Ultraviolet radiation suppresses obesity and symptoms of metabolic syndrome independently of vitamin d in mice fed a high-fat diet

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The role of vitamin D in curtailing the development of obesity and comorbidities such as 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. Long-term suberythemal and erythemal UVR significantly suppressed weight gain, glucose intolerance, insulin resistance, nonalcoholic 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 (NO) 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 such as NO.

Obesity has significant effects on our health and well-being: obese people have increased comorbidities 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 to 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 Earthman et al. [2] and Autier et al. [3]). Vitamin D is synthesized 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).

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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 (5) suggest that a higher BMI leads to reduced circulating 25(OH)D levels. Furthermore, randomized controlled trials that test the efficacy of vitamin D supplementation for weight loss (2) or for 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 levels (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. Long-term sunlight exposure (particularly suberythemal UVR) itself may be beneficial for obesity and MetS outcomes like type 2 diabetes (12) and nonalcoholic fatty liver disease (NAFLD) (13).

In this article, 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 long-term UVR and/or dietary vitamin D to alter the development of obesity using a physiologically relevant model induced by feeding a high-fat diet to C57BL/6 male mice. Our previous studies have shown that long-term UVR exposure does not modify serum 25(OH)D levels in male mice (14), allowing us to investigate the ability of UVR to modulate obesity and MetS independent of circulating 25(OH)D levels. Here, long-term UVR exposure but not dietary vitamin D suppressed weight gain and various measures of MetS (circulating cholesterol levels, glucose intolerance, and insulin resistance). Further, while vitamin D supplementation did improve NAFLD, UVR suppressed its development even more effectively. Vitamin D supplementation suppressed circulating tumor necrosis factor-α (TNF-α) levels, identifying a possible mechanism for the 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 level, 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 (Murdoch, Western Australia, Australia). The temperature and lighting were controlled, with a normal 12-h light/dark cycle to mimic day and night. Mice were housed under Perspex-filtered fluorescent lighting, which emitted no detectable UVR B as measured using an ultraviolet (UV) radiometer (UVX Digital Radiometer; Ultraviolet Products Inc., Upland, CA). Mice were allowed access to food and acidified water ad libitum.

Diet

All diets were obtained from Specialty Feeds (Glen Forrest, Western Australia, Australia) and included two semipure low-fat diets (5% fat; canola oil), which were supplemented with vitamin D_3 (2,280 or 0 IU vitamin D_3/kg) (LF-D^+) or not (LF-D^-) and two high-fat diets (23%; lard [20.7%] and canola oil [2.9%]) that were supplemented with vitamin D_3 (2,280 or 0 IU vitamin D_3/kg) (HF-D^+) or were not (HF-D^-). 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.

UVR and Topical Skin Treatments

A bank of six 40-W lamps (TL UV-B; Philips, 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 suberythemal (1 kJ/m^2) (15) or erythemal (4 or 8 kJ/m^2) UVR onto a clean-shaven 8-cm^2 dorsal skin area, as previously described (16). Alternatively, skin was treated with 0.1 mmoles *S*-nitroso-*N*-acetylpenicillamine (SNAP; Sigma-Aldrich) (17), a NO donor. In other treatments, a NO scavenger, carboxy-PTIO potassium salt (cPTIO; 0.1 mmoles; Sigma-Aldrich) (18), or 1,25$(OH)_2$D (11.4 pmol/cm^2; Sigma-Aldrich) (19) were applied immediately after delivery of suberythemal UVR (1 kJ/m^2). This dose of 1,25$(OH)_2$D was previously reported to not induce hypercalcemia (19). All topical reagents were diluted with 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 (>0.1 g sensitivity; Scout; Ohaus). The percentage weight gain was calculated from 8 weeks of age.

Glucose and Insulin Tolerance Tests

Mice were fasted for 5 h and then intraperitoneally challenged with either 1 g/kg glucose (Phebra, Lane Cove, New South Wales, Australia), for glucose tolerance tests

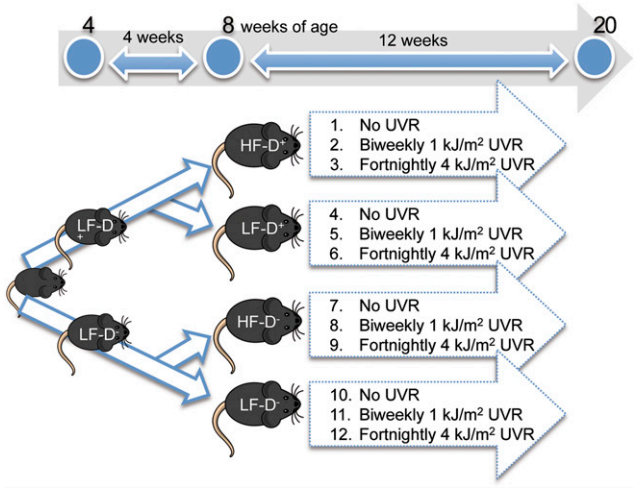

Figure 1—The experimental approach. The 4-week-old C57BL/6 male mice were fed a low-fat diet (either LF-D$^+$ or LF-D$^-$) for 4 weeks. At 8 weeks of age, mice were either continued on these diets or switched to an HF-D$^+$ or an HF-D$^-$. At the same time, each dietary group was further divided into three treatment groups of mice that received long-term irradiation with suberythemal UVR (1 kJ/m^2 twice a week [biweekly]), erythemal UVR (4 kJ/m^2 once a fortnight [fortnightly]), or no UVR. Mice were fed these diets and irradiated with these UVR regimens for a further 12 weeks until mice were 20 weeks of age. There were a total of 12 treatments, with 18 mice per treatment. The experiment was performed two times.

