Vitamin A supplementation induces adipose tissue loss through apoptosis in lean but not in obese rats of the WNIN/Ob strain

S M Jeyakumar, A Vajreswari, B Sesikeran[1] and N V Giridharan[2]

Department of Biochemistry, [1]Department of Pathology, [2]National Centre for Laboratory Animal Sciences, National Institute of Nutrition, Jamai-Osmania PO, Hyderabad-500 007, Andhra Pradesh, India

(Requests for offprints should be addressed to A Vajreswari; Email: avresgr@yahoo.co.in)

Abstract

Vitamin A is a known regulator of adipose tissue growth. In this paper, we report the possible role of dietary vitamin A supplementation in the regulation of adipose tissue mass, using a novel obese rat model of the WNIN/Ob strain developed at the National Centre for Laboratory Animal Sciences of the National Institute of Nutrition, India. Twenty-four male lean and obese rats of the WNIN/Ob strain were broadly divided into two groups at 7 months of age; each group was subdivided into two subgroups consisting of six lean and six obese rats and they were given diets containing either 2·6 mg or 129 mg vitamin A/kg diet for 2 months. Feeding a high but non-toxic dose of vitamin A (129 mg/kg diet) resulted in a significant reduction in the adiposity index and retroperitoneal white adipose tissue (RPWAT) weight in obese rats while a marginal reduction was observed in lean rats. Further, this treatment resulted in a significantly increased RPWAT apoptotic index and Bax protein expression and a decreased expression of Bcl2 in the lean rats. However, no such changes were observed in the RPWAT of the obese rats subjected to identical treatment. Thus, our data suggests that chronic dietary vitamin A supplementation at a high dose effectively regulates adipose tissue mass both in the lean and obese phenotypes of the WNIN/Ob rat strain, perhaps through different mechanisms.

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Introduction

Obesity is a condition where excess energy is stored as triglycerides in white adipose tissue (WAT) (Palou *et al.* 2000). WAT, the major component of the adipose organ, is widely distributed in the mammalian body in large quantities and serves as a reservoir of energy. On demand, it supplies energy in the form of free fatty acids (FFAs), which are released by a lipolytic process and utilized by various tissues (Frayn *et al.* 2003). Adipose tissue mass is determined by both size and number of adipocytes. The latter in turn is determined by adipocyte apoptosis and differentiation (Prins & O'Rahilly 1997).

Apoptosis, an evolutionarily conserved intracellular pathway, allows the organism to control cell number and tissue size very tightly, and protects it from rogue cells that threaten tissue homeostasis (Reed 2002). Apoptosis has been described both in WAT and brown adipose tissue (BAT) (Prins *et al.* 1994). Several studies have demonstrated *in vitro* and *in vivo* adipocyte apoptosis under various conditions such as growth factor deprivation, dietary conjugated linoleic acid supplementation and streptozotocin administration (Prins *et al.* 1997, Loftus *et al.* 1998, Miner *et al.* 2001, Hargrave *et al.* 2002). Even in patients with malignancy-associated weight loss, apoptosis is detected in abdominal, subcutaneous and omental fat (Prins *et al.* 1994). Thus, emerging evidence suggests that adipose tissue is an active organ with continuous recruitment of newer cells and elimination of adipocytes by apoptosis under normal physiological conditions as well as disease conditions.

Vitamin A, an important micronutrient, has an unusually wide range of vital physiological actions in mammals (e.g. morphogenesis, vision, embryonic development, reproduction and immune function etc.) (Villarroya *et al.* 1999). Although liver is the major organ involved in the storage and homeostasis of retinoids, adipose tissues contain substantial amounts of retinol and retinyl esters, which account for 15–20% of the total body retinoid stores (Tsutsumi *et al.* 1992). Moreover, adipose tissue is considered to be a potential site for retinoic acid (RA) action by expressing retinoid receptor subfamilies, namely RA receptor (RAR) and retinoid X receptor (RXR) (Bonet *et al.* 2003). It is very well documented that vitamin A and its active metabolite RA are positive regulators of uncoupling protein-1 (UCP1) (Kumar & Scarpace 1998, Bonet *et al.* 2003). Studies from brown adipocyte cell cultures and an *in vivo* system have demonstrated the role of RA as a transcriptional activator of UCP1 gene expression and its implication in

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the control of body adiposity (Puigserver *et al.* 1996, Bonet *et al.* 2000). Furthermore, the role of vitamin A and its metabolite status on body fat regulation in a rodent model has also been well documented (Bonet *et al.* 2003, Felipe *et al.* 2003).

Many *in vitro* studies have reported that RA inhibits adipocyte differentiation at a high concentration (Kuri-Harcuch 1982, Hernandez *et al.* 1993). In addition, vitamin A supplementation at high doses results in decreased adiposity in rats (Kumar *et al.* 1999). On the contrary, the feeding of a vitamin A-deficient diet to rats resulted in increased adiposity and body weight gain (Bonet *et al.* 2000, Ribot *et al.* 2001). Furthermore, all-*trans*-RA, a vitamin A metabolite, has been shown to be a potent inducer of apoptosis in rat stromal–vascular cells (Kim *et al.* 2000). All these studies indicate that vitamin A status can modulate the adipose tissue mass in rodents. The present study was therefore undertaken to gain insight into the *in vivo* modulation of adipose tissue mass by vitamin A, using a novel obese mutant rat model developed at our institute.

