Article

Characterising nitric oxide-mediated metabolic benefits of low-dose ultraviolet radiation in the mouse: a focus on brown adipose tissue

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Nitric oxide release sparked by #UVlight reduces signs of #T2D & liver disease & unhealthy changes to fatty tissues linked with #obesity. @telethonkids research in @DiabetologiaJnl explores mechanisms behind these benefits. @DiabetesWA @ANUPopHealth
(Research in context box)

Abstract

Aims/hypothesis Exposure to sunlight has the potential to suppress metabolic dysfunction and obesity. We previously demonstrated that regular exposure to low-doses of ultraviolet radiation (UVR) reduced weight gain and signs of diabetes in male mice fed a high-fat diet, in part via release of nitric oxide from skin. Here, we explore further mechanistic pathways through which low-dose UVR exerts these beneficial effects.

Methods We fed mice with a luciferase-tagged *Ucp1* gene, referred to here as the uncoupling protein-1 (*Ucp1*) luciferase transgenic mouse ('Thermomouse') a high-fat diet and examined the effects of repeated exposure to low-dose UVR on weight gain and development of metabolic dysfunction as well as UCP1-dependent thermogenesis in interscapular brown adipose tissue (iBAT).

Results Repeated exposure to low-dose UVR suppressed the development of glucose intolerance and hepatic lipid accumulation via dermal release of nitric oxide while also reducing circulating IL-6 (compared with mice fed a high-fat diet only). Dietary nitrate supplementation did not mimic the effects of low-dose UVR. A single low dose of UVR increased UCP-1 expression (more than twofold) in iBAT of mice fed a low-fat diet, 24 h after exposure. However, in mice fed a high-fat diet, there was no effect of UVR on UCP-1 expression in iBAT (compared with mock-treated mice) when measured at regular intervals over 12 weeks. More extensive circadian studies did not identify any substantial shifts in UCP-1 expression in mice exposed to low-dose UVR, although skin temperature at the interscapular site was reduced in UVR-exposed mice. The appearance of cells with a white adipocyte phenotype ('whitening') in iBAT induced by consuming the high-fat diet was suppressed by exposure to low-dose UVR in a nitric oxide-dependent fashion. Significant shifts in the expression of important core gene regulators of BAT function (*Dio2*, increased more than twofold), fatty acid transport (increased *Fatp2* [also known as *Slc27a2*]), lipolysis (decreased

Atgl [also known as *Pnpla2]*), lipogenesis (decreased *Fasn*) and inflammation (decreased *Tnf*), and proportions of macrophages (increased twofold) were observed in iBAT of mice exposed to low-dose UVR. These effects were independent of nitric oxide released from skin.

Conclusions/interpretation Our results suggest that non-burning (low-dose) UVR suppresses the BAT 'whitening', steatotic and pro-diabetic effects of consuming a high-fat diet through skin release of nitric oxide, with some metabolic and immune pathways in iBAT regulated by UVR independently of nitric oxide.

Keywords Brown adipose tissue; Circadian rhythm; Hepatic steatosis; High-fat diet; Metabolic dysfunction; Mice; Nitric oxide; Ultraviolet radiation; Uncoupling protein-1; Whitening

Research in context

What is already known about this subject?

- Exposure of mice fed a high-fat diet to low-dose ultraviolet radiation (UVR) reduces weight gain and associated metabolic dysfunction.
- These effects are partially dependent on nitric oxide released from irradiated skin.

What is the key question?

- Do metabolically beneficial effects of UV-induced nitric oxide occur through regulation of heat production in brown adipose tissue (BAT)?

What are the new findings?

- While regular exposure to UVR of mice fed a high-fat diet had metabolically beneficial effects (some dependent on nitric oxide), there were no substantial shifts in the daily patterns of markers for heat production (uncoupling protein-1) in BAT lying beneath the irradiated skin site.

- Skin temperature of irradiated skin, and ‘whitening’ of BAT, were reduced via UV-induced nitric oxide, while nitric oxide-independent effects on important metabolic and immune pathways in BAT were observed following exposure to UVR.
- These findings point to novel mechanisms, some involving whole body temperature regulation, through which UVR impairs the development of metabolic dysfunction.

How might this impact on clinical practice in the foreseeable future?

- These findings emphasise the importance of considering novel mediators (e.g. nitric oxide) induced by sun exposure in regulating biological function, significant in light of ongoing clinical trials assessing metabolic benefits of other molecules induced by UVR (e.g. vitamin D).

Abbreviations

BAT	Brown adipose tissue
cPTIO	2-(4-Carboxyphenyl)-4,4,5,5-tetramethylimidazoline-1-oxyl-3-oxide potassium salt
25(OH)D	25-Hydroxyvitamin D
iBAT	Interscapular BAT
NAFLD	Non-alcoholic fatty liver disease
SNAP	*S*-nitroso-*N*-acetyl-D,L-penicillamine
UVR	Ultraviolet radiation
WAT	White adipose tissue
ZT	Zeitgeber time

Introduction

Exposure to sunlight or ultraviolet radiation (UVR) has the potential to curb metabolic dysfunction [1]. Repeated exposure to low-dose (non-burning) UVR reduced weight gain and the development of glucose intolerance, insulin resistance, liver steatosis and inflammation in mice fed a high-fat diet [2-4]. Only limited benefits of dietary vitamin D supplementation were observed, with effects of UVR independent of changes to serum 25-hydroxyvitamin D (25(OH)D) [3]. Instead, nitric oxide was a mediator partially responsible for some observed metabolic benefits of UVR [2, 3]. Photolabile nitric oxide (e.g. nitrite) is mobilised from skin stores by exposure to UVR, delivered into the circulation as nitrite [3, 5, 6], and linked to hypotensive effects of UVR in humans [7].

Metabolically important deposits of brown adipose tissue (BAT, supraclavicular) lie adjacent to skin often exposed to sunlight (neck, shoulders). BAT contributes to metabolic and temperature homeostasis [8]. BAT dissipates energy as heat (thermogenesis), through uncoupling protein-1 (UCP-1), which uncouples respiration in mitochondria [9]. UCP-1 is highly expressed in BAT and may be induced by a high-fat diet [10]. *Ucp1* mRNA levels in BAT exhibit circadian (or daily) rhythms that may be entrained by external cues [11-13]. These are linked with the expression of the glucose transporter *Glut4* (also known as *Slc2a4*) and reciprocal changes in molecular clock genes [14]. NEFA [12] and glucose [15] uptake by BAT may also exhibit daily biorhythms. Genetic deletion of *Ucp1* shifts circadian rhythms and reduces energy expenditure in mice fed a high-fat diet [16]. These studies suggest that circadian rhythms of UCP-1 expression in BAT may influence thermogenesis and energy expenditure in response to dietary influences.

