INTRODUCTION

Obesity is an excessive accumulation of body fat that poses a real threat to health. It is characterized by either an increase in the number (hyperplasia) or the enlargement (hypertrophy) of adipocytes (Jequier, 2002). Cellular events can cause obesity only if they affect energy balance. This means that changes in the expression of genes which control the differentiation and the development of adipocytes are not primarily responsible for the gain in body weight (Spiegelman and Flier, 2001). The amount of adipose tissue in the body is determined by a complex interaction of genes and the environment (Ravussin and Gautier, 1999). A minor imbalance between energy intake and energy expenditure may lead to severe obesity. Jequier (2002) provided an effective equation to determine such balance as follows:

Energy stored = energy intake - energy lost in feces and urine - energy expenditure.

The prevalence of obesity is increasing globally, particularly among children and adolescents, and nearly half a billion of the world's population (estimated to be 6.5 billions) is now considered to be overweight or obese (Rossner, 2002). The prevalence of obesity in the Saudi population has been studied by a vast number of researchers interested in community health aspects (Binhemd *et al*., 1991; Al-Rehaimi and Björntorp, 1992; Al-Shammari *et al*., 1994; Rasheed *et al*., 1994; Soyannwo *et al*., 1998; Al-Mahroos and Al- Roomi, 1999). Overweight and obesity seem to be prevalent among Saudi children and adolescents as indicated by increased BMIs (Abalkhail 2002; Al-Rukban, 2003; Al-Hazzaa, 2007a,b; Mahfouz *et al*., 2008).

The body must have a mechanism for signaling the level of body fat stores to the brain so that peripheral adiposity signals can be centrally processed to affect the eating behavior and other

processes of energy homeostasis (English and Wilding, 2006). Both neural and hormonal factors were reported to have a role in regulatory energy homeostasis. Neural control is achieved via signals traveling from digestive tract detectors to satiety centers in the hypothalamus or brain stem (Carlson, 2001). Higher centers than the hypothalamus also play a role in the control of appetite (English and Wilding, 2006). Hormonal signals via integrated neuropeptide pathways also lead to a number of outputs that are directly related to energy homeostasis. These include neuroendocrine activation from the pituitary gland, motor behavior (eating, exercise, etc.), and autonomic activity (Woods and Seeley, 2002).

Hormonal signals include two main hormones, insulin and leptin, besides the peptide hormone cholecystokinin (CCK) secreted from the digestive tract (Carlson, 2001; English and Wilding, 2006). Insulin and leptin circulate in the blood at concentrations proportional to the body fat content and enter the central nervous system in proportion to their plasma levels. Leptin is secreted from well-nourished adipose tissue; acts by increasing the metabolic rate and decreasing the food intake by increasing the brain's sensitivity to short-term satiety signals such as CCK (Carlson, 2001). Although leptin is secreted from white adipocytes, its secretion is not dependent on the size of the fat mass (Woods and Seeley, 2002). The rate of insulin-stimulated glucose utilization, a process affected by changes in energy balance, appears to be a key factor in the relationship between leptin and adipose tissue mass (English and Wilding, 2006).

Pancreatic insulin secretion is directly proportional to the size of the fat mass. Obese people secrete more insulin after meals, although the blood glucose level is identical in obese and lean individuals (Polonsky *et al*., 1988). Administering either leptin or insulin directly into the brain causes a dose-dependent reduction of the food intake, increased energy expenditure, and decreased body weight. Conversely, reducing the amount of insulin or leptin uniquely in the brain causes an

increased food intake, decreased energy expenditure and increased body weight. The lack of leptin or leptin receptors, in both animals and humans, leads to obesity (Woods and Seeley, 2002). Fig.1.1, summarize the different mechanisms and pathways controlling the food intake and adiposity signals.