Materials and methods

Animals and experimental design

The WNIN/Ob mutant rat strain developed from an 80-year-old Wistar inbred rat stock colony at the National Centre for Laboratory Animal Sciences (NCLAS) of the National Institute of Nutrition (NIN), Hyderabad, India, has three phenotypes, namely lean (+/+), carrier (+/−) and obese (−/−). Rats of the obese phenotype are hyperphagic, hyperinsulinemic, hypertriglyceridemic and hypercholesteremic (Giridharan *et al.* 1996). In addition, these rats are also characterized by hyperleptinemia (Vajreswari A, Harishanker N and Giridharan NV, unpublished data).

Male, 7-month-old obese rats of the WNIN/Ob strain were obtained from NCLAS and broadly divided into two groups, A and B, each consisting of 12 lean and obese rats and each further divided into two subgroups (A I, A II and B I, B II) consisting of six rats in each subgroup. Subgroups A I and B I received the stock diet, which provided 2·6 mg vitamin A/kg diet, while subgroups A II and B II received a high vitamin A-containing diet (129 mg vitamin A/kg diet) (as retinyl palmitate). The stock diet and the high vitamin A-containing diets are identical with regard to the nature and concentrations of all ingredients except the concentrations of vitamin A. The study was approved by the Institutional Animal Ethical Committee. The animals were maintained on their respective diets for a period of 2 months. Food and water were provided *ad libitum*. Daily food intake and weekly body weights were recorded.

Rats were housed individually at an ambient temperature of 22·0 ± 1 °C with relative humidity of 50–60% in a 12 h light:12 h darkness cycle and animals were cared for in accordance with the principle of the Guide to the Care and Use of Experimental Animals formulated by the CPC SEA (Committee for the Purpose of Control and Supervision on Experiments on Animals), Government of India. At the end of 2 months, the rats were killed after 12 h fasting. Various adipose tissues were excised, weighed, rapidly frozen in liquid nitrogen and stored at −80 °C until analysis.

Determination of adiposity index

Adiposity index was determined by the sum of the weights of WATs (retroperitoneal, epididymal, subcutaneous and omental) divided by body weight × 100 (Taylor & Phillips 1996).

Adipose tissue cell density and apoptotic index measurements

Retroperitoneal WATs (RPWATs) were collected from the various experimental groups and fixed in 10% formalin. Tissues were then processed and slides were prepared and stained by hematoxylin and eosin by employing routine histopathological procedures for microscopic examination. To evaluate the adipocyte cell density, fat cell density was measured in a calibrated microscope eyepiece graticule at a uniform magnification of × 250. The number of cells within the marked area was counted and expressed as cells/mm^2. To evaluate the apoptotic index, adipose tissue apoptotic cells were counted based on the following criteria: (1) fragmented nuclei, (2) 50% smaller in size than normal cells and (3) irregular outer membrane shape, and expressed as per cent (apoptotic index (%)=no. of apoptotic cells/total no. of cells × 100).

DNA fragmentation analysis

RPWAT samples (100 mg) were homogenized in 10 mmol/l Tris–HCl (pH 7·5), 0·32 mol/l sucrose, 5 mmol/l $MgCl_2$ and 0·5% lauryl sarcosyl containing 200 mg/l proteinase K, and then incubated at 55 °C for 1 h. DNA was subsequently precipitated overnight with ethanol. DNA (20 μl) was loaded onto a 1·5% agarose gel, which was stained with ethidium bromide after migration (Gong *et al.* 2003).

Western blot analysis of pro- and anti-apoptotic proteins

Retroperitoneal adipose tissue was homogenized with lysis buffer (50 mM Tris (pH 8·0), 150 mM NaCl, 0·02% sodium azide, 1% SDS and 5% protease inhibitor cocktail (0·5% deoxycholate; Sigma Chemical Co.),

Table 1 Effect of high vitamin A supplementation on physical parameters. Data represent the means±S.E. of six rats from each group

	Pretreatment body weight (g)	Post-treatment body weight (g)	Body weight gain (g)	Adiposity index (%)	RPWAT weight (g/100 g body weight)	Daily food intake (g)
A I	374±13·3[a]	405±14·1[a]	31·3±7·1[a]	6·3±1·7[a]	2·58±0·64[a]	14·7±0·7[a]
A II	373±10·7[a]	402±13·9[a]	29·0±6·6[a]	3·4±0·51[a]	1·68±0·38[a]	15·8±0·6[a]
B I	613±35·3[b]	801±39·9[b]	187±10·6[b]	33·6±1·7[b]	10·3±1·0[b]	23·6±0·8[b]
B II	612±18·3[b]	723±21·7[b]	111±18·2[c]	27·6±1·6[c]	6·12±0·8[c]	22·0±1·0[b]
F ratio						
Group	121·3*	241·7*	102·6*	5·4*	60·9*	106·0*
Treatment	0·001	3·09	11·4†	0·73†	10·6†	0·62
Interaction	0·000	2·6	10·0‡	6·9	4·4†	1·9

Mean values without a common superscript are significant at $P<0·05$. The F ratios are significant at the $P\leq0·05$ level (*, † and ‡ denote group, treatment and interaction respectively) (by two-way ANOVA).

