Title: Ultraviolet radiation suppresses obesity and symptoms of metabolic syndrome independently of vitamin D in mice fed a high fat diet

Short running title: UV inhibits obesity independently of vitamin D

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Abbreviations:

1,25-Dihydroxyvitamin D ($1,25(OH)_2D$)

25-Hydroxyvitamin D (25(OH)D)

4,5-Diaminofluorescein Diacetate (DAF-2DA)

Carboxy-PTIO potassium salt (cPTIO)

glucose tolerance test (GTT)

high fat diet supplemented with vitamin D_3 ($HF\text{-}D^+$)

high fat diet not supplemented with vitamin D_3 ($HF\text{-}D^-$)

interleukin- (IL-)

insulin tolerance test (ITT)

low fat diet supplemented with vitamin D_3 ($LF\text{-}D^+$)

low fat diet not supplemented with vitamin D_3 ($LF\text{-}D^-$)

metabolic syndrome (MetS)

nitric oxide (NO)

non-alcoholic fatty liver disease (NAFLD)

non-alcoholic steatohepatitis (NASH)

S-nitroso-N-acetyl-D,L-penicillamine (SNAP)

tumour necrosis factor (TNF)

ultraviolet radiation (UVR)

white adipose tissue (WAT)

vitamin D receptor (VDR)

Abstract

The role of vitamin D in curtailing the development of obesity and comorbidities like the metabolic syndrome (MetS) and type-2 diabetes has received much attention recently. However, clinical trials have failed to conclusively demonstrate the benefits of vitamin D supplementation. In most studies, serum 25-hydroxyvitamin D (25(OH)D) decreases with increasing BMI above normal weight. These low 25(OH)D levels may also be a proxy for reduced exposure to sunlight-derived ultraviolet radiation (UVR). Here we investigate whether UVR and/or vitamin D supplementation modifies the development of obesity and type-2 diabetes in a murine model of obesity. Chronic sub-erythemal and erythemal UVR significantly suppressed weight gain, glucose intolerance, insulin resistance, non-alcoholic fatty-liver disease measures and serum levels of fasting insulin, glucose and cholesterol in C57Bl/6 male mice fed a high fat diet. However, many of the benefits of UVR were not reproduced by vitamin D supplementation. In further mechanistic studies, skin induction of the UVR-induced mediator, nitric oxide reproduced many of the effects of UVR. These studies suggest that UVR (sunlight exposure) may be an effective means of suppressing the development of obesity and MetS, through mechanisms that are independent of vitamin D but dependent on other UVR-induced mediators like nitric oxide.

Introduction

Obesity has significant effects on our health and wellbeing: obese people have increased co-morbidities resulting from cardiovascular disease, type-2 diabetes, breast and colon cancers, dementia and depression. Vitamin D deficiency is recognized as a health problem affecting many individuals worldwide (1) and may contribute toward the development of obesity. Insufficient levels of vitamin D are associated with obesity, and obese people are more likely than others to be vitamin D deficient (reviewed in (2; 3)). Vitamin D is synthesised from dermal 7-dehydrocholesterol after cutaneous exposure to the ultraviolet radiation (UVR) of sunlight. Vitamin D is transported to the liver bound to the vitamin D-binding protein for conversion into the storage form, 25-hydroxyvitamin D (25(OH)D), before further conversion into the active form, 1,25-dihydroxyvitamin D ($1,25(OH)_2D$) in the kidneys. Many cells in other tissues express the enzymatic machinery required to convert 25(OH)D into active $1,25(OH)_2D$ (2).

It is not known whether vitamin D deficiency is a causal pathway for the development of obesity and the metabolic syndrome (MetS). Serum 25(OH)D levels generally decrease with increasing BMI above normal weight (4) and results from a genetic association study suggest that higher BMI leads to reduced circulating 25(OH)D levels (5). Furthermore, randomized controlled trials that test the efficacy of vitamin D supplementation for weight loss (2) or curbing MetS-related diseases like type-2 diabetes and cardiovascular disease (3; 6; 7) have had little success. Even so, there is currently much interest in vitamin D supplementation as a clinical means of controlling obesity and MetS, with >100 clinical trials underway assessing vitamin D supplementation (ClinicalTrials.gov).

Increased storage of fat-soluble vitamin D in obese individuals may reduce circulating 25(OH)D (8). Also, obese people exercise less and spend less time in the sun (9). Our increasingly ‘indoor’ lifestyles, coupled with concerns about rising skin cancer rates for light-skinned populations, have

resulted in concomitant decreases in sun exposure (10) and increased prevalence of vitamin D deficiency (11) worldwide including countries like Australia, which experiences some of the highest obesity rates in the world. Chronic sunlight exposure (particularly sub-erythemal UVR) itself may be beneficial for obesity and MetS outcomes like type-2 diabetes (12) and non-alcoholic fatty liver disease (NAFLD) (13).

In this paper, we present data further defining the role of sunlight-induced vitamin D in modulating the development of obesity and aberrant metabolic outputs including glucose intolerance, insulin resistance, and NAFLD. We directly compared the abilities of chronic UVR and/or dietary vitamin D to alter the development of obesity using a physiologically relevant model induced by feeding C57Bl/6 male mice a high fat diet. Our previous studies have shown that chronic UVR does not modify serum 25(OH)D in male mice (14), allowing us to investigate the ability of UVR to modulate obesity and MetS independently of circulating 25(OH)D. Here, chronic UVR but not dietary vitamin D suppressed weight gain and various measures of MetS (circulating cholesterol levels, glucose intolerance, insulin resistance). Further, while vitamin D supplementation did improve NAFLD, UVR suppressed its development even more effectively. Vitamin D supplementation suppressed circulating TNFα levels, identifying a possible mechanism for control of NAFLD. In further mechanistic studies, UVR-induced nitric oxide (NO) significantly suppressed some measures of obesity and MetS development, including weight, white adipose tissue (WAT) accumulation, fasting glucose, the development of insulin resistance and NAFLD. These studies suggest that while vitamin D supplementation may be useful for preventing NAFLD development, sunlight exposure may be more effective, and have the added benefits of suppressing obesity and MetS through NO-dependent pathways.

Research Design and Methods

Mice. All experiments were performed according to the ethical guidelines of the National Health and Medical Research Council of Australia and with approval from the Telethon Institute for Child Health Research Animal Ethics Committee. C57Bl/6 male mice were purchased from the Animal Resources Centre, Western Australia. The temperature and lighting were controlled, with a normal 12-hour light/dark cycle to mimic day and night. Mice were housed under perspex-filtered fluorescent lighting, which emitted no detectable ultraviolet (UV) B radiation as measured using a UV radiometer (UVX Digital Radiometer, Ultraviolet Products Inc., Upland, CA, USA). Mice were allowed access to food and acidified water *ad libitum*.

Diet. All diets were obtained from Specialty Feeds (Glen Forrest, Western Australia) and included two semi-pure low fat diets (5% fat; canola oil), which were supplemented (**LF-D$^+$**) or not (**LF-D$^-$**) with vitamin D_3 (2280 or 0 IU vitamin D_3/kg) and two high fat diets (23%; lard (20.7%) and canola oil (2.9%)) that were (**HF-D$^+$**) or were not (**HF-D$^-$**) supplemented with vitamin D_3 (2280 or 0 IU vitamin D_3/kg). Mice that started on a vitamin D_3-supplemented diet were continued on diets supplemented with vitamin D_3 throughout. The LF-D$^-$ and HF-D$^-$ were also supplemented with 2% calcium (vs 1% for the LF-D$^+$ and HF-D$^+$) to ensure normocalcemia.

UV radiation and topical skin treatments. A bank of six 40 W lamps (Philips TL UV-B, Eindhoven, The Netherlands) emitting broadband UVR, 250–360 nm, with 65% of the output in the UVB range (280–315 nm), was used to irradiate mice to deliver sub-erythemal (1 kJ/m^2; (15)) or erythemal (4 or 8 kJ/m^2) UVR onto clean-shaven 8 cm^2 dorsal skin as previously described (16). Alternatively, skin was treated with 0.1 mmoles SNAP (S-nitroso-N-acetyl-D,L-penicillamine, Sigma, (17)), a nitric oxide (NO) donor. In other treatments, a NO scavenger, cPTIO (Carboxy-PTIO potassium salt, Sigma (18), 0.1 mmoles), or 1,25$(OH)_2$D (1,25-dihydroxyvitamin D, Sigma (19), 11.4

pmoles/cm^2) were applied immediately following delivery of sub-erythemal UVR (1 kJ/m^2). This dose of $1,25(OH)_2D$ was previously reported to not induce hypercalcemia (19). All topical reagents were diluted a vehicle consisting of ethanol, propylene glycol and water (2:1:1, (20)). All topical treatments were performed in the morning.

Measuring weight gain. Mice were weighed weekly on the same day in the morning using a digital scale (Ohaus Scout, >0.1g sensitivity). Percentage weight gain was calculated from 8 weeks of age.

Glucose and insulin tolerance tests. Mice were fasted for 5 hours and then intraperitoneally challenged with either 1 g/kg glucose (Phebra, Lane Cove, NSW) (for glucose tolerance tests, GTT) or 0.5-0.75 IU/kg insulin (Lilly, Indianapolis, IN) (for insulin tolerance tests, ITT). Glucose levels were recorded at 0, 15, 30, 45, 60 and 90 minutes post-injection using the Accu-Chek Performa glucometer (Roche).

Serum metabolites. Serum 25(OH)D levels were measured using IDS EIA kits (Immunodiagnostic Systems Ltd, Fountain Hills, AZ) as described by the manufacturer (limit of detection=5-7 nmol/L, CV=0.08 for internal controls). For confirmation, 25(OH)D levels in selected samples were measured using a liquid chromatography-tandem mass spectrometry method (21), which significantly correlated with immunoassay 25(OH)D levels ($n=8$; $r=0.99$, $p\leq0.0001$). Serum calcium, cholesterol, HDL-cholesterol, LDL-cholesterol and triglyceride were detected by standard colormetric reactions using the Architect c16000 Analyser (Abbott Diagnostics, Abbott Park, USA). Glucose, insulin, adiponectin and leptin were measured in serum after fasting mice for 5 h. Fasting glucose was measured using the Accu-Chek Performa glucometer (Roche, Castle Hill, NSW). Fasting insulin, adiponectin and leptin were measured using rat/mouse insulin, mouse adiponectin and mouse leptin ELISA kits, respectively, as described by the manufacturer (EMD Millipore Corporation, Billerica MA). Serum IL-6, TNFα and IL-10 concentrations were measured

in serum using ELISA as previously described (15; 22) with antibody pairs supplied by BD Biosciences (Franklin Lakes, NJ, USA). The levels of detection for the IL-6, TNFα and IL-10 assays were 12, 3 and 14 pg/ml, respectively. Serum nitrite and nitrate was measured as previously described (23).

