available at www.sciencedirect.com

ScienceDirect

journal homepage: www.elsevier.com/locate/biochempharm

Over-the-counter analgesics normalize blood glucose and body composition in mice fed a high fat diet

Eric L. Kendig [a], *Scott N. Schneider* [a], *Deborah J. Clegg* [b], *Mary Beth Genter* [a], *Howard G. Shertzer* [a,*]

[a] *Department of Environmental Health and Center for Environmental Genetics, University of Cincinnati College of Medicine, Cincinnati, OH, USA*

[b] *Department of Internal Medicine, Touchstone Diabetes Center, University of Texas Southwestern Medical Center, Dallas, TX, USA*

ARTICLE INFO

Article history:
Received 20 December 2007
Accepted 1 May 2008

Keywords:
Analgesic drugs
Diabetes
High fat diet
Mice
NADPH oxidase
Oxidative stress

ABSTRACT

Type 2 diabetes (noninsulin-dependent diabetes mellitus) develops from a pre-diabetic condition that is characterized by insulin resistance and glucose intolerance, and is exacerbated by obesity. In this study, we compared the ability of over-the-counter analgesic drugs (OTCAD) [acetaminophen (APAP); ibuprofen (IBU); naproxen (NAP); aspirin (ASA)], to protect against the development of a pre-diabetic state in mice fed a high fat diet. After 10 weeks on the high fat diet, mice had normal fasting blood glucose (FBG) levels, but exhibited impaired glucose tolerance. Treatment with 20 mg OTCADs/kg body weight improved glucose tolerance, with the order of efficacy, APAP = ASA > IBU, while NAP proved ineffective. Mice fed the high fat diet also exhibited increases in weight gain associated with an increase in body fat. OTCADs prevented in part this increase in body fat, in the order of efficacy, APAP = IBU > NAP = ASA. In isolated liver mitochondria, OTCADs inhibited succinate-dependent H_2O_2 production, while in white adipose tissue, APAP inhibited NADPH-oxidase mediated H_2O_2 production and lipid peroxidation. Thus, OTCADs diminish pro-oxidant processes that might otherwise exacerbate inflammation and a pre-diabetic state. We conclude that OTCADs, especially APAP and IBU, may be valuable tools to delay or prevent the development of type 2 diabetes from a pre-diabetic condition.

1. Introduction

Obesity and diabetes are at epidemic proportions in American and Western populations [1]. Type 2 diabetes (T2DM; non-insulin-dependent diabetes mellitus) usually occurs later in life and is typically preceded by a pre-diabetic condition that includes impaired glucose tolerance and insulin resistance. As the pre-diabetic state develops into frank T2DM, there is usually significant loss of pancreatic β-cell mass, and the normal functional reserve for insulin secretion is greatly reduced. Gain in body weight and visceral fat, as well as systemic inflammation, are primary risk factors for T2DM [2–4]. Hyperglycemia, the hallmark of diabetes, is the major risk factor for development of diabetic microvascular disease, including cardiomyopathy, nephropathy, retinopathy and peripheral neuropathy [5–9]. There are several mechanisms

* *Corresponding author at*: Department of Environmental Health, University of Cincinnati College of Medicine, 3223 Eden Avenue, Cincinnati, OH 45267-0056, USA. Tel.: +1 513 558 0522; fax: +1 513 558 0925.
E-mail address: shertzhg@ucmail.uc.edu (H.G. Shertzer).

Abbreviations: APAP, acetaminophen, *N*-(4-hydroxyphenyl)acetamide; ASA, aspirin, 2-acetylsalicylic acid, 2-acetoxybenzoic acid; FBG, fasting blood glucose; IBU, ibuprofen, 2-[4-(2-methylpropyl)phenyl]propanoic acid; NAP, naproxen, (S)-6-methoxy-α-methyl-2-naphthaleneacetic acid; T2DM, type 2 diabetes mellitus; NSAID, non-steroidal anti-inflammatory drug; OTCAD, over-the-counter analgesic drug.

0006-2952/$ – see front matter
doi:10.1016/j.bcp.2008.05.001

associated with the development and the etiology of hyperglycemic disease that provide major targets for therapy [10], especially those pathways related to inflammation, cellular oxidative stress and mitochondrial reactive oxygen production [11–13].

Many animal and human studies have examined the potential for analgesics and anti-inflammatory drugs to block or reverse cardiovascular disease, the most life-threatening complication of T2DM. It is therefore, surprising that few if any studies have examined the ability of such compounds to intervene in the development of diabetic hyperglycemia, with the exception of aspirin (ASA) [14–16]. Even in the case of ASA, almost all studies have focused on reducing the risk for developing cardiovascular disease, rather than reducing the severity of a primary underlying cause, hyperglycemia. We have recently shown that the analgesic acetaminophen (APAP) reduces the increase in body fat and the development of T2DM in mice fed a high fat diet [17]. This study suggests that APAP, and perhaps other over-the-counter analgesic drugs (OTCADs), might be useful in delaying the onset or preventing the development of T2DM. We therefore, examined the potential for widely used OTCADs [APAP; ibuprofen (IBU); naproxen (NAP); ASA] to prevent the development of factors associated with the risk of developing diabetes. We chose to use a standard dosage of OTCADs (20 mg/(kg d)), which approximates the analgesic dose range used routinely and safely by many individuals.

2. Materials and methods

2.1. Chemicals

All chemicals and reagents were obtained from Sigma–Aldrich Chemical Company (St. Louis, MO) as the highest available grades.

2.2. Animals and treatment

All experiments involving mice were conducted in accordance with the National Institutes of Health standards for care and use of experimental animals and the University of Cincinnati Institutional Animal Care and Use Committee (IACUC). Female C57BL/6J mice, 8–10 weeks of age, were purchased from Jackson Laboratory (Bar Harbor, ME), and conditioned in the animal facility for 1 week. Animals were group-housed, maintained on a 12-h light/dark cycle, and had access to rodent chow and water ad libitum. Mean body weight of each treatment group was the same at the beginning of the study. The chow consisted of either a normal or a high fat diet that was pelleted, semi-purified, and nutritionally complete. The normal diet (AIN-93M, Dyets, Bethlehem, PA) contained 3 g of butter oil and 1 g of soybean oil/100 g diet, supplying 16.12 kJ/g diet, with 1.29 kJ from fat [18]. High-fat diet-fed mice received a modified AIN-93M diet containing 19 g butter oil and 1 g of soybean oil/100 g diet, supplying 19.34 kJ/g diet, with 7.74 kJ from fat. Both diets contained the same amount of protein, minerals and vitamins [19].

