Dexamethasone Reduces Energy Expenditure And Increases Susceptibility To Diet-Induced Obesity In Mice

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Abstract

Objective—To investigate how long-term treatment with dexamethasone affects energy expenditure and adiposity in mice and whether this is influenced by feeding on a high fat diet (HFD).

Design and Methods—Mice were placed on a HFD for 2 weeks and started on dexamethasone at 5mg/kg every other day during the next 7 weeks.

Results—Treatment with dexamethasone increased body fat, an effect that was more pronounced in the animals kept on HFD; dexamethasone treatment also worsened liver steatosis caused by the HFD. At the same time, treatment with dexamethasone lowered the RQ in chow-fed animals and slowed nightly metabolic rate in the animals kept on HFD. In addition, the acute VO2 acceleration in response to β3 adrenergic-stimulation was significantly limited in the dexamethasone-treated animals, as a result of marked decrease in UCP-1 mRNA observed in the BAT of these animals.

Conclusions—Long-term treatment with dexamethasone in a mouse model of diet-induced obesity decreases BAT thermogenesis and exaggerates adiposity and liver steatosis.

Keywords

Dexamethasone; brown adipose tissue; metabolism; high fat diet

INTRODUCTION

Cortisol is the main naturally occurring glucocorticoid in humans and its excess increases the total mass of adipose tissue, redistributing the fat from peripheral to central depots (1). In fact, long-term glucocorticoid treatment is associated with central obesity in humans (2, 3), which is also typically observed in most patients with Cushing's syndrome (4). However, obese patients do not exhibit elevated serum cortisol concentrations. Instead, higher cortisol levels have been reported within their adipose tissue as a result of increased 11β-

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hydroxysteroid dehydrogenase type 1 (11β-HSD 1) activity, an enzyme that converts cortisone to cortisol (5).

The mechanisms by which exogenous glucocorticoids alter metabolism and induce weight gain are poorly understood. Despite stimulating leptin synthesis and secretion (directly or through insulin) by adipocytes in humans (6, 7), glucocorticoids increase appetite by limiting leptin-induced satiety and favoring NPY orexygenic activity (8, 9). For example, it has been suggested that an increase in dietary energy intake explains the increase in body weight and fat mass observed in healthy female volunteers treated with glucocorticoids (10). On the other hand, glucocorticoids are stress hormones with well-known lipolytic effects; these steroids are thought to be involved in cancer-associated cachexia through increased lipid mobilization from adipose tissue (11). Furthermore, the involvement of other systems is also likely as illustrated in the recent report that osteoblasts mediate many of the adverse effects of exogenous glucocorticoids on fuel metabolism (12).

One possible mechanism by which exogenous glucocorticoids affect adiposity and the size of various white adipose tissue depots is by slowing down the rate of energy expenditure (EE). This includes the resting EE and the inducible EE that is associated with physical activity, feeding on a high fat diet (HFD) or triggered by environmental stimuli such as cold exposure (13). Whereas muscle contraction and ATP breakdown explains the EE linked to physical activity, mitochondrial uncoupling in the brown adipose tissue (BAT) underlies a substantial fraction of the diet- and cold-inducible EE (13). However, glucocorticoids do not seem to play a major role in regulating resting EE. For example, there is poor correlation between basal EE and corticosterone levels in mice (14) and in humans EE is not altered with acute inhibition of glucocorticoid action (15) or glucocorticoid excess, e.g. in patients with Cushing's syndrome (16).

Little is known about inducible EE and exogenous glucocorticoids, with short-term studies in healthy male subjects reporting a significant increase in EE and accelerated fat oxidation during exercise (17, 18) while in other similar studies inducible EE was not affected by glucocorticoids (19). However, there is consensus that acute administration of glucocorticoids inhibits the expression of uncoupling protein-1 (UCP-1), the key mitochondrial protein involved in BAT energy expenditure (20, 21). Administration of corticosterone in rats reduced UCP-1 mRNA and protein levels as well as responsiveness to cold exposure or epinephrine administration (21). Also, ob/ob mice, which exhibit higher levels of corticosterone, have reduced levels of UCP-1 and decreased EE (22). Notably, if the glucocorticoid-mediated reduction in UCP-1 expression were to slow down the rate of EE then it would then explain why chronic treatment with dexamethasone aggravates diet-induced obesity and dyslipidemia in mice (23). This is potentially of great interest in view of the observation that BAT is retained by humans during adulthood (24, 25, 26).