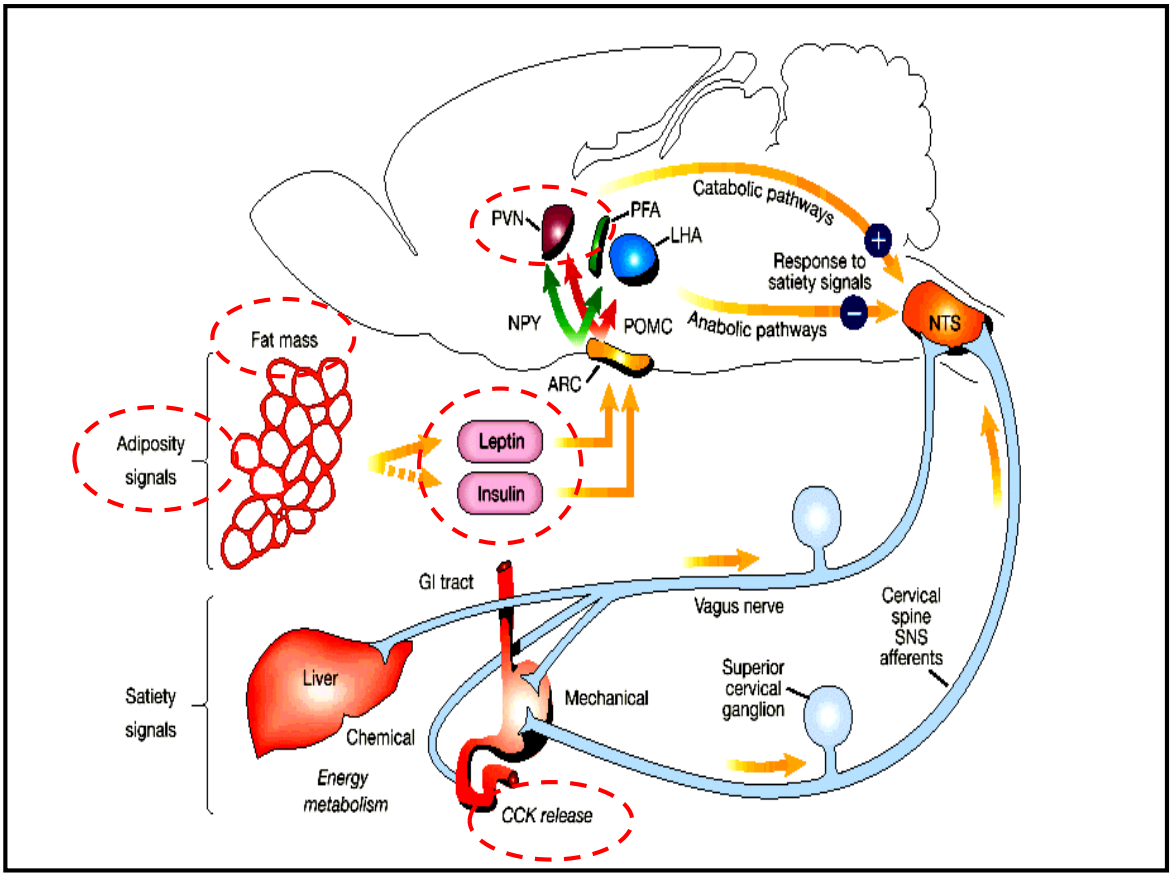


Fig. 1.1 Diagram to illustrate the role of leptin, insulin and CCK (dotted red circles) in energy homeostasis (English and Wilding, 2006).

1.1.1 Causes of obesity

The causes of obesity are multifactor, with environmental influences acting on genetic or biological predispositions (English and Wilding, 2006). Obesity is closely associated with a sedentary lifestyle, as illustrated by the inverse relationship between body weight and the amount

of physical activity (Al-Shammari *et al*., 1994; Jequier, 2002; Rossner, 2002; Tremblay and Willms, 2003; Popkin and Gordon-Larsen, 2004; Marti *et al*., 2004; Stettler *et al*., 2004; Al-Hazzaa and Al-Rasheedi, 2007; Al-Nozha *et al*., 2007). Other factors that lead to obesity include a low metabolic rate (Ferraro *et al.*, 1992), hereditary factors and genes (Eriksson *et al.*, 2003; Marti *et al*., 2004; Snyder *et al*., 2004), social and educational factors (Al-Malki *et al*., 2003; Eriksson *et al.*, 2003; Al-Shammari *et al*., 1994; Huot *et al*., 2004), illness (Dingemans *et al*., 2002; De Zwaan, 2001), neurogenic abnormalities (Guyton and Hall, 1996), medications (Aronne, 2002), age (Al-Malki *et al*., 2003; Huot *et al*., 2004; Al-Shammari *et al*., 1994; Al-Nuaim *et al*., 1997), gender (Al-Nuaim *et al*., 1997; Lovejoy, 1998; Harvie *et al*., 2005), emotions (Canetti *et al*., 2002), childhood overnutrition (Guyton and Hall, 1996), smoking cessation (Strauss and Mir, 2001), and pregnancy (Rooney and Schauberger, 2002).

1.1.2 Obesity-related disorders

Obesity has significant co-morbidities that are associated with substantial health care and social costs. It is estimated that more people will die from complications of overnutrition than starvation. Scientists must take action to deal with the obesity problem. Prevention should be the primary target, but it is also important to develop strategies to treat those already affected with obesity (Rossner, 2002).

Overweight and obesity increase the risk of multiple conditions such as heart disease (Al-Mahroos and Al-Roomi, 1999; Rexrode *et al*., 2001; Sharma, 2002), stroke (Gillum *et al*., 2001), hypertension (Al-Mahroos and Al-Roomi, 1999; Sovannwo *et al*., 1998; Schunkert, 2002; Rossner, 2002), type 2 diabetes (Al-Mahroos and Al-Roomi, 1999; Sovannwo *et al*., 1998;

Fujioka, 2002; Felber and Golay, 2002), cancer (Aronne, 2002; Rossner, 2002), gallbladder disease (Tsai *et al*., 2004; Siener *et al*., 2004), osteoarthritis (Coggon *et al*., 2001; Andersen *et al*., 2003), gout (Bult *et al*., 2008), sleep apnea (Resta *et al*., 2001), dyslipidemia (Montani *et al*., 2002), complications of pregnancy (Crane *et al*., 1997), psychological and social effects, such as depression and discrimination (Dong *et al*., 2004), and metabolic syndrome (Fujioka, 2002; Al-Nozha *et al*., 2005).

1.1.3 Treatment of obesity

The initial treatment of obesity should focus on a diet and exercise program that has been individualized to the patient's lifestyle and physical needs. Behavioral therapy should be implemented as an adjunct to this program (Fujioka, 2002). However, obese patients and overweight individuals who cannot achieve sufficient weight loss through lifestyle and behavioral modifications are urged to use one of several anti-obesity agents (including drugs, nutritional supplements, and herbal dietary supplements) to control their body weight (Table 1.1).