and centrifuged at 23 000 ***g*** for 1 h. Equal amounts of protein were separated on an SDS-12% polyacrylamide gel and transferred to a nitrocellulose membrane (Hybond-C extra; Amersham Pharmacia Biotech, Amersham, Bucks, UK). Equal loading of protein and transfer were ensured by staining membranes with Ponseau S (Sigma Chemical Co.). Blots were then blocked for 2 h at room temperature with PBS–0·02% Tween-20 containing 5% non-fat dry milk powder prior to incubation with 1:1000 diluted rabbit polyclonal antibodies to Bcl2 and Bax (Santa Cruz Biotechnology Inc., Santa Cruz, CA, USA). Blots were washed and then incubated with goat anti-rabbit IgG antibodies (1:20 000) conjugated with alkaline phosphatase (Sigma). After extensive washing, BCIP/NBT substrate (Sigma) was added, and the intensity of the developed bands was read and quantified by using a scanning densitometer with automatic calibration (GS-710 Imaging Densitometer; Bio-Rad, Hercules, CA, USA).

Statistical analysis

Results are expressed as means ± S.E. Statistical significance was determined by two-way ANOVA and the F ratios were considered significant at the $P\leq0·05$ level.

Results

Effect of high vitamin A supplementation on body weight gain, adiposity index and RPWAT weight

A significant reduction in body weight gain of the high vitamin A-supplemented obese group (B II) compared with the stock diet-fed obese group (B I) was observed without any alteration in their food intake. On the other hand, no such effect was seen in the lean rats receiving high doses of dietary vitamin A (A II) when compared with their lean counterparts maintained on the stock diet with normal levels of vitamin A (2·6 mg vitamin A/kg diet) (A I). Interestingly, this treatment resulted in a significantly decreased adiposity index and RPWAT weight in obese rats supplemented with high doses of vitamin A (B II). However, such effects were not seen in lean rats fed on a high vitamin A-containing diet (A II) (Table 1).

Significant interactions between phenotype and treatment were observed for body weight gain and RPWAT weight while no interactions were observed for other parameters (food intake and adiposity index).

Effect of high vitamin A supplementation on RPWAT cell density and apoptotic index

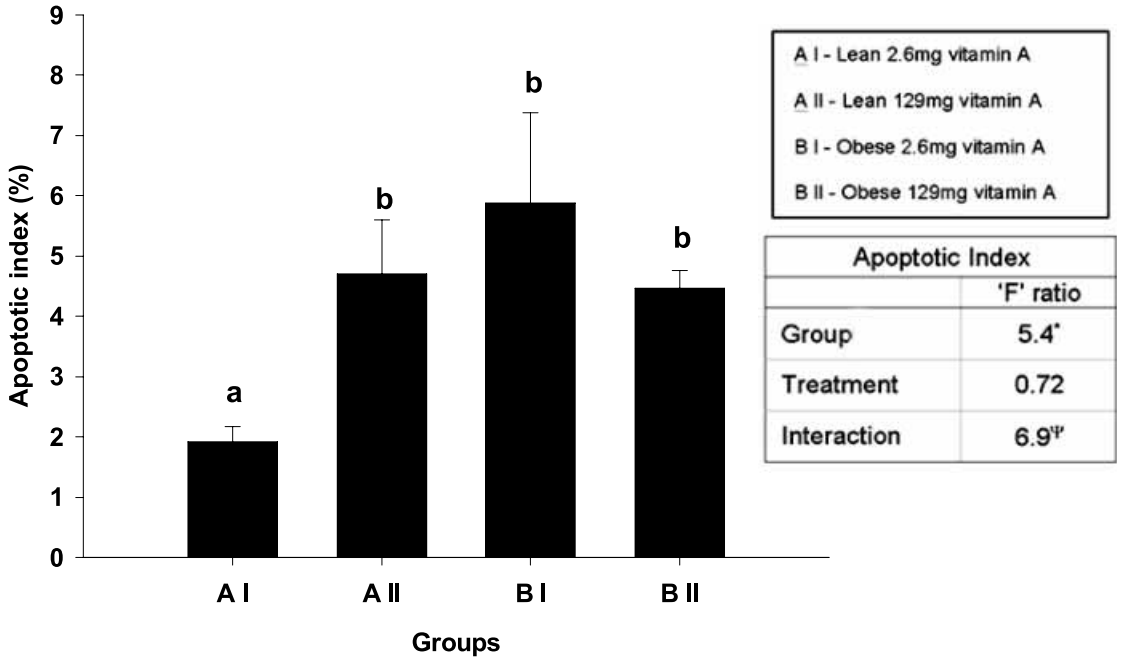
The cell density of RPWAT of the stock diet-treated obese rats (B I) was significantly low compared with their lean counterparts (A I). However, chronic dietary challenging with high doses of vitamin A did not bring about any change in the cell density of adipose tissue of the lean and obese rats (A II and B II respectively) (data not shown).

Obese WNIN/Ob rats had an increased RPWAT apoptotic index compared with their lean counterparts. Vitamin A supplementation resulted in a significant increment in this parameter in the lean but not the obese animals (Fig. 1).

In addition, interaction between phenotype and treatment significantly affected the apoptotic index, but not the cell density.

