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High-fat diet increases NF- κ B signaling in the prostate of reporter mice

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Abstract

Background—High-fat diet (HFD) is considered as a major risk factor for benign prostatic diseases and cancer in the Western world. Studies have shown an association between oxidative stress and prostatic diseases. NF- κ B has been implicated in stress response and is deregulated in prostate disorders; therefore, we sought to determine whether HFD could induce oxidative stress in the prostate which could contribute to prostatic diseases.

Methods—Transgenic NF- κ B-Luc-Tag mice were either fed with regular diet (RD) or HFD for 12 weeks. Serial, non-invasive molecular imaging was performed to study NF- κ B activation in the whole body, and in various organs including thymus, spleen, and prostate. Western blotting was used to determine the expression of NF- κ B, its upstream and downstream targets in the prostate.

Results—Two-fold increase in whole body NF- κ B activity *in vivo* and 2–3 fold up-regulated prostate NF- κ B activity *ex vivo* were observed after HFD intake compared with RD controls. HFD-induced NF- κ B activity was elevated remarkably in the abdominal cavity, thymus, spleen, and prostate with increase in prostate weight. In the prostate, an increase in the protein expression of gp91^{phox}, p22^{phox}, and p47^{phox} NADPH oxidase subunits was observed suggesting the involvement of HFD in causing oxidative stress. Nuclear extracts from the prostate tissue showed an increased expression of p65/RelA that corresponded with elevated cytosolic levels of p-I κ B α , along with increased expression of downstream targets of NF- κ B, nitric oxide synthase and cyclooxygenase-2.

Conclusions—Our findings suggest that HFD-mediated oxidative stress and deregulation of NADPH oxidase leads to NF- κ B activation in the prostate.

Keywords

oxidative stress; high-fat diet; prostate; obesity; NF- κ B

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INTRODUCTION

Several risk factors attribute towards prostatic diseases including prostate cancer. These are ethnicity, age, family history and lifestyle factors. Diet is considered as one of the most important lifestyle factor that has potential influence in determining the risk of prostate diseases [1]. Studies ascertaining the risk of prostate diseases have shown that high intake of total fat, saturated fat and cholesterol were positively associated with an increased risk of prostate cancer and benign prostatic hyperplasia (BPH) after adjustment of confounding factors [2, 3]. Saturated fat, in particular, omega-6 fatty acids and cholesterol have been shown to be a risk factor for prostate cancer [4]. Consistent with this possibility, health professional studies have shown that the long-term use of cholesterol-lowering drugs, 'statins' reduces prostate cancer risk by 20–50% [5, 6]. Levels of circulating cholesterol have been reported to cause relapse after treatment of localized prostate cancer and promote prostate cancer cell proliferation [7].

A number of intriguing hypothesis for the association between fatty acids and prostatic diseases have been proposed [8, 9]. These include mechanisms involving inflammation, oxidative stress, peroxidation of lipids and accumulation of 8-hydroxy-2'-deoxyguanosine and androgen synthesis driving the growth of the prostate [10–13]. However, most of these existing studies assume that the effects of high-dietary fat on prostate growth results primarily from lipogenesis, inflammation and oxidative stress, [11] and evidence of direct effects of diet on prostate is still lacking. Chronic inflammation and oxidative stress are thought to play an essential role in prostatic diseases as well as aging [10 and 14], so the possibility exists that high fat diet could induce intraprostatic inflammation and oxidative stress to increase vulnerability of the prostate to numerous prostatic diseases and to age-associated deficits. Therefore, it is important to determine whether high dietary fat can induce oxidative stress and inflammation directly in the prostate.