(GTTs), or 0.5–0.75 IU/kg insulin (Lilly, Indianapolis, IN), for insulin tolerance tests (ITTs). Glucose levels were recorded at 0, 15, 30, 45, 60, and 90 min postinjection 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; coefficient of variation 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, $P \leq$ 0.0001). Serum calcium, cholesterol, HDL cholesterol, LDL cholesterol, and triglyceride levels were measured by standard colorimetric reactions using the Architect c16000 Analyzer (Abbott Diagnostics, Abbott Park, IL). Glucose, insulin, adiponectin, and leptin levels were measured in serum after fasting mice for 5 h. Fasting glucose level was measured using the Accu-Chek Performa glucometer (Roche, Castle Hill, New South Wales, Australia). Fasting insulin, adiponectin, and leptin levels 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 interleukin (IL)-6, TNF-α, and IL-10 concentrations were measured in serum using ELISA as previously described (15,22) with antibody pairs supplied by

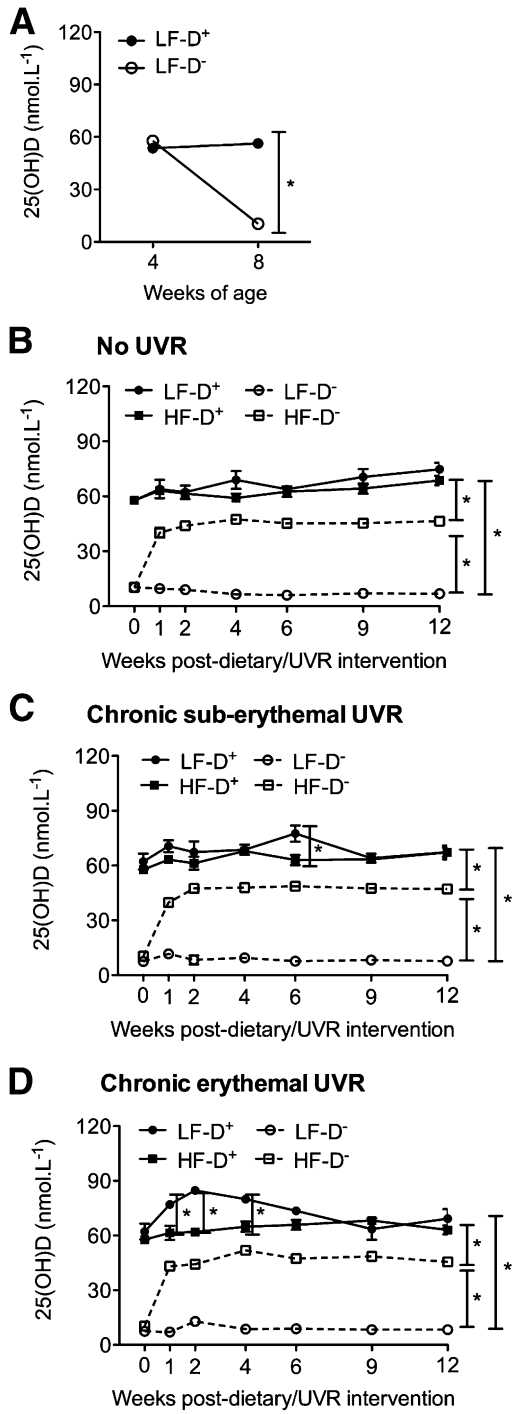

Figure 2—The effects of long-term skin exposure to UVR, dietary vitamin D, and a high-fat diet on serum 25(OH)D levels. *A*: The 4-week-old C57BL/6 male mice were fed a low-fat diet (either LF-D$^+$ or LF-D$^-$) for 4 weeks. *B–D*: At 8 weeks of age (week 0), mice were either continued on these diets or switched to an HF-D$^+$ or an HF-D$^-$. At the same time, each dietary group was further divided into three treatment groups of mice that received long-term irradiation with no UVR (*B*), suberythemal UVR (1 kJ/m^2 twice a week) (*C*), or erythemal UVR (4 kJ/m^2 once a fortnight) (*D*) 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 the mean ± SEM for n = 4–9 mice at each time, pooled from two independent experiments (*P < 0.05).