We hypothesised that regular skin exposure to low-dose UVR activates thermogenic processes in underlying BAT to suppress metabolic dysfunction. A large depot of BAT (interscapular BAT, iBAT) lies directly beneath skin exposed to UVR in our model [2, 3]. To test our hypothesis, we examined the effects of exposure to low-dose UVR in *Ucp1* luciferase transgenic (‘Thermomouse’) mice fed a high-fat diet, in which UCP-1 expression in iBAT was tracked, with detailed circadian analyses. We compared the effects of UVR to dietary nitrate, which may promote beiging [17], and skin application of a nitric oxide donor. We used a nitric oxide scavenger to determine whether any effects of UVR were dependent on skin release of nitric oxide.

Methods

See electronic supplementary material (ESM) Methods for more methodological details.

Mice Experiments were performed according to ethical guidelines of National Health & Medical and Research Council (Australia) with approval from the Telethon Kids Institute Animal Ethics Committee (AEC numbers 286, 315). Tg(*Ucp1*-luc2,-tdTomato)1Kajim/J transgenic mice, also known as Thermomouse, were obtained from the Jackson Laboratory (stock no. 026690; Bar Harbor, ME, USA) and were housed and bred at the Telethon Kids Institute (Subiaco, WA). They are referred to throughout this article as *Ucp-1* luciferase transgenic mice. Mice were housed under Perspex-filtered fluorescent lighting, with a normal 12 h light/dark cycle (with lights on at 06:00 h) with chipped Aspen bedding, tissues, crinkled paper and PVC piping for nesting in filter-topped cages. These lights do not emit any detectable UVR, as measured using an ultraviolet radiometer (UVX Digital Radiometer, UVP, Upland, CA, USA). Mice had free access to food and water.

Diet Mice were fed either high-fat (23% lard with canola oil) or low-fat (5% canola oil) diets (ESM Table 1). As previously [3], the lard fraction of the high-fat diet probably contained vitamin D; however, the precise amount is unknown.

UVR Mice with shaved dorsal skin were irradiated with sub-erythemal/-oedemal UVB radiation (1 kJ/m^2; 3.2 ± 0.3 min [mean ± SD]) or mock-irradiated.

Topical skin treatments Shaved dorsal skin was treated with 0.1 mmol SNAP (*S*-nitroso-*N*-acetyl-D,L-penicillamine; nitric oxide donor) alone, or 0.1 mmol cPTIO (2-(4-carboxyphenyl)-4,4,5,5-tetramethylimidazoline-1-oxyl-3-oxide potassium salt; nitric oxide scavenger) or vehicle (100 µl) immediately after UVR.

Dietary nitrate supplementation Nitrate ($NaNO_3$) was administered through drinking water (0.06 mmol kg^{-1} day^{-1} $NaNO_3$).

Experiment 1 design Four-week-old *Ucp1* luciferase transgenic male mice were fed the low-fat diet for 4 weeks (Fig. 1). From 8 weeks of age, mice in treatment 1 were fed the low-fat diet, while remaining mice were fed the high-fat diet (treatments 2-6). Mice were treated twice a week with: mock-irradiation then vehicle (treatments 1 and 2); UVR then vehicle (treatment 3); mock-irradiation then SNAP (treatment 4); or UVR then cPTIO (UVR + cPTIO, treatment 5). Mice in treatment 6 were mock-irradiated (with vehicle) and fed nitrate through drinking water. Mice were fed diets and administered treatments for 12 weeks until 20 weeks of age.

Experiment 2 design Four-week-old mice were individually housed and fed a low-fat diet for 4 weeks (Fig. 5a). From 8 weeks of age, mice in treatment 1 were fed the low-fat diet, while remaining mice were fed the high-fat diet (treatments 2-4). Mice were treated twice a week with: mock-irradiation (treatments 1 and 2) with vehicle; UVR then vehicle (treatment 3); or UVR then cPTIO (UVR + cPTIO, treatment 4). Mice were fed diets and administered treatments for 12 weeks until 20 weeks of age.

Measuring weight gain, tissue weights and food intake Weekly percentage weight gain was calculated from body weight at 8 weeks of age, with liver, gonadal WAT and iBAT weights determined at the end of experiments. Food and energy intake was determined in Experiment 1, by weighing diet present in food hoppers.

Glucose and insulin tolerance tests Fasted mice were intraperitoneally challenged with glucose or insulin for glucose or insulin tolerance tests (GTT/ITT). Glucose was measured before injection and 15, 30, 45, 60, 90 and 120 min post injection.

Serum lipids and metabolites Serum (total, HDL-, and LDL-) cholesterol, triacylglycerol, calcium, 25(OH)D, IL-6 and TNF-α, and plasma insulin and adiponectin (both from fasting mice) were quantified as detailed in ESM Methods. Serum nitrite and nitrate levels were measured as previously described [18].

Histopathological assessment of liver and iBAT Liver sections were stained with either H&E, Masson's trichrome or Oil Red O with signs of non-alcoholic fatty liver disease (NAFLD) and lipid content quantified in a blinded fashion. Sections of iBAT were stained with H&E and blindly scored for an increasing white adipocyte phenotype ('whitening').

UCP-1 expression In a preliminary experiment, real-time measurement of UCP1 expression (bioluminescence, measured post luciferin injection) was quantified in iBAT at 0, 0.25 (15 min), 3, 24, 48 and 192 h after 8-week-old mice fed a low-fat diet were exposed to 1 kJ/m^2 UVR. In Experiment 1 (Fig. 1a), UCP-1 expression was quantified in iBAT at baseline (8 weeks of age), prior to diet and skin treatments, and at 1, 4, 8 and 12 weeks post intervention. In Experiment 2 (Fig. 5a), daily UCP-1 expression (7 times in 28 h) was quantified in iBAT at baseline (8 weeks of age), prior to beginning (and 24 h following) diet and skin treatments, and at 6 and 12 weeks post intervention.

Interscapular skin temperature In Experiment 2, an infrared skin thermometer was used to measure the temperature at the shaved interscapular skin site above the iBAT deposit.

Blood glucose In Experiment 2, immediately prior to each luciferin injection, glucose concentrations were determined in a drop of blood from the tail vein.

Mitochondria in iBAT At the end of Experiment 1, mitochondrial content (amount) and activity (membrane potential) was quantified using confocal microscopy.

Immune cells in vascular stromal compartment of iBAT At the end of Experiment 1, macrophages and regulatory T cells were phenotyped using flow cytometry.

Detection of mRNA At the end of Experiment 2, mRNA levels were quantified in iBAT using real-time PCR for the following gene targets: *Eef1α* (also known as *Eef1a1-ps1*) (housekeeping), *Atgl*, *Bmp7*, *Cd36*, *Cidea*, *Dio2*, *Ebf2*, *Fasn*, *Fatp2*, *Fgf21*, *Glut4*, *Il6*, *Pgc-1α* (also known as *Ppargc1a*), *Pla2*, *Pparγ* (also known as *Pparg*), *Prdm16*, *Tnf*, *Ucp1* and *Zic1*.