In these studies, mice were treated by gastric gavage with either saline vehicle, or with 20 mg/(kg d) of one of the four OTCADs, once per day between 10 a.m. and 12 noon. An alternate route of administration for APAP was via drinking water at 0.2 mg APAP/ml drinking water. A 25 g mouse consuming 3 ml water/d would consume 24 mg APAP/kg body weight/d. We chose to use this standard dosage, which is in the range of conventional human analgesic usage for APAP, IBU and ASA, and slightly higher for NAP (Table 1). Although, it would have been optimal to use a range of dosages for each analgesic, logistics made this impossible. OTCAD treatment was started 7 days before changing to the high fat diet, and measurements were made, or mice were sacrificed, 20–24 h after the last OTCAD treatment.

2.3. Fasting blood glucose (FBG) and glucose tolerance test

Blood glucose concentration was determined with a handheld glucometer (Ascensia Contour glucometer, Bayer) [19]. Bi-weekly samples of blood (5 μl) from 8 h fasted mice were applied directly to the glucose strip to measure fasting levels of blood glucose. Glucose tolerance tests were performed after an 8-h fast. After initial blood glucose determinations, 1.5 mg D-glucose/g body weight was administered by i.p. injection, followed by glucose determinations at 20 min intervals for 120 min.

2.4. Body composition and plasma lipids

Body weights were measured and food and water consumption were estimated twice weekly. Body composition was assessed in live, unanesthetized mice by nuclear magnetic resonance (NMR) (EchoMRI; EchoMedical Systems, Houston TX, http://www.echomri.com). This method provides estimates of total fat tissue, lean tissue (muscle), and water [20–22]. In order to analyze plasma lipid levels, freshly

Table 1 – Comparison of dosages of OTCADs recommended for analgesic or anti-inflammatory human use, compared to those used in this study, adjusted for human body weight

	Mouse dosage, adjusted (mg)	Recommended single human dosage	Recommended maximal daily dosage (mg)
APAP	1300	325–650 mg/4–6 h	4000
IBU	1300	200 400 mg/4–6 h	1200
NAP	1300	220 mg/8 h	660
ASA	1300	650 mg/4 h[a]	4000

Mice were treated by gavage to a single daily dosage of 20 mg/kg d. For a 65 kg human, this is 1300 mg/d. Values for human usage were extracted from [58].

[a] A low dose of ASA of 81 mg/d is recommended for anticoagulant usage.

collected heparinized blood was centrifuged at 5000 × *g* for 5 min and stored at −80 °C for 1 week. Samples were thawed on ice, and triglycerides (Liquicolor R procedure no. 2200; Stanbio Laboratories, Boerne, TX) and total cholesterol (Cholesterol procedure no. 1015; Stanbio Laboratories, Boerne, TX) were determined.

2.5. Hepatic mitochondrial energy coupling and reactive oxygen production

Mice were killed by carbon dioxide asphyxiation, followed by cervical dislocation. Liver was excised and mitochondria were isolated, washed and suspended in respiratory buffer (140 mM KCl, 1.0 mM EDTA, 2.5 mM KH_2PO_4, 2.5 mM $MgCl_2$, 0.1% bovine serum albumin, and 5 mM HEPES, pH 7.4) [23]. Mitochondrial oxygen consumption was measured polarographically with a Clark-type oxygen electrode (Hansatech Instruments, Norfolk, England). Briefly summarized, 0.5 ml of respiratory buffer and 50 mg of mitochondrial protein were equilibrated at 37 °C with stirring. The rate of State 4 respiration (ADP-limited) was determined in the presence of 6 mM succinate. Following the addition of 0.2 mM ADP, the rate of State 3 respiration was measured. The respiratory control ratio (RCR) was calculated as the ratio of State 3 to State 4 respiration. H_2O_2 was monitored in freshly prepared mitochondria as luminol (5-amino-2,3-dihydro-1,4-phthalazinedione) chemiluminescence [23].

2.6. Oxygen metabolism in white adipose tissue (WAT)

A 10% whole homogenate of visceral white adipose fat was prepared in respiratory buffer, producing an emulsion. Aliquots of this whole homogenate emulsion were removed for analysis of lipid peroxides (malondialdehyde and 4-hydroxyalkenals) using the chromogenic probe, methylphenylindole (Diagnostic kit Bioxytech LPO 586, OxisResearch, OXIS Health Products Inc.). The remaining emulsion was broken by centrifuging at 1000 × *g* for 10 min. The upper fat layer was removed, and the post-nuclear homogenate evaluated for O_2 uptake and H_2O_2 production by polarography and luminol chemiluminescence, respectively, as described above.

2.7. Statistics

Statistical significance of the differences between group sample mean values was determined by one-way analysis of variance, followed by the Student–Newman–Keuls test for pairwise comparison of means. Statistics were performed using SigmaStat Statistical Analysis software (SPSS Inc., Chicago, IL).

3. Results

3.1. OTCADs protect against the high fat diet-induced loss of glucose homeostasis

Mice that were fed the high fat diet for 10 weeks showed no changes in FBG, either with or without OTCAD treatment (Fig. 1, zero-time values for each panel). High fat diet mice, however, displayed impaired glucose tolerance following an i.p. injection of 1.5 mg glucose/g body weight, with blood glucose area-under-the-curve (AUC) values about 50% higher (27,990 ± 2050 min mg glucose/dl blood; $P < 0.05$) than for normal diet mice (AUC = 18,350 ± 1375 min mg glucose/dl blood). Pretreatment with OTCADs for 7 days before initiating the high fat diet, and subsequent daily treatment, had varying effects on glucose tolerance. In mice fed the normal diet, APAP, ASA and IBU elicited an approximate 15% reduction in cumulative blood glucose (AUC values of 15,000–16,000 min mg glucose/dl blood) after a glucose challenge. In mice fed the high fat diet, APAP, ASA and IBU improved glucose tolerance, such that glucose levels for mice fed normal or the high fat diet were comparable. NAP produced a 24% increase in blood glucose in mice fed the normal diet (AUC = 23,903 ± 2190 min mg glucose/dl blood), and was ineffective in lowering blood glucose levels in mice receiving the high fat diet (AUC = 25,430 ± 1960 min mg glucose/dl blood).