Nuclear Factor-kappa B (NF- κ B), a family of transcription factors is activated as a result of inflammatory and stress responses in most mammalian cell types. It regulates various cellular processes like apoptosis, cell growth, angiogenesis, cell survival and its involvement in cancer is well recorded [15, 16]. In fact, NF- κ B has been reported to be constitutively upregulated in prostate cancer [17, 18]. Apart from cancer, NF- κ B is also associated with wide range of human diseases like rheumatism, inflammatory bowel diseases, neurodegenerative conditions, asthma and chronic obstructive pulmonary disease [19–22]. Over-expression of NF- κ B in these inflammatory diseases has been associated with increase presence of reactive oxygen species (ROS) and NADPH oxidase activity [23]. Upregulation of NF- κ B during inflammation also results in the recruitment of inflammatory cells leading to the production of various pro-inflammatory cytokines, such as IL-1, IL-6, IL-8 at the site of inflammation [24, 25]. Studies have shown an association between prostate inflammation with BPH and prostate cancer [26]. Studies from our laboratory have reported that IL-1 β -induced NF- κ B activity contributes in chemoattractant urging initial phase of prostatic inflammation that might have a capability for maintaining the chronic inflammation and proliferative inflammatory atrophy (PIA) in the prostate, which are recognized as putative precursor lesions in the development of prostate cancer [27]. Recently it has been shown

that high-fat diet (HFD) is a stimulator for NF- κ B responses in diet-induced obese mice [28].

In the present study we have investigated the consequence of HFD on the prostate using NF- κ B-Luciferase transgenic mice. We observed that HFD increased the activation of NF- κ B along with elevated levels of NADPH oxidase components. We report for the first time that HFD could initiate oxidative stress of the prostate that involves both NF- κ B and NADPH oxidase activity which might lead to intraprostatic inflammation and be a potential cause of prostatic diseases including BPH and prostate cancer.

MATERIALS AND METHODS

Animals

Forty eight males from the colony of NF- κ B-Luciferase transgenic mice (Jackson Laboratory, Bar Harbor, Maine) were used for these experiments. The animals were bred and maintained at the AAA-LAC accredited Animal Resource Facility of Case Western Reserve University. All animal experiments were performed according to institutional guidelines for animal care. The mice were matched according to age and divided into two groups. One group was fed with regular diet (RD) and the other was subjected to high fat diet (HFD) typical of 'Western style' high-fat diet. The HFD formula consisted of rodent chow containing of 23% fat, 43% carbohydrate, and 20% of protein. In contrast, RD consisted of standard rodent chow containing 4.5% fat, 56% complex carbohydrate, and 23% protein (all from Harlan Labs Inc., Madison, WI). The animals were kept on these diets for 4, 8 and 12 weeks respectively, sacrificed and used for *in vivo* and *ex vivo* experiments.

Materials

Antibodies for gp91^{phox}, p22^{phox}, and p47^{phox}, p65/RelA, I κ B α , iNOS, COX-2 Oct-1 and β -actin were purchased from Santa Cruz Biotechnology (Santa Cruz, CA). Phospho-I κ B α (Ser32/36) and phospho-IKK α / β (p-IKK α , Ser180; IKK β , Ser181) were purchased from Cell Signaling Technologies (Danvers, MA). Secondary antibodies for mouse and rabbit (horseradish peroxidase conjugates) were purchased from Santa Cruz Biotechnology (Santa Cruz, CA). Enhanced Chemiluminescence Kit (ECL) for chemiluminescence was purchased from GE Healthcare Biosciences (Piscataway, NJ).

Imaging of NF- κ B, *in vivo* luciferase activity

Imaging of transgenic mice was performed according to our previous publication [29]. The images of mice were taken after each time interval of feeding. The transgenic mice NF- κ B-dependent luciferase activity both *in vivo* and *ex vivo* was measured as described previously with some modifications [29].

Western blotting analysis

Prostate tissue from NF- κ B-Luc transgenic male mice fed with RD and HFD were processed for total, cytosolic and nuclear lysates. The whole prostate was sliced into two portions. The smaller portion was used for total lysate. 200 μ L of buffer [(50 mM TRIS-HCl (pH7.4), 150 mM NaCl, 1mM EDTA, 1mM EGTA, 20mM NaF, 1mM DTT, 0.5mM PMSF

100mM Na₃VO₄, and protease inhibitor mixture (Roche Molecular Biochemicals)] was added to the sliced tissue stored in eppendorff tubes and incubated for 30 min on ice, homogenized, followed by incubation for additional 30 min and centrifuged at 14000Xg for 15 min at 4°C. The supernatant was collected in pre-cooled micro-centrifuged tubes and stored at -80 °C.