Table 1.1 Agents used in the treatment of obesity

Drugs	
Name	**Mechanism**
Amphetamine derivatives	They decrease the degree of hunger by inhibiting the feeding centers in the brain, but they simultaneously overexcite the

	central nervous system, and make the person nervous and elevate the blood pressure (Guyton and Hall, 1996).
Sibutramine (Reductil or Meridia)	This drug reduces the food intake by increasing the content of hormonal substances serotonin and noradrenaline. These substances suppress the food intake by blocking signals of hunger coming to the brain (Barkeling *et al*., 2003). It has various side effects including dry mouth, insomnia, headache, fatigue, and increased blood pressure, and heart rate (Kim *et al*., 2003).
Orlistat (Xenical)	It is a lipase inhibitor. Orlistat is not an appetite suppressant and does not affect the metabolism. It did not alter short-term physiological or behavioral measures of satiety in response to high-fat meals in healthy, non obese subjects (Goedecke *et al*., 2003). The treatment with Orlistat resulted in reducing risk factors

Table 1.1 continued (Agents used in the treatment of obesity)

Drugs	
Name	**Mechanism**

Orlistat (Xenical)	for coronary heart disease (Krempf *et al*., 2003). Orlistat administration for 2 years promotes weight loss and minimizes weight regain. Additionally, Orlistat therapy improves lipid profile, blood pressure, and quality of life (Rossner *et al*., 2000; Muls *et al*., 2001).
Phentermine	It is an appetite suppressant (Kim, 2006; Mancini and Halpern, 2006)
Nutritional supplements	
Name	**Mechanism**
Spirulina	It is a type of cyanobacteria and a rich source of protein, vitamins, minerals, and essential fatty acids. Although spirulina has been promoted as a weight-loss aid, the scientific evidence supporting its use for this purpose is weak (Mascher *et al*., 2006).
5-hydroxytryptophan (5-HTP)	The precursor to the neurotransmitter serotonin has been shown in three short-term controlled trials to reduce appetite and to promote weight loss (Cangiano *et al*., 1992).

Table 1.1 continued (Agents used in the treatment of obesity)

Nutritional supplements	
Name	**Mechanism**
L-carnitine (a non-protein amino acid)	It promotes weight loss and is used in fat burners because it helps in converting stored fat into energy. L-carnitine is known to transport fatty acids to the innermost section of the mitochondria (the powerhouse of the cell), where they are used to create energy resources adenosine triphosphate (ATP), (Müller *et al*., 2002; Wutzke and Lorenz, 2004).
Pyruvate	Animal studies suggest that pyruvate supplementation leads to weight loss by increasing the resting metabolic rate (Ivy *et al*., 1994).
Chromium picolinate	It works well in combination with inositol to decrease low density lipoprotein (LDL) levels and increase high density lipoprotein (HDL) levels. Research has shown that chromium picolinate can burn fat and enhance muscle growth even without any exercise or a special diet. It plays an essential role in the metabolism of carbohydrates, fats and proteins and in the action of insulin (Anderson, 1998; Pittler *et al*., 2003; Sharpe *et al*., 2006).

Table 1.1 continued (Agents used in the treatment of obesity)

Nutritional supplements	
Name	**Mechanism**
(-)Hydroxycitric acid (HCA) extracted from the rind of the *Garcinia cambogia* fruit grown in Southeast Asia	HCA may be a useful weight loss aid by reducing the conversion of carbohydrates into stored fat by inhibiting certain enzyme processes (citrate cleavage enzyme). Animal research indicates that HCA suppresses appetite and induces weight loss (Lowenstein, 1971; Sullivan *et al*., 1972). HCA treatment reduced 24-hour energy intake in humans while satiety was sustained (Westerterp-Plantenga and Kovacs, 2002).
Dehydroepiandrosterone (DHEA)	DHEA supplementation lowers the fat mass without reducing total body weight (Vogiatzi *et al*., 1996; Richards *et al*., 2000). The reduction in the fat mass occurred in men but not in women (Vogiatzi *et al*., 1996).
Herbal dietary supplements	
Name	**Mechanism**
Ephedra sinica commonly known as Ma Huang	It is a central nervous system stimulant. Ephedra, particularly when combined with caffeine, promoted body weight and body fat reduction and improved blood lipids without significant adverse events (Boozer *et al*., 2002; Kalman *et al*., 2002; Coffey *et al*., 2004).

Table 1.1 continued (Agents used in the treatment of obesity)

Herbal dietary supplements	
Name	**Mechanism**
Cayenne pepper *Capsicum annuum*	It has modest reductions in appetite, increases metabolism of dietary fats, and causes an increase in sympathetic nervous system activities (Yoshioka *et al*., 1998). Capsaicin is the major pungent ingredient in cayenne.
Guarana *Paullinia cupana* (contains caffeine, theobromine, and theophylline)	These compounds may curb appetite and increase weight loss. Caffeine's effects are well known and include central nervous system stimulation, an increased metabolic rate, and a mild diuretic effect (Sharpe *et al*., 2006). The mixture of Ma Huang and Guarana effectively promoted short-term weight loss (Boozer *et al*., 2001).
Caffeine	It appears to be a safe thermogenic agent for weight control. Exercising, eating a low-fat diet, and consuming large amounts of caffeine slightly enhance weight loss. But when taken in large doses, caffeine can cause jitters, irritability, insomnia, and high blood pressure (Lopez-Garcia *et al*., 2006; Diepvens *et al*., 2007). Caffeine was found to be a suppressor of fat absorption (Shimoda *et al*., 2006).

Table 1.1 continued (Agents used in the treatment of obesity)

Herbal dietary supplements	
Name	**Mechanism**
Guggul *Commiphora mukul* (guggul extracts contain 5–10% guggulsterones)	A combination of guggul, phosphate salts, hydroxycitrate, and tyrosine has been shown to improve mood with a slight tendency to improve weight loss in overweight adults (Antonio *et al*., 1999).
Coleus forskohlii (the herb contains forskolin)	It is a substance that stimulates the lipolysis of fat that was deposited long ago. Forskolin activates adipocytes to respond more effectively upon the hormonal stimulus to cleavage fat (Han *et al*., 2005).
Green tea *Camellia sinensis* extract rich in polyphenols (epigallocatechin gallate, or EGCG)	It increases energy expenditure (Diepvens *et al*., 2007). The green tea-caffeine mixture improved weight maintenance through thermogenesis and fat oxidation (Westerterp-plantenga *et al*., 2005).
St. John's wort *Hypericum perforatum*	A combination of bitter orange extract *(Citrus aurantium)*, caffeine, and St. John's wort has been shown to be superior to placebo or no treatment in promoting weight loss in obese healthy adults who eat a low-fat diet (Colker *et al*., 1999).