Statistical analyses p values <0.05 were considered statistically significant. Generally, unless otherwise stated, data were compared using ANOVA with Tukey's post hoc (if normally distributed) or Kruskal–Wallis test with Dunn's post hoc (if not normally distributed) analyses to define differences between treatments.

Results

Chronic exposure to low-dose UVR did not modify weight gain or WAT weight in mice fed high-fat diet In the group-housed mice in Experiment 1, *Ucp1* luciferase transgenic male mice fed a high-fat diet weighed more (by mean 2.4 g, p=0.02) and had greater weight gain (by 7.5%, p=0.004) after 10 weeks compared with mice fed a low-fat diet (Fig. 1b,c, Table 1). There was no effect of repeated exposure to UVR, or any other treatments, on body weight or weight gain or gonadal WAT weight compared with mice fed the high-fat diet only.

Repeated exposure to UVR suppressed glucose intolerance After 10 weeks, fasting glucose levels were elevated by 43% in mice fed a high-fat diet compared with the low-fat diet (p=0.01), with no effect of UVR (Table 2). Increased glucose intolerance observed in mice fed the high-fat diet (p=0.002) was suppressed by UVR (p=0.04, Fig. 1d,e). These effects of UVR were reversed by topical cPTIO, a nitric oxide scavenger (p=0.03, Fig. 1d,e). Topical SNAP (nitric oxide donor) did not affect glucose levels in mice fed the high-fat diet (p=0.99, Fig. 1d,e). Thus, low-dose UVR suppressed glucose intolerance through skin release of nitric oxide.

UVR effects on insulin resistance in mice fed high-fat diet Increased insulin resistance (AUC; week 11) was observed in mice fed the high-fat diet compared with low-fat diet (p=0.001, Table 2). UVR reduced, but not significantly, the degree of insulin resistance observed in mice fed the high-fat diet (p=0.06). Fasting insulin or adiponectin levels (week 9) were increased by the high-fat diet (compared with low-fat diet, Mann–Whitney U test, $p \leq 0.01$), but were not affected by UVR or SNAP (Table 2).

UVR did not alter serum lipid levels or food intake At the end of Experiment 1, total- and HDL-cholesterol levels were increased in mice fed a high-fat diet compared with low-fat diet (by ~20%, $p \leq 0.0001$). There was no effect of UVR or SNAP on serum lipids compared with mice fed the high-fat diet only (p>0.16, Table 3). Food/energy intake was not significantly modified by exposure to UVR in mice fed the high-fat diet (p>0.1, ESM Table 2).

UVR suppressed serum IL-6 levels and improved vascular nitric oxide IL-6 (but not TNF-α) measured at week 12 was increased in mice fed the high-fat diet compared with low-fat diet (by 69%, p=0.03, Student's t test) and reduced by UVR or SNAP (by 58%, p=0.02, Table 3).

The effects of UVR were not reversed by cPTIO. Serum 25(OH)D levels were increased in mice fed the high-fat diet (ESM Table 3), as previously observed [3]. UVR did not increase serum 25(OH)D as previously observed and extensively characterised in male mice fed low- or high-fat diets supplemented (or not) with vitamin D [3, 19]. Similarly, in previous studies, 1,25-dihydroxyvitamin D levels were not increased in irradiated skin of male mice exposed to UVR, although a lack of response could have been due to the limits of detection of the immunoassay used [19]. A small increase in serum calcium occurred in UVR-exposed mice (by 4%, p=0.04). Circulating nitrite concentrations were reduced in mice fed a high-fat diet compared with low-fat diet (by 38%, p<0.0001, Student's t test), indicative of impaired vascular nitric oxide production and endothelial dysfunction [20]. UVR, SNAP and dietary nitrate all tended to normalise nitrite concentrations (only significantly for SNAP, p=0.04, ESM Table 3).

Low-dose UVR suppressed signs of NAFLD in mice fed high-fat diet Livers of mice fed a high-fat diet had increased lipid content (by 95%, p=0.02, Fig. 2b, m), which was reduced by UVR (by 80%, p=0.02, Fig. 2c, m); an effect reversed by cPTIO (p=0.02, Fig. 2h, m). SNAP had suppressive effects on liver lipid content similar to UVR (although not significant, p=0.07, Fig. 2g, m). Similar findings were obtained when the extent of steatosis, hepatocellular ballooning and fibrosis were compared (Fig. 2 d-f, j-l, n).

Dietary nitrate did not reproduce suppressive effects of low-dose UVR Dietary nitrate did not reproduce any of the suppressive effects of UVR (or SNAP) on metabolic outcomes described in Figs 1-2, and Tables 1-3, with liver weights increased compared with mice fed a high-fat diet (p=0.01, Table 1). Serum levels of nitrate (not nitrite) were substantially increased by dietary nitrate (~six–ninefold, p<0.0001, ESM Table 3). Together, these findings suggest that skin release of nitric oxide (induced by UVR, or chemically) may be a more potent way of regulating metabolic dysfunction than dietary nitrate.

UVR did not modify UCP-1 expression in iBAT in mice fed high-fat diet In a preliminary experiment, UCP-1 expression peaked in iBAT 24 h after exposure of adult mice (fed the low-fat diet) to a single 1 kJ/m^2 dose of UVR (by >twofold, p=0.03, Fig. 3). At week 12 in Experiment 1 (Fig. 1a), there was some evidence for increased UCP-1 expression in iBAT of mice fed a high-fat diet, compared with low-fat diet (Table 4; p=0.07, Student's t test). In mice fed the high-fat diet, exposure to low-dose UVR did not modulate UCP-1 expression in iBAT

(Table 4). Interestingly, UCP-1 expression was reduced in the iBAT of mice topically treated with cPTIO at week 12 (Table 4). Treatment with dietary nitrate transiently increased UCP-1 levels at the subcutaneous WAT site (lower back, inguinal), with increased levels observed at week 8 only (low-fat diet=2.9±0.3; high-fat diet=3.2±0.4; UVR=3.3±0.3; SNAP=2.4±0.1; UVR + cPTIO=3.8±0.7; nitrate=8.8±0.2; mean±SEM $x10^3$ peak radiance, $p<0.05$), but not other times (data not shown). We observed no effect of feeding mice a high-fat diet or UVR on mitochondrial content or membrane potential in iBAT at week 12 (ESM Fig. 1).