UCP-1 is a cAMP-inducible inner-membrane mitochondrial protein that disrupts the proton gradient across this membrane, thus rapidly accelerating the oxidation of energy substrates uncoupled from ATP synthesis in BAT. Both UCP-1 and the adrenergic stimulation are critical for diet-induced EE in the BAT, as mice with the UCP-1 knock out (27), triple adrenergic receptor knock out (28) or knock out of the β1 adrenergic receptor (29) exhibit

defective BAT thermogenesis, developing severe cold intolerance and obesity. However, the key question addressed by the present studies is whether the reduction in UCP-1 caused by the administration of glucocorticoids can significantly slow down basal or inducible BAT-mediated EE and hence contribute to metabolic dysregulation in a mouse model of diet-induced obesity.

METHODS AND PROCEDURES

Animals

The animal Care and Use Committee of the Miami Miller School of Medicine approved all procedures. Two-month-old male C57BL/6J mice were obtained from Jackson Laboratory (Bar Harbor, ME) and were housed under standard conditions with food and water *ad libitum*, at 22C and a 12h light/dark cycle. Animals were treated with high fat diet (HFD: 4.5 kcal/g; 42.7% carbohydrate, 15.3% protein, 42% fat; TD95121; Harlan Teklad, Indianapolis, IN) or remained on standard chow diet (3.5 kcal/g; 58.5% carbohydrate, 28.8% protein, 12.7% fat; 5010; PMI Nutrition, Richmond IN) for a total of 10 weeks. Mice were allowed to adjust to the environment for at least one week before starting the diet. After 2 weeks of diet, animals were treated with intraperitoneal (i.p.) injections of dexamethasone sodium phosphate (APP Pharmaceutical, LLC; 5 mg/kg) or 0.9% sodium chloride (Baxter, Deerfield, IL) on alternate days until the end of the experimental period. As indicated, data are presented as (i) the variance between baseline and end of the experimental period (pre and post-dexa) or (ii) cross-comparison between saline- or dexamethasone-treated groups at the end of the experimental period.

Metabolic parameters

Body weight was measured every other day until euthanasia. The caloric intake was measured individually during 8-day period after two weeks of dexamethasone or saline treatment and the results expressed as a function of body weight (kcal/g BW·day). Body composition (lean body mass, fat % and fat mass) was measured in mice fasted overnight and anesthetized with ketamine and xylazine (200 mg/kg and 7–20 mg/kg) using Dual-Energy X-ray Absorptiometry (DXA, Lunar Pixi, Janesville, WI) at selected time points.

Indirect calorimetry and β3-selective adrenergic stimulation

For these studies, animals were admitted to a comprehensive lab animal monitoring system (C.L.A.M.S.; Oxymax System, Columbus Instruments, Columbus OH), in individual cages and allowed to acclimatize for two days. 24h-metabolic profiles were obtained during the following five days. Equipment was calibrated against a defined gas mixture of O2 and CO2 (Airgas, Tampa, FL). Respiratory quotient (RQ) was calculated as ratio between CO2 production (liters) and O2 consumption (liters). Oxygen consumption (VO2) was expressed in ml O2/min and normalized by lean body mass as defined by DXA. VO2 was also assessed for 4h before and after the subcutaneous (s.c.) injections of a β3-selective adrenergic receptor agonist- CL 316243 (Sigma Chemical Co. St Louis, MO; 1mg/kg).

Euthanasia and post-mortem analysis

At the end of the experimental period, overnight fasted mice were killed by cardiac puncture after brief exposure to CO_2. Interscapular BAT and liver were removed, weighted and rapidly frozen in liquid nitrogen. The tissue weight was normalized by femoral length measured using an electronic digital caliper (Control Company, Friendswood, TX purchased through VWR). Liver triglycerides were measured after extraction with chloroform:methanol (2:1) and 0.05% sulfuric acid from a 0.2 g sample of frozen liver using a commercially available kit (Sigma-Aldrich, St. Louis, Mo), as previously published (30).