DNA fragmentation assay

DNA fragmentation was not observed in the RPWAT of the lean rats of group A I which received the stock diet. Interestingly, the same adipose tissue of the stock



Apoptotic Index	
	'F' ratio
Group	5.4*
Treatment	0.72
Interaction	6.9ψ

Figure 1 Effect of high vitamin A on the RPWAT apoptotic index. Values are means±S.E. for three to four rats. Mean values without common superscripts are significant at $P \leq 0{\cdot}05$. The F ratios are significant at the $P \leq 0{\cdot}05$ level (*,† and ‡ denote group, treatment and interaction respectively) (by two-way ANOVA).

diet-fed obese rats (B I) and also the lean and the obese rats maintained on a high vitamin A dietary regimen (A II and B II groups) exhibited nucleosomal DNA fragmentation (Fig. 2).

Effect of high vitamin A supplementation on apoptosis-related proteins

The obese rats of group B I and B II (irrespective of the vitamin A content in their diets) exhibited an under-expression of an important anti-apoptotic protein Bcl2 in RPWAT (Fig. 3A) compared with that observed in lean rats receiving stock diet (A I). Further, chronic challenging with the high vitamin A diet resulted in a significant reduction in RPWAT Bcl2 expression in the lean rats (A II), when compared with their stock diet-fed lean counterparts (A1). On the other hand, no such effect was seen in obese rats (B II), when compared with the expression observed in their respective lean and obese counterparts consuming stock diet (A I and B I respectively). In addition, this particular dietary regimen (129 mg vitamin A/kg diet) also resulted in over-expression of Bax, a pro-apoptotic protein, in lean rats (A II) compared with their respective control rats fed on a normal vitamin A diet (A I). However, no such over-expression was observed in obese rats (B II) compared with their obese counterparts fed on stock diet (normal dose of vitamin A) (B I) (Fig. 3A).

The ratio of Bcl2–Bax of RPWAT was significantly lower in the lean rats maintained on the high vitamin A diet (A II) as compared with lean rats receiving the stock diet (2·6 mg vitamin A/kg diet). However, the ratio was not altered in obese rats fed on an identical dietary regimen (129 mg vitamin A/kg diet) when compared with their respective control rats fed on the stock diet (having 2·6 mg vitamin A/kg diet) (B I) (Fig. 3B). Furthermore, the Bcl2–Bax ratio was significantly influenced by interaction between phenotype and treatment.

Discussion

Adipose tissue mass is tightly regulated by both the size and/or number of the adipocytes and the latter, in turn, is regulated by pre-adipocyte recruitment, differentiation and adipocyte apoptosis (Ailhaud 1990, Bjorntorp 1991). Further, recent studies substantiate the concept that adipocyte deletion by apoptosis is a significant contributor to the regulation of adipose tissue mass and its loss during weight reduction (Della-Fera *et al.* 2001, Hargrave *et al.* 2002, Fischer-Posovszky *et al.* 2004, Kim *et al.* 2004, Sun & Zemel 2004). Our retroperitoneal adipose tissue cell density data clearly showed that chronic vitamin A challenging through diet had no impact on cell size. This formed the basis for our hypothesis that vitamin A-mediated loss of adipose tissue

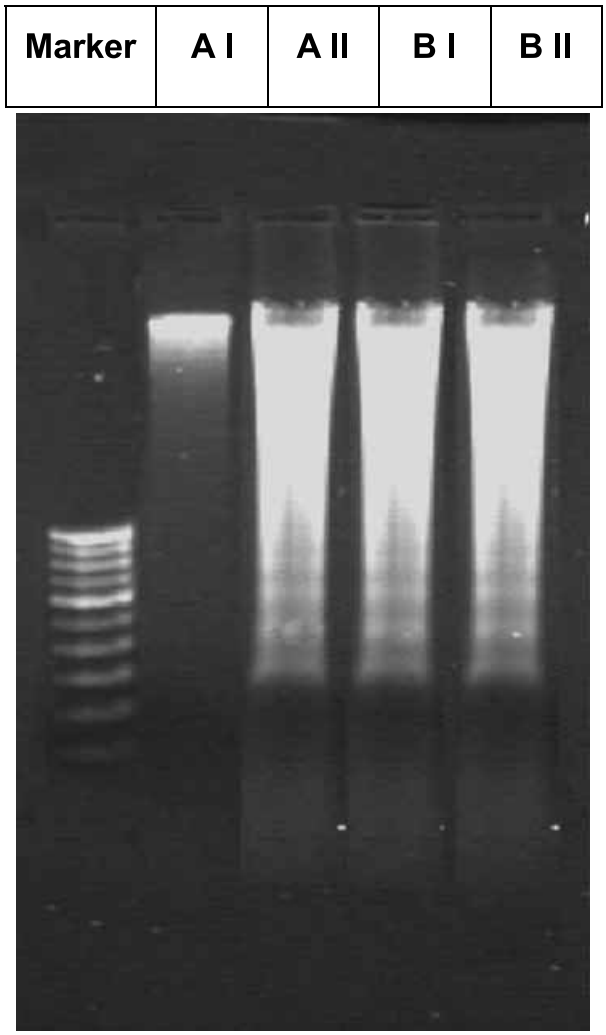


Figure 2 Nucleosomal DNA fragmentation: A I, lean rats fed on stock diet, A II, lean rats fed on a high vitamin A diet, B I, obese rats fed on stock diet and B II, obese rats fed on a high vitamin A diet.

could be through altered (decreased) adipocyte number rather than size.