Histopathological assessment of liver pathology. The severity of NAFLD was assessed by grading formalin-fixed and H&E-stained liver sections. Steatosis and hepatocellular ballooning were scored using a scoring system based on the NASH (non-alcoholic steatohepatitis) scoring system (24). A separate score was given for steatosis (0-3) and hepatocellular ballooning (0-3). These scores were added together for an overall score (≤ 6).

Measurement of skin nitric oxide levels. Formation of NO in the skin was measured by a non-invasive *in vivo* assay using the substrate DAF-2 (applied in the form of the membrane-permeable precursor 4,5-Diaminofluorescein Diacetate, DAF-2DA; Millipore, is cleaved by intracellular esterases to generate DAF-2; this then chemically reacts with NO to form the highly fluorescent compound DAF-2T; (25)). DAF-2DA (1 µmole in an ethanol, propylene glycol and water (2:1:1, vehicle, (20)) was applied to shaved dorsal skin for absorption for 1 h prior to skin treatment with UVR and/or the topical reagent. Serial images of skin fluorescence (excitation at 488 nm, emission at 515 nm) were taken every 5 min over 20 min using the IVIS Spectrum Bioimager (Perkin Elmer).

Statistical analyses. Area under the curve (AUC) was calculated for GTT and ITT using GraphPad Prism (v5) using 0 as the baseline. Student's t-tests and Analysis of Variance (ANOVA) were used to compare treatments with Tukey post-hoc analyses. Due to a significantly greater variance in weight gain among HFD mice, the effects of vitamin D intake and UV treatment (and their

interaction) on weight gain were analysed separately to the LFD mice using SPSS (v21.0.0). Results were considered as statistically significant for p-values < 0.05.

Results

Tracking the effects of chronic UVR and dietary fat on serum 25(OH)D

To confirm our previous findings that UVR does not modify serum 25(OH)D in male mice (14), vitamin D-deficient male or female C57Bl/6 mice were exposed to a single erythemal dose (4 or 8 kJ/m^2) of UVR, and serum 25(OH)D levels tracked over 17 days. Serum 25(OH)D levels were raised in a dose-related fashion by skin exposure to erythemal UVR in female but not male mice (Supp. Fig. 1). To determine the relative roles of dietary vitamin D and/or UVR-induced vitamin D in the regulation of obesity and related cardiometabolic disease outcomes, we performed the following experiment using C57Bl/6 mice (Fig. 1). Male mice were fed a vitamin D-supplemented or non-supplemented (low fat) diet from 4 until 8 weeks of age to establish vitamin D-sufficiency or -deficiency (Fig. 2*A*). From 8 weeks of age, mice were continued on the supplemented or non-supplemented diets, but some were switched to a diet that was high in fat. Each of these four dietary treatments were further divided into three, with the shaved skin of mice chronically irradiated with no UVR, sub-erythemal UVR (1 kJ/m^2, biweekly) or erythemal UVR (4 kJ/m^2 fortnightly) as indicated in Fig. 1. Mice were treated from 8 until 20 weeks of age with these UVR and dietary interventions. A high fat diet significantly increased serum 25(OH)D levels in mice fed diets not specifically supplemented with vitamin D (HF-D$^-$, LF-D$^-$; Fig. 2*B*). Mice fed either the diets further supplemented with vitamin D (HF-D$^+$, LF-D$^+$) had significantly greater serum 25(OH)D than those mice fed the diets not supplemented with vitamin D (Fig. 2*B*). There was no additive effect of a high fat diet and vitamin D supplementation on serum 25(OH)D (Fig. 2*B*). Although not observed in our preliminary (Supp. Fig. 1) and past investigations (14), chronic sub-erythemal (Fig. 2*C*) or erythemal (Fig. 2*D*) UVR significantly (but transiently) enhanced serum 25(OH)D, when

administered to mice fed a LF-D^+ diet (but not HF-D^+, LF-D^-, or HF-D^-; see also Supp. Fig. 2). The effects were more pronounced for mice administered the chronic erythemal UVR, but returned to baseline levels after 6 weeks of UVR/dietary intervention (Fig. 2*D*; Supp. Fig. 2*B*).

Chronic UVR exposure suppressed weight gain in mice fed a vitamin D-non-supplemented diet

There was no effect of vitamin D supplementation on weight gain (Fig. 3*A*,*B*). Both chronic sub-erythemal (1 kJ/m^2 biweekly) and erythemal UVR (4 kJ/m^2 fortnightly) suppressed weight gain in mice fed the HF-D^- (Fig. 3*A*) by ≥40%. Chronic erythemal UVR also suppressed weight gain in mice fed the LF-D^- (Fig. 3*B*). The effects of chronic skin exposure to UVR were less effective for mice fed the vitamin D-supplemented diet, where UVR suppressed weight gain in a transient fashion in mice fed the HF-D^+ (Suppl. Fig. 3*A*). At the end of the UVR/dietary intervention period (12 weeks), gonadal fat pad weights were not affected by dietary vitamin D supplementation but were significantly suppressed in mice irradiated with UVR and fed the HF-D^- (Fig. 3*D*).

Chronic UVR exposure suppressed glucose intolerance and insulin resistance in mice fed a vitamin D-non-supplemented diet

After 10 and 11 weeks of UVR/dietary intervention, GTTs and ITTs were performed (Table 1). Mice fed the high fat diets developed glucose intolerance (Supp. Fig. 3*B*) and insulin resistance (Supp. Fig. 3*C*), with no suppressive effect of vitamin D supplementation (Supp. Fig. 3*B* and 3*C;* Table 1 for AUC). Both measures were suppressed in mice chronically irradiated with UVR (either sub-erythemal or erythemal) fed the HF-D^- (Table 1). Glucose intolerance was significantly suppressed by chronic sub-erythemal UVR in mice fed the HF-D^+ only (Table 1). In addition, fasting glucose and insulin levels were also reduced by either UVR treatment in mice fed the HF-D^-, with fasting leptin also suppressed in mice that were chronically irradiated with erythemal UVR

(Table 1). There were no effects of chronic UVR (or dietary vitamin D) on fasting adiponectin levels (Table 1).

Chronic erythemal UVR exposure suppressed circulating cholesterol levels in mice fed a high fat diet not supplemented with vitamin D

After 12 weeks of UVR/dietary intervention, circulating levels of triglycerides and cholesterol (HDL, LDL and total) were measured (Table 2). Triglyceride levels were not modified by vitamin D supplementation or chronic UVR (Table 2). HDL-, LDL- and total-cholesterol were suppressed in mice fed the HF-D^- also chronically irradiated with erythemal UVR (Table 2).

Chronic UVR exposure more effectively suppressed the development of non-alcoholic fatty-liver disease than vitamin D supplementation

The development of markers of NAFLD was measured by analysing the degree of liver steatosis and lobular ballooning after 12 weeks of UVR/dietary intervention (Fig. 4, Fig. 5*A*). Chronic skin exposure to UVR substantially suppressed liver histopathology in mice fed the high fat diets (Fig. 4*A*-*C* HF-D^+; Fig. 4*G*-*I* HF-D^-; Fig. 5*A*) to a greater degree than achieved by dietary vitamin D supplementation alone (Fig. 4*A* HF-D^+; Fig. 4*G* HF-D^-; Fig. 5*A*). Vitamin D supplementation had no effect on liver weight, while chronic erythemal UVR suppressed liver weight in mice fed the HF-D^- (Fig. 5*B*).

Vitamin D supplementation prevented the suppressive effects of UVR upon weight gain and markers of MetS

The results presented above suggest that many of the effects of UVR were more prominent in mice not further supplemented with vitamin D. We used a general linear model to assess whether there may be interactions within the high fat diet treatments, such that dietary vitamin D may have inhibited the suppressive ability of UVR. Significant interactions between dietary vitamin D and chronic UVR exposure were detected for weight gain (Fig. 3C; $p=0.05$), gonadal fatpad weights (Fig. 3D; $p=0.03$) and fasting glucose (Table 1, $p=0.01$), but not the other measures including liver histopathology (Fig. 4, Fig. 5*A*, $p>0.05$).

Serum vitamin D or calcium were not related to weight loss or suppression of MetS in UVR-irradiated mice

Chronic UVR exposure suppressed aspects of weight gain and measures of MetS, independently of changes to circulating 25(OH)D levels (Fig. 2, Supp. Fig. 2). Therefore it is unlikely that the mechanism through which UVR acted was dependent on vitamin D. As calcium levels can be modified by vitamin D and have been associated with weight loss (26), we also assessed circulating calcium levels after 12 weeks of UVR/dietary intervention, but observed no significant effects of dietary vitamin D or chronic skin exposure to UVR in mice fed the high fat diets (Fig. 5*C*). Chronic skin exposure to UVR reduced calcium levels in mice fed a low fat diet (Fig. 5*C*).

Circulating TNFα was linked with improved markers of NAFLD in the absence of dietary vitamin D supplementation but not skin exposure to UVR

The ability of phototherapy to suppress the development of NAFLD has been associated with reduced expression of TNFα (13). However, chronic UVR did not modify serum TNFα levels after 12 weeks of UVR/dietary intervention in mice fed a high fat diet (Fig. 5*D*). Vitamin D supplementation reduced circulating TNFα levels in mice fed a HF-D^+ when compared to those fed a HF-D^- (Fig. 5*D*). Serum levels of IL-6 and IL-10 were below the level of detection of the ELISA.

UV-induced nitric oxide suppresses the development of obesity and symptoms of MetS

A role for nitric oxide (NO), an alternate (non-vitamin D) mediator induced by UVR was examined. Skin levels of NO increased from as early as 5 min post-UVR/SNAP (Fig. 6*A*, *B*) treatment as determined using DAF-2. To examine a role for UVR-induced NO in modulating obesity and MetS symptoms, four week-old C57Bl/6 male mice were fed a LF-D^- for four weeks. From eight weeks of age, mice were either continued on this diet or switched to the HF-D^-, with HF-D^--fed mice further divided into five treatments, where their dorsal skin was treated with (i) vehicle only, (ii) sub-erythemal UVR (1 kJ/m^2) and then vehicle, (iii) SNAP, (iv) sub-erythemal UVR and then cPTIO or (v) sub-erythemal UVR and then 1,25$(OH)_2$D. This final treatment was selected to test whether active 1,25$(OH)_2$D could prevent the suppressive effects of UVR on obesity and MetS development (like dietary vitamin D in Suppl. Fig. 3*A*) through inhibition of skin-induced NO. Indeed, vitamin D may repair UV-induced DNA damage in skin by suppressing NO (27).