Measurements of UCP-1 expression

Total RNA was extracted using the QuiagenRNeasy kit according to manufacturer's protocol (Qiagen, Valencia, CA) and the concentration determined with a Nanodrop spectrophotometer. cDNA was prepared from 1 μg of mRNA by using High capacity cDNA reverse Transcription Kit according to the manufacturer's protocol (Applied Biosystems, Foster City, CA). The gene of interest was assessed by RT-qPCR against a calibration curve of pooled mouse samples (Bio-Rad iCycleriQ Real-Time PCR Detection System) and normalized by the housekeeping gene cyclophilin A, as previously described (31). The primer sequences used are: UCP-1: 5′AGGTGTGGCAGTGTTCATTGG-3′, 3′ CTGTCCTGGGAGAGAGTTGATG-5′. Cyclo-A: 5′ GCCGATGACGAGCCCTTG-3′, 3′ TGCCGCCAGTGCCATTATG-5′.

Data Analysis

Throughout the manuscript, all data are expressed as mean ± standard error of the mean (SEM). Data were analyzed by PRISM Parallel GraphPad software (San Diego, CA). Student's t-test was used to compare the differences between two independent groups. Statistical significance was considered at a level of $p<0.05$. One-way analysis of variance (ANOVA) was used to compare more than two groups, followed by the student-Newman-Keuls post-test to detect differences between groups.

RESULTS

Treatment with dexamethasone increases adiposity

To define the effects of dexamethasone on metabolic parameters of mice kept on a HFD, mice that had been on chow or HFD for 2 weeks were treated with saline or dexamethasone for an additional 7 weeks while remaining on the same diets. As expected, the body weight gain was approximately doubled in mice kept on a HFD (Fig. 1A). Body fat mass (Fig. 1B), body percentage of fat (Fig. 1C) and liver triglyceride content (Fig. 1D) were also substantially increased; no significant effects of HFD were observed on caloric intake (0.50±0.06 vs. 0.42±0.01 kcal/g BW/day; chow diet versus HFD; n=3/group) in the saline-treated animals as measured on the fifth week of the experiment.

Treatment with dexamethasone reduced by almost half the body weight gain of the animals kept on a HFD (Fig. 1A), while increasing body fat mass (Fig. 1B) and percentage of fat (Fig. 1C), on both types of diet; however, the effects were more pronounced in the animals kept on the HFD (Figs. 1B–C). Likewise, only in dexamethasone-treated mice kept on the

HFD was hepatic triglyceride content approximately doubled (Fig. 1D). Notably, at least part of the effect of dexamethasone on reducing body weight gain was due to a decrease in linear growth, evidenced by the reduced femoral length (14.6 ± 0.20 vs. 15.5 ± 0.04 mm; n=3/group; p<0.05). Treatment with dexamethasone did not affect caloric intake, regardless of the type of diet (0.46 ± 0.02 vs. 0.50 ± 0.06 kcal/g BW/day in chow diet; n=3/group; 0.43 ± 0.02 vs. 0.42 ± 0.01 kcal/g BW/day in HFD; n=3/group).

VO2 and RQ are reduced by treatment with dexamethasone

The analysis of the 24h-indirect calorimetry indicated that treatment with dexamethasone did not affect VO2 in the animals kept on chow diet (nightly data shown in Fig. 2A) but it did significantly reduce the nightly RQ (Fig. 2C–D). This is an indication that fatty acid oxidation was accelerated by treatment with dexamethasone. However, in the animals kept on the HFD, treatment with dexamethasone promoted a marked reduction in VO2 that reached statistical significance during the nightly period (Fig. 2A–B). This, however, was not associated with a further reduction in RQ probably because fatty acid oxidation was already accelerated by keeping the animals on the HFD (Fig. 2C).

Treatment with dexamethasone slows down β3 agonist-induced VO2 acceleration

β3-induced acceleration of VO2 reflects the potential for adaptive thermogenesis and is thought to take place predominantly in BAT (13, 32). Notably, the absolute and relative BAT weights were substantially increased by treatment with dexamethasone (Table 1). However, treatment with dexamethasone resulted in a marked drop in UCP1 mRNA levels, both in chow and in high fat-fed animals (Table 1), indicating that the potential for adaptive thermogenesis is reduced in these animals.