The observed differences between the lean and the obese phenotype with regard to various obesity (body weight gain and adiposity index) and apoptosis-related parameters (apoptotic index, Bcl2 and Bax expression) could be explained by the differences in their genetic composition. Despite identical treatments (vitamin A supplementation), the two phenotypes responded differently (with regard to the above-mentioned parameters) and this cannot be explained solely by genetic differences. Hence, the role of complex interactions between the phenotype (genetic make-up) and nutrient involved should be considered. In view of this, the data were subjected to two-way ANOVA and the results are presented.

The observed DNA fragmentation, high apoptotic index and marginal reduction in adiposity, especially RPWAT mass, in high vitamin A-treated lean rats could be attributed to decreased Bcl2, enhanced Bax expressions and decreased Bcl2 to Bax ratio. Bcl2 is a prominent regulator of apoptosis and helps in prolonging cell survival. It is also known that over-expression of one of the apoptotic effector genes, Bax, is sufficient to antagonize the function of Bcl2 and enhance the cellular susceptibility to apoptosis (Korsmeyer *et al.* 1993, Rosse *et al.* 1998, Murphy *et al.* 2000). Based on this, the ratio of Bcl2 to Bax is deemed important in determining cell survival or death.

Further, several *in vitro* studies have clearly established the role of RA, an important metabolite of vitamin A, on Bcl2 expression. All-*trans*-RA has been reported to induce cell death in a variety of cell lines including 3T3-L1 preadipocytes (Thaller & Eichele 1987, Martin *et al.* 1990, Chawla & Lazar 1994, Li *et al.* 1999, Kim *et al.* 2000). However, to our knowledge, this is the first report wherein the effect of feeding high doses of vitamin A on RPWAT apoptosis has been demonstrated in a lean phenotype of a genetically obese rat model.

In contrast to lean rats fed on the stock diet (2·6 mg vitamin A/kg diet), obese rats maintained on an identical diet displayed a higher apoptotic index in RPWAT despite their higher adiposity, which could be explained by lower Bcl2 expression and decreased Bcl2 to Bax ratio (due to unaltered Bax expression) in this tissue. In addition, FFAs, especially saturated fatty acids, are known to mediate apoptosis in pancreatic β cells, hepatocytes, human granuloma cells and brain tumors (Shimabukuro *et al.* 1998, Williams *et al.* 1998, Wu & Cederbaum 2000, Mu *et al.* 2001). These fatty acids may be inducing apoptosis either by reducing Bcl2 expression or hetero-dimerization of Bax proteins. In Zucker diabetic fatty rats, increased ceramide generation or nitric oxide production has been implicated in FFA-induced β-cell apoptosis (Shimabukuro *et al.* 1998). Interestingly, obese rats of the WNIN/Ob strain have increased plasma FFAs concentration and high levels of saturated fatty acids, particularly palmitic and stearic acids, in RPWAT (due to enhanced lipogenesis), which could be responsible for the higher apoptotic index and decreased Bcl2 expression observed (Vajreswari A, Jeyakumar SM and Giridharan NV, unpublished data).

Surprisingly, high vitamin A treatment of obese rats had no effect on any of the above-mentioned parameters (apoptotic index, Bcl2 and Bax expressions, Bcl2 to Bax ratio and FFA concentrations), thereby ruling out the possible role of vitamin A-mediated apoptosis in lowering retroperitoneal adipose tissue mass/adiposity. The most significant finding of this study is that obese rats have abundant retroperitoneal adipose depots despite the occurrence of apoptosis in the very same