After 12 weeks of feeding mice the HF-D^-, skin NO levels were assessed 10 min following a final treatment with one of the five topical treatments detailed above. Skin NO levels increased with UVR or SNAP (Fig. 6*C*). The NO scavenger cPTIO reduced levels of NO in skin post-UVR

treatment but unexpectedly, 1,25$(OH)_2$D did not. Serum nitrite/nitrate concentrations, measured 20 min after the final skin treatment, were not altered by chronic low-dose UVR or SNAP (data not shown). Chronic UVR suppressed weight gain and the accumulation of WAT after 12 weeks of the HF-D^- (Fig. 6*D*). Chronic SNAP treatment also effectively suppressed mouse weights (although not weight gain) and WAT accumulation (Fig. 6*D*). However, neither the NO scavenger cPTIO, nor 1,25$(OH)_2$D reversed the suppressive effects of UVR on weight gain or WAT accumulation. Indeed the UVR and 1,25$(OH)_2$D treatment was more effective than UVR alone but this observation may reflect the hypercalcemia observed early on with topical 1,25$(OH)_2$D (4 weeks post-UVR (2.4±0.03) or -UVR+1,25$(OH)_2$D (3.5±0.07); serum calcium, *$p<0.001$). In response to these observations, we halved the dose of 1,25$(OH)_2$D administered and mice were treated only once per week from 4 weeks of intervention. Despite this change, 1,25$(OH)_2$D-treated mice were still modestly hypercalcemic at the end of the experiment (12 weeks post-UVR (2.4±0.03) or -UVR+1,25$(OH)_2$D (2.7±0.07); serum calcium, *$p<0.001$).

As observed previously, chronic UVR suppressed fasting glucose, insulin and the development of glucose intolerance and insulin resistance (Fig. 6*E*, *F*). Here, chronic SNAP also suppressed the development of insulin resistance (Fig. 6*F*). Furthermore, cPTIO treatment following UVR reversed the suppressive effects of UVR alone upon fasting glucose levels (Fig. 6*E*). Finally, both chronic UVR and SNAP suppressed the development of NAFLD, while cPTIO reversed the effects of UVR upon liver histopathology (Fig. 6*G*). Cumulatively, these data suggest that UVR-induced NO may play an important role in modulating the development of obesity and MetS through effects on weight, WAT accumulation, fasting glucose, the development of insulin resistance and NAFLD.

Discussion

Here we present evidence that chronic skin exposure to low (sub-erythemal) and high (erythemal) dose UVR suppresses the development of obesity and measures of MetS in mice fed a high fat diet. Vitamin D supplementation alone did not reproduce these effects. In addition, the suppressive effects of UVR on obesity and MetS development were not observed to the same degree in mice that were further supplemented with vitamin D (HF-D^+). For mice fed a high fat diet, serum 25(OH)D levels were not enhanced by chronic UVR exposure, suggesting that any effects induced by UVR in these mice were independent of circulating 25(OH)D levels. The HF-D^- increased circulating 25(OH)D; it is likely that this diet contains vitamin D, perhaps within the lard-derived fat fraction. Supplementation of this diet with vitamin D (i.e., the HF-D^+) further increased serum 25(OH)D levels. Both UV irradiation and vitamin D supplementation reduced the severity of NAFLD, suggesting that vitamin D can recapitulate the effects of UVR for the prevention of certain obesity-related pathologies. We also showed that some of the effects of UVR may occur through NO production. In particular, it is likely that UVR-induced NO may have profound effects on the development of NAFLD, as topical SNAP suppressed liver pathology, and cPTIO antagonised the effects of UVR. Various non-vitamin D immunomodulators induced by UVR, like NO (28), may be important for regulation of immunity (29) and obesity/MetS development (30). Skin exposure to UVR releases NO from skin (28) and could control obesity through NO-dependent effects on mitochondria biogenesis within brown adipose tissue (31). We have recently shown that UVR-induced NO reduces blood pressure in healthy human volunteers (28). NO may also be a crucial modulator of insulin and glucose transport, and inhibition of NO may cause insulin resistance (32). Combined with our results, these studies point to topically-induced NO as a potentially important clinical means to suppress obesity and type-2 diabetes development.

The capacity of chronic UVR to suppress the development of obesity and metrics of MetS was less effective in mice orally supplemented with vitamin D (but not topical $1,25(OH)_2D$). This was an

unexpected finding but could be explained by potential interactions of UVR-induced mediators and dietary vitamin D, including NO (27). The different effects of dietary vitamin D and topical $1,25(OH)_2D$ could be accounted for by the hypercalcemia induced by chronic topical $1,25(OH)_2D$. In addition, after 12 weeks of treatment, serum 25(OH)D levels were significantly reduced by topical $1,25(OH)_2D$ but not the other treatments (data not shown). Others have also observed that vitamin D suppressed weight gain *in vivo* following intraperitoneal injections of $1,25(OH)_2D$ (5 µg/kg every two days), although the effects on circulating calcium (and 25(OH)D) were not reported (33). Others have shown that UVR may increase cortisol production in skin, which has potential to impact the hypothalamic-pituitary-adrenal axis (34). While this might be hypothesised to alter physical activity, no obvious behavioural effects were observed in this study. However, we cannot exclude the possibility that UVR alters neuroendocrine signalling networks in the skin (35) that might have systemic impact.

Nakano *et al* showed that phototherapy suppressed NAFLD but failed to reduce obesity, steatosis and blood glucose levels Zucker *fa-fa* rats (13). These results may differ from our own through significant differences in the phototherapies delivered and the mouse model of obesity. Dietary vitamin D has also previously been shown to suppress development of NAFLD in Sprague-Dawley rats fed a 'westernised' (high fat/fructose) diet (36) and Lewis rats a choline-deficient and iron-supplemented L-amino acid-defined (CDAA) diet (13). We also observed that dietary vitamin D suppressed circulating TNFα levels in mice fed a high fat diet. UVR did not suppress serum TNFα, suggesting that dietary vitamin D and UVR may suppress NAFLD through differing mechanisms. For control of NAFLD, the role of other players within the vitamin D pathway is worthy of further consideration. For example, circulating levels of the vitamin D binding protein (GC) inversely correlate with liver steatosis, and may determine the ability of vitamin D to modulate the development of NAFLD (37). In addition, $1,25(OH)_2D$ may act through the VDR to improve insulin sensitivity (38).

Our observations suggest that not all of the effects of UVR on disease prevention can be achieved through dietary vitamin D and that role of other UV-induced mediators like NO deserve further consideration. Furthermore, by using a mouse modelling approach we were able to remove the confounding effects of activity out-of-doors, which might explain the observed associations of reduced obesity and increased serum 25(OH)D. A caveat is that while mice have conserved the ability to synthesize vitamin D and NO in the skin and systemically post-UVR, as fur-covered nocturnal animals they are not usually exposed to much sunlight. Further studies are required to translate the findings of our murine studies for humans. However, our results support recent calls for clinical trials that test the efficacy of skin exposure to sunlight or UVR for the control of chronic diseases like multiple sclerosis (39) and depression (40) that, like obesity and MetS, may take years to develop. In conclusion, our studies show that chronic low dose sunlight exposure may be an effective means of suppressing obesity and MetS in mice fed a high fat diet, through pathways that are independent of vitamin D and at least partially dependent on skin-derived NO.

Author Contributions

S. Geldenhuys performed the majority of the experiments and statistical analyses, and reviewed/edited the manuscript. P. H. contributed to the discussion and reviewed/edited the manuscript. R.E. helped optimize the skin nitric oxide assay and reviewed/edited the manuscript. P. J. provided the statistical expertise for the experimental design and data analysis. M. F. helped design the study, supervised the analysis of serum nitric oxide metabolites and reviewed/edited the manuscript. R. B. W. helped design the study, and reviewed/edited the manuscript. V. M. helped design the study, contributed to the discussion and reviewed/edited the manuscript. S. Gorman envisaged and designed the study, and wrote the manuscript. The authors report no conflicts of interest.

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References

1. Holick MF: Vitamin D deficiency in 2010: health benefits of vitamin D and sunlight: a D-bate. Nat Rev Endocrinol 2011;7:73-75

2. Earthman CP, Beckman LM, Masodkar K, Sibley SD: The link between obesity and low circulating 25-hydroxyvitamin D concentrations: considerations and implications. Int J Obes (Lond) 2012;36:387-396

3. Autier P, Boniol M, Pizot C, Mullie P: Vitamin D status and ill health: a systematic review. The Lancet - Diabetes-Endocrinology 2014;ePub Dec 6 2013

4. Daly RM, Gagnon C, Lu ZX, Magliano DJ, Dunstan DW, Sikaris KA, Zimmet PZ, Ebeling PR, Shaw JE: Prevalence of vitamin D deficiency and its determinants in Australian adults aged 25 years and older: a national, population-based study. Clin Endocrinol (Oxf) 2012;77:26-35

5. Vimaleswaran KS, Berry DJ, Lu C, Tikkanen E, Pilz S, Hiraki LT, Cooper JD, Dastani Z, Li R, Houston DK, Wood AR, Michaelsson K, Vandenput L, Zgaga L, Yerges-Armstrong LM, McCarthy MI, Dupuis J, Kaakinen M, Kleber ME, Jameson K, Arden N, Raitakari O, Viikari J, Lohman KK, Ferrucci L, Melhus H, Ingelsson E, Byberg L, Lind L, Lorentzon M, Salomaa V, Campbell H, Dunlop M, Mitchell BD, Herzig KH, Pouta A, Hartikainen AL, Streeten EA, Theodoratou E, Jula A, Wareham NJ, Ohlsson C, Frayling TM, Kritchevsky SB, Spector TD, Richards JB, Lehtimaki T, Ouwehand WH, Kraft P, Cooper C, Marz W, Power C, Loos RJ, Wang TJ, Jarvelin MR, Whittaker JC, Hingorani AD, Hypponen E: Causal relationship between obesity and vitamin D status: bi-directional Mendelian randomization analysis of multiple cohorts. PLoS Med 2013;10:e1001383