Cytosolic and nuclear extract were obtained from the larger portion of the minced prostate tissue. The tissue was suspended in 400µL of ice-cold hypotonic buffer [10mM HEPES, pH 7.9, 10 mM KCl, 0.1 mM EDTA, 0.1 mM EGTA, 1mM DTT, 0.5mM PMSF 100mM Na₃VO₄, and protease inhibitor mixture (Roche Molecular Biochemicals)]. The samples were incubated for 30 min on ice, homogenized, with 30 min additional incubation followed by the addition of 10% NP-40. The samples were vortexed and centrifuged for 1min at 14000Xg at 4°C and the supernatant constituted the cytosolic fraction. The pellet constituted the nuclear fraction and was extracted with 100µL of buffer containing, 200mM HEPES, pH 7.9, 400mM NaCl, 1mM EDTA, 1mM EGTA, 1mM DTT, 0.5mM PMSF and protease inhibitor (Roche Molecular Biochemicals). After 30 minute incubation with intermittent vortex to ensure that the pellet was suspended uniformly, the suspension was centrifuged at 14000Xg at 4°C for 5 minutes. The collected supernatant constituted the nuclear fraction. All the fractions were stored at -80°C and were used for Western blotting.

Total tissue lysate, cytosolic and nuclear fractions were used for Western blotting by loading 40µg proteins resolved over 4–20% Tris-glycine polyacrylamide gel and then transferred onto the nitrocellulose membrane. The blots were blocked using 5% nonfat dry milk and probed using appropriate primary antibodies overnight at 4°C. The membrane was then incubated with appropriate secondary antibody horseradish peroxidase conjugate (Santa Cruz Biotech) followed by detection using chemiluminescence ECL kit (GE Healthcare Biosciences). For equal loading of proteins, the membrane was probed with appropriate loading controls. Densitometric measurements of the bands in Western blot analysis were done using digitalized scientific software program using Kodak 2000R imaging system.

Statistical Analysis

The values are expressed as mean ± SD. The significance of measured values between the groups with RD and HFD were performed by Student's t-test for Western blotting densitometric analysis and by one-way analysis of variance (ANOVA) with 95% confidence limits for bio-luminescence data analysis. The *p* values less than 0.05 were taken as significant in experiments.

RESULTS

HFD increases whole body NF-κB activity in NF-κB-Luc mice

Recent reports showed that HFD stimulate NF-κB in obese mice [28]. In our study we used non-obese NF-κB-Luc transgenic mice fed with HFD for 4, 8, and 12 weeks, and compared with mice fed on RD, respectively. In order to know if there was a change in the NF-κB-dependent luminescence *in vivo* we accessed the photon flux of the whole body from animals with different diets. Following this procedure, we found that *in vivo* imaging at each

time period revealed significant difference in NF- κ B-dependent luminescence between the animals kept on RD and HFD, especially in organs located in abdominal cavity (Figure 1A). Unexpectedly, a twofold increase of NF- κ B-dependent luminescence of whole body of HFD mice was noted after 4 weeks of HFD. This up-regulated NF- κ B dependent luminescence in the mice fed with HFD was steady at 8 week with an increase at 12 week as compared to the animals with RD (Figure 1B). Though an increase in the body weight was observed in both the groups over time, mice fed with HFD gained weight that were markedly higher than the individuals in the control group (Figure 1C). These results suggest that HFD feeding increases NF- κ B activity by fourth week that remained elevated during the 8 and 12 weeks.