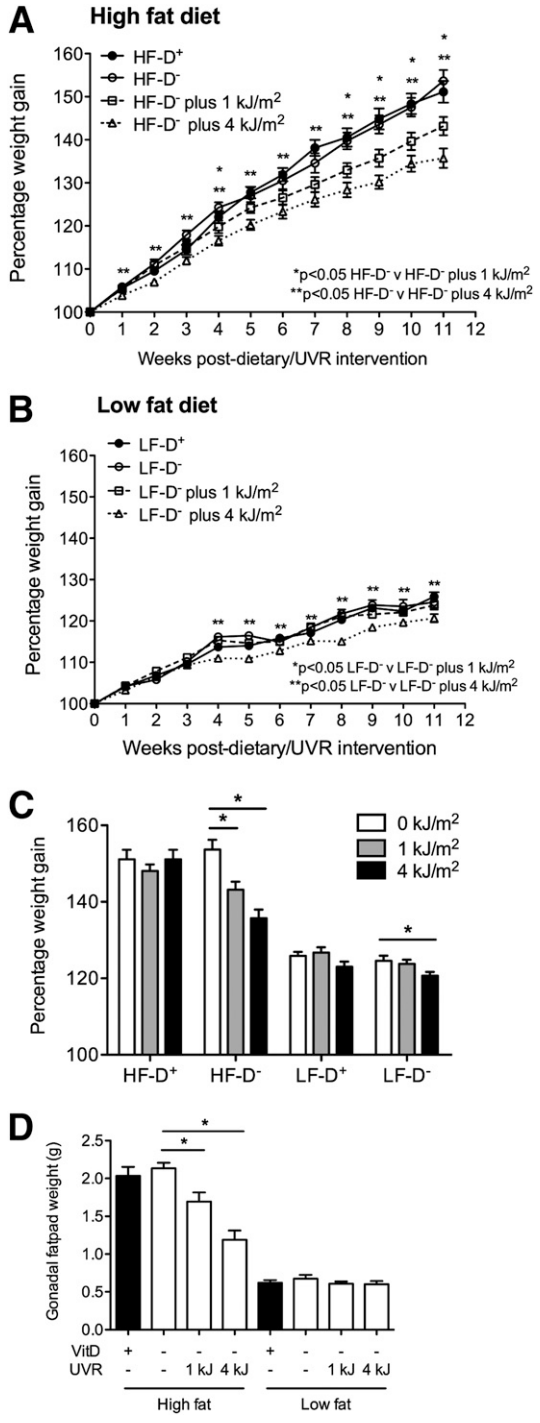

Figure 3—Long-term UVR exposure suppressed weight gain in mice fed high-fat or low-fat diets not supplemented with vitamin D (VitD). The 4-week-old C57BL/6 male mice were fed a low-fat diet (either LF-D$^+$ or LF-D$^-$) for 4 weeks. *A* and *B*: At 8 weeks of age (week 0), mice were either continued on these diets or switched to an HF-D$^+$ or an HF-D$^-$. At the same time, each dietary group was further divided into three treatment groups of mice that received long-term irradiation with no UVR, suberythemal UVR (1 kJ/m^2 twice a week), or erythemal UVR (4 kJ/m^2 once a fortnight). The 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 high-fat diet (*A*) or a low-fat diet (*B*). Data are shown as the mean ± SEM for n = 18 mice/treatment from a representative of two independent experiments. *C*: Total weight gain after 12 weeks of these UVR/dietary interventions (at 20 weeks of age) is shown for all treatments (mean ± SEM). *D*: After 12 weeks of these UVR/dietary interventions (at 20 weeks of age) gonadal fat-pad (n = 18/treatment) weights were measured. Data are representative of two independent experiments (mean + SEM). *P < 0.05.

BD Biosciences (Franklin Lakes, NJ). The levels of detection for the IL-6, TNF-α, and IL-10 assays were 12, 3, and 14 pg/mL, respectively. Serum nitrite and nitrate levels were measured as previously described (23).

Histopathological Assessment of Liver Pathology

The severity of NAFLD was assessed by grading formalin-fixed and hematoxylin-eosin–stained liver sections. Steatosis and hepatocellular ballooning were scored using a scoring system based on the nonalcoholic steatohepatitis (NASH) 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 NO 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 [cleaved by intracellular esterases to generate DAF-2, which 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 (PerkinElmer).

Statistical Analyses

Area under the curve (AUC) was calculated for GTT and ITT using GraphPad Prism (version 5) using 0 as the baseline. Student t tests and ANOVA were used to compare treatments with Tukey post hoc analyses. Because of a significantly greater variance in weight gain among high-fat diet–fed mice, the effects of vitamin D intake and UVR treatment (and their interaction) on weight gain were analyzed separately from the low-fat diet–fed mice using SPSS (version 21.0.0). Results were considered to be statistically significant for P values <0.05.