3.2. OTCADs protect against the high fat diet-induced increase in body fat

A loss of glucose tolerance is one component of a pre-diabetic condition in humans. Often this state is accompanied by

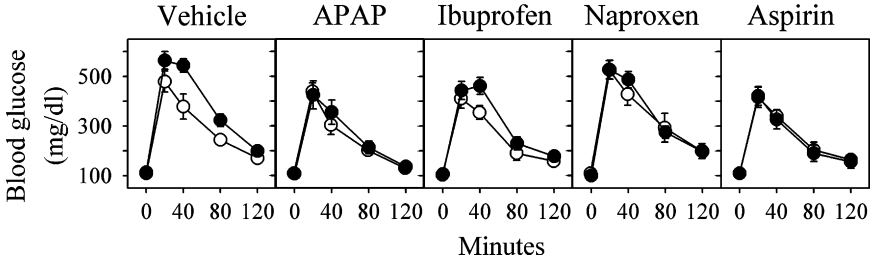

Fig. 1 – OTCADs protect against hyperglycemia in mice fed a high fat diet. Mice were gavaged daily with 20 mg OTCADs/kg body weight, or with the equivalent volume of isotonic saline vehicle, beginning 7 days before changing to a high fat diet. Data for mice fed a normal diet are shown with open circles, while data from mice fed the high fat diet are shown with black circles. After 10 weeks, FBG levels were determined followed by an i.p. injection of glucose. Blood glucose was then determined at 20 min intervals for 120 min. Mean values ± S.E.M. (N = 4) are shown for each group. Areas under the curves (AUC, in units of min mg glucose/dl blood) were approximated for each treatment group using the trapezoidal rule. Mean AUC values ± S.E.M. are given in the text.

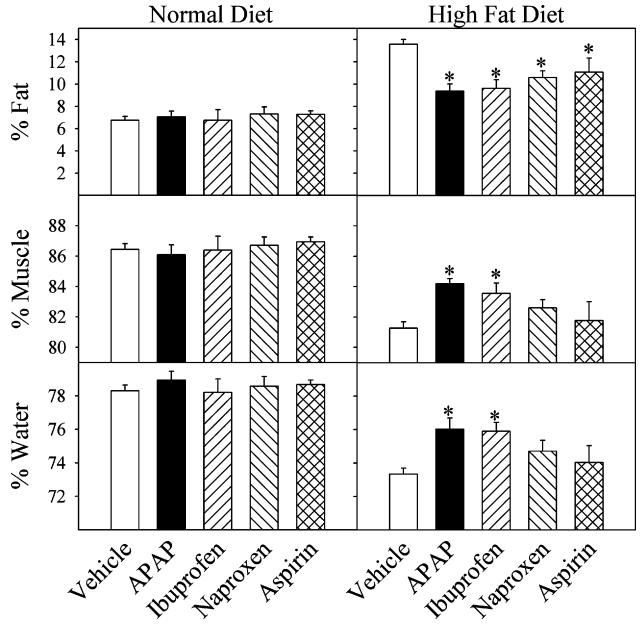

Fig. 2 – Effect of high fat diet and OTCADs on body composition. Mice were fed a normal diet (left panel of figures), or a high fat diet (right panel of figures) for 10 weeks. Mice were gavaged daily with 20 mg OTCADs/kg body weight (filled bars, as indicated), or with the equivalent volume of isotonic saline vehicle (open bars), beginning 7 days before changing to a high fat diet. The bars represent mean values for the parameters indicated on the abscissas ± S.E.M. (N = 4). *Significantly different mean value from vehicle-treated group (P < 0.05). While the figure depicts body component percentages of body weight, the actual weights of body fat, lean muscle and water, independent of body weight, are shown in Table 2.

changes in body composition, especially an increase in the amount of body fat. We evaluated body composition in mice fed the high fat diet for 10 weeks, with and without OTCAD intervention. Initially, body weights were matched for the different experimental groups. Consumption of the high fat diet resulted in a doubling in the percentage of total body fat (Fig. 2, top panels). Pretreatment and daily treatment during the period of high dietary fat consumption with either APAP or IBU significantly prevented about 70% of the gain in fat, while NAP and ASA had a lesser effect on percentage of body fat gain. The gain in percentage of body fat for mice consuming the high fat diet was accompanied by a decrease in percentage of body muscle (Fig. 2, center panels) and water (Fig. 2, lower panels), effects that were prevented by the different OTCADs to the extent that each prevented the increase in body fat.

In order to evaluate whether the changes in body fat composition were the result of changes in caloric intake, we measured food and water consumption, relative to weight gain. Although the percentage of body muscle and water decreased in mice receiving the high fat diet, the actual weights of muscle and water did not change (Table 2). Most of the increase in body weight (0.044 g/d for mice on normal diet, and 0.067 g/d for mice on high fat diet) was due to fat accumulation. Animals treated with OTCADs all showed lower fat accumulation during high fat feeding, with the order of effectiveness APAP = IBU > NAP = ASA. It is of interest that the mice appeared to self-regulate caloric intake; mice consumed about 20% less food by weight of the high fat diet, yet since the high fat diet contained 20% more energy/g diet, the energy consumption was the same on both diets. Therefore, the normal and the high fat diets were isocaloric in these studies. None of the OTCADs affected food or water consumption with either diet (data not shown). Thus, feeding efficiency (conversion of food to body mass in units of g body weight gain/energy content of food consumed) was higher in mice fed the high fat diet (1.9 mg body weight/kJ) than with the normal diet (1.1 mg body weight/kJ). None of the OTCADs had any effect on feeding efficiency (data not shown).

Table 2 – Actual weights for body composition (fat, lean muscle and water)

	Vehicle	APAP	Ibuprofen	Naproxen	Aspirin
BW (final) (g)					
Normal diet	18.4 ± 0.3	18.2 ± 0.2	19.2 ± 0.2[a]	18.3 ± 0.3	18.5 ± 0.2
High fat diet	19.5 ± 0.3[b]	19.3 ± 0.3[b]	20.0 ± 0.3[b]	19.2 ± 0.2[b]	19.6 ± 0.2[b]
Body fat (g)					
Normal diet	1.23 ± 0.06	1.33 ± 0.10	1.30 ± 0.18	1.34 ± 0.12	1.34 ± 0.06
High fat diet	2.64 ± 0.09[b]	1.80 ± 0.13[a,b]	1.92 ± 0.15[a,b]	2.03 ± 0.12[a,b]	2.17 ± 0.25[a,b]
Muscle (g)					
Normal diet	15.73 ± 0.07	16.19 ± 0.12	16.59 ± 0.18	15.87 ± 0.10	16.08 ± 0.06
High fat diet	15.85 ± 0.08	16.25 ± 0.06	16.71 ± 0.14	15.86 ± 0.10	16.03 ± 0.24
Water (g)					
Normal diet	14.25 ± 0.06	14.84 ± 0.10	15.02 ± 0.15	14.38 ± 0.11	14.56 ± 0.05
High fat diet	14.30 ± 0.07	14.67 ± 0.13	15.18 ± 0.11	14.34 ± 0.13	14.51 ± 0.20

Mice were fed a normal diet or a high fat diet for 10 weeks. Mice were gavaged daily with 20 mg OTCAD/kg body weight, or with the equivalent volume of isotonic saline vehicle, beginning 7 days before changing to a high fat diet. The table shows mean values for the parameters indicated ± S.E.M. (N = 4). The percentages body fat, lean muscle and water, based on body weight, are shown in Fig. 2.