UVR reduced 'whitening' of iBAT induced by high-fat diet At week 12, iBAT obtained from mice fed a high-fat diet weighed more (Table 1) and exhibited increased 'whitening' (white adipocyte phenotype of BAT [21], Fig. 4b,g) compared with mice fed a low-fat diet ($p=0.006$, Fig. 4a,g). UVR reduced iBAT weights ($p=0.02$, Table 1) and degree of whitening of iBAT ($p<0.0001$, Fig. 4c,g) in mice fed the high-fat diet. Effects of UVR were reversed by cPTIO (Table 1; Fig. 4e,g), but not reproduced by SNAP (Fig. 4d) or dietary nitrate (Fig. 4f). There was a moderate and significant linear correlation between the hepatic lipid content and iBAT whitening (Spearman's correlation, $r=0.428$, $p=0.002$). These data suggest that exposure to low-dose UVR prevented fat accumulation in the liver and the whitening of iBAT, through skin release of nitric oxide. There was also an increase (by >twofold) in proportions of macrophages ($F4/80^+$MHC class II^+ cells [22]) in iBAT of mice exposed to UVR or SNAP, compared with mice fed the high-fat diet only (ESM Fig. 2). More substantial increases in $F4/80^+$MHC class II^+ cells were observed in mice supplemented with nitrate, with a (non-significantly) reduced percentage expressing CD86, and less CD11c and CD301 (macrophage type-1 and -2 markers [23]) per cell (ESM Fig. 2). There were no differences between treatments in proportions of regulatory $CD4^+$ T cells expressing FOXP3, CD25 and/or IL-10 in iBAT (data not shown).

Determining the effects of low-dose UVR on biorhythms of UCP-1 In Experiment 1, we measured UCP-1 expression at a single time of day (between 10:00 and 14:00) at each timepoint. Thus, our inability to detect robust changes in UCP-1 expression in response to dietary (high-fat) or skin (UVR) treatment (Table 4) may have been because we did not consider circadian rhythm. In Experiment 2, we examined the daily biorhythms of UCP-1 expression in iBAT prior to, and after, 6 and 12 weeks of treatments, with data collected across 28 h at each timepoint and mice exposed to UVR (or high-fat diet at baseline) at Zeitgeber time (ZT)3 (09:00) (Fig. 5a).

High-fat diet increased adiposity and signs of NAFLD in mice (housed as singletons) Increased body weight ($p=0.001$, Fig. 5b) and weight gain ($p=0.001$, Fig. 5c) were observed after 1 week of feeding mice the high-fat diet, which also had greater WAT depot weights at week 12 (Table 5). There were some transient reductions in body weight and weight gain in response to each circadian rhythm analysis (Fig. 5b,c). Worse liver histopathology was observed in mice fed the high-fat diet (Fig. 5d). However, there was limited evidence for metabolic dysfunction when mice were housed as singletons with the high-fat diet not increasing fasting insulin or glucose, or glucose levels when measured during the GTT or ITT (Table 6, Fig. 5e). However, in the circadian analyses, (non-fasting) blood glucose levels were increased in mice fed a high-fat diet (Fig. 6e: from ZT5, week 6).

Low-dose UVR suppressed gonadal WAT and iBAT weights and signs of NAFLD in mice (housed as singletons) While UVR did not affect body weight (Fig. 5b) or weight gain (Fig. 5c), gonadal WAT and iBAT weights (Table 5), and liver histopathology (Fig. 5d) was reduced in mice exposed to UVR, compared to mice only fed the high-fat diet. These effects of UVR on liver histopathology (Fig. 5d), but not WAT or iBAT weights (Table 5), were reversed by cPTIO, pointing towards nitric oxide-dependent and -independent effects.

UCP-1 expression in iBAT increased by high-fat diet in mice housed as singletons There were minimal effects of eating a high-fat diet on iBAT UCP-1 expression at baseline (Fig. 6a) and 6 weeks (Fig. 6d). By 12 weeks, UCP-1 levels were increased in iBAT of mice fed a high-fat diet at nearly all times (except ZT9, 15:00, Fig. 6g, *****$p<0.05$). Similarly, there were more distinct increases in blood glucose levels after 12 weeks (Fig. 6h, *****$p<0.05$) of high-fat diet feeding, than at baseline (Fig. 6b), or 6 weeks (Fig. 6e). While no significant cosinor rhythms in the expression of UCP-1 in iBAT or glucose levels in blood were detected (ESM Table 4), levels varied across the day; e.g. in mice fed a high-fat diet for 12 weeks, UCP-1 was increased at ZT3 (24 h post UVR), compared with ZT9 and ZT13 (Fig. 6g, one-way ANOVA, $p<0.05$).

Low-dose UVR had minimal effects on UCP-1 expression in iBAT and blood glucose (in mice housed as singletons) There was no effect of low-dose UVR on the expression of UCP-1 in iBAT or blood glucose levels in mice fed a high-fat diet at any time, except at week 12 at ZT3 (24 h post UVR), when UCP-1 levels were reduced (Fig. 6g, $p<0.05$). This effect was not reversed by cPTIO (Fig. 6g). These findings suggest that the capacity for UVR to reduce

gonadal WAT (Table 5) and prevent liver histopathology (Fig. 5d) were unlikely to occur through changes in UCP1 mediated thermogenesis in iBAT, or pathways that regulate blood glucose.

Low-dose UVR reduced interscapular skin temperature in mice fed high-fat diet After 6 (Fig. 6f) and 12 (Fig. 6i) weeks (but not at baseline, Fig. 6c), interscapular skin temperatures were reduced in mice fed a low-fat diet (week 6 at ZT13, ZT3 [24 h post UVR]; week 12, ZT23; $p<0.05$), and mice exposed to low-dose UVR (week 6 at ZT13, $p<0.05$), compared with mice fed a high-fat diet (only). Interscapular skin temperatures were reduced in mice exposed to UVR, compared with those treated with cPTIO (Fig. 6f, week 6 at ZT13; Fig. 6i, week 12, ZT1, ZT9, ZT23; $p<0.05$). At 12 weeks, there were no differences in interscapular skin temperature measured between mice fed the low-fat diet, and those exposed to UVR (Fig. 6i; $p>0.05$). There were no significant linear correlations ($p>0.05$) between UCP-1 expression in iBAT, and interscapular skin temperature in mice of any treatment. These data suggest repeated exposure of mice fed a high-fat diet to low-dose UVR reduced interscapular skin temperatures (at the irradiated site), through mechanisms dependent on skin release of nitric oxide.