RESULTS

Tracking the Effects of Long-term UVR Exposure and Dietary Fat on Serum 25(OH)D

To confirm our previous findings that UVR does not modify serum 25(OH)D levels 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 were 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

Table 1—AUC values for GTTs and ITTs, and fasting glucose, insulin, leptin, and adiponectin levels measured 9–11 weeks after UVR/dietary intervention

Treatment	Diet	UVR (kJ/m^2)	GTT (AUC, % basal glucose)	ITT (AUC, % basal glucose)	Fasting glucose (mmol/L)	Fasting insulin (ng/mL)	Fasting leptin (ng/mL)	Fasting adiponectin (ng/mL)
1	HF-D$^+$	0	2,190 ± 83	1,200 ± 63	9.8 ± 0.5	8.2 ± 3.5	36.7 ± 3.0	10.4 ± 0.3
2	HF-D$^+$	1	1,770 ± 49*	1,060 ± 46	8.8 ± 0.4	7.1 ± 0.4	29.8 ± 5.7	11.9 ± 1.8
3	HF-D$^+$	4	1,880 ± 180	1,370 ± 34	10.2 ± 0.4	3.6 ± 1.1	19.7 ± 7.3	15.8 ± 3.9
4	LF-D$^+$	0	1,470 ± 67	800 ± 38	7.9 ± 0.3	1.0 ± 0.4	1.5 ± 0.6	12.9 ± 2.8
5	LF-D$^+$	1	1,510 ± 65	760 ± 37	8.0 ± 0.4	4.9 ± 2.8	2.6 ± 1.1	8.8 ± 2.5
6	LF-D$^+$	4	1,390 ± 56	770 ± 79	7.8 ± 0.4	1.8 ± 1.0	2.2 ± 0.7	11.9 ± 1.0
7	HF-D$^-$	0	2,120 ± 130	1,230 ± 15	9.8 ± 0.3	11.1 ± 1.9	29.8 ± 3.5	13.0 ± 2.6
8	HF-D$^-$	1	1,760 ± 65†	1,050 ± 43†	8.7 ± 0.3†	3.8 ± 1.1†	32.6 ± 5.6	11.3 ± 0.9
9	HF-D$^-$	4	1,690 ± 73†	960 ± 72†	8.1 ± 0.4†	3.9 ± 2.8†	14.0 ± 5.3†	13.0 ± 1.1
10	LF-D$^-$	0	1,260 ± 51	680 ± 48	6.3 ± 0.2	3.4 ± 1.6	5.9 ± 2.5	16.6 ± 6.2
11	LF-D$^-$	1	1,280 ± 102	600 ± 27	6.0 ± 0.2	1.6 ± 1.1	1.0 ± 0.5	10.8 ± 0.6
12	LF-D$^-$	4	1,480 ± 36	760 ± 60	7.7 ± 0.4	4.3 ± 1.8	1.9 ± 0.2	11.7 ± 1.9

Data are the mean ± SEM; n = 4–8 mice/treatment. *$P < 0.05$ vs. no UVR and HF-D$^+$ with data representative of two experiments. †$P < 0.05$ relative to no UVR and HF-D$^-$ with data representative of two experiments.

(Supplementary 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 nonsupplemented (low-fat) diet from 4 to 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 nonsupplemented diets, but some were switched to a diet that was high in fat. Each of these four dietary treatments were further divided into three treatments, with the shaved skin of mice exposed to long-term irradiation with no UVR, suberythemal UVR (1 kJ/m^2 twice a week) or erythemal UVR (4 kJ/m^2 once a fortnight), as indicated in Fig. 1. Mice were treated from 8 to 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 diet that was further supplemented with vitamin D (HF-D$^+$, LF-D$^+$) had significantly higher serum 25(OH)D levels than those mice fed a diet that was 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 level (Fig. 2*B*). Although not observed in our preliminary (Supplementary

Table 2—Circulating triglyceride and cholesterol levels at 12 weeks after dietary and UVR interventions

Treatment	Diet	UVR (kJ/m^2)	Triglycerides (mmol/L)	HDL cholesterol (mmol/L)	LDL cholesterol (mmol/L)	Total cholesterol (mmol/L)
1	HF-D$^+$	0	0.7 ± 0.1	2.1 ± 0.2	0.3 ± 0.0	4.2 ± 0.4
2	HF-D$^+$	1	0.6 ± 0.0	2.0 ± 0.2	0.2 ± 0.0	3.8 ± 0.4
3	HF-D$^+$	4	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	1.2 ± 0.1	1.8 ± 0.1	0.2 ± 0.0	2.9 ± 0.1
6	LF-D$^+$	4	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	0.6 ± 0.0	2.1 ± 0.0	0.3 ± 0.0	4.2 ± 0.2
9	HF-D$^-$	4	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	0.9 ± 0.1	1.4 ± 0.1	0.1 ± 0.0	2.0 ± 0.1
12	LF-D$^-$	4	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 and HF-D$^-$ with data representative of two experiments.