HFD augments NF- κ B activity in various organs

In order to determine how HFD affected the NF- κ B activity in various organs or body regions of the mice we measured the luminescence photon flux from the abdominal cavity (consisting of the alimentary canal and GU apparatus), thymus and the spleen. The total photon flux was significantly increased ($P<0.01$) by 12 weeks from the organ of abdominal cavity, but no change was observed in the 4 or 8 weeks of HFD feeding. In the thymus, the NF- κ B activity increased by 4 weeks, but showed no change during the 8 week feeding. Interestingly, 12 week HFD feeding caused a significant increase in the total flux of NF- κ B-dependent luminescence ($P<0.01$) in the thymus. In the spleen, HFD feeding caused a modest increase in flux by 4 weeks, compared to RD mice spleen, however showed a marked increase in the photon flux by 8 and 12 weeks (Figure 2).

HFD increases prostate weight and NF- κ B activity

We evaluated the effect of HFD on the prostate weight and NF- κ B activity. HFD intake caused a significant elevation of the prostate weight by 4 week which further increase at 8 week with a plateau till 12 week, as compared to the prostate of RD mice. As a result of this, no significant change was observed in the weight of prostate at 12 weeks of age between the HFD and RD mice. In *ex vivo* studies, we observed that HFD feeding increases NF- κ B-dependent luminance in the prostate gland that led to an increased total photon flux which peaked at 8 weeks, and correlated with increase in prostate weight at same time interval (Figure 3).

HFD increases intraprostatic expression of NADPH oxidase components

Studies have demonstrated that high-fat diet induces NADPH-oxidase associated oxidative stress in the cerebral cortex of rats that led to inflammation and NF- κ B activation [30]. Therefore, we investigated the effect of HFD on the levels of NADPH oxidase components in the prostate since we observed an increase in NF- κ B activation. We used prostate samples from mice that were treated with HFD for 4 and 8 week and compared them with the RD. Using Western blotting we probed for gp91^{phox}, p22^{phox} and p47^{phox} expression. The protein levels of gp91^{phox} were increased after 4 weeks of HFD by 3.0 fold ($P<0.01$) which further increased by 6.0 folds ($P<0.01$) at 8 weeks. Similarly, expression of p47^{phox} were significantly increased by 2.25 fold ($P<0.005$), and p22^{phox} by 2.6 folds ($P<0.005$) after 8 weeks for HFD feeding (Figure 4).

HFD causes intraprostatic NF- κ B activation

To examine changes in nuclear levels of NF- κ B, we performed Western blotting using the nuclear extracts from prostate tissues from mice fed with RD and HFD. Our data showed that the nuclear levels of p65/RelA significantly increased to two fold ($p<0.05$) by four weeks of HFD feeding which persisted to the same levels at 8 weeks ($p<0.01$), suggesting increased translocation of p65/RelA into the nucleus as a result of HFD intake. Since inactivated p65/RelA is bound to I κ B α in the cytosol along with p50 subunit and upon activation I κ B α is phosphorylated at Ser32/36 releasing p65/RelA that translocates into the nucleus, therefore we examined the total and phosphorylated of I κ B α in the cytosol of various groups. Feeding HFD caused a significant increase in the phosphorylation of I κ B α after 4 and 8 weeks of feeding (4 weeks, $P<0.01$; 8 weeks, $P<0.05$). Furthermore, HFD intake resulted in increased p-IKK expression, the upstream kinase that phosphorylates the NF- κ B complex, at 4 and 8 weeks ($P<0.01$), respectively.

HFD modulates intraprostatic NF- κ B-regulated genes

Next we probed for iNOS and COX-2 to find out if HFD caused any alterations in the levels of these proteins. Interestingly, iNOS levels were significantly increased to 4-fold ($P<0.001$) after 4 weeks of HFD intake, and 2-fold increase ($P<0.05$) was observed at 8 weeks. Similarly, COX-2 levels also increased (2-fold) at 4 weeks and 1.6-fold increase at 8 weeks of HFD intake.