UVR increased *Dio2, Glut4* and *Fatp2* and reduced *Atgl, Fasn* and *Tnf* mRNAs in iBAT At the end of Experiment 2, exposure to low-dose UVR 'normalised' mRNA levels of some core gene regulators of BAT function, namely *Pgc1α, Pparγ* and *Ucp1,* with levels similar to those observed in mice fed the low-fat diet (ESM Fig. 3). Levels of other core gene regulators, *Prdm16, Ebf2, Bmp7* and *Zic1,* were reduced by high-fat diet and not modulated by UVR, with similar findings for genes regulating fatty acid storage (*Cidea*) and transport (*Cd36*), and a batokine (*Fgf21*). However, mRNAs of a core gene regulator (*Dio2,* deiodinase, iodothyronine, type II) and glucose and fatty acid transporters (*Glut4*, solute carrier family 2 [facilitated glucose transporter], member 4; *Fatp2,* solute carrier family 2 [facilitated glucose transporter], member 2) were increased in iBAT of mice exposed to UVR. Levels of *Fasn* (fatty acid synthase, a lipogenesis gene), *Atgl* (adipose triglyceride lipase, a lipolysis gene) and *Tnf* mRNAs were reduced in mice exposed to UVR, compared with those only fed the high-fat diet. These observations were independent of nitric oxide release from UV-irradiated skin.

Discussion

Here we provide evidence for metabolic benefits of low-dose UVR, which promoted glucose tolerance, and reduced hepatic fat accumulation, whitening of iBAT, and blood IL-6 levels. Some effects of low-dose UVR were different in mice housed as singletons (e.g. reduced gonadal WAT weights compared with co-housed mice). Dermal release of nitric oxide was responsible for the protective effects of UVR in reducing glucose intolerance and hepatic fat accumulation (and whitening of iBAT for co-housed mice). Exposure to UVR enhanced expression of *Dio2* in iBAT of mice fed the high-fat diet. This gene can be activated via bile acids produced by the liver, promote expression of PGC1α (Peroxisome proliferator-activated receptor-γ cofactor-1α), and mitochondrial biogenesis in BAT (reviewed by [24]). These findings suggest that there may be novel mechanistic links between the liver and BAT regulated by UVR. While increased UCP-1 levels in iBAT were observed 24 h after exposure to low-dose UVR in mice fed a low-fat diet, low-dose UVR did not raise UCP-1 levels in iBAT at any time in our detailed circadian analyses in mice fed a high-fat diet. Instead, chronic exposure to low-dose UVR reduced interscapular skin temperatures, an effect that was reversed by topical cPTIO, suggesting a role for UVR-induced nitric oxide.

Dermal interscapular temperatures may only partially reflect thermogenesis in iBAT [25, 26], with further contributions by local vasomotor tone [26], which can influence thermoregulation in rodents [27]. Dermal vasomotor activity depends partially on core body temperature [26] as suggested in our study with skin temperatures >36ºC. However, UVR increased forearm blood flow in healthy young men, independently of dermal skin temperature [28]. Both diet and sun exposure may alter vascularisation of the dermis, which is increased by caloric restriction [29], while reduced lymphatic vessel density occurs in sun-damaged skin [30]. Although we did not observe any signs of increased UCP1-mediated thermogenesis with UVR, there was reduced whitening of iBAT, and lower *Tnf* mRNA levels, but more macrophages. Whitening of BAT may induce crown-like structures and macrophage infiltration [31]. Other unexpected findings were that mRNAs of lipolysis and lipogenesis genes, *Atgl* and *Fasn*, respectively, were reduced, while *Glut4* and *Fatp2* mRNAs increased with UVR. More studies are needed to further characterise the effects of UVR on these pathways in iBAT, and to establish if there are links to the anti-inflammatory effects of UVR.

As we demonstrate here, to gain better biological insights into processes that govern thermogenesis, some consideration of variability induced by time of day is needed. Other important considerations include: non-thermoneutral conditions [10]; cage density; experimental stress; sex (the *Ucp1* luciferase transgene is inserted into the Y chromosome [9]); and genetic background (e.g. FVB/NJ mice have restricted weight gain [32]). Our findings in *Ucp1* luciferase transgenic mice (FVB/NJ background) are mostly reminiscent of previous observations in C57Bl/6J mice [3], suggesting that the effects of low-dose UVR are not limited to a single mouse strain, and may be effective in genetically diverse humans. While proton (H^+) leak in mitochondria may be mediated by proteins other than UCP1 [33], this process is thought to be the main driver of heat production by iBAT [34]. Additional mechanisms may include proton leak and/or OH^- channel activation through allosteric binding of long-chain fatty acids to UCP1 [34], and UCP1-independent pathways, such as creatinine cycling, the glycerol-3-phosphate shuttle and the sarco/endoplasmic reticulum Ca^{2+}-ATPase (SERCA) pathway [35].

In conclusion, we report beneficial effects of low-dose UVR on metabolic outcomes in *Ucp1* luciferase transgenic mice fed a high-fat diet. The suppressive effects of UVR on glucose intolerance, hepatic fat accumulation, whitening of iBAT and interscapular skin temperatures were dependent on release of nitric oxide from irradiated skin. Detailed circadian analyses did not reveal substantial differences in UCP-1 expression in iBAT, suggesting that beneficial metabolic effects of UVR are mediated via another route. We also observed that dermal release of nitric oxide may be a more effective means of reducing metabolic dysfunction than dietary manipulation. Further studies examining the effects of exposure to UVR on glucose and lipid metabolic pathways in iBAT and other tissues, and its effects on vascular tone and temperature of the dermis, are needed.

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Data availability Data are available on request to authors.

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Duality of interest MF and RBW are members of the Scientific Advisory Board of AOBiome LLC, a company commercialising ammonia-oxidising bacteria for use in inflammatory skin disease. RBW is also a director of, and MF a scientific advisor for, RelaxSol Ltd, a company developing novel sunscreen and skincare products. All other authors declare that there is no duality of interest associated with their contribution to this manuscript.

Contribution statement SG conceived and designed this study with input from GKD, KP, NJF, KC, MF, PHH, RML, RBW and VM. GD and KP acquired and analysed the data for the study with help from NJF, RCC, PJM and SG. TNA performed the confocal microscopy, while MKA performed the Oil Red O staining of liver samples, and MM and BOF measured serum nitrite and nitrate levels. All authors contributed towards the interpretation of findings from this study, played a role in drafting the article or revising it critically for its intellectual content,

and gave their final approval for this version of the paper to be published. SG is the guarantor of this work.