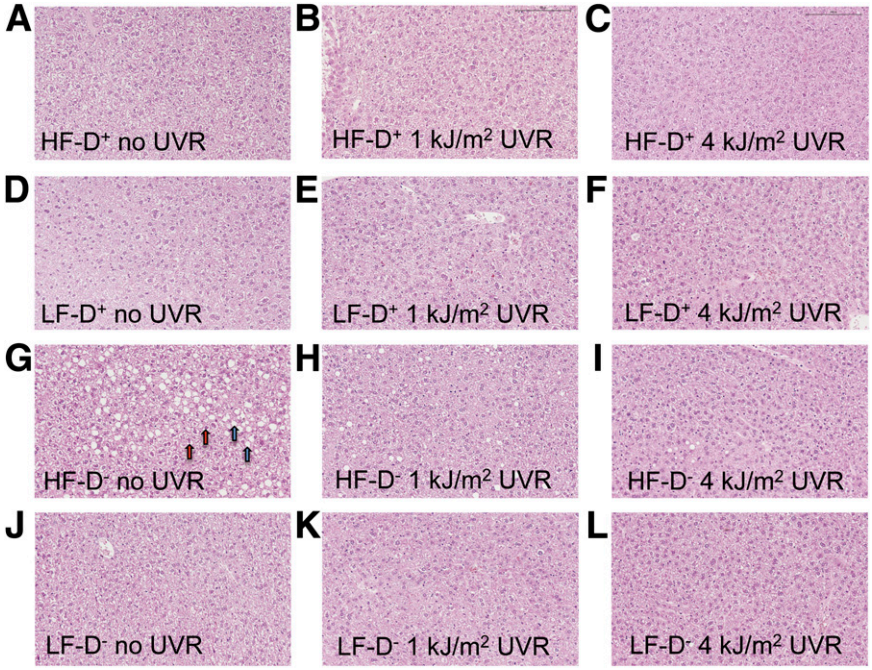

Figure 4—Long-term UVR significantly reduced the extent of liver steatosis and lobular ballooning in mice fed a high-fat diet. The 4-week-old C57BL/6 male mice were fed a low-fat diet (either LF-D$^+$ or LF-D$^-$) for 4 weeks. At 8 weeks of age, mice were either continued on these diets or switched to an HF-D$^+$ or an HF-D$^-$. At the same time, each dietary group was further divided into three treatment groups of mice that received long-term irradiation with no UVR (*A*, *D*, *G*, and *J*), suberythemal UVR (1 kJ/m^2 twice a week; *B*, *E*, *H*, *K*), or erythemal UVR (4 kJ/m^2 once a fortnight; *C*, *F*, *I*, and *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). *A*–*L*: Representative hematoxylin-eosin–stained sections of liver for each treatment (*B* and *C*, original magnification ×20 [equivalent to 150 μm]). Examples of liver steatosis (blue arrow) and lobular ballooning (red arrow) are shown in *G*.

Fig. 1) and past investigations (14), long-term suberythemal (Fig. 2*C*) or erythemal (Fig. 2*D*) UVR exposure significantly (but transiently) enhanced serum 25(OH)D levels, when administered to mice fed an LF-D$^+$ (but not HF-D$^+$, LF-D$^-$, or HF-D$^-$) (Supplementary Fig. 2). The effects were more pronounced for mice administered the long-term erythemal UVR, but returned to baseline levels after 6 weeks of UVR/dietary intervention (Fig. 2*D* and Supplementary Fig. 2B).

Long-term UVR Exposure Suppressed Weight Gain in Mice Fed a Vitamin D–Nonsupplemented Diet

There was no effect of vitamin D supplementation on weight gain (Fig. 3*A* and *B*). Both long-term suberythemal UVR (1 kJ/m^2 twice a week) and erythemal UVR (4 kJ/m^2 once a fortnight) treatment suppressed weight gain in mice fed the HF-D$^-$ (Fig. 3*A*) by ≥40%. Long-term erythemal UVR exposure also suppressed weight gain in mice fed the LF-D$^-$ (Fig. 3*B*). The effects of long-term skin exposure to UVR were less apparent for mice fed the vitamin D–supplemented diet, where UVR exposure suppressed weight gain in a transient fashion in mice fed the HF-D$^+$ (Supplementary 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*).

Long-term UVR Exposure Suppressed Glucose Intolerance and Insulin Resistance in Mice Fed a Vitamin D–Nonsupplemented 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 (Supplementary Fig. 3*B*) and insulin resistance (Supplementary Fig. 3*C*), with no suppressive effect of vitamin D supplementation (Supplementary Fig. 3*B* and *C*; Table 1 for AUC). Both measures were suppressed in mice receiving long-term irradiation with UVR (either suberythemal or erythemal) and fed the HF-D$^-$ (Table 1). Glucose intolerance was significantly suppressed by long-term suberythemal UVR in mice fed the HF-D$^+$ only (Table 1). In addition, fasting glucose and insulin levels were also reduced by UVR treatment in mice fed the HF-D$^-$, with fasting leptin levels also suppressed in mice that received long-term irradiation with erythemal UVR (Table 1). There were no effects of long-term UVR (or dietary vitamin D) on fasting adiponectin levels (Table 1).