To test this hypothesis, we acutely injected the β3-adrenergic receptor agonist CL316243 (1 mg/kg BW) to activate adaptive thermogenesis and subsequently followed the VO2 acceleration during the next 4h. In chow-fed control animals, administration of CL316243 led to an immediate VO2 acceleration, jumping from a baseline of $2,635.7\pm53.9$ ml/Kg/h to $3,811.4\pm53.3$ ml/kg/h; n=3/group; p<0.0001 (Fig. 3A). This was associated with a drop in RQ from 0.83 ± 0.007 to 0.79 ± 0.006; n=3/group (Fig. 3C). Treatment with dexamethasone did not substantially affect the VO2 response to β3 agonist administration (Fig. 3A) as the integrated response (area under the curve) remained largely unaffected (Fig. 3B); dexamethasone only slightly limited the peak response to CL316243 at one time-point (Fig. 3A). At the same time, the drop in RQ that normally followed the β3 agonist administration (before treatment with dexamethasone) was only marginal in these animals given that the baseline was already reduced by treatment with dexamethasone (Fig. 3C).

In contrast, in the animals kept on a HFD, treatment with dexamethasone slowed down the VO2 acceleration caused by administration of CL316243 (Fig. 3D). This was observed at every time point that followed the β3 agonist injection (Fig. 3D) as well as when the VO2 response was expressed in terms of integrated area under the curve (Fig. 3E). The drop in RQ that followed CL316243 administration in the animals fed with a HFD was not affected by treatment with dexamethasone (Fig. 3F).

DISCUSSION

The present study demonstrates that long-term treatment with dexamethasone in mice is associated with slower nightly EE (Fig. 2A–B) and, in chow-fed animals, accelerated fatty acid oxidation (lowered the RQ) (Fig. 2C–D). Whereas glucocorticoids were known for disrupting cold- or norepinephrine-induced UCP-1 expression (21), slowing down basal and inducible EE during high fat feeding is to our knowledge a novel finding. In fact, treatment with dexamethasone reduced β3-stimulated EE in the HFD animals (Fig. 3A–B, D–E), explained by the marked decrease in UCP-1 mRNA observed in the BAT of these animals (Table 1). These effects of dexamethasone treatment on diet-induced EE provide new insights into the mechanisms by which glucocorticoids aggravate diet-induced obesity and dyslipidemia in mice (23) and promote their well known increase in the percentage of body fat (Fig. 1B–C) and liver steatosis (Fig. 1D).

The mechanisms by which dexamethasone dysregulates fuel metabolism are not completely understood. Whereas glucocorticoids exhibit orexigenic effects by acting directly in the central nervous system (8, 9), no significant changes in caloric intake were observed in the dexamethasone-treated animals in the present study. This indicates that changes in EE are likely to play a major role in worsening the metabolic phenotype of mice placed on HFD. In fact, a key observation that is likely to contribute to increased body fat is the reduction in nightly EE during treatment with dexamethasone, when mice are awake and eating, particularly while on a HFD (Fig. 2A–B). While this should largely reflect the basal metabolic rate, it also reflects adaptive EE given that mice were kept at room temperature, a condition previously shown to activate BAT (30). At the same time, dexamethasone also inhibited β3-stimulated EE (Fig. 3D–E), which largely reflects BAT activation (33).

Despite the slower rate of EE, treatment with dexamethasone accelerated fatty acid oxidation, as evidenced in the present study by the drop in RQ (Fig. 2C–D), which is explained by the fact that glucocorticoids promote the availability of circulating energy substrates such as fatty acids and glucose that are to be used during the fight-flight response to stress (34, 35). Nonetheless, even though fatty acid oxidation is increased, the net metabolic effect of long-term treatment with dexamethasone is an increase in body fat (Fig. 1B–C) and liver steatosis (Fig. 1D) (36).

In small rodents such as the mouse, most the acceleration in the rate of EE caused by the high fat feeding takes place in the BAT. Thus, it is notable that UCP-1 gene expression was substantially decreased by prolonged treatment with dexamethasone (Table 1), which turns out to have an important metabolic impact, i.e. the limited β3-stimulated EE. In fact, short-term administration of corticosterone in rats inhibits BAT UCP-1 expression, both at the basal level and in response to adrenergic stimuli such as exposure to cold (21); similar results were also observed in the ob/ob mouse model (37). *In vitro* studies on a brown adipocyte cell line have shown a direct inhibitory effect of glucocorticoids on UCP-1 gene expression (38). In addition to these directs effects at the BAT level, acute central administration of dexamethasone in rats was shown to increase BW gain and 11βHSD1 expression in subcutaneous adipose tissue, while at the same time decreasing BAT UCP-1; at variance, peripheral dexamethasone administration decreased BW gain and 11βHSD

expression (39). This is in agreement with the present observation of reduced BW gain in mice treated with dexamethasone (Fig. 1A), an effect that others have linked to the type II glucocorticoid receptor (40).