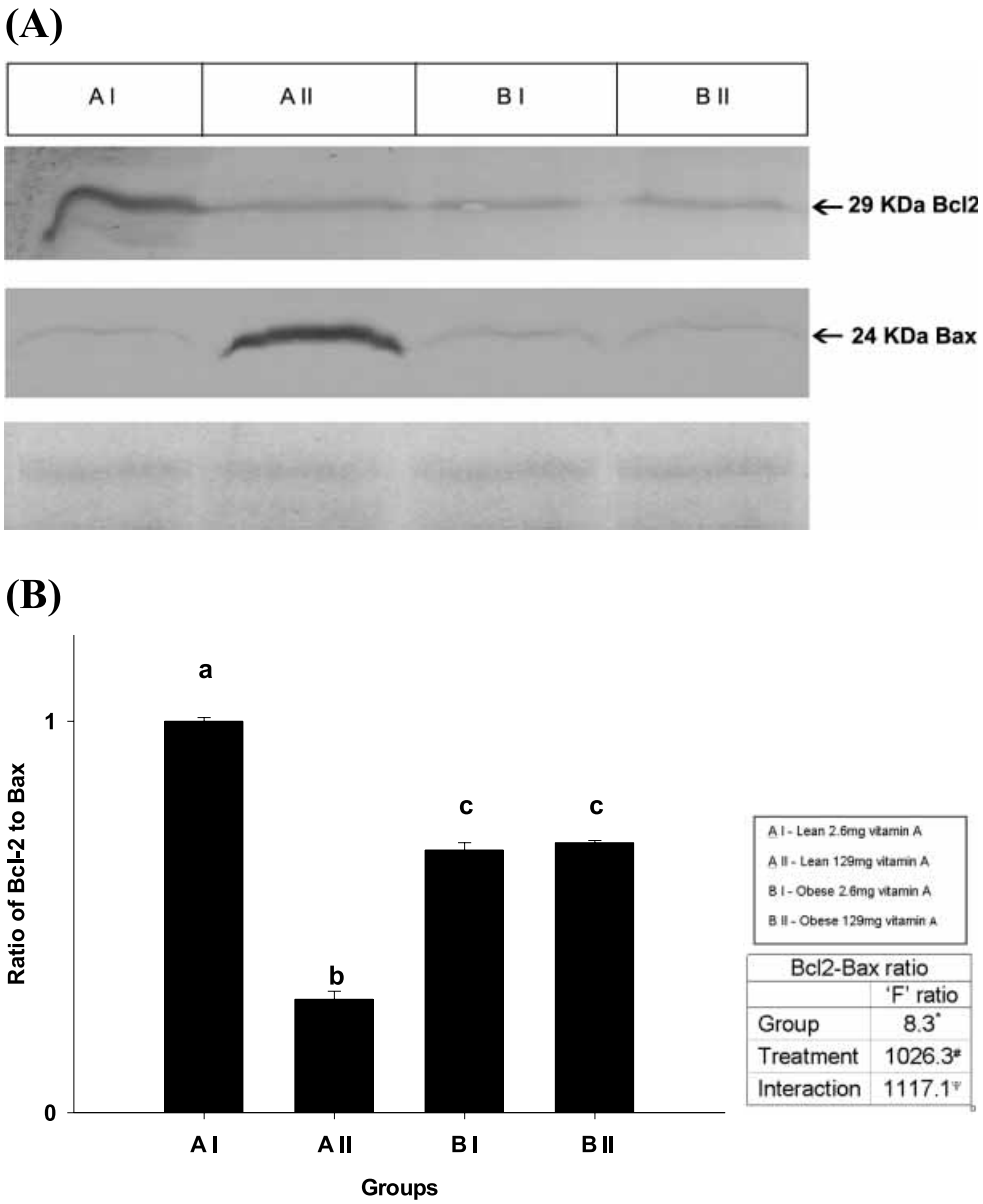


Figure 3 Effect of high vitamin A on RPWAT apoptotsis-related proteins expression. (A) The upper and middle panels are representative western blots of RPWAT Bcl-2 and Bax protein expression respectively and the lower panel shows the Ponseau-S-stained blot for the equal loading control. (B) RPWAT Bcl2–Bax ratio quantified densitometric values are expressed relative to a value of 1 for A I (lean) as control. Values are means±S.E. for three to four rats. Mean values without common superscripts are significant at $P \leq 0{\cdot}05$. The F ratios are significant at the $P \leq 0{\cdot}05$ level (*, † and ‡ for group, treatment and interaction respectively) (by two-way ANOVA).

tissue. However, the mechanism underlying these paradoxical findings is unclear.

Although, in general, decreased apoptosis contributes to obesity, this may not be true for this particular obese rat model (WNIN/Ob rat strain), thereby implicating the role of non-apoptotic pathways, namely preadipocyte recruitment, differentiation and the thermogenic pathway, in manifesting obesity. This derives further support from the concept that adipocyte number is not predetermined at the time of birth, but increases even in adulthood (Kawada *et al.* 2001). Interestingly, vitamin A metabolites, besides their effects on apoptosis,

impact two important non-apoptotic pathways, namely preadipocyte differentiation and the thermogenic pathway. Although the former aspect was not addressed in this particular study, the latter has been studied in detail and reported elsewhere (Jeyakumar *et al.* 2005).

UCP1 expression in the BAT of these rats indicated that obese rats receiving normal levels of vitamin A had lower UCP1 mRNA levels compared with their lean counterparts maintained on identical diets. Further, vitamin A supplementation resulted in significant overexpression in the obese but not in the lean phenotype (Jeyakumar *et al.* 2005). This particular observation and several other *in vitro* studies and those employing experimental models of obesity highlight the role of the non-apoptotic (thermogenic) pathway in the regulation of adiposity/body weight gain (Alvarez *et al.* 1995, Puigserver *et al.* 1996, Kumar & Scarpace 1998, Kumar *et al.* 1999, Bonet *et al.* 2000, Villarroya *et al.* 2004).

RA induces thermogenic activity by enhancing the expression of BAT UCP1 through its nuclear receptors RXR and RAR (Villarroya *et al.* 1999, Felipe *et al.*, and Bonet *et al.* 2003). The RAR receptors homodimerize or heterodimerize with RXR and ligand dependently bring about specific gene activation (BAT UCP1). On the other hand, RXR homodimerize or heterodimerize with various members of the nuclear hormone superfamily receptors (Peroxisome proliferator-activated receptor/Thyroid hormone receptor/Vitamin D receptor) RAR and ligand dependently activate several other genes and elicit various physiological responses (Bonet *et al.* 2003).

It has recently been shown that the administration of a synthetic RXR agonist (LG268) through oral and intracerbral routes to Zucker fa/fa rats induces anorexia, decreases food intake, body weight gain and adiposity and activates apoptotic pathway without affecting lean body mass, possibly by activating RXR receptors in the central nervous system (Ogilvie *et al.* 2004). However, in the present study, vitamin A feeding, although resulting in reduced adiposity and body weight gain in obese rats, had no effect on food intake and retroperitoneal adipose tissue apoptosis. These effects in obese rats of the WNIN/Ob strain could obviously be due to the activation of non-apoptotic pathways.