6. Lavie CJ, Lee JH, Milani RV: Vitamin D and cardiovascular disease will it live up to its hype? J Am Coll Cardiol 2011;58:1547-1556

7. Maxwell CS, Wood RJ: Update on vitamin D and type 2 diabetes. Nutr Rev 2011;69:291-295

8. Brouwer DA, van Beek J, Ferwerda H, Brugman AM, van der Klis FR, van der Heiden HJ, Muskiet FA: Rat adipose tissue rapidly accumulates and slowly releases an orally-administered high vitamin D dose. Br J Nutr 1998;79:527-532

9. Kull M, Kallikorm R, Lember M: Body mass index determines sunbathing habits: implications on vitamin D levels. Intern Med J 2009;39:256-258

10. Lucas RM, Ponsonby AL, Dear K, Valery P, Taylor BV, van der Mei I, McMichael AJ, Pender MP, Chapman C, Coulthard A, Kilpatrick T, Stankovich J, Williams D, Dwyer T: Vitamin D status: multifactorial contribution of environment, genes and other factors in healthy Australian adults across a latitude gradient. J Steroid Biochem Mol Biol 2013;136:300-308

11. Ginde AA, Liu MC, Camargo CA, Jr.: Demographic differences and trends of vitamin D insufficiency in the US population, 1988-2004. Arch Intern Med 2009;169:626-632

12. Lindqvist PG, Olsson H, Landin-Olsson M: Are active sun exposure habits related to lowering risk of type 2 diabetes mellitus in women, a prospective cohort study? Diabetes Res Clin Pract 2010;90:109-114

13. Nakano T, Cheng YF, Lai CY, Hsu LW, Chang YC, Deng JY, Huang YZ, Honda H, Chen KD, Wang CC, Chiu KW, Jawan B, Eng HL, Goto S, Chen CL: Impact of artificial sunlight therapy on the progress of non-alcoholic fatty liver disease in rats. J Hepatol 2011;55:415-425

14. Gorman S, Scott NM, Tan DH, Weeden CE, Tuckey RC, Bisley JL, Grimbaldeston MA, Hart PH: Acute erythemal ultraviolet radiation causes systemic immunosuppression in the absence of increased 25-hydroxyvitamin D3 levels in male mice. PLoS One 2012;7:e46006

15. McGlade JP, Gorman S, Zosky GR, Larcombe AN, Sly PD, Finlay-Jones JJ, Turner DJ, Hart PH: Suppression of the asthmatic phenotype by ultraviolet B-induced, antigen-specific regulatory cells. Clin Exp Allergy 2007;37:1267-1276

16. McGlade JP, Gorman S, Lenzo JC, Tan JW, Watanabe T, Finlay-Jones JJ, Thomas WR, Hart PH: Effect of both ultraviolet B irradiation and histamine receptor function on allergic responses to an inhaled antigen. J Immunol 2007;178:2794-2802

17. Ikeyama K, Fuziwara S, Denda M: Topical application of neuronal nitric oxide synthase inhibitor accelerates cutaneous barrier recovery and prevents epidermal hyperplasia induced by barrier disruption. J Invest Dermatol 2007;127:1713-1719

18. Yasukawa K, Tokuda H, Tun X, Utsumi H, Yamada K: The detrimental effect of nitric oxide on tissue is associated with inflammatory events in the vascular endothelium and neutrophils in mice with dextran sodium sulfate-induced colitis. Free Radic Res 2012;46:1427-1436
19. Dixon KM, Norman AW, Sequeira VB, Mohan R, Rybchyn MS, Reeve VE, Halliday GM, Mason RS: 1alpha,25(OH)(2)-vitamin D and a nongenomic vitamin D analogue inhibit ultraviolet radiation-induced skin carcinogenesis. Cancer Prev Res (Phila) 2011;4:1485-1494
20. Gorman S, Kuritzky LA, Judge MA, Dixon KM, McGlade JP, Mason RS, Finlay-Jones JJ, Hart PH: Topically applied 1,25-dihydroxyvitamin D3 enhances the suppressive activity of CD4+CD25+ cells in the draining lymph nodes. J Immunol 2007;179:6273-6283
21. Clarke MW, Tuckey RC, Gorman S, Holt B, Hart PH: Optimized 25 hydroxy vitamin D analysis using liquid-liquid extraction with 2D separation with LCMS/MS detection, provides superior precision compared to conventional assays. Metabolomics 2013;Accepted 14th March 2013
22. Gorman S, Judge MA, Hart PH: Immune-modifying properties of topical vitamin D: Focus on dendritic cells and T cells. J Steroid Biochem Mol Biol 2010;121:247-249
23. Milsom AB, Fernandez BO, Garcia-Saura MF, Rodriguez J, Feelisch M: Contributions of nitric oxide synthases, dietary nitrite/nitrate, and other sources to the formation of NO signaling products. Antioxid Redox Signal 2012;17:422-432
24. Kleiner DE, Brunt EM, Van Natta M, Behling C, Contos MJ, Cummings OW, Ferrell LD, Liu YC, Torbenson MS, Unalp-Arida A, Yeh M, McCullough AJ, Sanyal AJ: Design and validation of a histological scoring system for nonalcoholic fatty liver disease. Hepatology 2005;41:1313-1321
25. Rodriguez J, Specian V, Maloney R, Jourd'heuil D, Feelisch M: Performance of diamino fluorophores for the localization of sources and targets of nitric oxide. Free radical biology & medicine 2005;38:356-368
26. Zemel MB, Shi H, Greer B, Dirienzo D, Zemel PC: Regulation of adiposity by dietary calcium. FASEB J 2000;14:1132-1138
27. Mason RS, Sequeira VB, Dixon KM, Gordon-Thomson C, Pobre K, Dilley A, Mizwicki MT, Norman AW, Feldman D, Halliday GM, Reeve VE: Photoprotection by 1alpha,25-dihydroxyvitamin D and analogs: further studies on mechanisms and implications for UV-damage. J Steroid Biochem Mol Biol 2010;121:164-168
28. Liu D, Fernandez BO, Hamilton MB, Lang NN, Gallagher JMC, Newby DE, Feelisch M, Weller RB: UVA Irradiation of Human Skin Vasodilates Arterial Vasculature and Lowers Blood Pressure Independently of Nitric Oxide Synthase. J Invest Dermatol 2014;ePub Jan 20 2014
29. Zosky GR, Berry LJ, Elliot JG, James AL, Gorman S, Hart PH: Vitamin D deficiency causes deficits in lung function and alters lung structure. Am J Respir Crit Care Med 2011;183:1336-1343
30. Sessa WC: A new approach to weight loss: just activate endothelial NO synthase! Circulation research 2012;111:1111-1112
31. Knott AB, Bossy-Wetzel E: Impact of nitric oxide on metabolism in health and age-related disease. Diabetes Obes Metab 2010;12 Suppl 2:126-133
32. Sydow K, Mondon CE, Cooke JP: Insulin resistance: potential role of the endogenous nitric oxide synthase inhibitor ADMA. Vasc Med 2005;10 Suppl 1:S35-43
33. Yin Y, Yu Z, Xia M, Luo X, Lu X, Ling W: Vitamin D attenuates high fat diet-induced hepatic steatosis in rats by modulating lipid metabolism. Eur J Clin Invest 2012;42:1189-1196
34. Skobowiat C, Sayre RM, Dowdy JC, Slominski AT: Ultraviolet radiation regulates cortisol activity in a waveband-dependent manner in human skin ex vivo. The British journal of dermatology 2013;168:595-601
35. Zmijewski MA, Slominski AT: Neuroendocrinology of the skin: An overview and selective analysis. Dermato-endocrinology 2011;3:3-10
36. Roth CL, Elfers CT, Figlewicz DP, Melhorn SJ, Morton GJ, Hoofnagle A, Yeh MM, Nelson JE, Kowdley KV: Vitamin D deficiency in obese rats exacerbates nonalcoholic fatty liver disease and increases hepatic resistin and Toll-like receptor activation. Hepatology 2012;55:1103-1111

37. Adams LA, White SW, Marsh JA, Lye SJ, Connor KL, Maganga R, Ayonrinde OT, Olynyk JK, Mori TA, Beilin LJ, Palmer LJ, Hamdorf JM, Pennell CE: Association between liver-specific gene polymorphisms and their expression levels with nonalcoholic fatty liver disease. Hepatology 2013;57:590-600
38. Takiishi T, Gysemans C, Bouillon R, Mathieu C: Vitamin D and Diabetes. Endocrinol Metab Clin N Am 2010;39:419–446
39. Correale J, Farez MF: Modulation of multiple sclerosis by sunlight exposure: role of cis-urocanic acid. J Neuroimmunol 2013;261:134-140
40. Knippenberg S, Damoiseaux J, Bol Y, Hupperts R, Taylor BV, Ponsonby AL, Dwyer T, Simpson S, van der Mei IA: Higher levels of reported sun exposure, and not vitamin D status, are associated with less depressive symptoms and fatigue in multiple sclerosis. Acta Neurol Scand 2014;129:123-131

Table 1: Area under the curve values for glucose and insulin tolerance tests (GTT, ITT) and fasting glucose, insulin, leptin and adiponectin levels measured 9-11 weeks post-UVR/dietary intervention.