[a] Significantly different mean value from vehicle control of the same diet (P < 0.05).

[b] Significantly different mean value from normal diet mice receiving the same treatment (P < 0.05).

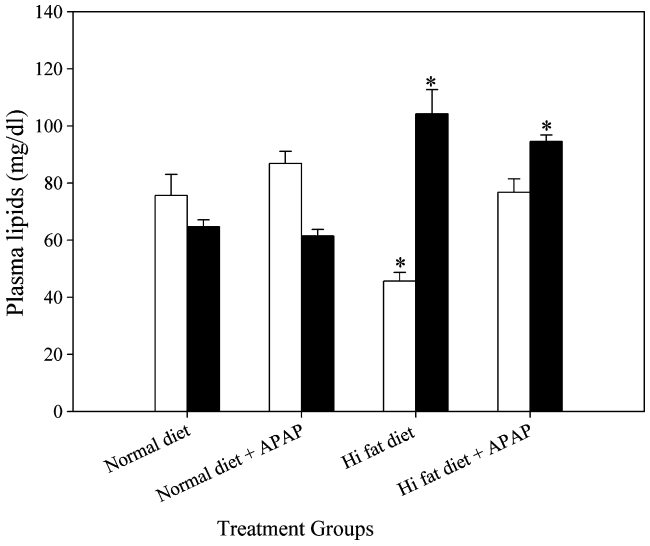

Fig. 3 – Effect of high fat diet and APAP on plasma lipids. Mice were fed a normal diet or a high fat diet for 10 weeks. Mice were treated with 0.2 mg APAP/ml drinking water (~24 mg APAP/kg body weight), or with drinking water alone, beginning 7 days before changing to a high fat diet. The bars are mean values ± S.E.M. (N = 4). Open bars represent plasma triglycerides, and the shaded bars represent total cholesterol. *Significantly different mean value from normal diet control (P < 0.01).

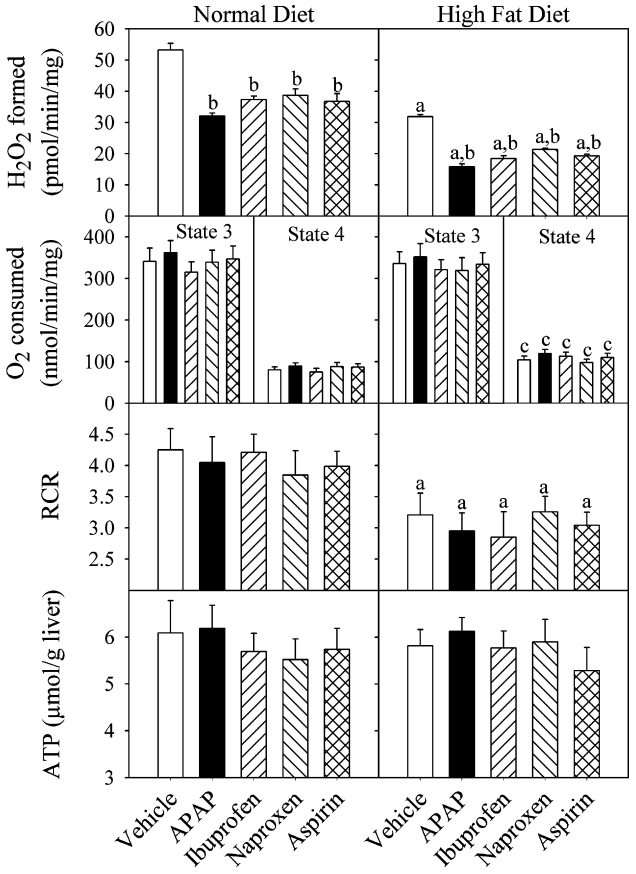

Fig. 4 – Effect of treating mice with OTCADs on liver mitochondrial energy metabolism. Mice were fed a normal diet (left panels), or a high fat diet (right panels) for 10 weeks. Mice were gavaged daily with 20 mg OTCADs/kg body weight, or with the equivalent volume of isotonic saline vehicle, beginning 7 days before changing to a high fat diet where indicated. The bar fills are open for vehicle, black for APAP, ascending hatch for IBU, descending hatch for NAP and cross-hatch for ASP. Mitochondria were prepared from livers of 8 h fasted mice, and parameters of mitochondrial activity were assayed. Succinate-dependent H_2O_2 production was assayed under State 4 respiration conditions (top panels). Oxygen consumed was measured as State 3 respiration (left bar of each pair of bars) or State 4 ADP-limited respiration (right bar of each pair of bars second from top). Respiratory control ratio (RCR) was calculated for individual mouse mitochondria as ratios of State 3 to State 4 respiration (third panels from the top). ATP levels are shown for liver tissue (bottom panels). Mean values are shown for the parameters indicated ± S.E.M. (N = 4). [a]Significantly lower mean value than for normal diet of corresponding treatment group (P < 0.05). [b]Significantly lower mean value than for vehicle-treated mice fed the same diet (P < 0.05). [c]Significantly greater mean value than for diet of corresponding treatment group (P < 0.05).

3.3. *APAP moderates the high fat diet-induced changes in blood lipids*

Since elevated plasma lipids are considered a risk factor for microvascular diseases, we measured plasma triglycerides and cholesterol (Fig. 3). Cholesterol levels were increased by the high fat diet, and APAP appeared to prevent a small portion of that increase. Triglycerides, however, displayed a significant decrease in plasma levels, an effect prevented by APAP. Such a decrease in blood triglycerides associated with an isocaloric high fat diet has been observed previously in both rodents [24] and humans [25].

3.4. *OTCADs, especially APAP, inhibit mitochondrial and cytosolic oxidative stress associated with a high fat diet*

With mitochondrial oxidative stress involved in the etiology of the pre-diabetic state leading to T2DM, we evaluated the ability of OTCADs, administered in vivo, to quench the production of mitochondria-derived reactive oxygen. In liver mitochondria isolated 20–24 h after the last OTCAD treatment, the succinate-dependent production of H_2O_2 was lower in mitochondria from OTCAD-treated mice by about one-third, with APAP more effective than the other compounds tested (Fig. 4, top panels). Although mitochondrial H_2O_2 production was lower in mice fed the high fat diet, there were no differences in the effect of OTCAD treatment on H_2O_2 production between mice fed the normal or the high fat diet. We next examined energy coupling, since this parameter is associated with mitochondrial reactive oxygen production. Mice fed a high fat diet exhibited higher rates of State 4 (ADP-limited) respiration, with no change in State 3 respiration (Fig. 4, second panels down). The resulting decrease in RCR (State 3/State 4 respiration, third panels down) indicates that