Table 1.1 continued (Agents used in the treatment of obesity)

Herbal dietary supplements	
Name	**Mechanism**
Psylliym *Plantago ovata*	Consuming it before a meal was associated with a decreased intake of fat and increased feelings of fullness following a meal (Turnbull and Thomas, 1995).
Chitosan	This is a dietary supplement made from chitin, a starch found in the skeleton of shrimp, crab and other shellfish (Gades and Stern, 2002). The efficacy of chitosan supplements are insufficiently documented efficacy to recommend their use (Aronne, 2002). Chitosan did not increase the fecal fat content and therefore did not block fat absorption (Gades and Stern, 2002).
Usnic acid (UA)	Health food supplements containing UA have been promoted for use in weight reduction because of its thermogenic effect (Ingolfsdottir, 2002).
Conjugated linoleic acid (CLA)	CLA reduces body fat accumulation in animal models and has been suggested to have significant effects on lipid and glucose metabolism in animals , humans, and cell culture (Smedman and Vessby, 2001; Evans *et al*., 2002a; Riserus *et al*., 2003).

A detailed review concerning the fat burner effect of both UA and CLA is reported in this chapter.

1.2.1 Usnic acid (UA) as a weight loss agent

Usnic acid is a complex polycyclic chemical compound produced naturally as a secondary metabolite by certain lichen species; it was first isolated by Knop in 1844 (Frankos, 2005). The ingredient names are usnic acid, sodium usniate, or sodium usneate. Labeling names are usnic acid, sodium usniate, *usnea* lichen, or extract *usnea barbata* (Frankos, 2005). *Usnea*, also known as old man's beard, is not a plant but a lichen (a symbiotic relationship between an algae and a fungus). *Usnea sp.* (Usneaceae) is the main source, but it is found in other genera of lichens, including *Cladonia* (Cladoniaceae), *Lecanora* (Lecanoraceae), *Ramalina* (Ramalinaceae), *Evernia*, *Parmelia* (Parmeliaceae), and other lichen genera. *Alectoria* (Alectoriaceae) species are often rich sources of UA with yields of up to 6% (Proksa *et al*., 1996; Ingolfsdottir, 2002).

The globally distributed consortium of lichens is developed through the symbiosis between the green algae and cyanobacteria (photobionts), which produce carbohydrates by photosynthesis for themselves and for their dominant fungal partners (mycobionts) that provide physical protection, water, and minerals in exchange. Lichens can colonize on rocks (foliose and crustose lichens*)* or on tree trunks, soil, or several other diversified substrata (fruticose lichens) (Fig. 1.2). It is estimated that lichens cover approximately 8% of the earth's surface (Cocchietto, 2002).

Out of more than 20,000 known species of lichens, a number of them have been used for diversified purposes, such as dyeing, pollution monitoring, perfumery, and floral decoration, and as therapeutic agents (Romagni *et al*., 2000; Ingolfsdottir, 2002).

In Saudi Arabia, Kürschner (1984) reported 13 species of lichens from Asir mountains (southwestern region bordering the Red Sea), where Abu-Zinada *et al*. (1986) studied the flora of

central, southern, and western regions while recording about 67 lichen species belonging to 38 genera. Additional 12 species belonging to eight genera were identified by Bokhary *et al*. (1993).

It is generally believed that the production of UA is exclusively restricted to lichens (Correche *et al*., 1998; Ingolfdottir, 2002). In a few unconfirmed isolated cases, this compound was also reported in non-lichens ascomycetes and isolated mycobionts (Bondarenko *et al*., 1969; Komiya and Shibata, 1969). In addition, closely related compounds such as phytotoxin mycousnine, cercosporamide, and UA amide are found in non-lichen fungi (Sassa and Igarashi, 1990; Conover *et al.*, 1992).

1.2.2 Traditional uses of *Usnea* species

Usnea species have been used in homeopathic and traditional medicine in China, Pacific Islands, and New Zealand. Reportedly, Hippocrates used some of these lichens to treat urinary conditions (Ingolfdottir, 2002). Many other lichens have been used as medicines, and it is estimated that most of all lichen species possess antibiotic properties (Ghione *et al*., 1988; Marcano *et al*., 1999).

The crude extracts of UA rich lichens (e.g., *Usnea* species) have been used throughout the world to treat various clinical conditions such as pulmonary tuberculosis, pain, fever, wounds, athlete's foot, and other dermal lesions; they have also been used as an expectorant, deodorants, and herbal tinctures (Okuyama *et al*., 1995; Correche *et al*., 1998; Ingolfsdottir, 2002).