Treatment	Diet	UVR	GTT (AUC, %basal glucose)	ITT (AUC, %basal glucose)	Fasting glucose (mM)	Fasting insulin (ng/ml)	Fasting leptin (ng/ml)	Fasting adiponectin (ng/ml)
1	HF-D^+	0	2190±83	1200±63	9.8±0.5	8.2±3.5	36.7±3.0	10.4±0.3
2	HF-D^+	1 kJ/m^2	**1770±49****	1060±46	8.8±0.4	7.1±0.4	29.8±5.7	11.9±1.8
3	HF-D^+	4 kJ/m^2	1880±180	1370±34	10.2±0.4	3.6±1.1	19.7±7.3	15.8±3.9
4	LF-D^+	0	1470±67	800±38	7.9±0.3	1.0±0.4	1.5±0.6	12.9±2.8
5	LF-D^+	1 kJ/m^2	1510±65	760±37	8.0±0.4	4.9±2.8	2.6±1.1	8.8±2.5
6	LF-D^+	4 kJ/m^2	1390±56	770±79	7.8±0.4	1.8±1.0	2.2±0.7	11.9±1.0
7	HF-D^-	0	2120±130	1230±15	9.8±0.3	11.1±1.9	29.8±3.5	13.0±2.6
8	HF-D^-	1 kJ/m^2	**1760±65***	**1050±43***	**8.7±0.3***	**3.8±1.1***	32.6±5.6	11.3±0.9
9	HF-D^-	4 kJ/m^2	**1690±73***	**960±72***	**8.1±0.4***	**3.9±2.8***	**14.0±5.3***	13.0±1.1
10	LF-D^-	0	1260±51	680±48	6.3±0.2	3.4±1.6	5.9±2.5	16.6±6.2
11	LF-D^-	1 kJ/m^2	1280±102	600±27	6.0±0.2	1.6±1.1	1.0±0.5	10.8±0.6
12	LF-D^-	4 kJ/m^2	1480±36	760±60	7.7±0.4	4.3±1.8	1.9±0.2	11.7±1.9

(n=4-8 mice/treatment, *$p<0.05$ relative to no UVR HF-D^-; **$p<0.05$ relative to no UVR HF-D^+ with data representative of two experiments)

Table 2: Circulating triglyceride and cholesterol levels at 12 weeks post-dietary and UVR-intervention.

Treatment	Diet	UVR	Triglyceride (mM)	HDL-cholesterol (mM)	LDL-cholesterol (mM)	Total-cholesterol (mM)
1	HF-D^+	0	0.7±0.1	2.1±0.2	0.3±0.0	4.2±0.4
2	HF-D^+	1 kJ/m^2	0.6±0.0	2.0±0.2	0.2±0.0	3.8±0.4
3	HF-D^+	4 kJ/m^2	0.8±0.1	2.1±0.1	0.2±0.0	4.3±0.2
4	LF-D^+	0	1.0±0.1	1.5±0.1	0.2±0.0	2.5±0.2
5	LF-D^+	1 kJ/m^2	1.2±0.1	1.8±0.1	0.2±0.0	2.9±0.1
6	LF-D^+	4 kJ/m^2	1.1±0.3	1.3±0.2	0.1±0.0	2.2±0.3
7	HF-D^-	0	0.9±0.1	2.1±0.1	0.4±0.0	4.3±0.1
8	HF-D^-	1 kJ/m^2	0.6±0.0	2.1±0.0	0.3±0.0	4.2±0.2
9	HF-D^-	4 kJ/m^2	0.9±0.1	**1.5±0.2***	**0.2±0.0***	**2.6±0.3***
10	LF-D^-	0	1.2±0.1	1.6±0.3	0.1±0.0	2.4±0.4
11	LF-D^-	1 kJ/m^2	0.9±0.1	1.4±0.1	0.1±0.0	2.0±0.1
12	LF-D^-	4 kJ/m^2	1.1±0.1	1.5±0.1	0.1±0.0	2.3±0.1

(n=4 mice/treatment, *$p<0.05$ relative to no UVR HF-D^- with data a representative of two experiments)

Figure legends

Supplementary Figure 1: *Erythemal UVR increased serum 25(OH)D levels in initially vitamin D-deficient female but not male mice.* Four week-old C57Bl/6 mice were fed a low fat diet not supplemented with vitamin D for four weeks to reduce serum 25(OH)D levels to less than 20 nmol.L^{-1} (dotted line). Serum 25(OH)D levels were measured following the exposure of the shaved dorsal skin of the initially vitamin D-deficient mice to 4 or 8 kJ/m^2 UVR. Data is shown as mean ± SEM (n=4-8 mice/treatment/time point, *$p<0.05$ relative to initial serum 25(OH)D levels).

Figure 1: *The experimental approach.* Four week-old C57Bl/6 male mice were fed a low fat diet supplemented with vitamin D (LF-D^+) or not (LF-D^-) for four weeks. At eight weeks of age, mice were either continued on these diets or switched to a diet that was high in fat and supplemented (HF-D^+) or not (HF-D^-) with vitamin D. At the same time, each dietary group was further divided into three treatments of mice that were chronically irradiated with sub-erythemal UVR (biweekly, 1 kJ/m^2), erythemal UVR (fortnightly, 4 kJ/m^2) or no UVR. Mice were fed these diets and irradiated with these UVR regimens for a further 12 weeks until mice were twenty weeks of age. There were a total of 12 treatments, with 18 mice per treatment. The experiment was performed two times.

Figure 2: *The effects of chronic skin exposure to UVR, dietary vitamin D and a high fat diet on serum 25(OH)D.* In (A), four week-old C57Bl/6 male mice were fed a low fat diet supplemented with vitamin D (LF-D^+) or not (LF-D^-) for four weeks. At eight weeks of age (week 0 for Fig. 2*B – D*), mice were either continued on these diets or switched to a diet that was high in fat and supplemented (HF-D^+) or not (HF-D^-) with vitamin D. At the same time, each dietary group was further divided into three treatments of mice that were chronically irradiated with (B) no UVR, (C) sub-erythemal UVR (biweekly, 1 kJ/m^2), or (D) erythemal UVR (fortnightly, 4 kJ/m^2) for a further 12 weeks. In (B) – (D), serum 25(OH)D levels are depicted for mice that underwent these

UVR/dietary interventions for 12 weeks. Data are shown as mean ± SEM for n=4-9 mice at each time, pooled from two independent experiments (*p<0.05).

Supplementary Figure 2: *The effects of chronic skin exposure to UVR, dietary vitamin D and a high fat diet on serum 25(OH)D.* Data from Figure 2 are shown in an alternate fashion, comparing the effects of no UVR, or, chronic skin exposure to sub-erythemal UVR (biweekly, 1 kJ/m^2) or erythemal UVR (fortnightly, 4 kJ/m^2) on serum levels of 25(OH)D for mice fed a (A) high (HF-D$^+$) or (B) low fat (LF-D$^+$) diet supplemented with vitamin D, or, (C) high (HF-D$^-$) or (D) low fat (LF-D$^-$) diet not supplemented with vitamin D. Data are shown as mean ± SEM for n=4-9 mice at each time, pooled from two independent experiments (*p<0.05).

Figure 3: *Chronic UVR suppressed weight gain in mice fed high or low fat diets not supplemented with vitamin D.* Four week-old C57Bl/6 male mice were fed a low fat diet supplemented with vitamin D (LF-D$^+$) or not (LF-D$^-$) with vitamin D for four weeks. At eight weeks of age (week 0 for Fig. 3*A – B*), mice were either continued on these diets or switched to a diet that was high in fat and supplemented (HF-D$^+$) or not (HF-D$^-$) with vitamin D. At the same time, each dietary group was further divided into three treatments of mice that were chronically irradiated with no UVR, sub-erythemal UVR (biweekly, 1 kJ/m^2), or erythemal UVR (fortnightly, 4 kJ/m^2). In (A) – (B), percentage weight gain is shown for mice that underwent these UVR/dietary interventions for 12 weeks (until 20 weeks of age) for mice fed a (A) high fat or (B) low fat diet. Data are shown as mean ± SEM for n=18 mice/treatment from a representative of two independent experiments. In (C) total weight gain after 12 weeks of these UVR/dietary interventions (at 20 weeks of age) is shown for all treatments (mean + SEM). In (D), after 12 weeks of these UVR/dietary interventions (at 20 weeks of age) gonadal fatpad (n=18/treatment) weights were measured. Data are representative of two independent experiments (mean + SEM) *p<0.05).

Supplementary Figure 3: *Dietary vitamin D did not affect weight gain or the development of glucose tolerance or insulin resistance for mice fed a high or low fat diet.* Four week-old C57Bl/6 male mice were fed a low fat diet supplemented with vitamin D (LF-D^+) or not (LF-D^-) for four weeks. At eight weeks of age (week 0 for Suppl. Fig. 3*A*), mice were either continued on these diets or switched to a diet that was high in fat and supplemented (HF-D^+) or not (HF-D^-) with vitamin D. At the same time, each dietary group was further divided into three treatments of mice that were chronically irradiated with no UVR, sub-erythemal UVR (biweekly, 1 kJ/m^2), or erythemal UVR (fortnightly, 4 kJ/m^2) for 12 weeks until 20 weeks of age. In (A), percentage weight gain is shown (n=18 mice/treatment) for mice fed the HF-D^+ or LF-D^+. In (B), a glucose tolerance test was performed after 10 weeks of the UVR/dietary interventions (at 18 weeks of age, n=8 mice/treatment), with data shown for non-irradiated mice. In (C), an insulin tolerance test was performed after 11 weeks of the UVR/dietary interventions (at 19 weeks of age, n=8 mice/treatment) with data shown for non-irradiated mice. Data are shown as mean ± SEM from a representative of two independent experiments (*$p<0.05$).

Figure 4: *Chronic UVR significantly reduced the extent of liver steatosis and lobular ballooning in mice fed a high fat diet.* Four week-old C57Bl/6 male mice were fed a low fat diet supplemented with vitamin D (LF-D^+) or not (LF-D^-) for four weeks. At eight weeks of age, mice were either continued on these diets or switched to a diet that was high in fat and supplemented (HF-D^+) or not (HF-D^-) with vitamin D. At the same time, each dietary group was further divided into three treatments of mice that were chronically irradiated with no UVR (A, D, G, J), sub-erythemal UVR (biweekly, 1 kJ/m^2; B, E, H, K), or erythemal UVR (fortnightly, 4 kJ/m^2; C, F, I, L). After 12 weeks of these UVR/dietary interventions (at 20 weeks of age), the extent of liver histopathology was measured in liver specimens (n=10/treatment for data pooled from two independent experiments). Shown in A-L are representative H&E-stained sections of liver for each treatment

(20xmagnification with the scales in B and C equivalent to 150 μm). Examples of liver steatosis (blue arrow) and lobular ballooning (red arrow) are shown in section (G).

Figure 5: *Chronic UVR significantly reduced the extent of liver histopathology in mice fed a high fat diet.* Four week-old C57Bl/6 male mice were fed a low fat diet supplemented with vitamin D (LF-D^+) or not (LF-D^-) for four weeks. At eight weeks of age, mice were either continued on these diets or switched to a diet that was high in fat and supplemented (HF-D^+) or not (HF-D^-) with vitamin D. At the same time, each dietary group was further divided into three treatments of mice that were chronically irradiated with no UVR, sub-erythemal UVR (biweekly, 1 kJ/m^2), or erythemal UVR (fortnightly, 4 kJ/m^2). After 12 weeks of these UVR/dietary interventions (at 20 weeks of age), the (A) extent of liver histopathology (n=10/treatment for data pooled from two independent experiments), (B) liver weights (n=18/treatment for data from a representative experiment) and serum levels serum levels of (C) calcium (n=4-8/treatment for data pooled from two independent experiments) and (D) TNFα (n=12-18/treatment for data pooled from two independent experiments) are shown. Data are shown as mean + SEM (*$p<0.05$).