Table 3 – Parameters of oxidative stress in white adipose tissue (WAT)

Substrate	Parameters	Normal diet		High fat diet	
		Vehicle	APAP	Vehicle	APAP
–	Malondialdehyde	0.16 ± 0.05	0.12 ± 0.05	0.23 ± 0.06	0.19 ± 0.04
–	4-Hydroxyalkenals	0.31 ± 0.08	0.23 ± 0.09	0.59 ± 0.12**	0.37 ± 0.10*
Succinate	O_2 uptake	5.6 ± 1.0	5.2 ± 1.2	4.8 ± 0.9	4.9 ± 1.1
Succinate	H_2O_2	0.07 ± 0.04	0.1 ± 0.06	0.02 ± 0.02	0.03 ± 0.02
NADPH	O_2 uptake	14.7 ± 1.2	7.1 ± 1.1*	22.6 ± 3.1**	11.8 ± 1.5*,**
NADPH	H_2O_2	1.2 ± 0.11	0.5 ± 0.07*	2.5 ± 0.21**	0.8 ± 0.10*
NADPH + DPI	H_2O_2	0.23 ± 0.04	0.14 ± 0.02	0.36 ± 0.06	0.3 ± 0.05**

Mice were fed either a normal or a high fat diet for 10 weeks. Mice were provided drinking water, or water containing APAP, such that their daily dosage was approximately 20 mg/kg body weight. Parameters were determined using visceral WAT, as described in Section 2. Mean values ± S.E.M. (N = 4) are indicated. Succinate-dependent O_2 uptake and H_2O_2 production (nmol min/g fat) were determined under State 4 (ADP-limiting) conditions. Concentrations of substrates was 6 mM succinate, 0.4 mM NADPH and 25 μM DPI (diphenyleneiodonium chloride). Units for the products of lipid peroxidation, malondialdehyde and 4-hydroxyalkenals are nmol/g fat.

* Significantly different mean value from vehicle control of the same diet ($P < 0.05$).

** Significantly different mean value from normal diet mice receiving the same treatment ($P < 0.05$).

liver mitochondria from mice fed a high fat diet were slightly uncoupled, and none of the OTCADs affected this parameter. Most importantly for sustained tissue viability, the partial uncoupling did not affect tissue levels of ATP, which remained normal in mice consuming the high fat diet and with OTCAD treatment (Fig. 4, bottom panels).

WAT is involved in oxidative and inflammatory pathways leading to obesity-related disease. We therefore, evaluated the effects of APAP, the most effective OTCAD, on oxygen metabolism in WAT (Table 3). The high fat diet doubled the content of WAT 4-hydroxyalkenals, which are products of lipid peroxidation. APAP prevented most of this increase. Malondialdehyde content of WAT was low and not altered by the high fat diet, probably because of the rapid rate of malondialdehyde metabolism. In WAT, succinate-dependent mitochondrial oxygen consumption and H_2O_2 production were very low relative to parameters observed in liver. In contrast, NADPH supported higher levels of oxygen consumption and H_2O_2 production in WAT from mice fed a high fat diet, most of which was prevented by APAP treatment. Furthermore, NADPH-dependent oxygen consumption and H_2O_2 production were inhibited by the NADPH oxidase inhibitor, diphenyleneiodonium chloride (DPI), suggesting the involvement of this pro-oxidant enzyme in the oxidative stress response in WAT of mice fed a high fat diet.

4. Discussion

The inability to maintain blood glucose homeostasis is the hallmark of diabetes, yet frank T2DM ensues only after years of a slowly developing pre-diabetic state involving obesity, progressive insulin resistance and loss of glucose tolerance [26,27]. The induction of T2DM by a high fat diet in C57BL/6J mice [28] is a highly relevant animal model to study the relationship between diet and the development of T2DM. In mice, as in humans, a high fat diet leads to obesity, accompanied by insulin resistance and impaired glucose tolerance. Eventual loss of pancreatic β-cell mass leads to frank diabetes, characterized by fasting hyperglycemia [28,29]. Although FBG levels are used clinically to diagnose diabetes, non-fasting blood glucose levels are also important, and even transient hyperglycemia can produce oxidative tissue damage, such as neuronal cell death [30]. Thus, insulin resistance and impaired glucose tolerance pose substantial risks for development of hyperglycemia-derived pathology.

Hyperlipidemia is another important risk factor for microvascular disease resulting from a high fat diet and obesity. A hypercaloric high fat diet is often associated with a increase in triglyceride levels. However, in rats under fasting conditions, an isocaloric high fat diet has been shown to decrease plasma triglycerides while having little effect on total cholesterol [24]. This appears to be the case in the present study, since mice fed the high fat diet reduced food consumption such that their caloric intake was similar to mice eating the normal diet. The reason for a reduction in fasting blood triglyceride levels in mice fed a high fat diet may be related to circulating levels of very low density lipoproteins (VLDL), which contain both triglycerides and cholesterol. While the high fat diet tends to increase VLDL levels under non-fasting conditions, fasting conditions promote the delivery of triglycerides to peripheral tissues via the VLDL receptor [31]. Following the removal of triglycerides, the high levels of residual VLDL remnants represent a risk factor for atherosclerosis because of their high cholesterol content.

Although ASA has been used to treat diabetic hyperglycemia for many years [14,32,33], the mechanism of action for ASA is poorly understood, and there are few reports regarding the potential for the use of other NSAIDs or analgesics for the prevention or treatment of diabetics. A small cohort study (58 men and 19 women) revealed that neither ASA nor IBU at conventional analgesic dosages were effective in lowering FBG levels in T2DM patients with elevated FBG [34]. These results suggest that OTCADs would more likely be useful in preventing the onset of T2DM than in treating frank diabetes. In keeping with this possibility, we recently reported that the analgesic APAP ameliorated hyperglycemia and conditions leading to hyperglycemia in a mouse model [17]. Although the mechanisms for the ability of APAP [17] or NSAIDs (IBU, NAP, and ASA) to prevent the loss of glucose and insulin home-

ostasis, and reduce fat accumulation in mice fed a high fat diet are not known, it is likely related to the general ability of these compounds to reduce oxidative stress and inflammation.