DISCUSSION

The present study provides evidence that high dietary fat intake promoted oxidative stress and inflammation in the prostate gland. Our major findings revealed that HFD intake enhanced ROS generation through elevated expression of NADPH oxidase subunits causing NF- κ B activation and induction of NF- κ B regulated genes COX-2 and iNOS. These results demonstrate that NF- κ B is important downstream effectors of both oxidative stress and inflammatory pathways and can be activated by consuming HFD. Our studies strengthen the link between the 'Western style' high-fat diet and increased risk of prostatic diseases including BPH and prostate cancer.

An increasing scientific literature provides abundant evidence that overproduction of ROS can induce cellular damage *via* oxidation of critical cellular components such as membrane lipids, proteins and DNA. ROS are generated by both mitochondrial electron transport and by cytosolic enzymes such as the NOX family of NADPH oxidases [31, 32]. NADPH oxidases consists of at least six subunits, the membrane bound gp91^{phox} and p22^{phox} homodimer, the cytoplasmic complex of p40^{phox}, p47^{phox} and p67^{phox} and small GTPase Rac1 & 2, respectively in non-phagocytic cells as well as in various epithelial cells [33, 34]. In prostate cancer cells, NADPH oxidase system is a major source of production of reactive oxygen species where NOX1 (homolog of the catalytic subunit of the superoxide-generating NADPH oxidase of phagocytes, gp91^{phox}) and NOX5 (Ca²⁺ concentration-responsive gp91^{phox} homolog) exert major effects on prostate tumor growth and angiogenesis [35]. In the present study, we determined the effect of HFD on the NADPH-oxidase components that are considered to be upstream of NF- κ B. Our findings suggests that HFD intake elevated the

levels of gp91^{phox} significantly at 4 and 8 weeks of feeding, while p22^{phox} and p47^{phox} were elevated at 8 weeks, suggesting that high levels of dietary fatty acids might induce oxidative stress in the prostate *via* the NADPH oxidase pathway. However, precise signaling mechanism after HFD intake in the prostate needs further investigation.

Researchers have linked an association between obesity, HFD and NF- κ B. Recent studies connect the role of HFD in potentiating NF- κ B activity in obese mice, and the activity is possibly derived from the abdominal tissue fat deposits [28]. HFD is suggested to increase the risk of aggressive prostate tumors in older males [36] and high content of saturated fat in the HFD has been linked to increased mortality in prostate cancer patients [37]. In the present study, we hypothesized that HFD augmented NF- κ B activation leading to inflammatory condition of the prostate in NF- κ B-Luc mice. We observed that HFD caused an increased NF- κ B dependent luminance which was not uniform in all the regions of the body of mice that harbor the three binding sites for NF- κ B coupled to the luciferase gene. We further observed that HFD caused the activation of NF- κ B in the abdominal cavity, and lymphoid organs such as spleen and thymus. In the abdominal cavity, the NF- κ B activity was significantly higher at 12 weeks of HFD feeding, while in the thymus activity increased after 4 weeks and 12 weeks of feeding. In the spleen, HFD intake caused an increase in NF- κ B activity at 8 and 12 weeks. This is in concurrence with previous report that shows that HFD-evoked NF- κ B activity was non-uniform and sex-dependent. In the males the NF- κ B signals were localized to the abdomen and in the females the activity was more in the thoracic region [28].

NF- κ B is a family of transcription factors that play an important role in regulating the expression of genes involved in inflammation, stress response, proliferation and apoptosis [20, 38]. NF- κ B is a heterodimer composed of p50 and p65 subunits and, under resting conditions, the dimer is retained in the cytoplasm in an inactive state through interaction with I κ B. NF- κ B can be rapidly activated in response to a variety of inflammatory stimuli that lead to degradation of I κ B [39]. Earlier reports suggest that HFD induces neural oxidative stress, inflammation and NF- κ B activation in rat cerebral cortex that leads to dementia [48]. Our results demonstrate that high-fat diet intake enhanced I κ B phosphorylation and p65/RelA subunit activation in the prostate, which supports our observation of HFD-induced oxidative stress and inflammation in the prostate.