In summary, the present findings indicate that chronic treatment with dexamethasone slows down EE and β3 agonist-inducible EE, particularly in animals on a HFD, largely due to a reduction in BAT UCP-1 mRNA. This is likely to contribute to the increased body fat and liver steatosis observed during treatment with dexamethasone.

Acknowledgments

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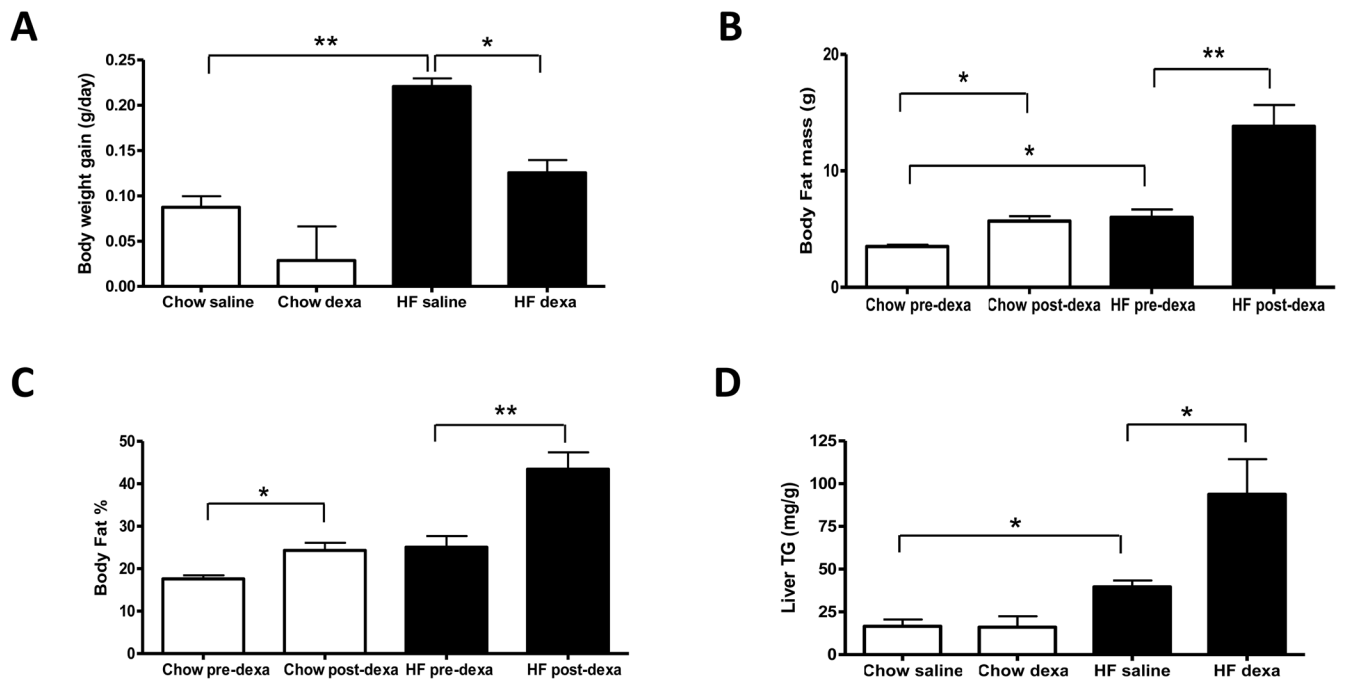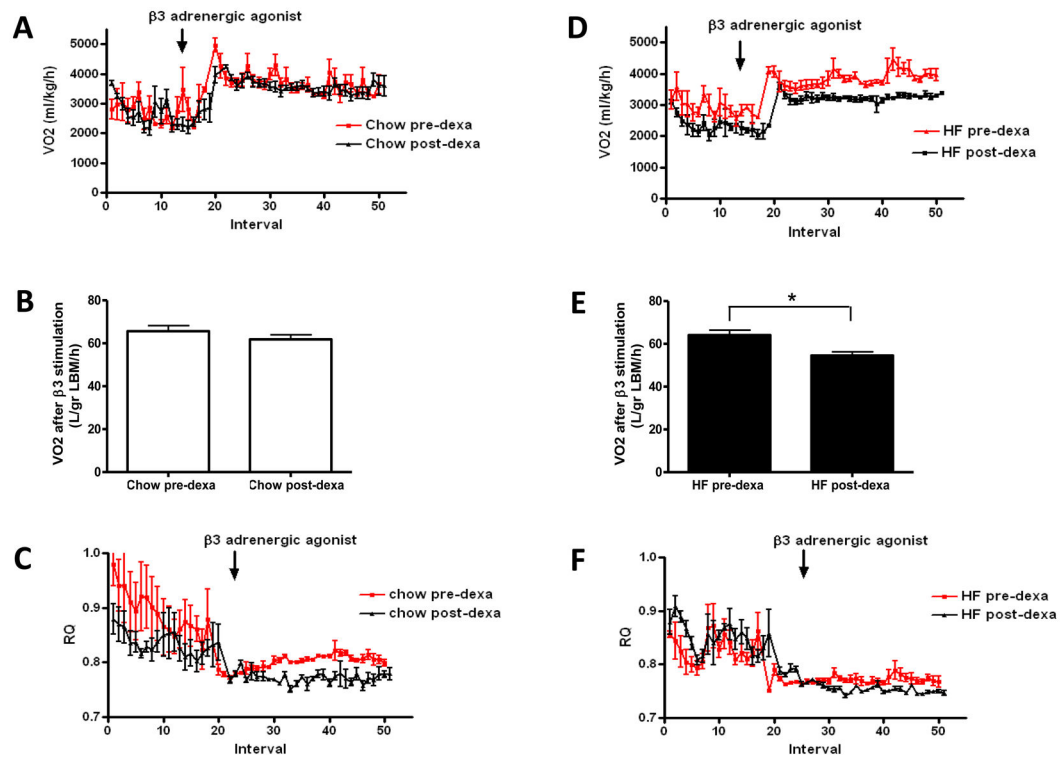
Figure 1. Effect of treatment with dexamethasone (DEXA) on different metabolic parameters in chow diet and high fat diet mice