A primary mode of analgesic and anti-inflammatory action for OTCADs is to inhibit one or more prostaglandin synthases (PTGS; cyclooxygenases), which convert arachidonic acid to prostaglandin G_2 (cyclooxygenase activity) and the subsequent reduction of prostaglandin G_2 to prostaglandin H_2 (peroxidase activity). Analgesic, antipyretic, and anti-inflammatory activities are achieved principally through PTGS2 inhibition. Each OTCAD used in this study, APAP and the NSAIDs, differs in its pharmacokinetics, specificity for specific PTGS, other pharmacological effects, and side effects. APAP is distinct in its mode of action from NSAIDS in that it is a poor inhibitor of *peripheral* PTGS2, while it quite effectively inhibits vascular endothelial PTGS1, and possibly neuronal PTGS1b [35]. Furthermore, NSAIDs preferentially inhibit the cyclooxygenase activity of PTGS, while APAP preferentially inhibits peroxidase activity. The result is that APAP is an analgesic and antipyretic, without the peripheral anti-inflammatory and anti-platelet thrombosis properties of NSAIDs [35]. IBU and NAP inhibit both PTGS1 and PTGS2, and ASA is preferential for PTGS1. While IBU and NAP are similar in inhibiting PTGS1 and PTGS2, the results from this study show that IBU is highly effective in promoting glucose tolerance, while NAP has no effect. Furthermore, APAP is highly effective in promoting glucose tolerance and inhibiting gain in body fat, yet is a poor inhibitor of PTGS1 and PTGS2. These results are not consistent with PTGS inhibition as a major factor in OTCAD effect on blood glucose or body fat gain in mice fed a high fat diet. A similar conclusion was reached in human studies showing that ASA treatment and PTGS1 activity levels were not related to the severity of T2DM [36].

A related mechanism of action by NSAIDs to prevent dysfunction of pancreatic β-cells during the development of T2DM is the ability of NSAIDs to inhibit the conversion of pancreatic islet amyloid polypeptide into β sheet polypeptide fibrils [16]. Such amyloid deposits are cytotoxic to β-cells. However, this mechanism appeared to function through PTGS1, with PTGS2 inhibitors less effective. Furthermore, since APAP had no effect on amylin fibrillogenesis, yet was highly effective in the present study, amyloid fibrillar deposition is not considered relevant as a mechanism of action for OTCADs described in this paper.

Another potential mechanism of action by OTCADs is a direct inhibition of mitochondrial oxidative stress, which appears to be an important pathway in the pathology associated with hyperglycemia [37]. In the present study, treatment of mice with OTCADs slightly increased State 4 respiration in isolated mitochondria, resulting in a slight decrease in the respiratory control ratio (RCR; State 3/State 4), suggesting that OTCADs partially uncoupled mitochondrial ATP synthesis from respiration. Via this partial uncoupling pathway, OTCADs inhibit the production of reactive oxygen, similar to the effect of Ca^{2+} at low concentrations [38]. This appears to be the mechanism responsible for the decrease in H_2O_2 production in mice fed a high fat diet. In the high fat diet mouse, higher levels of free fatty acids such as oleic and linoleic may increase inner membrane permeability via opening the permeability transition pore, resulting in a lower membrane potential associated with decreased succinate-dependent reactive oxygen production [38]. In addition to the effect of the high fat diet, each of the OTCADs examined inhibited mitochondrial succinate-dependent H_2O_2 production. The inhibition of reactive oxygen production by each OTCAD was not associated with a decrease in RCR, so partial uncoupling should not be considered as the responsible mechanism. A possible mechanism of action for APAP to inhibit mitochondrial-derived reactive oxygen levels is for APAP to act directly as a phenolic radical scavenger, similar to α-tocopherol. For this mechanism to apply to the NSAIDs, the aromatic rings would need to become phenolic. In the case of NAP and ASA, the major metabolites are phenolic aromatics, 6-O-desmethyl naproxen [39] and salicylic acid [40], respectively. This postulated mechanism is problematic for IBU, since the major site of hydroxylation is not the ring, but the 2-position carbon alpha to the carboxylate moiety [41]. Nevertheless, NAP and salicylate have both been shown to inhibit rat liver microsomal H_2O_2 production, at mM concentrations [42].

Among the OTCADs examined in the current study, only NAP was ineffective in lowering FBG levels in mice receiving the high fat diet, and even appeared to elevate FBG in mice fed the normal diet. While NAP was the least effective drug examined for its ability to reduce mitochondrial reactive oxygen production, it was still able to scavenge about 50% of the H_2O_2 generated in mitochondria from mice fed either the normal or high fat diet. Thus, the hyperglycemic effect of NAP is not likely associated with the production of H_2O_2. Rather, in keeping with oxidative stress as a major factor in the etiology of T2DM, this effect of NAP could be associated with the loss in the ability of tissues to scavenge reactive oxygen. This is a likely possibility, considering that NAP has been shown to inhibit antioxidant enzyme activities of the glutathione-linked enzymes, glutathione S-transferase and glutathione peroxidase [43].

Recent studies have shown that ASA and other salicylates lower FBG, triglycerides and improve insulin resistance in humans and in model systems [44,45]. Several laboratories have reported that inhibition of serine kinase IKKβ may be a common mechanism of action of salicylates in anti-inflammation and restoration of lipid and glucose homeostasis [44–46]. IKKβ phosphorylates IκB which leads to the activation and nuclear translocation of nuclear factor kappa B (NFκB). In addition to the inhibition of NFκB by IKKβ, NFκB activity can be decreased by inhibiting tumor necrosis factor-alpha (TNFα) [47], loss-of-function mutation in Toll-like receptor-4 (TLR4) [48], use of dietary antioxidants (e.g., curcumin [49]), or inhibiting NFκB activating scaffold proteins [50]. NFκB transcriptionally upregulates a battery of inducible genes involved in inflammation and oxidative stress responses, leading to insulin resistance, loss of glucose tolerance, and other manifestations of the pre-diabetic and T2DM condition [51]. It is therefore, likely that NFκB is involved in the protection against the high fat diet-induced loss of glucose tolerance observed with the OTCADs examined in the present study, since APAP [52] and IBU [53], but not NAP [54] inhibit NFκB activity. Consistent with a role for NFκB in maintaining glucose homeostasis, we found that NAP was the only OTCAD tested that did not improve glucose tolerance in high fat-fed mice.

A potentially important pathway by which APAP, a highly effective OTCAD in this study, may reduce reactive oxygen production and inflammation associated with a high fat diet is through NADPH oxidase. Normally, this enzyme is involved in important activities in WAT, such as oxygen signaling of pre-adipocyte differentiation to mature adipocytes [55]. However, high NADPH oxidase activities can also generate an oxidative stress response in adipocytes, activating MAP kinase pathways, decreasing the availability of NO, and increase protein nitrosylation and lipid peroxidation [56]. Obesity associated with a high fat diet may also generate metabolic syndrome through oxidative stress pathways involving the increased expression of WAT NADPH oxidase [57]. Thus, the present finding that APAP reduces NADPH oxidase-dependent production of H_2O_2 may be clinically relevant.