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Table 1 Final body and tissue weights (Experiment 1: group-housed mice)

Treatment Group[a]	Body weight (g)	Weight gain (%)	WAT weight (g)	Liver weight (g)	iBAT weight (g)
LFD	31.2 ± 0.3	117 ± 1.3‡	0.81 ± 0.04	1.29 ± 0.03‡	0.24 ± 0.01‡
HFD	33.5 ± 0.5*	124 ± 1.4*	1.11 ± 0.05*	1.28 ± 0.03‡	0.30 ± 0.01*
UVR	32.7 ± 0.5	121 ± 1.5‡	1.03 ± 0.05*	1.29 ± 0.03‡	0.23 ± 0.01†, ‡
SNAP	33.7 ± 0.5*	120 ± 1.4‡	1.14 ± 0.04*	1.34 ± 0.03	0.29 ± 0.02
UVR + cPTIO	33.4 ± 0.4*	123 ± 1.1*	1.19 ± 0.04*	1.32 ± 0.02	0.28 ± 0.02‡
Nitrate	35.2 ± 1.2*	129 ± 3.2*	1.03 ± 0.08	1.45 ± 0.05*, †	0.34 ± 0.03*

Data are presented as mean ± SEM (*n*=21-49 per treatment)

[a]Four-week old male *Ucp1* luciferase transgenic mice were fed LFD for 4 weeks, until 8 weeks of age. At 8 weeks of age mice were separated into one of six treatment groups:(1) LFD; (2) HFD; (3) HFD with UVR (1 kJ/m^2 UVB radiation, twice a week; UVR); (4) HFD with topical SNAP (0.1 mmol, twice a week; SNAP); (5) HFD with UVR followed by topical cPTIO (0.1 mmol, twice a week; UVR + cPTIO); or (6) HFD with nitrate (0.06 mmol $NaNO_3$/kg/day) in drinking water (Nitrate)

Data were compared using one-way ANOVA with Tukey's post hoc analysis

*p<0.05 relative to LFD, †p<0.05 relative to HFD, ‡p<0.05 relative to nitrate

HFD, high-fat diet; LFD, low-fat diet

Table 2 Fasting blood glucose (week 10), fasting plasma levels of insulin and adiponectin (week 9), and AUC values for ITTs (week 11) (Experiment 1: group-housed mice)

Treatment Group[a]	**Fasting glucose** (mmol/l) (***n***=10)	**ITT** (AUC, mmol/l x min) (***n***=**7**-16**)**	**Fasting insulin** (pmol/l) (***n***=7–14)	**Fasting adiponectin** (µg/ml) (n=7–14)
LFD	6.1 ± 0.4	521 ± 37	126 ± 25	7.7 ± 0.3
HFD	8.7 ± 0.7*	861 ± 68*	385 ± 102*	8.5 ± 0.2*
UVR	7.4 ± 0.5	621 ± 29	350 ± 123	8.2 ± 0.2
SNAP	7.5 ± 0.4	696 ± 63	343 ± 109*	8.4 ± 0.2*
UVR + cPTIO	7.6 ± 0.5	712 ± 45	609 ± 126*	7.8 ± 0.3
Nitrate	8.3 ± 1.3	692 ± 168	291 ± 112	7.8 ± 0.4

Data are presented as mean ± SEM

[a]Four-week-old male *Ucp1* luciferase transgenic mice were fed LFD for 4 weeks, until 8 weeks of age. At 8 weeks of age mice were separated into one of six treatment groups: (1) LFD; (2) HFD; (3) HFD with UVR (1 kJ/m^2 UVB radiation, twice a week; UVR); (4) HFD with topical SNAP (0.1 mmol, twice a week; SNAP); (5) HFD with UVR followed by topical cPTIO (0.1 mmol, twice a week; UVR + cPTIO); or (6) HFD with nitrate (0.06 mmol kg^{-1} day^{-1} $NaNO_3$) in drinking water (nitrate)

Data were compared using one-way ANOVA with Tukey's post hoc analysis, or Mann–Whitney *U* test

*p<0.05 relative to LFD

HFD, high-fat diet; LFD, low-fat diet

Table 3 Circulating lipid and cytokine levels (Experiment 1: group-housed mice)

Treatment group[a]	**Total cholesterol** (mmol/l) (*n*=5–10)	**HDL-cholesterol** (mmol/l) (*n*=5–10)	**LDL-cholesterol** (mmol/l) (*n*=5–10)	**Triacylglycerol** (mmol/l) (*n*=5–10)	**IL-6**[b] (pg/ml) (*n*=6–12)	**TNF-α**[b] (pg/ml) (*n*=6–12)
LFD	3.6 ± 0.1	2.2 ± 0.0	0.21 ± 0.04	1.8 ± 0.3	5.9 ± 1.6	4.1 ± 0.7
HFD	4.5 ± 0.1*	2.7 ± 0.1*	0.16 ± 0.01	1.2 ± 0.2	10.0 ± 0.8	6.5 ± 1.6
UVR	4.9 ± 0.1*	3.0 ± 0.1*	0.16 ± 0.00	1.6 ± 0.2	4.2 ± 1.1†,‡	3.2 ± 0.4
SNAP	4.5 ± 0.1*	2.8 ± 0.1*	0.17 ± 0.02	1.0 ± 0.1	1.6 ± 0.6†,‡	8.0 ± 1.1
UVR + cPTIO	4.7 ± 0.1*	2.8 ± 0.1*	0.18 ± 0.01	1.4 ± 0.2	6.4 ± 1.7	6.5 ± 1.1
Nitrate	5.1 ± 0.2*	3.1 ± 0.1*	0.16 ± 0.00	1.4 ± 0.4	12.2 ± 1.8	6.1 ± 2.7

Data are presented as mean ± SEM

[a]Four-week old male *Ucp1* luciferase transgenic mice were fed LFD for 4 weeks, until 8 weeks of age. At 8 weeks of age mice were separated into one of six treatment groups: (1) LFD; (2) HFD; (3) HFD with UVR (1 kJ/m^2 UVB radiation, twice a week; UVR); (4) HFD with topical SNAP (0.1 mmol, twice a week; SNAP); (5) HFD with UVR followed by topical cPTIO (0.1 mmol, twice a week; UVR + cPTIO); or (6) HFD with nitrate (0.06 mmol kg^{-1} day^{-1} $NaNO_3$) in drinking water (Nitrate)

[b]The levels of detection for the IL-6 and TNF-α assays were 1 and 3 pg/ml, respectively

Data were compared using one-way ANOVA with Tukey's post hoc analysis

*p<0.05 relative to LFD, †p<0.05 relative to HFD, ‡p<0.05 relative to nitrate

HFD, high-fat diet; LFD, low-fat diet

Table 4 Mean peak UCP1 radiance in iBAT (Experiment 1: group-housed mice)

Mean peak UCP1 radiance ($\times 10^3$)						
Weeks post intervention	Treatment group[a]					
	LFD	**HFD**	**UVR**	**SNAP**	**UVR + cPTIO**	**Nitrate**
Baseline	7.2 ± 0.8	7.0 ± 1.2	7.5 ± 1.2	7.4 ± 1.5	6.2 ± 0.8	8.4 ± 2.7
Week 1	7.0 ± 1.0	8.2 ± 1.6	4.5 ± 0.4	5.1 ± 0.8	11.3 ± 2.7	8.4 ± 3.1
Week 4	8.9 ±1.5	13.5 ± 2.9	7.6 ± 1.7	6.2 ± 1.7	6.2 ± 0.5	12.5± 3.4
Week 8	16.3 ± 3.6	11.9 ± 3.1	16.4 ± 2.2	16.0 ± 5.1	17.0 ± 5.6	12.9 ± 6.8
Week 12	10.4 ± 2.0	16.7 ± 2.6*	16.1 ± 3.4*	16.0 ± 5.1	5.1 ± 1.4	15.6 ± 3.8*