Figure 6: *The UVR-induced mediator, nitric oxide may regulate body weight, WAT accumulation, glucose metabolism and the development of NAFLD in mice fed a high fat diet.* In (A) and (B), using the DAF-2DA substrate, skin nitric oxide levels are shown for adult C57Bl/6 male mice fed a low fat diet not supplemented with vitamin D (LF-D^-), 5 min following skin treatment with vehicle, 1 kJ/m^2 UVR or the nitric oxide donor, SNAP; with a quantitative measure (photons/sec) shown in (A) and representative skin fluorescence shown in (B). Four week-old C57Bl/6 male mice were fed a LF-D^- for four weeks. At eight weeks of age, mice were either continued on these diets or switched to a diet that was high in fat, not supplemented with vitamin D (HF-D^-). Within the HF-D^- treatments, mice were further divided into five treatments. The shaved dorsal skin of these mice were; (i) treated with vehicle only, (ii) chronically irradiated with sub-erythemal UVR (biweekly, 1

kJ/m^2) and then vehicle, (iii) topically treated with SNAP, (iv) chronically irradiated with sub-erythemal UVR and then cPTIO or (v) chronically irradiated with sub-erythemal UVR and then $1,25(OH)_2D$. Mice were treated for 12 weeks with these skin/dietary interventions until 20 weeks of age. In (C), skin NO levels, 10 min post-skin treatment (n=8 mice/treatment); in (D), mouse weights, weight gain and WAT (WAT) weights (n=18 mice/treatment); in (E), fasting glucose and GTT AUC (area under the curve, n=8 mice/treatment); in (F), fasting insulin and ITT AUC (n=8 mice/treatment; and in (G), liver histopathology scores (n=8 mice/treatment). Data are shown as mean + SEM from one experiment (*$p<0.05$).

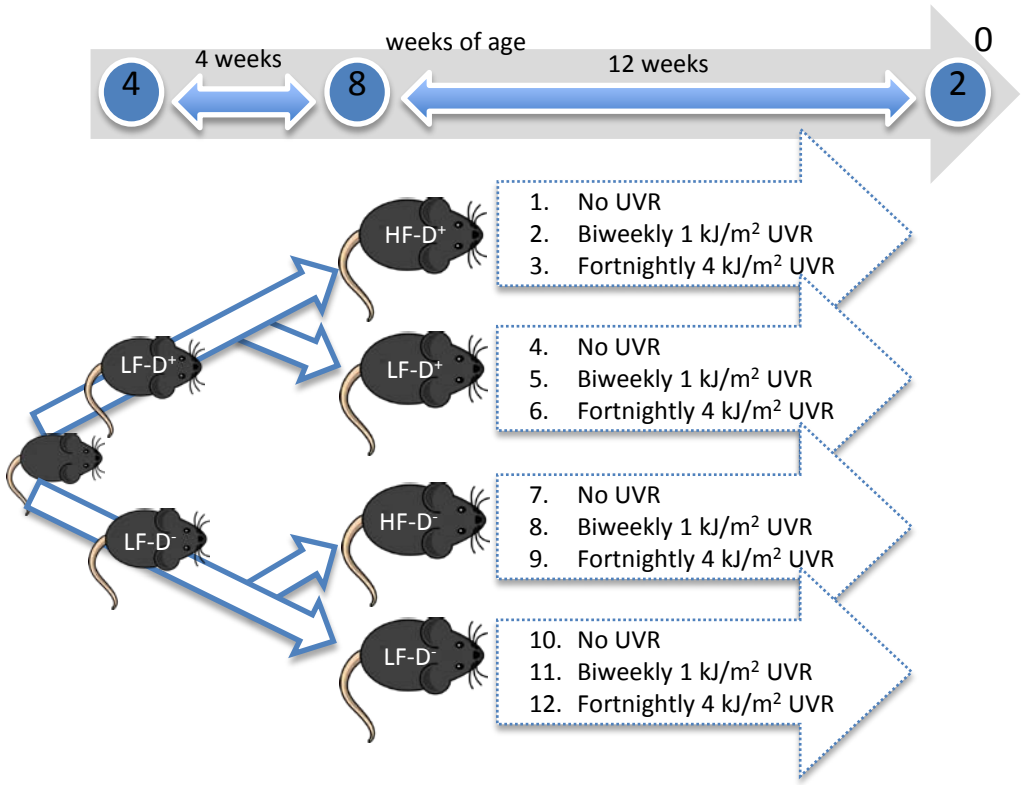

4
4 weeks
8
weeks of age
12 weeks
2
0
HF-D+
LF-D+
LF-D+
HF-D-
LF-D-
LF-D-
1. No UVR
2. Biweekly 1 kJ/m² UVR
3. Fortnightly 4 kJ/m² UVR
4. No UVR
5. Biweekly 1 kJ/m² UVR
6. Fortnightly 4 kJ/m² UVR
7. No UVR
8. Biweekly 1 kJ/m² UVR
9. Fortnightly 4 kJ/m² UVR
10. No UVR
11. Biweekly 1 kJ/m² UVR
12. Fortnightly 4 kJ/m² UVR

Figure 1

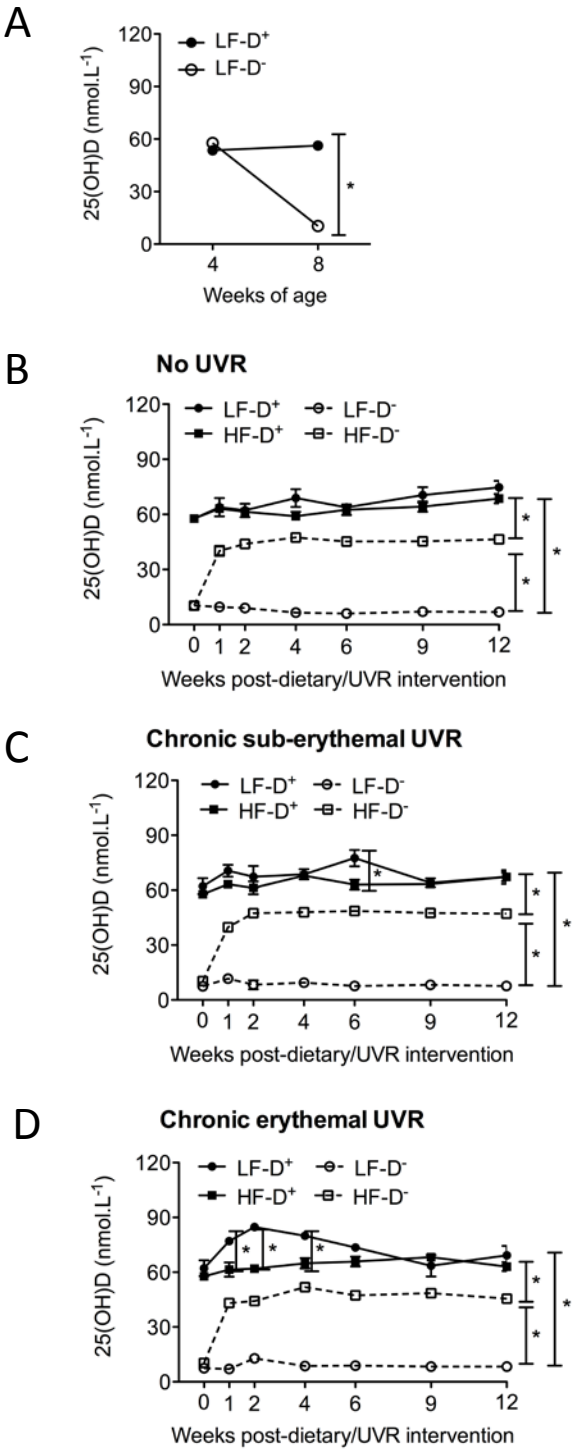

A
LF-D+
LF-D-
25(OH)D (nmol.L-1)
120
90
60
30
0
4
8
Weeks of age
*
B
No UVR
LF-D+
LF-D-
HF-D+
HF-D-
25(OH)D (nmol.L-1)
0 1 2 4 6 9 12
Weeks post-dietary/UVR intervention
C
Chronic sub-erythemal UVR
D
Chronic erythemal UVR