Diabetes is associated with a multitude of debilitating health effects, especially microvascular diseases, and is associated with obesity and metabolic diseases such as metabolic syndrome. It is likely that the protective action of OTCADs in this study occurs via multiple mechanisms, consistent with the complexity of the phenotype. This study has shown each of the OTCADs examined has the potential to either prevent the loss of glucose tolerance (APAP, IBU, ASA), or decrease the accumulation of body fat (especially APAP and IBU). Thus, over-the-counter OTCADs have the potential to delay or prevent the onset of T2DM in populations designated to be at-risk.

Acknowledgements

This study was supported in part by a research grant from McNeil Consumer Healthcare and NIEHS Center Grant P30 ES06096. We thank Kathy LaDow and Jennifer Schurdak for their technical assistance.

REFERENCES

[1] Allison DB, Fontaine KR, Manson JE, Stevens J, VanItallie TB. Annual deaths attributable to obesity in the United States. JAMA 1999;282:1530–8.
[2] Lazar MA. How obesity causes diabetes: not a tall tale. Science 2005;307:373–5.
[3] Wellen KE, Hotamisligil GS. Inflammation, stress, and diabetes. J Clin Invest 2005;115:1111–9.
[4] Dehghan A, van HM, Sijbrands EJ, Stijnen T, Hofman A, Witteman JC. Risk of type 2 diabetes attributable to C-reactive protein and other risk factors. Diabetes Care 2007;30:2695–9.
[5] Edelman SV, Morello CM. Strategies for insulin therapy in type 2 diabetes. South Med J 2005;98:363–71.
[6] Goralski KB, Sinal CJ. Type 2 diabetes and cardiovascular disease: getting to the fat of the matter. Can J Physiol Pharmacol 2007;85:113–32.
[7] Beisswenger PJ, Drummond KS, Nelson RG, Howell SK, Szwergold BS, Mauer M. Susceptibility to diabetic nephropathy is related to dicarbonyl and oxidative stress. Diabetes 2005;54:3274–81.
[8] Duby JJ, Campbell RK, Setter SM, White JR, Rasmussen KA. Diabetic neuropathy: an intensive review. Am J Health Syst Pharm 2004;61:160–73.
[9] Valeri C, Pozzilli P, Leslie D. Glucose control in diabetes. Diabetes Metab Res Rev 2004;20:S1–8.
[10] Hammes HP, Du X, Edelstein D, Taguchi T, Matsumura T, Ju Q, et al. Benfotiamine blocks three major pathways of hyperglycemic damage and prevents experimental diabetic retinopathy. Nat Med 2003;9:294–9.
[11] Robertson RP. Chronic oxidative stress as a central mechanism for glucose toxicity in pancreatic islet beta cells in diabetes. J Biol Chem 2004;279:42351–4.
[12] Pennathur S, Heinecke JW. Mechanisms for oxidative stress in diabetic cardiovascular disease. Antioxid Redox Signal 2007;9:955–69.
[13] Susztak K, Raff AC, Schiffer M, Bottinger EP. Glucose-induced reactive oxygen species cause apoptosis of podocytes and podocyte depletion at the onset of diabetic nephropathy. Diabetes 2006;55:225–33.
[14] Hopper AH, Tindall H, Davies JA. Effect of aspirin on glucose tolerance and insulin levels in patients with type 2 diabetes mellitus. Thromb Haemostasis 1985;54:895.
[15] Nobles-James C, James EA, Sowers JR. Prevention of cardiovascular complications of diabetes mellitus by aspirin. Cardiovasc Drug Rev 2004;22:215–26.
[16] Thomas T, Nadackal GT, Thomas K. Aspirin and diabetes: inhibition of amylin aggregation by nonsteroidal anti-inflammatory drugs. Exp Clin Endocrinol Diabetes 2003;111:8–11.
[17] Shertzer HG, Schneider SN, Kendig EL, Clegg DJ, D'Alessio DA, LaDow K, et al. Acetaminophen normalizes blood glucose in mouse models for diabetes. Biochem Pharmacol 2008;75:1402–10.
[18] Reeves PG, Nielsen FH, Fahey Jr GC. AIN-93 purified diets for laboratory rodents: final report of the American Institute of Nutrition ad hoc writing committee on the reformulation of the AIN-76A rodent diet. J Nutr 1993;123:1939–51.
[19] Woods SC, Seeley RJ, Rushing PA, D'Alessio D, Tso P. A controlled high-fat diet induces an obese syndrome in rats. J Nutr 2003;133:1081–7.
[20] Tinsley FC, Taicher GZ, Heiman ML. Evaluation of a quantitative magnetic resonance method for mouse whole body composition analysis. Obes Res 2004;12:150–60.
[21] Taicher GZ, Tinsley FC, Reiderman A, Heiman ML. Quantitative magnetic resonance (QMR) method for bone and whole-body-composition analysis. Anal Bioanal Chem 2003;377:990–1002.
[22] Clegg DJ, Brown LM, Woods SC, Benoit SC. Gonadal hormones determine sensitivity to central leptin and insulin. Diabetes 2006;55(4):978–87.
[23] Senft AP, Dalton TP, Nebert DW, Genter MB, Puga A, Hutchinson RJ, et al. Mitochondrial reactive oxygen production is dependent on the aromatic hydrocarbon receptor. Free Radical Biol Med 2002;33(9):1268–78. Nov 1.
[24] Middleton S, Schneeman BO. Rat plasma triglycerides and hepatic fatty acid synthetase mRNA, but not apolipoprotein B and A-IV mRNA, respond to dietary fat content. J Nutr 1996;126(6):1627–34.
[25] Jacobs B, De Angelis-Schierbaum G, Egert S, Assmann G, Kratz M. Individual serum triglyceride responses to high-fat and low-fat diets differ in men with modest and severe hypertriglyceridemia. J Nutr 2004;134:1400–5.
[26] Weir GC, Bonner-Weir S. Five stages of evolving beta-cell dysfunction during progression to diabetes. Diabetes 2004;53:S16–21.
[27] Ramlo-Halsted BA, Edelman SV. The natural history of type 2 diabetes. Implications for clinical practice. Primary Care 1999;26:771–89.
[28] Surwit RS, Kuhn CM, Cochrane C, McCubbin JA, Feinglos MN. Diet-induced type II diabetes in C57BL/6J mice. Diabetes 1988;37:1163–7.