(A) Body weight gain is shown. Body composition was assessed by DXA before (pre-dexa) and after (post-dexa) treatment with DEXA and expressed as: **(B)** Body fat mass, and **(C)** total body fat. **(D)** Liver triglycerides (TG) content. Results are expressed as mean ± SEM (n=3–6). HF, high fat diet. Dexa, dexamethasone. $^*P<0.05$; $^{**}P<0.001$.
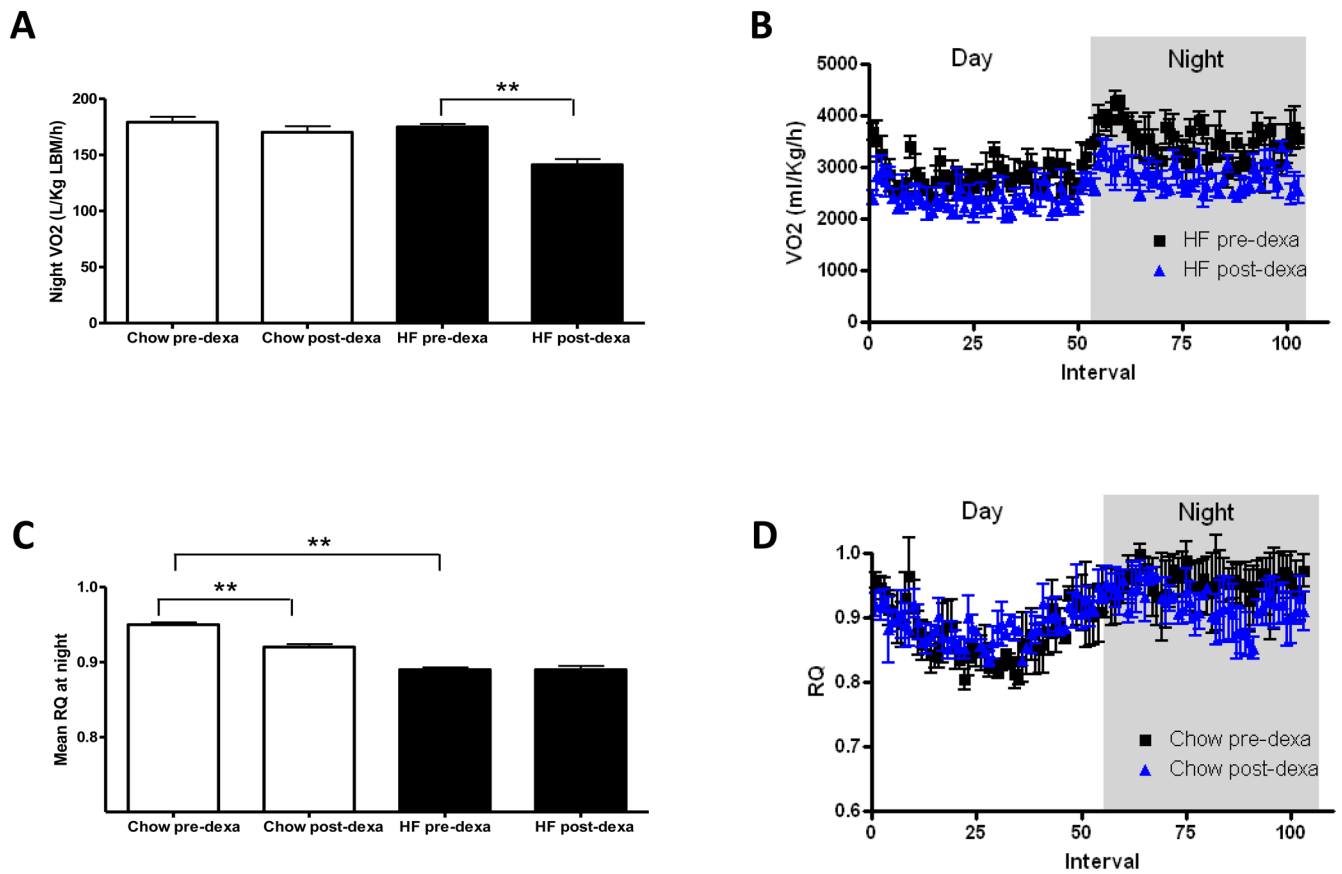
Figure 2. Impact of dexamethasone (DEXA) treatment in the metabolic profile
(A) Oxygen consumption in dark cycle before (pre-dexa) and after (post-dexa) DEXA treatment in chow diet and high fat diet mice. **(B)** VO2 was measured during a 24-hours period in mice on high fat diet, before and after treatment with DEXA. **(C)** Same as in (A), except that RQ is shown. **(D)** RQ in a 24-hours measure period in mice on chow diet, before and after treatment with DEXA. Results are expressed as mean ± SEM (n=3). HF, high fat diet. Dexa, dexamethasone. $^{*}P<0.05$; $^{**}P<0.001$.