Figure 2

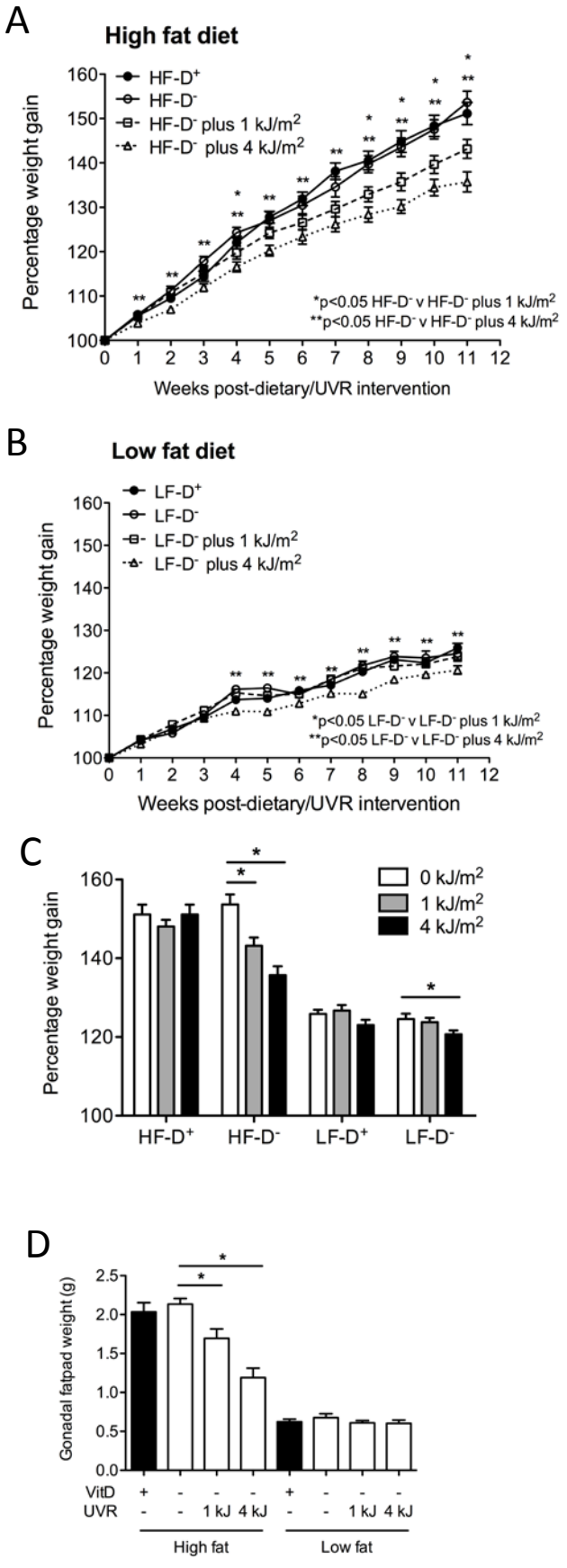

A
High fat diet
HF-D+
HF-D-
HF-D- plus 1 kJ/m2
HF-D- plus 4 kJ/m2
Percentage weight gain
*p<0.05 HF-D- v HF-D- plus 1 kJ/m2
**p<0.05 HF-D- v HF-D- plus 4 kJ/m2
Weeks post-dietary/UVR intervention
B
Low fat diet
LF-D+
LF-D-
LF-D- plus 1 kJ/m2
LF-D- plus 4 kJ/m2
Percentage weight gain
*p<0.05 LF-D- v LF-D- plus 1 kJ/m2
**p<0.05 LF-D- v LF-D- plus 4 kJ/m2
Weeks post-dietary/UVR intervention
C
0 kJ/m2
1 kJ/m2
4 kJ/m2
Percentage weight gain
HF-D+
HF-D-
LF-D+
LF-D-
D
Gonadal fatpad weight (g)
VitD
UVR
1 kJ
4 kJ
High fat
Low fat

Figure 3

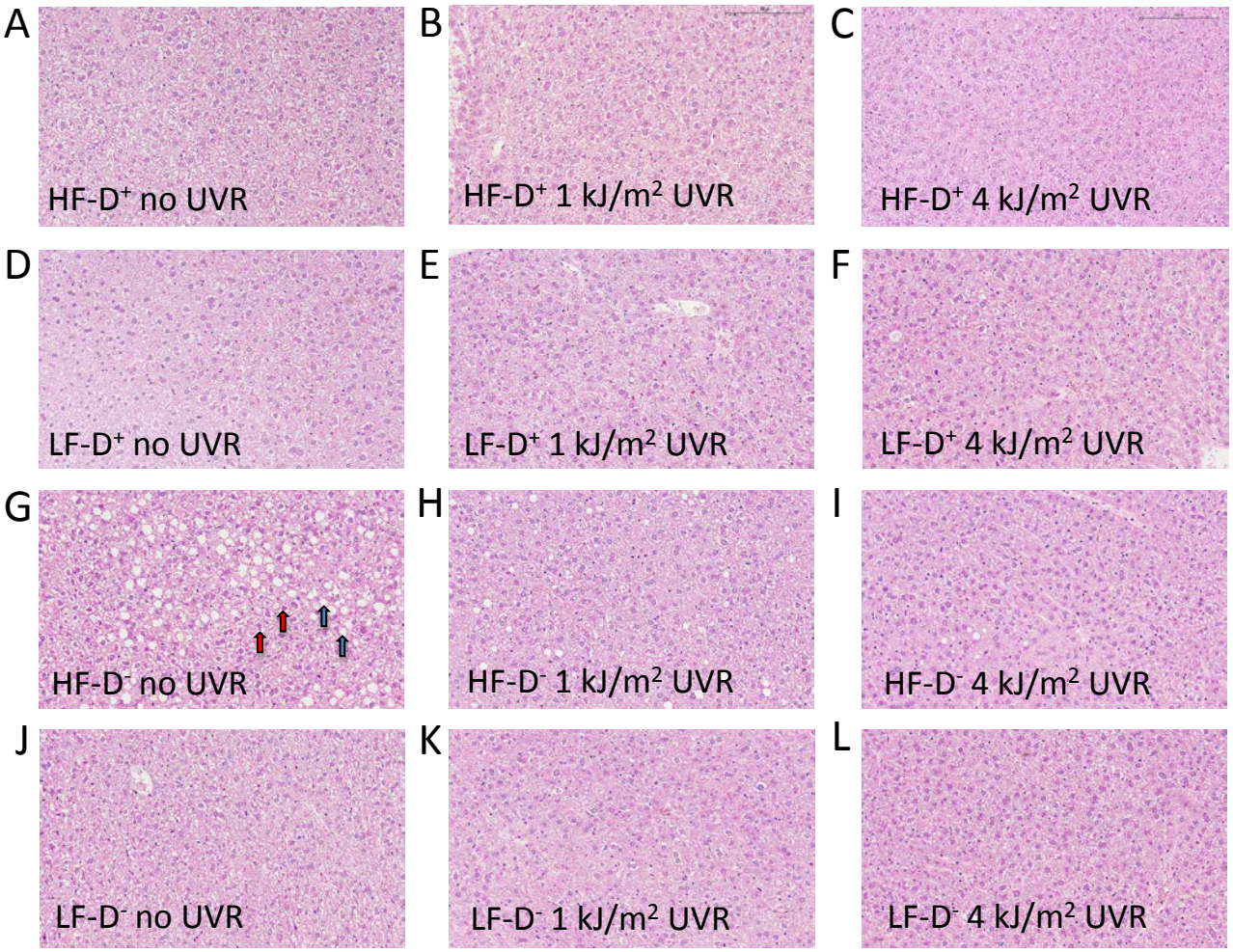

A
HF-D+ no UVR
B
HF-D+ 1 kJ/m2 UVR
C
HF-D+ 4 kJ/m2 UVR
D
LF-D+ no UVR
E
LF-D+ 1 kJ/m2 UVR
F
LF-D+ 4 kJ/m2 UVR
G
HF-D- no UVR
H
HF-D- 1 kJ/m2 UVR
I
HF-D- 4 kJ/m2 UVR
J
LF-D- no UVR
K
LF-D- 1 kJ/m2 UVR
L
LF-D- 4 kJ/m2 UVR

Figure 4

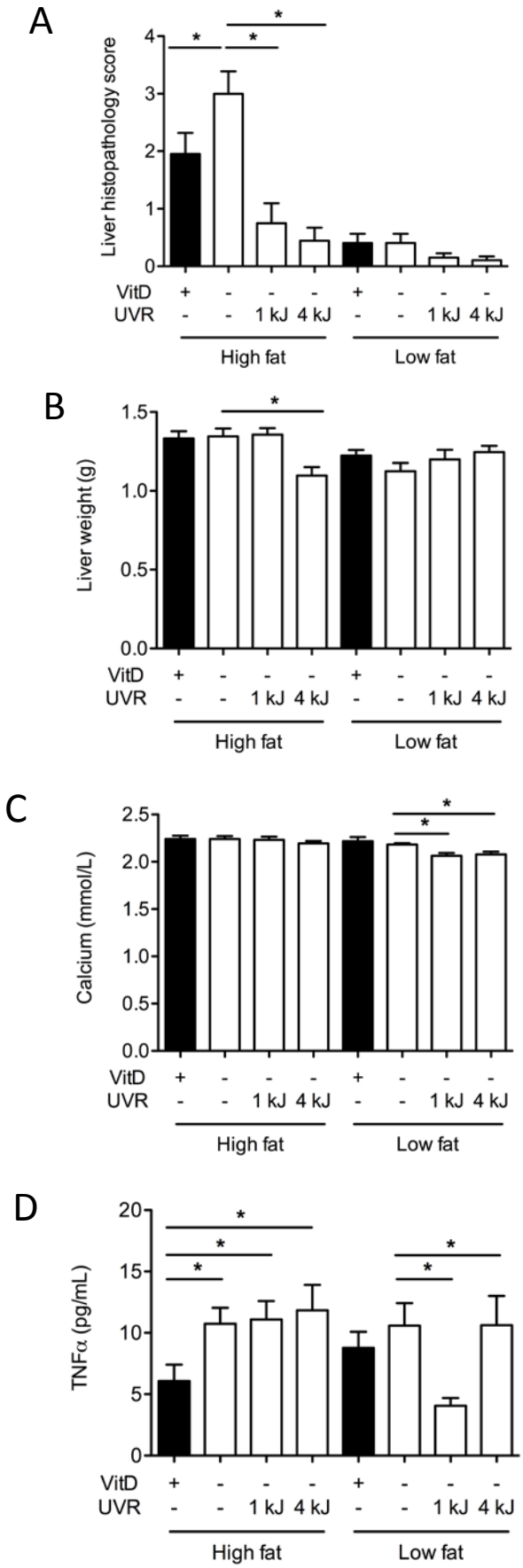

A
Liver histopathology score
0
1
2
3
4
*
*
*
VitD + - - - + - - -
UVR - - 1 kJ 4 kJ - - 1 kJ 4 kJ
High fat
Low fat
B
Liver weight (g)
0.0
0.5
1.0
1.5
*
VitD + - - - + - - -
UVR - - 1 kJ 4 kJ - - 1 kJ 4 kJ
High fat
Low fat
C
Calcium (mmol/L)
0.0
0.5
1.0
1.5
2.0
2.5
*
*
VitD + - - - + - - -
UVR - - 1 kJ 4 kJ - - 1 kJ 4 kJ
High fat
Low fat
D
TNFα (pg/mL)
0
5
10
15
20
*
*
*
*
*
VitD + - - - + - - -
UVR - - 1 kJ 4 kJ - - 1 kJ 4 kJ
High fat
Low fat