Long-term 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

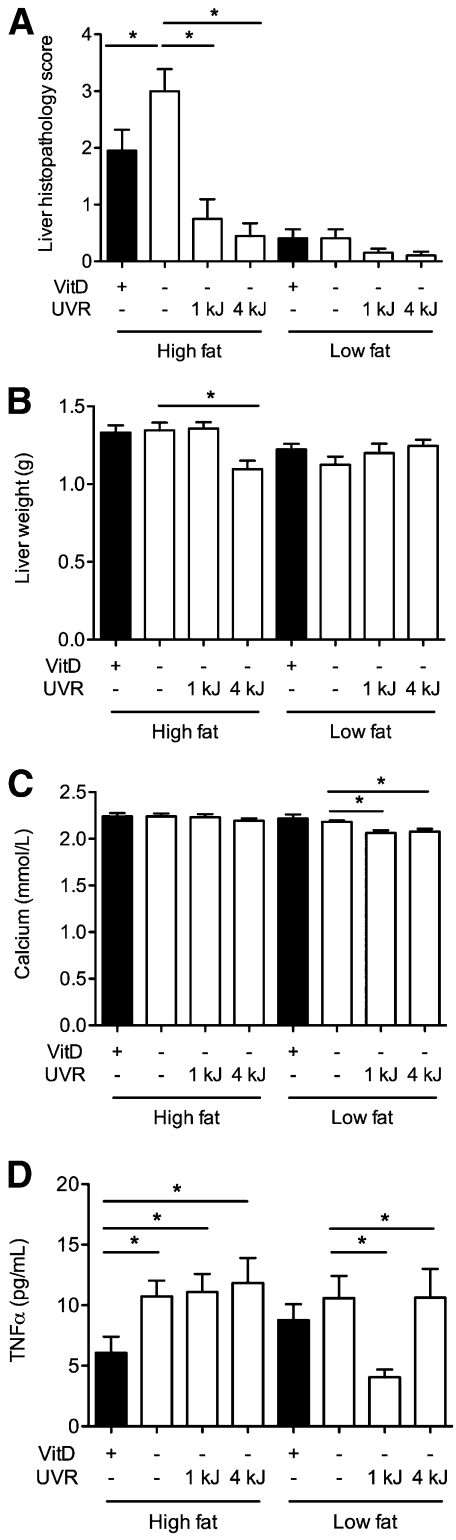

Figure 5—Long-term UVR exposure significantly reduced the extent of liver histopathology in mice fed a high-fat diet. The 4-week-old C57BL/6 male mice were fed a low-fat diet (either LF-D^+ or LF-D^-) for 4 weeks. At 8 weeks of age, mice were either continued on these diets or switched to an HF-D^+ or HF-D^-. At the same time, each dietary group was further divided into three treatment groups of mice that received long-term irradiation with no UVR, suberythemal UVR (1 kJ/m^2 twice a week), or erythemal UVR (4 kJ/m^2 once a fortnight). After 12 weeks of these UVR/dietary interventions (at 20 weeks of age), the extent of liver histopathology (*n* = 10/treatment for data pooled from two independent experiments) (*A*), liver weights (*n* = 18/treatment for data from a representative experiment) (*B*), and serum levels of calcium (*n* = 4–8/treatment for data pooled from two independent experiments) (*C*) and TNF-α (*n* = 12–18/treatment for data pooled from two independent experiments) (*D*) are shown. Data are shown as the mean ± SEM. *$P < 0.05$. VitD, vitamin D.

not modified by vitamin D supplementation or long-term UVR (Table 2). HDL, LDL, and total cholesterol levels were suppressed in mice fed the HF-D^- and also receiving long-term irradiation with erythemal UVR (Table 2).

Long-term UVR Exposure More Effectively Suppressed the Development of NAFLD Than Vitamin D Supplementation

The development of markers of NAFLD was measured by analyzing the degree of liver steatosis and lobular ballooning after 12 weeks of UVR/dietary intervention (Figs. 4 and 5*A*). Long-term 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 that 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, whereas long-term 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 long-term UVR exposure were detected for weight gain (Fig. 3*C*) ($P = 0.05$), gonadal fat-pad weights (Fig. 3*D*) ($P = 0.03$), and fasting glucose levels (Table 1) ($P = 0.01$), but not the other measures, including liver histopathology (Figs. 4 and 5*A*) ($P > 0.05$).

Serum Vitamin D or Calcium Levels Were Not Related to Weight Loss or Suppression of MetS in UVR-Irradiated Mice

Long-term UVR exposure suppressed aspects of weight gain and measures of MetS, independently of changes to circulating 25(OH)D levels (Fig. 2 and Supplementary 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 long-term skin exposure to UVR in mice fed the high-fat diets (Fig. 5*C*). Long-term skin exposure to UVR reduced calcium levels in mice fed a low-fat diet (Fig. 5*C*).