Figure 3. Effects of treatment with β3-adrenergic receptor agonist-CL-316243 during treatment with dexamethasone

Oxygen consumption **(A)** and RQ **(C)** before and after treatment with β3 stimulation on chow diet animals. **(B)** VO2 is shown as an average data of 4-hours period of β3 stimulation on chow diet animals. Oxygen consumption **(D)** and RQ **(F)** before and after treatment with β3 stimulation on HFD diet animals. **(E)** VO2 shown as an average data of 4-hours period of β3 stimulation on HFD diet animals. Results are expressed as mean ± SEM (n=3). HF, high fat diet. Dexa, dexamethasone. $^{*}P<0.05$.

Table 1

Interscapular BAT weight and UCP1 mRNA levels in mice placed on a high fat diet and treated with dexamethasone.

	High Fat Diet		Chow Diet	
	Control	Dexamethasone	Control	Dexamethasone
BAT weight (g)	0.13±0.018	0.21±0.005[*]	0.06±0.01	0.17±0.05
BAT/BW (mg/g BW)	4.3±0.3	8.0±0.5[*]	2.0±0.3	10±1.0[**]
UCP-1/Cyclo A mRNA (fold)	0.35±0.02	0.17±0.04[*]	0.77±0.08	0.17±0.03[**]

Data are expressed as mean ± SEM; n=3–6 per group.

[*] p<0.05 vs. control (saline) on high fat diet;

[**] p<0.05 vs. control (saline) on chow diet.