiant energy [J]	108

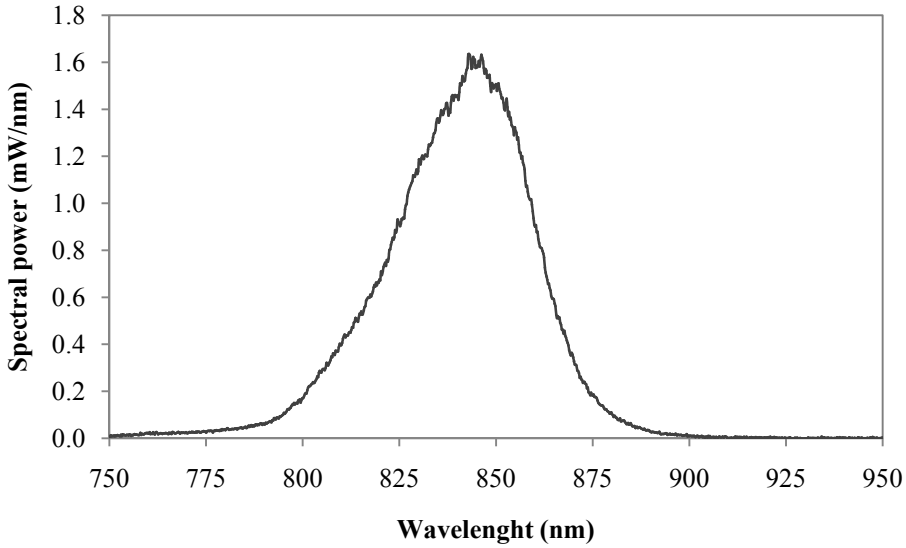

Figure 1: LED's spectral power obtained by using an OceanOptics HR2000 spectrometer

2.3 Metabolic parameters assessment

Blood glucose levels were assessed by a portable glucometer (OneTouch® Ultra®, Johnson & Johnson Medical Devices & Diagnostics) using the appropriate test strips. Blood samples were obtained via tail puncture after a 3 h daytime food restriction.

Total cholesterol (TC) and triglyceride (TG) blood levels were quantified using the Accutrend® Plus (Roche) system, after 3 h daytime food restriction. Blood samples (10 μL) from the retro-orbital plexus were collected with heparinized Pasteur pipettes.

Body mass data were collected during the experimental course.

2.4 Statistical analysis

Paired Student's *t*-test was performed to verify differences in body mass and blood glucose, TC and TG levels after the eight weeks period for MetS diet induction. In order to compare the global effects of LLLT, the areas under the curve were calculated using the variable's normalized values in relation to their initial values (at day 0, one day before the first irradiation session) plotted against time. Variance analysis (Lavene and Brown-Forsythe) were performed to attest normal distribution and means were compared using one-way ANOVA. Statistic relevance were considered for $p<0.05$. Analyses were conducted using OriginPro 8.5 (OriginLab Corporation, Northampton, MA, USA).

RESULTS AND DISCUSSION

3.1 Metabolic syndrome diet induction

After the experiment's first eight weeks, animals gained 9.48 g, which corresponds to a 32.16 % mass gain and an average final body mass of 38.78 g ± 3.91 g. This result is higher when compared to age-matched C57BL/6 fed with normal diet [36, 37], indicating significant increased mass gain.

Augmented fasting blood glucose levels were observed after the hypercaloric diet stage (Table 3). Although hyperinsulinemic-euglycemic glucose clamp is the gold-standard insulin sensitivity assessment method, it is rather complex and demands serial blood sample collection, which limits its application in small rodent models [38]. In

conjunction with the increased body mass, however, hyperglycemia can indirectly indicate a decreased responsiveness to the insulin's metabolic actions [39, 40], and we can infer an insulin resistance state settlement.

In relation to TC and TG blood levels, although a discrete but significant decrease in the first variable level, non substantial changes in magnitude were observed after the 8 weeks diet period (Table 3).

Table 3: metabolic parameters after eight weeks of hypercaloric diet.
* $p<0.05$

Variables	Before diet (week 0)	After diet (week 8)
Relative mass gain	1.0	1.3216 ± 0.0859*
Blood glucose	145.75 ± 29.01 mg/dL	165.92 ± 25.30 mg/dL*
Triglycerides	154.17 ± 22.07 mg/dL	175.92 ± 28.08 mg/dL
Total cholesterol	179.42 ± 05.76 mg/dL	172.83 ± 05.15 mg/dL*

3.2 Low level light therapy

As can be seen in Figure 2, both irradiated (LED) and non-irradiated (C) groups presented a similar behavior during the treatment period in relation to the assessed metabolic parameters, showing no differences after statistical analysis.

Approaching different LLLT targets, Aquino Junior et al [41] have found dissonant data after irradiating the paw muscles of high-fat fed rats. Sedentary rodents presented decreased TC and TG levels after 830 nm laser irradiation in a protocol that aimed to evaluate the laser's adjuvant effects on trained animals. This divergence might be due to the fact that our animals didn't show increased TG and TC levels after the eight weeks diet period, which could possibly be achieved if we had extended the period of MetS diet induction. Regarding the irradiation parameters, the mentioned authors preferred to use much higher energy density and power density, although delivering half of our total energy per session, 5 days/week, during 8 weeks. Our treatment encompassed a total of 6 sessions throughout 3 weeks, and prolonging its time course is to be considered.