Taken together, a balance between apoptotic and non-apoptotic pathways determines adipose tissue weight/homeostasis. Notably, nutrients like vitamin A have a modulatory role on these two opposite events which, in turn, are determined by the genetic make-up of the species. This is evident from the differential expression of some apoptosis-related proteins (Bcl2 and Bax) in the lean and obese phenotypes and their divergent response to the identical dose of vitamin A, in terms of the expression of these proteins and UCP1 expression in these two phenotypes.

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References

Ailhaud G 1990 Extracellular factors, signalling pathways and differentiation of adipose precursor cells. *Current Opinion in Cell Biology* **2** 1043–1049.

Alvarez R, de Andres J, Yubero P, Vinas O, Mampel T, Iglesias R, Giralt M & Villarroya F 1995 A novel regulatory pathway of brown fat thermogenesis. Retinoic acid is a transcriptional activator of the mitochondrial uncoupling protein gene. *Journal of Biological Chemistry* **270** 5666–5673.

Bjorntorp P 1991 Adipose tissue distribution and function. *International Journal of Obesity and Related Metabolic Disorders* **2** 67–81.

Bonet ML, Oliver J, Pico C, Felipe F, Ribot J, Cinti S & Palou A 2000 Opposite effects of feeding a vitamin A-deficient diet and retinoic acid treatment on brown adipose tissue uncoupling protein 1 (UCP1), UCP2 and leptin expression. *Journal of Endocrinology* **166** 511–517.

Bonet ML, Ribot J, Felipe E & Palou A 2003 Vitamin A and the regulation of fat reserves. *Cellular and Molecular Life Sciences* **60** 1311–1321.

Chawla A & Lazar MA 1994 Peroxisome proliferator and retinoid signaling pathways co-regulate preadipocyte phenotype and survival. *PNAS* **91** 1786–1790.

Della-Fera MA, Qian H & Baile CA 2001 Adipocyte apoptosis in the regulation of body fat mass by leptin. *Diabetes, Obesity and Metabolism* **3** 299–310.

Felipe F, Bonet ML, Ribot J & Palou A 2003 Up-regulation of muscle uncoupling protein 3 gene expression in mice following high fat diet, dietary vitamin A supplementation and acute retinoic acid-treatment. *International Journal of Obesity and Related Metabolic Disorders* **27** 60–69.

Fischer-Posovszky P, Tornqvist H, Debatin KM & Wabitsch M 2004 Inhibition of death-receptor mediated apoptosis in human adipocytes by the insulin-like growth factor I (IGF-I)/IGF-I receptor autocrine circuit. *Endocrinology* **145** 1849–1859.

Frayn KN, Karpe F, Fielding BA, Macdonald IA & Coppack SW 2003 Integrative physiology of human adipose tissue. *International Journal of Obesity and Related Metabolic Disorders* **27** 875–888.

Giridharan NV, Harishankar N & Satyavani M 1996 A new rat model for the study of obesity. *Scandinavian Journal of Laboratory Animal Sciences* **23** 131–139.

Gong HX, Guo XR, Fei L & Guo M, Liu QQ & Chen RH 2003 Lipolysis and apoptosis of adipocytes induced by neuropeptide Y-Y5 receptor antisense oligonucleotides in obese rats. *Acta Pharmacologica Sinica* **24** 569–575.

Hargrave KM, Li C, Meyer BJ, Kachman SD, Hartzell DL, Della-Fera MA, Miner JL & Baile CA 2002 Adipose depletion and apoptosis induced by trans-10, cis-12 conjugated linoleic acid in mice. *Obesity Research* **10** 1284–1290.

Hernandez A, Garcia-Jimenez C, Santisteban P & Obregon MJ 1993 Regulation of malic-enzyme-gene expression by cAMP and retinoic acid in differentiating brown adipocytes. *European Journal Biochemistry* **215** 285–290.

Jeyakumar SM, Vajreswari A & Giridharan NV 2005 Chronic dietary vitamin A supplementation regulates obesity: in obese mutant rat model of WNIN/Ob strain. *Obesity Research* (in press).

Kawada T, Takahashi N & Fushiki T 2001 Biochemical and physiological characteristics of fat cell. *Journal of Nutritional Science and Vitaminology* **47** 1–12.

Kim HS, Hausman DB, Compton MM, Dean RG, Martin RJ, Hausman GJ, Hartzell DL & Baile CA 2000 Induction of apoptosis by all-trans-retinoic acid and C2-ceramide treatment in rat stromal-vascular cultures. *Biochemical and Biophysical Research Communications* **270** 76–80.

Kim MS, Yoon CY, Jang PG, Park YJ, Shin CS, Park HS, Ryu JW, Pak YK, Park JY, Lee KU *et al.* 2004 The mitogenic and antiapoptotic actions of ghrelin in 3T3-L1 adipocytes. *Molecular Endocrinology* **18** 2291–2301.

Korsmeyer SJ, Shutter JR, Veis DJ, Merry DE & Oltvai ZN 1993 Bcl-2/Bax: a rheostat that regulates an anti-oxidant pathway and cell death. *Seminars in Cancer Biology* **4** 327–332.

Kumar MV & Scarpace PJ 1998 Differential effects of retinoic acid on uncoupling protein-1 and leptin gene expression. *Journal of Endocrinology* **157** 237–243.

Kumar MV, Sunvold GD & Scarpace PJ 1999 Dietary vitamin A supplementation in rats: suppression of leptin and induction of UCP1 mRNA. *Journal of Lipid Research* **40** 824–829.