HFD is considered as one of the risk factors for prostate cancer in the United States and other Western nations and has been shown to induce low-grade inflammation in adipose tissue [40]. Importantly, HFD intake typically induced NF- κ B activity levels to about 2–3 fold, which is much lower than 10–100 fold activation typically of acute inflammatory reaction. In fact, increase in low NF- κ B activity might contribute to intraprostatic inflammation with increased susceptibility to develop prostatic diseases including cancer. Furthermore, it has been reported that a high-fat, high-cholesterol diet induced considerably greater hepatic expression of several inflammatory and oxidative stress-responsive genes and significant NF- κ B activation that might cause longstanding inflammation in the prostate. We have previously reported that IL-1 β -induced NF- κ B has ability to maintain chronic inflammation and proliferative inflammatory atrophic lesions in the prostate [27].

Further studies are required to precisely understand the role of high-fat diet in intraprostatic inflammation.

It is well documented that NF- κ B plays a prominent role in inflammation [41] and inflammation driven cancers [16]. In inflammation-associated liver cancer models, tumor development progresses from chronic liver inflammation to dysplasia, carcinoma, and metastasis where NF- κ B plays a critical role during malignant conversion [38 and 42]. We have previously demonstrated that NF- κ B/p65/RelA is constitutively over-expressed in human prostate adenocarcinoma and correlates with disease progression affecting the downstream target genes related to inflammation, angiogenesis and cancer progression [17]. Therefore, next we determined whether HFD intake has an effect on the downstream targets of NF- κ B. We studied the levels of inducible nitric oxide synthase (iNOS) and cyclooxygenase-2 (COX-2), which are known downstream targets of NF- κ B. The level of iNOS and COX-2 were increased as a result of NF- κ B activation after HFD intake. Inflammation is reported to induce expression and enzyme activity of iNOS and COX-2, which produces pro-inflammatory mediators such as prostaglandin E₂ (PGE₂) and nitric oxide [43, 44, 45, 46 and 47]. We have previously demonstrated increased levels of iNOS, prostaglandins and COX-2 in prostate tumor tissue compared with non-cancerous benign tissue, suggesting the role of these two molecules in prostate tumorigenesis [17 and 48].

In summary, HFD might be an important risk factor for benign diseases and prostate cancer in the Western world. Our in-vivo molecular imaging studies suggest that HFD increased NADPH oxidase-related oxidative stress and NF- κ B activity with increased inflammatory response due to augmentation of COX-2 and iNOS. In the prostate, HFD appears to trigger growth signal causing an increase in weight of the prostate. We suggest HFD to be an important risk factor in causing inflammatory and oxidative stress that could be linked with the benign prostatic disease and cancer of the prostate gland.

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Abbreviations

HFD	High Fat Diet
RD	Regular Diet
NF-κB	Nuclear Factor-kappa B
iNOS	nitric oxide synthase
COX-2	cyclooxygenase-2
ROS	reactive oxygen species
PIA	proliferative inflammatory atrophy

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Figure 1. Imaging of NF-κB-Luc mice after treatment with regular (RD) and high-fat diet (HFD). Mice fed with RD or HFD for 4, 8 and 12 weeks were imaged at the end of each time interval. Imaging was performed using an ultra-sensitive camera on anesthetized mice that were placed in a light-sealed chamber connected to an image intensifier coupled to a CCD camera of IVIS 150 System Xenogen (Alameda, CA). Gray scale images were obtained before luminescence imaging for reference. **(A)** Representative figure of mice imaged for NF-κB-dependent luminescence after 12 weeks of RD and HFD feeding. **(B)** Data of NF-κB induced total proton flux in the whole body of mice, fed with RD or HFD after 4, 8 and 12 weeks. Black bars indicate RD and white bars indicate HFD. The SD is indicated, *p<0.05, **p<0.01, represents the significant changes in NF-κB activation by HFD compared to its respective RD treated controls. **(C)** Body weight changes in mice after RD and HFD feeding. NF-κB-Luc mice were either fed with RD (black circle) or HFD (white circle) and increase in body weight was recorded after the indicated time intervals.

Figure 2.