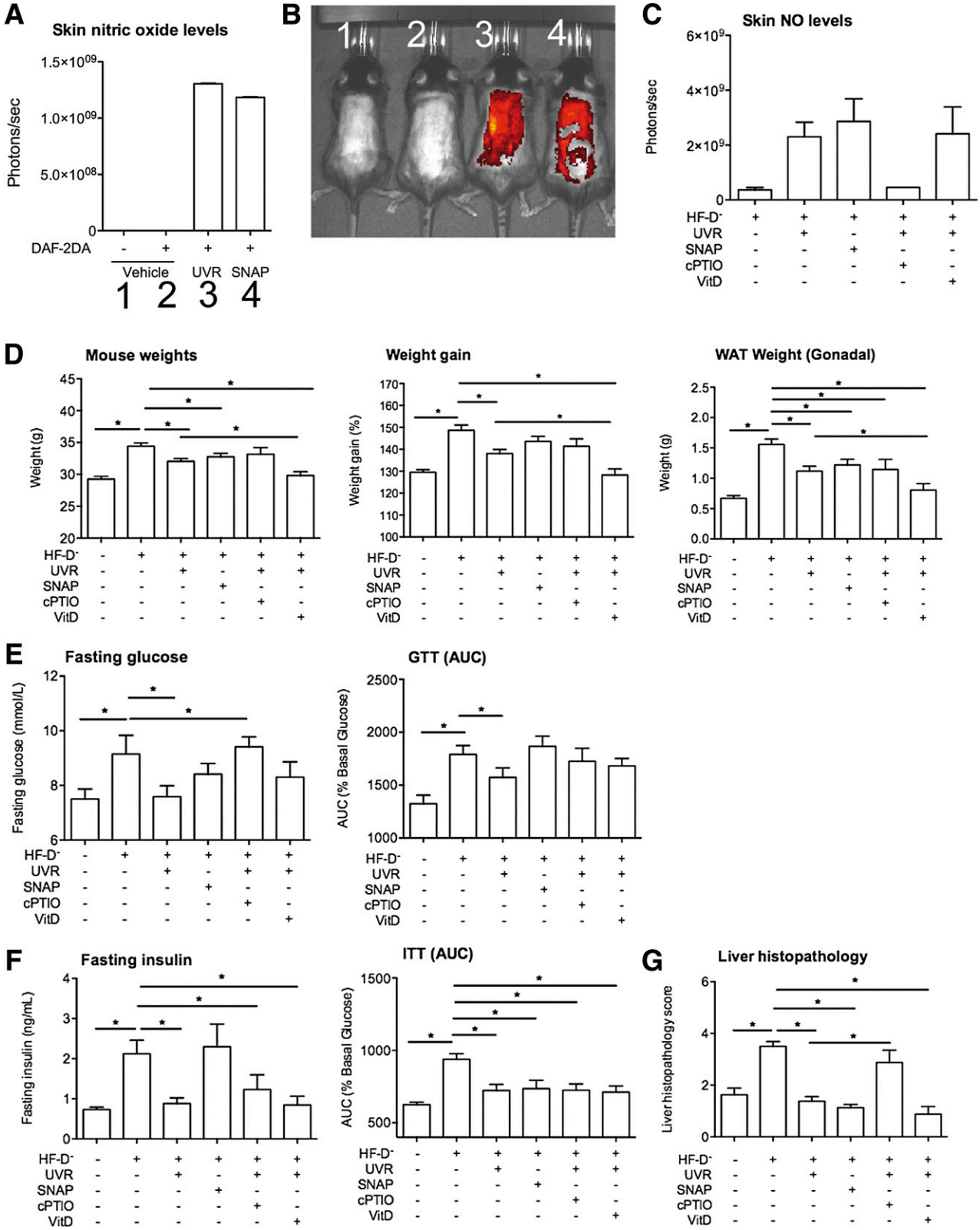

Figure 6—The UVR-induced mediator NO may regulate body weight, WAT accumulation, glucose metabolism, and the development of NAFLD in mice fed a high-fat diet. *A* and *B*: Using the DAF-2DA substrate, skin NO levels are shown for adult C57BL/6 male mice fed a low-fat diet (LF-D$^-$), 5 min after skin treatment with vehicle, 1 kJ/m^2 UVR, or the NO donor SNAP, with a quantitative measure (in photons per second) (*A*) and representative skin fluorescence (*B*) shown. The 4-week-old C57BL/6 male mice were fed an LF-D$^-$ for 4 weeks. At 8 weeks of age, mice were either continued on these diets or switched to the HF-D$^-$. Within the HF-D$^-$ treatments, mice were further divided into five treatment groups. The shaved dorsal skin of these mice *1*) was treated with vehicle only, *2*) received long-term irradiation with suberythemal UVR (1 kJ/m^2 twice a week) and then vehicle, *3*) was topically treated with SNAP, *4*) received long-term irradiation with

Circulating TNF-α Level 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 TNF-α (13). However, long-term UVR did not modify serum TNF-α levels after 12 weeks of UVR/dietary intervention in mice fed a high-fat diet (Fig. 5*D*). Vitamin D supplementation reduced circulating TNF-α levels in mice fed an HF-D$^+$ when compared with those fed an HF-D$^-$ (Fig. 5*D*). Serum levels of IL-6 and IL-10 were below the level of detection of the ELISA.