Kuri-Harcuch W 1982 Differentiation of 3T3-F442A cells into adipocytes is inhibited by retinoic acid. *Differentiation* **23** 164–169.

Li Y, Hashimoto Y, Agadir A, Kagechika H & Zhang X 1999 Identification of a novel class of retinoic acid receptor beta-selective retinoid antagonists and their inhibitory effects on AP-1 activity and retinoic acid-induced apoptosis in human breast cancer cells. *Journal of Biological Chemistry* **274** 15360–15366.

Loftus TM, Kuhadja FP & Lane MD 1998 Insulin depletion leads to adipose-specific cell death in obese but not lean mice. *PNAS* **24** 14168–14172.

Martin SJ, Bradley JG & Cotter TG 1990 HL-60 cells induced to differentiate towards neutrophils subsequently die via apoptosis. *Clinical and Experimental Immunology* **79** 448–453.

Miner JL, Cederberg CA, Nielsen, MK, Chen X & Baile CA 2001 Conjugated linoleic acid (CLA), body fat, and apoptosis. *Obesity Research* **9** 129–134.

Mu YM, Yanase T & Nishi Y 2001 Saturated FFAs, palmitic acid and stearic acid, induce apoptosis in human granulosa cells. *Endocrinology* **142** 3590–3597.

Murphy KM, Ranganathan V, Farnsworth ML, Kavallaris M & Lock RB 2000 Bcl-2 inhibits Bax translocation from cytosol to mitochondria during drug-induced apoptosis of human tumor cells. *Cell Death and Differentiation* **7** 102–111.

Ogilvie KM, Saladin R, Nagy TR, Urcan MS, Heyman RA & Leibowitz MD 2004 Activation of the retinoid X receptor suppresses appetite in the rat. *Endocrinology* **145** 565–573.

Palou A, Serra F, Bonet ML & Picó C 2000 Obesity: molecular bases of a multifactorial problem. *European Journal of Nutrition* **39** 127–144.

Prins JB & O'Rahilly S 1997 Regulation of adipose cell number in man. *Clinical Sciences* **92** 3–11.

Prins JB, Walker NI, Winterford CM & Cameron DP 1994 Apoptosis of human adipocytes *in vitro*. *Biochemical and Biophysical Research Communications* **202** 500–507.

Prins, JB, Niesler CU, Winterford CM, Bright NA, Siddle K, O'Rahilly S, Walker NI & Cameron DP 1997 Tumor necrosis factor-alpha induces apoptosis of human adipose cells. *Diabetes* **46** 1939–1944.

Puigserver P, Vazquez F, Bonet ML, Pico C & Palou A 1996 *In vitro* and *in vivo* induction of brown adipocyte uncoupling protein (thermogenin) by retinoic acid. *Biochemical Journal* **317** 827–833.

Reed JC 2002 *Bcl*-2 family proteins and the dysregulation of programmed cell death. In *Encyclopedia of Cancer*, edn 2, vol. 1, Ed JR Bertin. pp 179–181 San Diego: Academic Press.

Ribot J, Felipe F, Bonet ML & Palou A 2001 Changes of adiposity in response to vitamin A status correlate with changes of PPAR gamma 2 expression. *Obesity Research* **9** 500–509.

Rosse T, Olivier R, Monney L, Rager M Conus S, Fellay I, Jansen B & Borner C 1998 Bcl-2 prolongs cell survival after Bax-induced release of cytochrome C. *Nature* **391** 496–499.

Shimabukuro M, Zhou YT, Levi M & Unger RH 1998 Fatty acid-induced beta cell apoptosis: a link between obesity and diabetes. *PNAS* **95** 2498–2502.

Sun X & Zemel MB 2004 Role of uncoupling protein 2 (UCP2) expression and 1 alpha, 25-dihydroxyvitamin D3 in modulating adipocyte apoptosis. *FASEB Journal* **18** 1430–1432.

Taylor BA & Phillips SJ 1996 Detection of obesity QTLs on mouse chromosomes 1 and 7 by selective DNA pooling. *Genomics* **34** 389–398.

Thaller C & Eichele G 1987 Identification and spatial distribution of retinoids in developing chick limb bud. *Nature* **327** 625–628.

Tsutsumi C, Okuno M, Tannous L, Piantedosi R, Allan M, Goodman DS & Blaner WS 1992 Retinoids and retinoid-binding protein expression in rat adipocytes. *Journal of Biological Chemistry* **267** 1805–1810.

Villarroya F, Giralt M & Iglesias R 1999 Retinoids and adipose tissues: metabolism, cell differentiation and gene expression. *International Journal of Obesity and Related Metabolic Disorders* **23** 1–6.

Villarroya F, Iglesias R & Giralt M. 2004 Retinoids and retinoid receptors in the control of energy balance: novel pharmacological strategies in obesity and diabetes. *Current Medicinal Chemistry* **11** 795–805.

Williams JR, Leaver HA, Ironside JW, Miller EP, Whittle IR & Gregor A 1998 Apoptosis in human primary brain tumours: actions of arachidonic acid. *Prostaglandins Leukotrienes and Essential Fatty Acids* **58** 193–200.

Wu D & Cederbaum AI 2000 Ethanol and arachidonic acid produce toxicity in hepatocytes from pyrazole-treated rats with high levels of CYP2E1. *Molecular and Cellular Biochemistry* **204** 157–167.

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