Figure 5

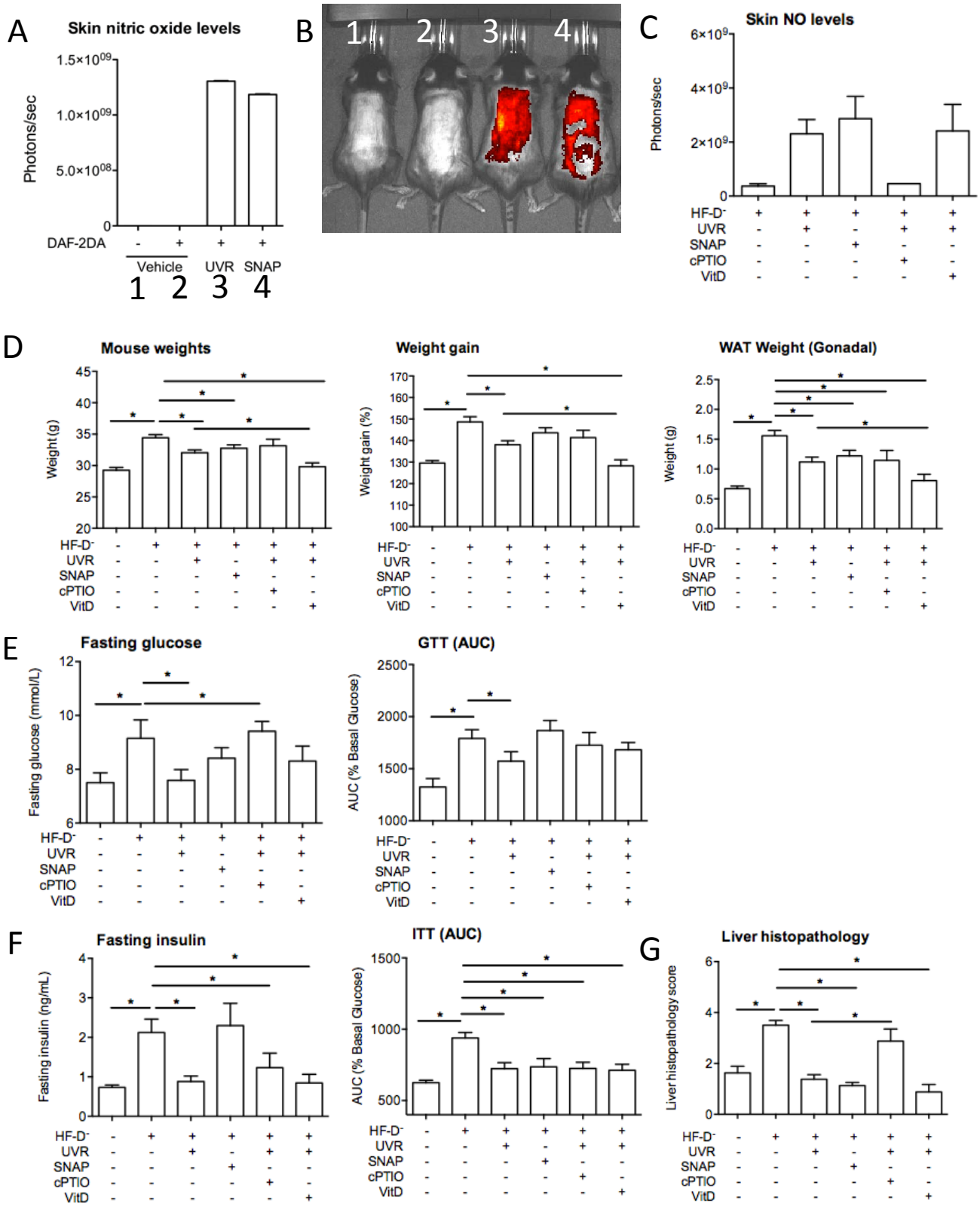
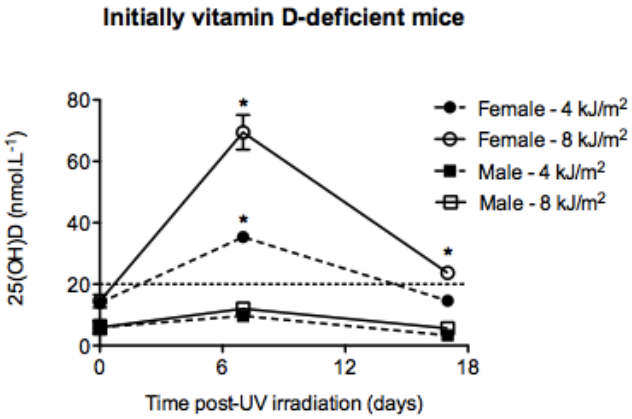
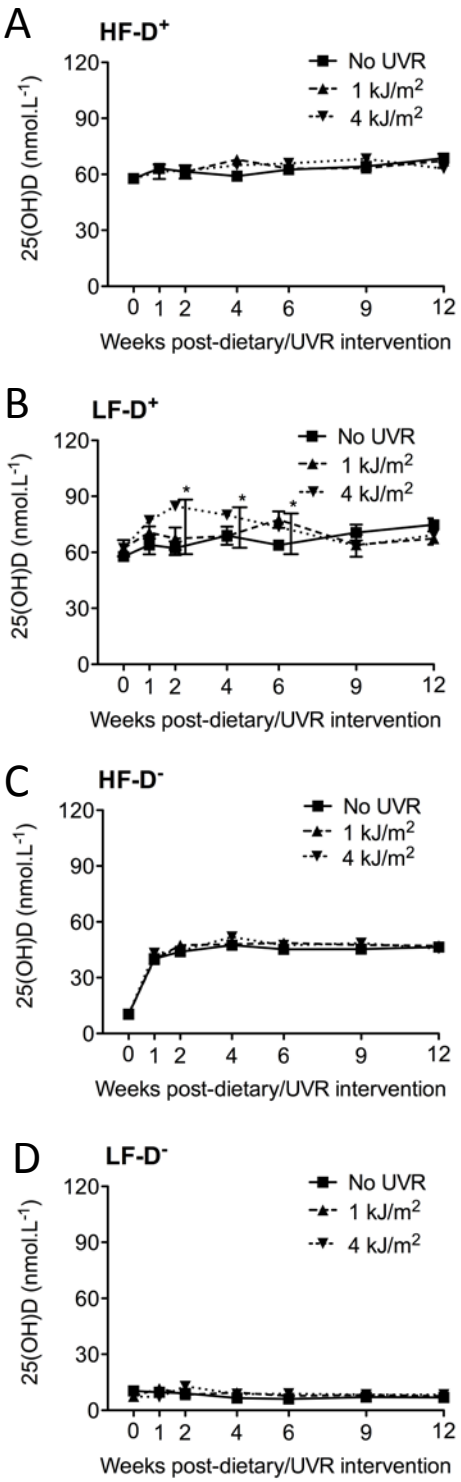

A
Skin nitric oxide levels
Photons/sec
1.5×10^09
1.0×10^09
5.0×10^08
0
DAF-2DA - + + +
Vehicle
UVR
SNAP
1 2 3 4
B
1 2 3 4
C
Skin NO levels
Photons/sec
6×10^9
4×10^9
2×10^9
0
HF-D
UVR
SNAP
cPTIO
VitD
D
Mouse weights
Weight (g)
Weight gain
Weight gain (%)
WAT Weight (Gonadal)
Weight (g)
E
Fasting glucose
Fasting glucose (mmol/L)
GTT (AUC)
AUC (% Basal Glucose)
F
Fasting insulin
Fasting insulin (ng/mL)
ITT (AUC)
AUC (% Basal Glucose)
G
Liver histopathology
Liver histopathology score

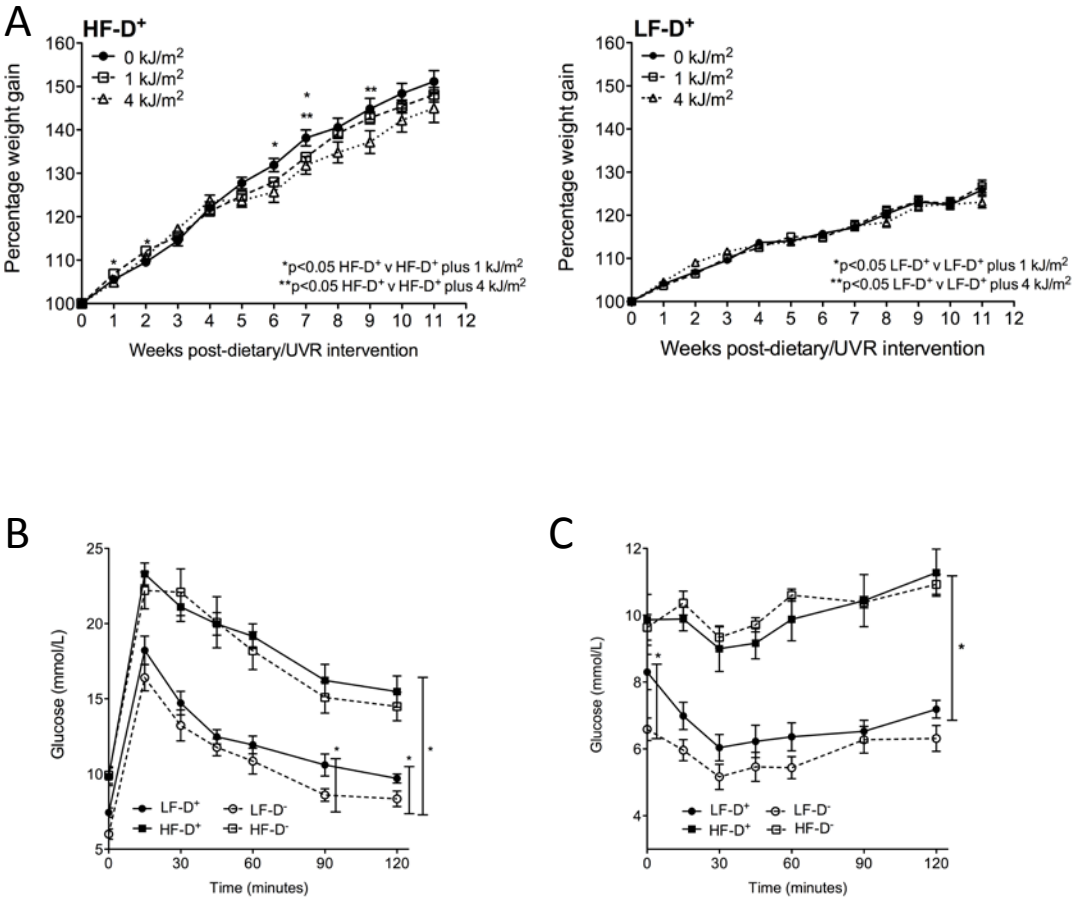
Figure 6



Supplementary Figure 1



Supplementary Figure 2



Supplementary Figure 3