Data are presented as mean ± SEM $\times 10^3$ (*n*=5-12 per treatment, units are photons s^{-1} cm^{-2} sr^{-1})

[a]Four-week-old male *Ucp1* luciferase transgenic mice were fed LFD for 4 weeks, until 8 weeks of age. At 8 weeks of age mice were separated into one of six treatment groups: (1) LFD; (2) HFD; (3) HFD with UVR (1 kJ/m^2 UVB radiation, twice a week; UVR); (4) HFD with topical SNAP (0.1 mmol, twice a week; SNAP); (5) HFD with UVR followed by topical cPTIO (0.1 mmol, twice a week; UVR + cPTIO); or (6) HFD with nitrate (0.06 mmol kg^{-1} day^{-1} $NaNO_3$) in drinking water (Nitrate)

Data were compared using one-way ANOVA with Tukey's post hoc analysis

*$p<0.05$ relative to UVR + cPTIO

HFD, high-fat diet; LFD, low-fat diet

Table 5 Final body and tissue weights (Experiment 2: individually housed mice)

Treatment Group[a]	**Body weight** (g)	**Weight gain** (%)	**WAT weight** (g)	**Liver weight** (g)	**iBAT weight** (g)
LFD	31.3 ± 0.3	113.6 ± 1.1	0.87 ± 0.03	1.31 ± 0.03	0.27 ± 0.01
HFD	34.6 ± 0.7*	124.1 ± 1.9*	1.26 ± 0.03*	1.25 ± 0.03	0.33 ± 0.02*
UVR	33.1 ± 0.7	119.2 ± 2.3	1.06 ± 0.06†	1.18 ± 0.03	0.26 ± 0.02†
UVR + cPTIO	33.4 ± 0.6	123.9 ± 1.9*	1.11 ± 0.04†	1.14 ± 0.03†	0.28 ± 0.01†

Data are presented as mean ± SEM (*n*=20 per treatment)

[a]Four-week old male *Ucp1* transgenic mice were fed LFD for 4 weeks, until 8 weeks of age. At 8 weeks of age mice were separated into one of four treatment groups: (1) LFD; (2) HFD; (3) HFD with UVR (1 kJ/m^2 UVB radiation, twice a week; UVR); (4) HFD with UVR followed by topical cPTIO (0.1 mmol, twice a week; UVR + cPTIO)

Data were compared using one-way ANOVA with Tukey's post hoc analysis

*$p<0.05$ relative to LFD, †$p<0.05$ relative to HFD

HFD, high-fat diet; LFD, low-fat diet

Table 6 Fasting plasma levels of insulin (9 weeks), blood glucose (10 weeks) and AUC values for ITTs (11 weeks) and serum cytokines (12 weeks) (Experiment 2: individually housed mice)

Treatment Group[a]	**Fasting insulin** (pmol/l) (*n*=7–8)	**Fasting glucose** (mmol/l) (*n*=8)	**ITT** (AUC, mmol/l x min (*n*=7-8)	**IL-6**[b] (pg/ml) (*n*=18)	**TNF-α**[b] (pg/ml) (*n*=18)
LFD	693 ± 179	8.4 ± 0.3	720 ± 61	<LoD[c]	<LoD
HFD	1054 ± 186	9.1 ± 0.4	873 ± 108	<LoD	<LoD
UVR	1103 ± 315	8.5 ± 0.3	859 ± 89	<LoD	<LoD
UVR + cPTIO	1068 ± 294	9.3 ± 0.4	818 ± 61	<LoD	<LoD

Data are presented as mean ± SEM, *n* as indicated

[a]Four-week-old male *Ucp1* transgenic mice were fed LFD for 4 weeks, until 8 weeks of age. At 8 weeks of age mice were separated into one of four treatment groups: (1) LFD; (2) HFD; (3) HFD with UVR (1 kJ/m^2 UVB radiation, twice a week; UVR); (4) HFD with UVR followed by topical cPTIO (0.1 mmol, twice a week; UVR + cPTIO)

[b]The levels of detection for the IL-6 and TNF-α assays were 1 and 3 pg/ml, respectively

[c]<LoD – below limit of detection of assay

Data were compared using one-way ANOVA with Tukey's post hoc analysis

HFD, high-fat diet; LFD, low-fat diet

Figure legends

Fig. 1 Low-dose UVR reduced glucose intolerance through skin release of nitric oxide but did not affect weight gain by *Ucp1* luciferase transgenic mice fed the HFD (group-housed). In Experiment 1, 4-week-old *Ucp1* luciferase transgenic male mice were fed a low-fat (non-vitamin D-supplemented) diet for 4 weeks. From 8 weeks of age, mice were fed the LFD (treatment 1) or switched to the HFD (treatments 2–6). From 8 weeks of age, the shaved dorsal skin of mice were treated twice a week with: mock-irradiation and then vehicle was applied topically to skin (treatments 1 and 2); sub-erythemal UVR (1 kJ/m^2 UVB) and then vehicle (treatment 3); mock-irradiation and then the nitric oxide donor SNAP (0.1 mmol) was applied topically to skin (SNAP, treatment 4); or, sub-erythemal UVR and then the nitric oxide scavenger cPTIO (0.1 mmol) was applied topically to skin (UVR + cPTIO, treatment 5). Mice that received treatment 6 were mock-irradiated and fed nitrate (0.06 mmol kg^{-1} day^{-1}) through their drinking water. Mice were fed the LFD or HFD and administered the treatments twice a week for 12 weeks until 20 weeks of age. (**a**) Overview of this experiment. (**b**, **c**) Mice were weighed weekly. Body weight (n≥21) (**b**) and weight gain (n≥21) (**c**) post intervention. (**d**, **e**) A GTT was conducted at 10 weeks post intervention, with blood glucose levels (n≥7) (**d**) and AUCs (**e**) determined. Data are shown as mean ± SEM; *p<0.05 (one-way ANOVA with Tukey's post hoc analysis) for mice fed the LFD vs all other treatments (**b–d**) and between the indicated groups (**e**). HFD, high-fat diet; LFD, low-fat diet