[29] Ikemoto S, Thompson KS, Takahashi M, Itakura H, Lane MD, Ezaki O. High fat diet-induced hyperglycemia: prevention by low-level expression of a glucose transporter (GLUT4) minigene in transgenic mice. Proc Natl Acad Sci USA 1995;92:3096–9.
[30] Vincent AM, McLean LL, Backus C, Feldman EL. Short-term hyperglycemia produces oxidative damage and apoptosis in neurons. FASEB J 2005;19:638–40.
[31] Tacken PJ, Teusink B, Jong MC, Harats D, Havekes LM, van Dijk KW, et al. LDL receptor deficiency unmasks altered VLDL triglyceride metabolism in VLDL receptor transgenic and knockout mice. J Lipid Res 2000;41:2055–62.
[32] Micossi P, Pontiroli AE, Baron SH, Tamayo RC, Lengel F, Bevilacqua M, et al. Aspirin stimulates insulin and glucagon secretion and increases glucose tolerance in normal and diabetic subjects. Diabetes 1978;27:1196–204.
[33] Seino Y, Usami M, Nakahara H, Takemura J, Nishi S, Ishida H, et al. Effect of acetylsalicylic acid on blood glucose and glucose regulatory hormones in mild diabetes. Prostag Leukotr Med 1982;8:49–53.
[34] Mork NL, Robertson RP. Effects of nonsteroidal antiinflammatory drugs in conventional dosage on glucose homeostasis in patients with diabetes. West J Med 1983;139:46–9.
[35] Aronoff DM, Oates JA, Boutaud O. New insights into the mechanism of action of acetaminophen: its clinical pharmacologic characteristics reflect its inhibition of the two prostaglandin H2 synthases. Clin Pharmacol Ther 2006;79:9–19.
[36] Ohmori T, Yatomi Y, Nonaka T, Kobayashi Y, Madoiwa S, Mimuro J, et al. Aspirin resistance detected with aggregometry cannot be explained by cyclooxygenase activity: involvement of other signaling pathway(s) in cardiovascular events of aspirin-treated patients. J Thromb Haemostasis 2006;4:1271–8.
[37] Nishikawa T, Edelstein D, Du XL, Yamagishi S, Matsumura T, Kaneda Y, et al. Normalizing mitochondrial superoxide production blocks three pathways of hyperglycaemic damage. Nature 2000;404:787–90.
[38] Shertzer HG, Genter MB, Shen D, Nebert DW, Chen Y, Dalton TP. TCDD decreases ATP levels and increases reactive oxygen production through changes in mitochondrial F(0)F(1)-ATP synthase and ubiquinone. Toxicol Appl Pharmacol 2006;217:363–74.
[39] Anttila M, Haataja M, Kasanen A. Pharmacokinetics of naproxen in subjects with normal and impaired renal function. Eur J Clin Pharmacol 1980;18:263–8.
[40] Summerbell J, Yelland C, Woodhouse K. The kinetics of plasma aspirin esterase in relation to old age and frailty. Age Ageing 1990;19:128–30.
[41] Tan SC, Patel BK, Jackson SH, Swift CG, Hutt AJ. Stereoselectivity of ibuprofen metabolism and pharmacokinetics following the administration of the racemate to healthy volunteers. Xenobiotica 2002;32:683–97.
[42] Ji B, Masubuchi Y, Horie T. A possible mechanism of naproxen-induced lipid peroxidation in rat liver microsomes. Pharmacol Toxicol 2001;89:43–8.
[43] Orhan H, Sahin G. In vitro effects of NSAIDS and paracetamol on oxidative stress-related parameters of human erythrocytes. Exp Toxicol Pathol 2001;53:133–40.
[44] Yuan M, Konstantopoulos N, Lee J, Hansen L, Li ZW, Karin M, et al. Reversal of obesity- and diet-induced insulin resistance with salicylates or targeted disruption of Ikkbeta. Science 2001;293:1673–7.
[45] Hundal RS, Petersen KF, Mayerson AB, Randhawa PS, Inzucchi S, Shoelson SE, et al. Mechanism by which high-dose aspirin improves glucose metabolism in type 2 diabetes. J Clin Invest 2002;109:1321–6.
[46] Yin MJ, Yamamoto Y, Gaynor RB. The anti-inflammatory agents aspirin and salicylate inhibit the activity of I(kappa)B kinase-beta. Nature 1998;396:77–80.
[47] Schutze S, Potthoff K, Machleidt T, Berkovic D, Wiegmann K, Kronke M. TNF activates NF-kappa B by phosphatidylcholine-specific phospholipase C-induced ''acidic'' sphingomyelin breakdown. Cell 1992;71:765–76.
[48] Tsukumo DM, Carvalho-Filho MA, Carvalheira JB, Prada PO, Hirabara SM, Schenka AA, et al. Loss-of-function mutation in TLR4 prevents diet-induced obesity and insulin resistance. Diabetes 2007;56:1986–98.
[49] Thaloor D, Miller KJ, Gephart J, Mitchell PO, Pavlath GK. Systemic administration of the NF-kappaB inhibitor curcumin stimulates muscle regeneration after traumatic injury. Am J Physiol 1999;277:C320–9.
[50] Chariot A, Meuwis MA, Bonif M, Leonardi A, Merville MP, Gielen J, et al. NF-kappaB activating scaffold proteins as signaling molecules and putative therapeutic targets. Curr Med Chem 2003;10:593–602.
[51] Sonnenberg GE, Krakower GR, Kissebah AH. A novel pathway to the manifestations of metabolic syndrome. Obes Res 2004;12:180–6.
[52] Mancini F, Landolfi C, Muzio M, Aquilini L, Soldo L, Coletta I, et al. Acetaminophen down-regulates interleukin-1beta-induced nuclear factor-kappaB nuclear translocation in a human astrocytic cell line. Neurosci Lett 2003;353:79–82.
[53] Stuhlmeier KM, Li H, Kao JJ. Ibuprofen: new explanation for an old phenomenon. Biochem Pharmacol 1999;57:313–20.
[54] Kazmi SM, Plante RK, Visconti V, Taylor GR, Zhou L, Lau CY. Suppression of NF kappa B activation and NF kappa B-dependent gene expression by tepoxalin, a dual inhibitor of cyclooxygenase and 5-lipoxygenase. J Cell Biochem 1995;57:299–310.
[55] Mouche S, Mkaddem SB, Wang W, Katic M, Tseng YH, Carnesecchi S, et al. Reduced expression of the NADPH oxidase NOX4 is a hallmark of adipocyte differentiation. Biochim Biophys Acta 2007;1773:1015–27.
[56] Sautin YY, Nakagawa T, Zharikov S, Johnson RJ. Adverse effects of the classic antioxidant uric acid in adipocytes: NADPH oxidase-mediated oxidative/nitrosative stress. Am J Physiol Cell Physiol 2007;293:C584–96.
[57] Furukawa S, Fujita T, Shimabukuro M, Iwaki M, Yamada Y, Nakajima Y, et al. Increased oxidative stress in obesity and its impact on metabolic syndrome. J Clin Invest 2004;114:1752–61.
[58] Ngo AL, Guiang R. Acute pain management. In: Boswell MV, Cole BE, editors. Weiner's pain management: a practical guide for clinicians. 7th ed., Boca Raton, FL: CRC Taylor & Francis Press; 2006. p. 285–96.