UV-Induced NO Suppresses the Development of Obesity and Symptoms of MetS

A role for NO, an alternate (non–vitamin D) mediator induced by UVR, was examined. Skin levels of NO increased from as early as 5 min after UVR/SNAP (Fig. 6*A* and *B*) treatment as determined using DAF-2. To examine a role for UVR-induced NO in modulating obesity and MetS symptoms, 4-week-old C57BL/6 male mice were fed an LF-D$^-$ for 4 weeks. From 8 weeks of age, mice were either continued on this diet or switched to the HF-D$^-$, with mice fed an HF-D$^-$ further divided into groups receiving the following five dorsal skin treatments: *1*) vehicle only; *2*) suberythemal UVR (1 kJ/m^2) and then vehicle; *3*) SNAP; *4*) suberythemal UVR and then cPTIO; or *5*) suberythemal 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 Supplementary 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 after 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 after 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 treatment with long-term low-dose UVR or SNAP (data not shown). Long-term UVR suppressed weight gain and the accumulation of WAT after 12 weeks of the HF-D$^-$ (Fig. 6*D*). Long-term 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 treatment alone, but this observation may reflect the hypercalcemia observed early on with topical 1,25(OH)$_2$D treatment (4 weeks post-UVR [2.4 ± 0.03 mmol/L] vs. post-UVR+1,25(OH)$_2$D [3.5 ± 0.07]; $^*P < 0.001$ for serum calcium). In response to these observations, we halved the dose of 1,25(OH)$_2$D administered, and mice were treated only once per week after 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] vs. post-UVR+1,25(OH)$_2$D [2.7 ± 0.07]; $^*P < 0.001$ for serum calcium).

As observed previously, long-term UVR exposure suppressed fasting glucose and insulin levels, and the development of glucose intolerance and insulin resistance (Fig. 6*E* and *F*). Here, long-term SNAP treatment also suppressed the development of insulin resistance (Fig. 6*F*). Furthermore, cPTIO treatment after UVR reversed the suppressive effects of UVR alone upon fasting glucose levels (Fig. 6*E*). Finally, both long-term UVR and SNAP treatment 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 level, and the development of insulin resistance and NAFLD.

DISCUSSION

Here we present evidence that long-term skin exposure to low-dose (suberythemal) and high-dose (erythemal) 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 (i.e., HF-D$^+$). For mice fed a high-fat diet, serum 25(OH)D levels were not enhanced by long-term 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 levels; 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

suberythemal UVR and then cPTIO, or *5*) received long-term irradiation with suberythemal UVR and then 1,25(OH)$_2$D. Mice were treated for 12 weeks with these skin/dietary interventions until 20 weeks of age. *C*: Skin NO levels, 10 min after skin treatment (n = 8 mice/treatment). *D*: Mouse weights, weight gain, and WAT weights (n = 18 mice/treatment). *E*: Fasting glucose and GTT AUC (n = 8 mice/treatment). *F*: Fasting insulin and ITT AUC (n = 8 mice/treatment). *G*: Liver histopathology scores (n = 8 mice/treatment). Data are shown as the mean ± SEM from one experiment. $^*P < 0.05$. VitD, vitamin D.

UVR-induced NO may have profound effects on the development of NAFLD, as topical SNAP suppressed liver pathology, and cPTIO antagonized the effects of UVR. Various non–vitamin D immunomodulators induced by UVR, like NO (28), may be important for the 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 long-term UVR to suppress the development of obesity and metrics of MetS was less effective in mice orally supplemented with vitamin D [but not with 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 long-term 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 by the other treatments (data not shown). Others have also observed (33) that vitamin D suppressed weight gain in vivo after intraperitoneal injections of $1,25(OH)_2D$ (5 μg/kg every 2 days), although the effects on circulating levels of calcium [and 25(OH)D] were not reported. Others have shown (34) that UVR may increase cortisol production in skin, which has the potential to impact the hypothalamic-pituitary-adrenal axis. While this might be hypothesized to alter physical activity, no obvious behavioral effects were observed in this study. However, we cannot exclude the possibility that UVR alters neuroendocrine signaling networks in the skin (35) that might have a systemic impact.

Nakano et al. (13) showed that phototherapy suppressed NAFLD but failed to reduce obesity, steatosis, and blood glucose levels in Zucker *fa-fa* rats. 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 the development of NAFLD in Sprague-Dawley rats fed a "westernized" (high-fat/fructose) diet (36), and in Lewis rats fed a choline-deficient and iron-supplemented L-amino acid–defined diet (13). We also observed that dietary vitamin D suppressed circulating TNF-α levels in mice fed a high-fat diet. UVR did not suppress serum TNF-α levels, 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 vitamin D receptor 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 the role of other UV-induced mediators like NO deserve further consideration. Furthermore, by using a mouse modeling 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 levels. 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 to 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), which, like obesity and MetS, may take years to develop. In conclusion, our studies show that long-term 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.

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