Changes in NF-κB-induced luminescence in the abdominal cavity, thymus and spleen of mice after RD and HFD feeding. NF-κB-Luc mice fed with RD or HFD for 4, 8 and 12 weeks were imaged at the end of each time interval. Total photon flux was measured to determine the extent of NF-κB activation. Data represents the mean value of total photon flux from the abdominal cavity, thymus and spleen after 4, 8 and 12 weeks of RD and HFD feeding. Black bars represent RD and white bars represent HFD. The SD is indicated, * $p < 0.05$, ** $p < 0.01$ represents the significant changes in HFD compared to its respective RD fed controls.

Figure 3.

HFD induced changes in the prostate of NF- κ B-Luc mice. **(A)** Changes in the weight of prostates after RD and HFD feeding at the end of the indicated time intervals. Data represents the mean value of prostate weight compared between RD and HFD after each time interval. Black bars represent RD and white bars represent HFD. The SD is indicated, ** $p < 0.01$ represents the significant changes in the weight of prostates from mice fed with HFD compared to its respective RD treated controls. **(B)** Data of NF- κ B-induced total proton flux from the prostate of mice, fed with RD or HFD after 4, 8 and 12 weeks. Black bars indicate RD and white bars indicate HFD. The SD is indicated, ** $p < 0.01$, represents the significant changes in HFD compared to its respective RD fed controls.

Figure 4.

Protein expressions of NADPH oxidase subunits in the prostrate of NF- κ B-Luc mice fed with RD and HFD. **(A)** Total cell lysates were prepared as described in the 'materials and methods' from the prostrate of mice fed with RD and HFD. The samples were subjected to SDS-PAGE gel electrophoresis. The blots were analyzed for the indicated antibodies and β -actin was used as the loading control. **(B)** Densitometric quantification represents the changes in the levels of gp91^{phox}, p22^{phox} and p47^{phox}. Black bars indicate RD and white bars indicate HFD. Data are a mean of $\pm$ SD and corrected for loading. The asterisk (**) indicates the significant changes ($p < 0.01$) in HFD compared to its respective RD fed controls.

Figure 5.

Protein expression of NF- κ B in the nuclear and cytosolic fractions extracted from the prostate of RD and HFD fed NF- κ B-Luc mice. **(A)** Nuclear extract were prepared as described in the 'materials and methods' from the prostate of mice fed with RD and HFD. The samples were subjected to SDS-PAGE gel electrophoresis. The blots were analyzed for p65/RelA; Oct-1 was used as the loading control. The densitometric quantification for p65/RelA was normalized with Oct-1. Black bars indicate RD and white bars indicate HFD. Data are a mean of $\pm$ SD and corrected for loading. The asterisk (**) indicates the significant changes ($p < 0.01$) in HFD compared to its respective RD fed controls. **(B)** Cytosolic fractions prepared as described in the 'materials and methods' from the prostate of mice fed with RD and HFD. The samples were subjected to SDS-PAGE gel electrophoresis. The blots were analyzed for the indicated antibodies and β -actin was used as the loading control. Densitometric quantification represents the ratio of I κ B α to p-I κ B α and p-IKK β levels. Black bars indicate RD and white bars indicate HFD. Data are a mean of $\pm$ SD and corrected for loading. The asterisk (**) indicates the significant changes ($p < 0.01$) in HFD compared to its respective RD fed controls.

Figure 6.

Protein expression of iNOS and COX-2 levels in the total cell lysate extracted from the prostate of RD and HFD fed NF- κ B-Luc mice. (A) Total cell lysates were prepared as described in the 'materials and methods' from the prostate of mice fed with RD and HFD. The samples were subjected to SDS-PAGE gel electrophoresis. The blots were analyzed for the indicated antibodies and β -actin was used as the loading control. Densitometric quantification represents the changes in the levels of iNOS and COX-2 after HFD. Black bars indicate RD and white bars indicate HFD. Data are a mean of $\pm$ SD and corrected for loading. The asterisk (**) indicates the significant changes (p < 0.01) in HFD compared to its respective RD fed controls.