As mentioned before, to our knowledge there are no studies using phototherapy to treat the inflammatory component of MetS. That being said, our irradiation parameters were based on LLLT application on chronic inflammatory conditions, which usually adopts lower energy density and power density [19, 21, 22, 24, 42]. We recognize opportunities for improvement and other irradiation protocols should be evaluated. Nevertheless it is important to highlight that after the last irradiation session, animals were euthanized and blood was collected for plasma inflammatory cytokine quantification and fresh *ex vivo* samples of liver and intra-abdominal adipose tissue were harvested for immunohistochemistry purposes. Further analyses are being conducted, and our investigations might find other LLLT effects in the context of MetS.

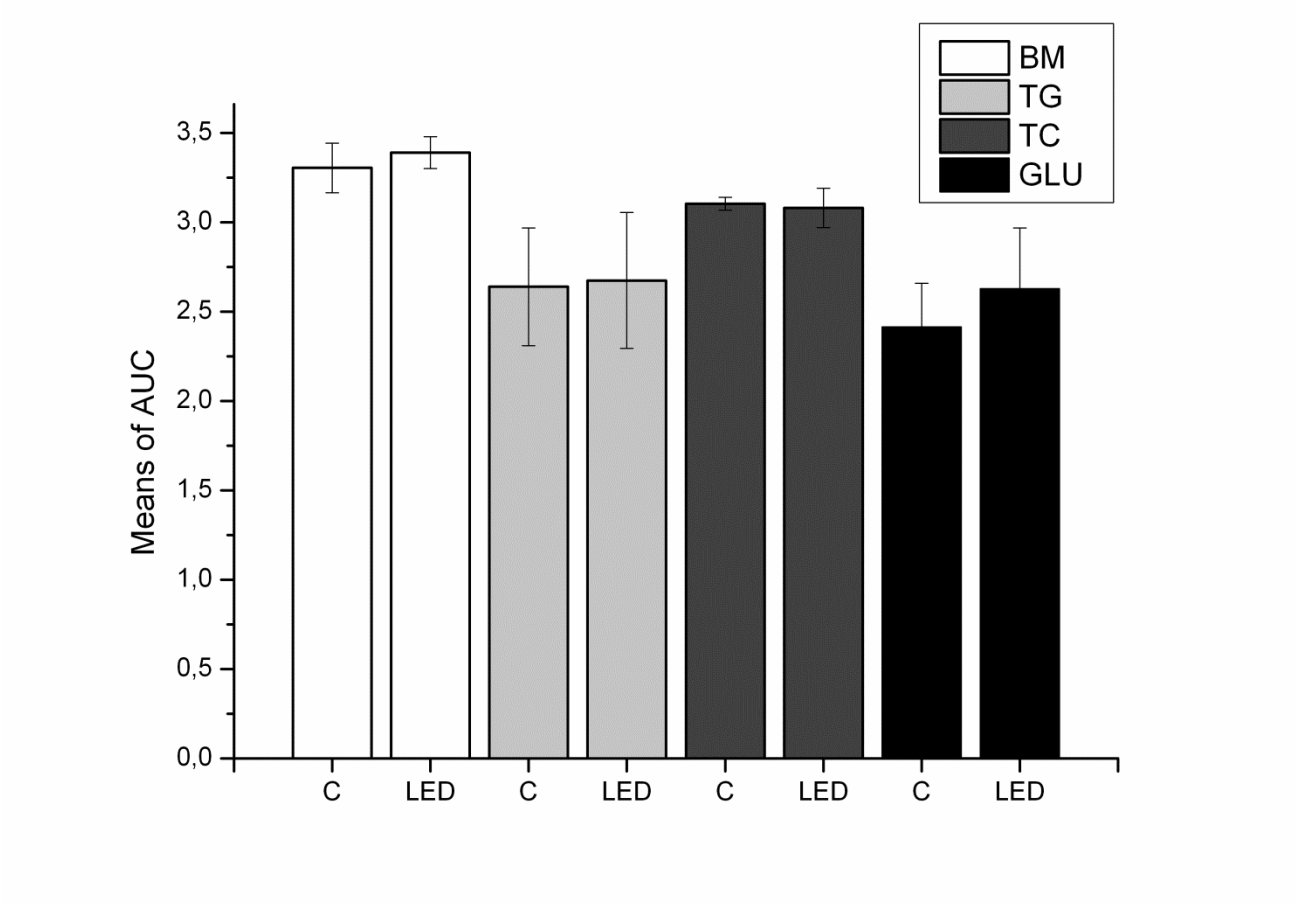

Figure 2: Comparison between irradiated (LED) and non-irradiated (C) groups in relation to the assessed metabolic parameters (BM = body mass, TG = triglycerides, TC = total cholesterol and GLU = blood glucose). Columns represent the means of area under the curve (AUC) and bars stand for Standard Deviation.

CONCLUSIONS

Our findings demonstrate that MetS was successfully induced. Considering the adopted irradiation parameters, LLLT showed no effects on body mass and blood glucose, total cholesterol and triglycerides levels of C57BL/6 mice fed with hyperchaloric, high-fat and fructose enriched diet during eight weeks for MetS induction.

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