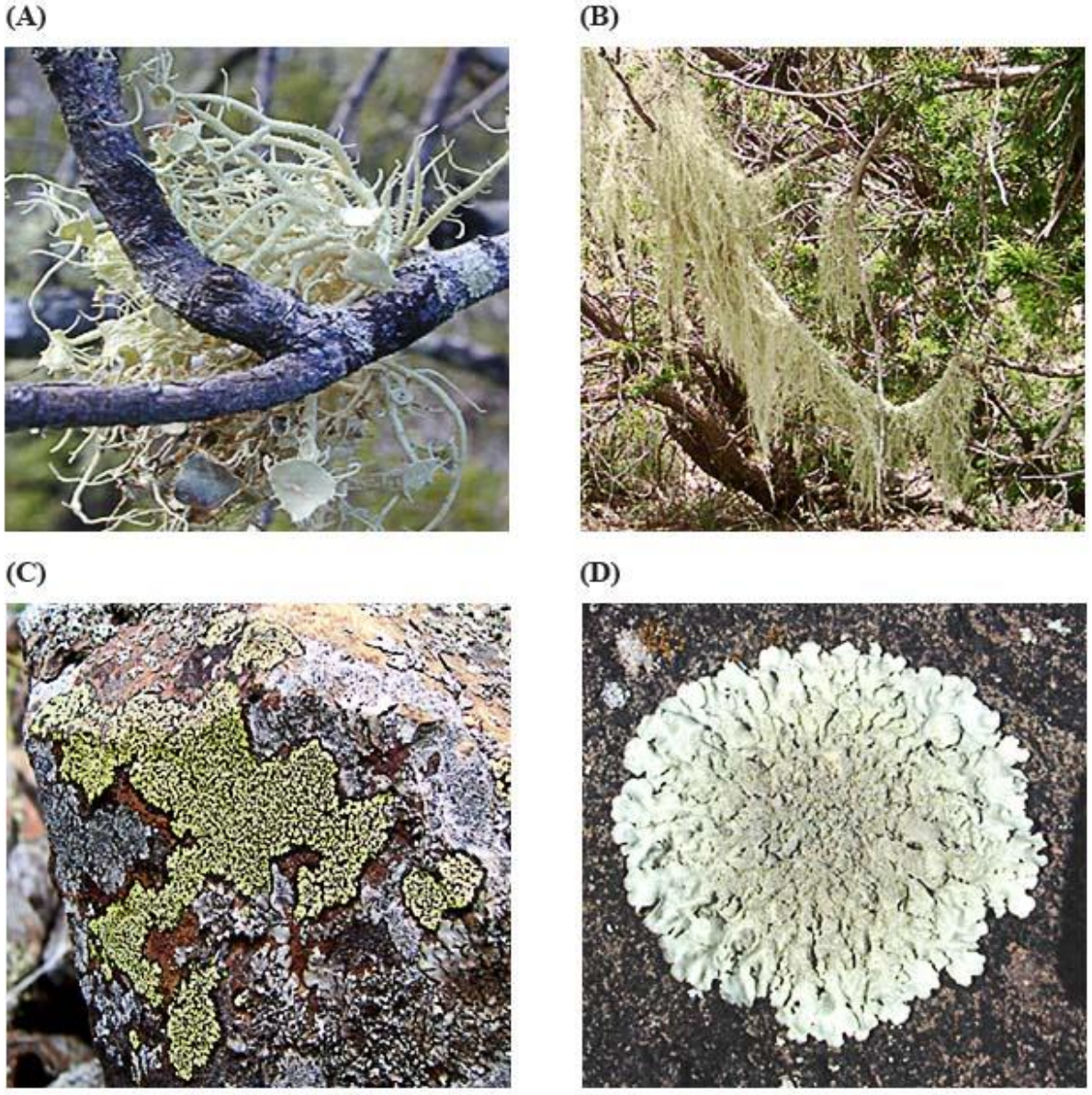
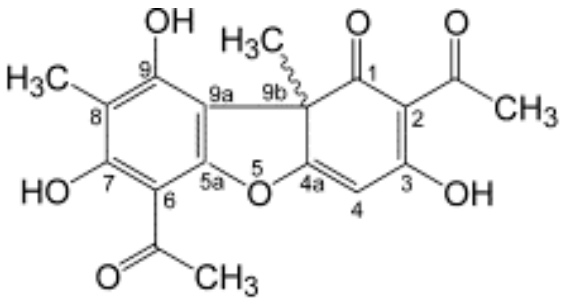


Fig. 1.2 Lichens: (A) *Usnea australis* and (B) *Usnea articulata* (a fruticose form, growing on tree branch) photographed from Al-Sawdah region KSA. (C) (*Rhizocarpon geographicum* (on rock) and (D) *Xanthoparmelia* cf. *lavicola* (a foliose lichen on basalt). (Researcher; Wikipedia, 2009).

1.2.3 Chemistry of UA

Usnic acid is a dibenzofuran derivative, [2,6-diacetyl-7,9-dihydroxy-8,9b-dimethyl-1,3(2H,9bH)-dibenzo-furandione; $C_{18}H_{16}O_7$], and it is a yellowish pigment (Fig.1.3). It occurs in two enantiomeric forms depending on the projection of the angular methyl group at the chiral 9b position determined by X-ray analyses to be R (Huneck *et al*., 1981; Ingolfsdottir, 2002). The three hydroxyls (3-OH enolic) present in the molecule have the strongest acidic character (p*K*a 4.4) due to an inductive effect of the keto group (Ingolfsdottir, 2002).



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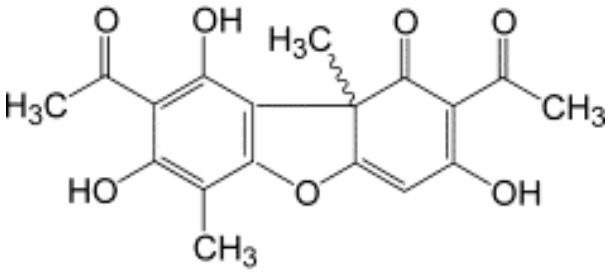

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Fig. 1.3 Structures of (+)-(9b-R)- and (-)-(9b-S)-usnic acids (1) and (+)-(9b-R)- and (-)- (9b-S)-isousnic acids (2). (Ingolfsdottir, 2002).