Fig. 2 Low-dose UVR prevented liver steatosis through a nitric oxide-dependent mechanism (in group-housed mice). In Experiment 1, 4-week-old male *Ucp1* luciferase transgenic mice were fed the LFD for 4 weeks. At 8 weeks of age mice were separated into one of six treatment groups: (1) LFD; (2) HFD; (3) HFD with UVR (UVR); (4) HFD with topical SNAP (SNAP); (5) HFD with UVR followed by topical cPTIO (UVR + cPTIO); or (6) HFD with nitrate in drinking water (Nitrate). After 12 weeks of treatment, livers were histopathologically assessed. Representative sections (×20 oil immersion objective; scale bar, 10 μm) are shown for Oil Red O-stained (rows 1 and 3) or Masson's trichrome-stained (rows 2 and 4) liver samples obtained from mice from the: (**a**, **d**) LFD; (**b**, **e**) HFD; (**c**, **f**) UVR; (**g**, **j**) SNAP; (**h**, **k**) UVR + cPTIO; and (**i**, **l**) Nitrate treatments. In (**m**), lipid content was scored following the staining of liver sections with Oil Red O (HFD vs SNAP; p=0.07). In (**n**), livers were histopathologically assessed following staining with H&E or Masson's trichrome with a combined score for steatosis, hepatocellular ballooning and fibrosis shown. Data in (**m**) and (**n**) are shown as mean

± SEM (n≥5/treatment); *p<0.05 (one-way ANOVA with Tukey's post hoc analysis). HFD, high-fat diet; LFD, low-fat diet

Fig. 3 UCP-1 expression in iBAT peaked 24 h after exposure of *Ucp1* luciferase transgenic mice to low-dose UVR. Four-week-old male *Ucp1* luciferase transgenic mice were fed the LFD. At 8 weeks of age, mice were exposed to a single dose of UVR (1 kJ/m^2) with UCP-1 levels in iBAT (red circles) tracked over 192 h. (**a**) Representative mice are shown at baseline, 0.25 (15 min), 3, 24, 48 and 192 h post exposure, with (**b**) the peak radiance levels of bioluminescence at the iBAT site. (**b**) Data are shown as mean ± SEM (n=8/treatment, units are photons s^{-1} cm^{-2} sr^{-1}); *p<0.05 compared with baseline (one-way ANOVA with Tukey's post hoc analysis). LFD, low-fat diet

Fig. 4 The extent of whitening of iBAT induced by feeding mice an HFD was suppressed by exposure to low-dose UVR. In Experiment 1, 4-week-old male *Ucp1* luciferase transgenic mice were fed an LFD for 4 weeks. At 8 weeks of age mice were separated into one of six treatment groups: (1) LFD; (2) HFD); (3) HFD with UVR (UVR); (4) HFD with topical SNAP (SNAP); (5) HFD with UVR followed by topical cPTIO (UVR + cPTIO); or (6) HFD with nitrate in drinking water (Nitrate). After 12 weeks of treatment, iBAT was histopathologically assessed for the extent of whitening following staining with H&E, with representative iBAT tissue sections shown for mice from the (**a**) LFD, (**b**) HFD, (**c**) UVR, (**d**) SNAP, (**e**) UVR + cPTIO, and (**f**) Nitrate treatments (×20 oil immersion objective; scale bar, 10 μm), and with histopathological scores for the extent of whitening (**g**), in which data are shown as mean ± SEM (n≥5/treatment); *p<0.05 (one-way ANOVA with Tukey's post hoc analysis). In (**b**) the blue arrows point to cells with a white adipocyte phenotype. HFD, high-fat diet; LFD, low-fat diet

Fig. 5 Low-dose UVR reduced NAFLD histopathology in *Ucp1* luciferase transgenic mice fed an HFD (individually housed, with data collected for circadian rhythm analysis). In Experiment 2, 4-week-old *Ucp1* luciferase transgenic male mice were fed a low-fat (non-vitamin D-supplemented) diet for 4 weeks. From 8 weeks of age, mice were fed the LFD (treatment 1) or switched to the HFD (treatments 2–4). From 8 weeks of age, the shaved dorsal skin of mice was treated twice a week with: mock-irradiation and then vehicle applied to skin (treatments 1 and 2); sub-erythemal UVR (1 kJ/m^2 UVB) and then vehicle (treatment 3); or sub-erythemal UVR and then the nitric oxide scavenger cPTIO (0.1 mmol) was applied to skin

(UVR + cPTIO, treatment 4). Mice were fed the LFD or HFD and administered the treatments twice a week for 12 weeks until 20 weeks of age. At baseline (prior to feeding mice the HFD or skin treatments), and after 6 and 12 weeks of treatment, data were collected for circadian analyses of UCP1 bioluminescence in iBAT, interscapular skin temperature and blood glucose, with these times shown in (**b**) and (**c**). (**a**) Overview of the experiment. (**b**, **c**) Mice were weighed weekly. Body weight (*n*=20) (**b**) and weight gain (*n*=20) (**c**) post intervention. (**d**) After 12 weeks of treatment, livers were histopathologically assessed following staining with H&E or Masson's trichrome with a combined score for steatosis, hepatocellular ballooning and fibrosis shown as mean ± SEM (*n*=10/treatment). A GTT was conducted at 10 weeks post intervention with (**e**) AUCs of the GTT (*n*=8/treatment) determined. Data are shown as mean ± SEM; **p*<0.05 (one-way ANOVA with Tukey's post hoc analysis) for mice fed an LFD vs all other treatments (**b–c**); and between indicated groups (**d**). HFD, high-fat diet; LFD, low-fat diet

Fig. 6 Detailed circadian expression analyses of UCP-1 in iBAT, interscapular skin temperature and blood glucose (in individually housed mice). In Experiment 2, 4-week-old male *Ucp1* luciferase transgenic mice were fed an LFD for 4 weeks. From 8 weeks of age mice were separated into one of four treatment groups: (1) LFD; (2) HFD; (3) HFD with UVR (UVR); or (4) HFD with UVR followed by topical cPTIO (UVR + cPTIO). At baseline (prior to feeding mice the HFD or skin treatments) (**a–c**), and after 6 (**d–f**) and 12 (**g–i**) weeks of treatment, data were collected for circadian analyses of UCP1 bioluminescence in iBAT (**a**, **d**, **g**), blood glucose (**b**, **e**, **h**) and interscapular skin temperature (**c**, **f**, **i**) at intervals of 4-6 h over 28 h. A yellow broken line depicts when the dietary and skin interventions began at baseline (**a–c**, HFD UVR box), and when a skin treatment (UVR box) was performed at weeks 6 (**d–f**) and 12 (**g–i**). Data are shown as mean ± SEM (*n*=12 for UCP1 bioluminescence, and *n*=20 for temperature and blood glucose per treatment); *****$p<0.05$ for HFD vs LFD; †$p<0.05$ for HFD vs UVR; ‡$p<0.05$ for UVR vs cPTIO (two-way ANOVA with Tukey's post hoc analysis). HFD, high-fat diet; LFD, low-fat diet