1.2.4 Commercial availability and use of UA

A highly purified form of UA isolated from the genus *Usnea* is commercially available. It is also prepared in tissue cultures using tiny segments of thalli from *Usnea* and *Ramalina* species. In its purified form, UA has been formulated into creams, toothpastes, mouthwashes, deodorants, antibiotic ointments, and sunscreen products (Ingolfsdottir, 2002). *Usnea barbata*, UA, and copper usnate have been produced as antimicrobial preparations. In Italy, UA has been used in vaginal creams, foot creams, powders, and hair shampoos (Rafanelli *et al*., 1995). In Argentina, Barba della Piedra *(Usnea densirostra)* has been sold to treat many ailments (Correch *et al*., 1998). In these preparations, UA is employed as an active agent as well as a preservative (Ingolfsdottir, 2002). In ecological applications, UA could also be used as a biomarker to assess pollution, as its concentration in lichens increases with the increased exposure to toxicants.

In recent years, UA and its salt form, sodium usniate, have been marketed in the U.S. as an ingredient in dietary supplement products (mostly with claims as weight-loss aids, though some as antimicrobial agents) (Frankos, 2005). The actual mechanism of UA as a weight loss agent is still unclear; however, it was reported that it acts through raising the body's metabolic rate (Durazo *et al*., 2004).

No observed adverse effect of UA is available. For dietary supplement use (potential long term and unsupervised use), doses lower than 2-3 g of the herb (equal to > 30 mg/day UA) would seem to be without serious effects (Frankos, 2005).

1.2.5 Biological activities of UA

Lichens grow very slowly and require chemical defense against microorganisms. This is probably the reason why lichens are able to live for hundreds of years. According to the available literature, it seems that the main biological role of UA is that of an antibiotic (Ghione *et al.*, 1988; Marcano *et al*., 1999; Campanella *et al*., 2002; Cocchietto *et al*., 2002).

Usnic acid was identified as an antiviral and cytotoxic component (Perry *et al*., 1999), an antituberculosis agent (Patiala, 1951), having antimicrobial and antifungal activities (Mitteilungen, 1950; Lauterwein *et al*., 1995; Ingolfsdottir *et al*., 1998; Cocchietto *et al*., 2002; Ingolfsdottir, 2002; Francolini *et al*., 2004; Yilmaz *et al*., 2004; Saenz *et al*., 2006; Weckesser *et al*., 2006), an antiproliferative agent (Kumar and Muller, 1999; Campanella *et al*., 2002; Bucar *et al*., 2004), and having antiprotozoal activities (Carvalho *et al*., 2005). UA also showed anti-inflammatory, antipyretic, and analgesic activities (Dobrescu *et al*., 1993; Vijayakumar *et al*., 2000) as well as antitumor and antimitotic effects (Cardarelli *et al*., 1997).

Usnic acid has ecological effects, such as antifeedant, antigrowth, antiherbivore, and insecticide properties (Emmerich *et al*. 1993; Cocchietto *et al*., 2002; Ingolfsdottir, 2002).

Santos *et al.* (2004) suggested that UA has immunostimulatory activity, while Odabasoglu *et al*. (2006) reported that UA has gastroprotective effects. The phytotoxicity of UA was determined by Romagni *et al.* (2000). They found that *P*-hydroxyphenylpyrovate dioxygenase was inhibited by UA. Lechowski *et al*. (2006) reported that UA reduced the content of the macroelements present in plant tissues.

Usnic acid was used in UV protection (photoprotective agent). Therefore, it may be useful as new filters in sun-screen preparations (Rancan *et al*., 2002). UA was also reported as an allergic

agent. Cases of occupational contact dermatitis from lichens in forestry workers have been known since the beginning of the twentieth century (Cocchietto *et al*., 2002). As reported in the literature, two forest workers affected with allergic contact dermatitis, which occurred only during work in forest areas, showed positive patch test reactions to lichens containing UA or isolated UA form (Mitchell and Vancouver, 1965).

Contact dermatitis can also be caused by the oak moss (*Evernia*) perfume. In a routine series, Dahlquist and Fregert (1980) reported that UA gave negative patch test reactions in those few subjects found to be allergic to lichen substances (1% of tested subjects). In another routine series, Sheu *et al*. (2006) described four cases of lichen acid allergy associated with the natural deodorant. In other two cases, dermatitis was caused by vaginal ovules and by a sunscreen containing oak moss (Rafanelli *et al*. 1995; Rademaker, 2000).

1.2.6 Toxicity and adverse effects of UA

Irrespective of a long history of using UA containing products, only a few animal studies were conducted to evaluate the clinical safety of UA (Pramyothin *et al*., 2004). Toxic reactions, including ataxia leading to paralysis and death, have been reported in animals ingesting lichens containing UA (Favreau *et al*., 2002). There is a total lack of systemic subchronic and chronic general toxicity studies. The conduct and quality of some of the available studies are also questionable.

At present, no scientifically sound data are available to support the safe oral use of UA products. Secondary studies could examine the toxicity of UA in the context of consumption of *Usnea* herb and the dermal toxicity of orally administered UA. There may also be pharmacokinetic

differences in how UA and sodium usneate are handled that would affect the toxicity. Pharmacokinetic studies on rabbits have proved that UA, after single oral or intravenous administration, reaches the systemic circulation without evident signs of toxicity (Krishna and Venkataramana, 1992).

In non-clinical trials, acute toxicity of UA was studied using larvae of an herbivore insect *(Spodoptera littoralis)* that received injections of both enantiomers of UA in the hemolymph. The (-)-form was found to be ten times more toxic than its (+)-form (LD_{50} 8.6 versus 90.8 µmol) (Emmerich *et al*., 1993).

Cytotoxic effects of UA were reported in literature, and it has been shown to have antiproliferative activity. (-) UA caused moderate cytotoxic activity on various cancer cell lines (IC_{50} = 6, 12.1, 15.8, 17.8, 8.2, and 6.8 µg/ml on L1210, 3LL, DU145, MCF7, K-562, and U251, respectively). This compound was also shown to induce apoptosis of murine leukemia L1210 cells in a dose- and time- dependent manner (Bezivin *et al*., 2004). (+) UA exhibited cytotoxic activity against human keratinocyte cell cultures (Kumar and Muller, 1999).

The intravenous toxic dose was found to be 25 mg/kg in mice, 30 mg/kg in rats and rabbits, and 40 mg/kg in dogs (Ingolfsdottir, 2002). While, the oral toxic dose in rats was reported to be 2000 mg/kg by Odabasoglu *et al*. (2006).

Chronic or subchronic effect of UA animal studies were not available in the literature. However, reproductive and developmental toxicity studies reported no adverse effect of 200 mg/kg/day of (+) UA on the number, motility, and structure of epididymal spermatozoa in a 35-day oral study in 5-6 weeks old male Swiss mice. Additionally, no quantitative differences in the content of testicular protein, RNA, DNA, and organ weights were recorded (AI-Bekairi *et al*., 1991).

Correché *et al*. (1998) demonstrated that slight molecular modifications of UA not only reduce *in vitro* antimicrobial activity but also produce an increase in cytotoxicity while completely inhibiting spleen lymphocyte growth.

Usnic acid has a slight inhibitory action against leukotriene biosynthesis in bovine polymorphonuclear leucocytes *in vitro* by a specific enzyme interaction rather than by acting as an antioxidant against the peroxidation process, as a scavenger, or even as a source of free radicals (Kumar and Muller, 1999).

As reported in the literature, UA is a hepatotoxic dietary supplement (Smolinske, 2005; Lewis *et al*., 2006). Han *et al*. (2004) suggested that the use of UA kills hepatocytes.

Severe hepatotoxicity is associated with the dietary supplements, containing UA, Lipokinetix (norephedrine hydrochloride, sodium usniate, 3,5-diiodothyronin, yohimbine hydrochloride and caffeine) and UCP-1 (150 mg of usnic acid, 525 mg of L-carnitine, and 1050 mg of calcium pyruvate) were reported by Favreau *et al*. (2002) and Sanchez *et al*. (2006).

Fulminant liver failure associated with the ingestion of pure UA in a 500 mg/day dose to lose weight was reported by Durazo *et al*. (2004). Gunawan and Kaplowitz (2004) reported that herbal and natural supplements containing UA like Lipokinetix and kambala tea were causing hepatotoxicity with increasing frequency as patients turn more and more to alternative medicine. Neff *et al*. (2004) reported fulminant hepatic failure due to weight-loss diet supplements containing Ma Huang and UA (marketed under several names, including Xenadrine, Excelerator, Hydroxycut, and Lipokinetix). Hsu *et al*. (2005) described acute hepatitis from the use of usnic acid as a 'fat burner'.

1.2.7 Role of mitochondria in energy and fat metabolism

In cell biology, a mitochondrion (plural mitochondria) is a membrane-enclosed organelle found in most eukaryotic cells. These organelles range from one to ten micrometers (μm) in size. Mitochondria are sometimes described as "cellular power plants" because they generate most of the cell's supply of adenosine triphosphate (ATP), which is used as a source of chemical energy. In addition to supplying cellular energy, mitochondria are involved in a range of other processes, such as signaling, cellular differentiation, cell death, as well as the control of the cell cycle and cell growth**.**

The organelle is composed of compartments that carry out specialized functions. These compartments or regions (Fig.1.4) include the outer membrane, the intermembrane space, the inner membrane, the cristae, and the matrix (Ross *et al*., 2003).

Because mitochondria generate ATP, they are more numerous in cells that use large amounts of energy, such as striated muscle cells, hepatocytes, and cells engaged in fluid and electrolyte transport. Mitochondria also localize at sites in the cell where energy is needed, as in the middle piece of the sperm, in the intermyofibrillar spaces in striated muscle cells, and adjacent to the basolateral plasma membrane infoldings in the cells of the proximal convoluted tubule of the kidney (Ross *et al*., 2003).

Mitochondria generate ATP in a variety of metabolic pathways including oxidative phosphorylation, the citric acid cycle, and β-oxidation of fatty acids (Ross *et al*., 2003).

Heat is produced under certain conditions, where protons re-enter the mitochondrial matrix without contributing to ATP synthesis. This process is known as the proton leak or mitochondrial uncoupling and is due to the facilitated diffusion of protons into the matrix (Fig. 1.4). The energy

produced by the transport of electrons is released as heat rather than being used to synthesize ATP (Champe *et al*., 2005).

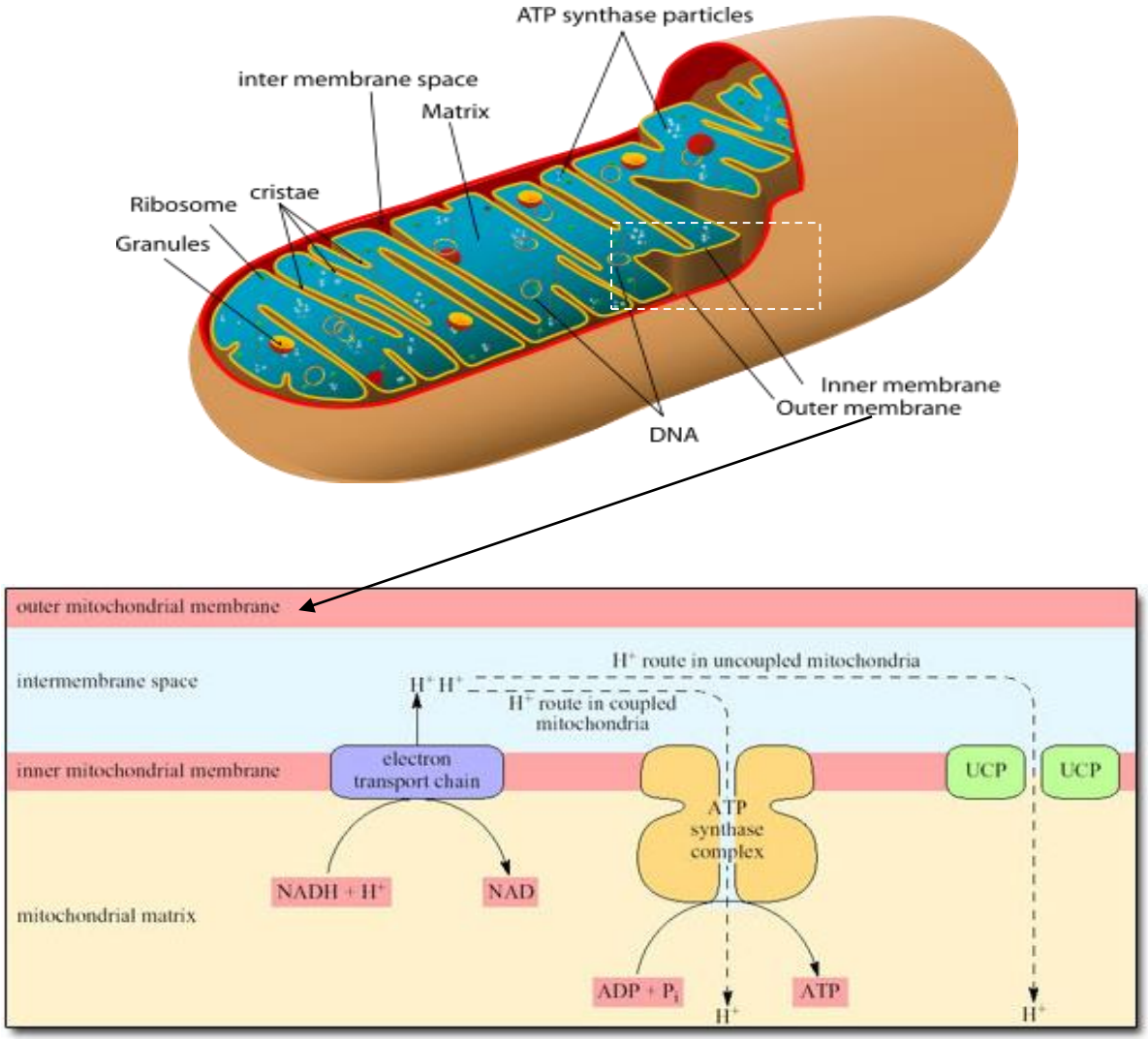


Fig. 1.4 A schematic drawing of mitochondrion and the process of proton translocation actions of uncoupling protein (UCP). (Wikipedia, 2009).

1.2.8 Usnic acid and mitochondrial function

As UA was reported to be a metabolic stimulant, the most probable mechanism is via affecting mitochondrial function. Several studies, using well-established methods, have studied the effects of UA on mitochondrial function *in vitro* (Han *et al*., 2004; Pramyothin *et al*., 2004).

Johnson and colleagues (1950), Abo-Khatwa *et al*. (1996), and Pramyothin *et al*. (2004) studied the effect of UA on mitochondrial function and O_2 consumption.

Johnson *et al*. (1950) reported that the uncoupling of oxidative phosphorylation by UA was confirmed by the decrease in the ratio of phosphates consumed (used to make ATP) to oxygens consumed (P/O ratio). This uncoupling occurred at concentrations of UA that did not interfere with rates of oxygen consumption. At concentrations of 50 µM or higher, UA inhibited oxygen consumption in the presence of a wide range of substrates, suggesting inhibition of the electron transport chain (ETC).

Abo-Khatwa *et al*. (1996) confirmed the uncoupling of oxidative phosphorylation by UA in mouse liver mitochondria. At concentrations as low as 0.75 µM, UA decreased the P/O ratio dramatically without inhibition of oxygen consumption. Stimulation of oxygen consumption by UA was observed in the presence of the ATP synthase inhibitor oligomycin, confirming that UA was acting to uncouple oxidative phosphorylation. Interestingly, concentrations of the classic uncoupler, 2,4-dinitrophenol, were required to be 5 µM to reproduce the uncoupling associated with the UA exposure. They reported inhibition of mitochondrial oxygen consumption at UA concentrations above 1µM, again suggesting that the adverse effects on mitochondrial function are not limited to uncoupling. Also, they noted that UA possessed physical properties like that of a "membrane disruptor", consistent with its uncoupling actions.

Abo-Khatwa *et al*. (2005) reported that mice injected with 200 mg/kg of (+) UA produced significant uncoupling of isolated hepatic mitochondria, hindering of ATP biosynthesis, and stimulation of Mg^{+2}-ATPase activity. Loss of ATP from the cell may lead to an increase of cystolic Ca^{+2} and simultaneous stoppage of Na^{+}/K^{+}-ATPase and other transporting systems (Stricker and Kumar, 2007). These changes are usually sufficient to cause adverse biochemical and pathological damages to most animal cells, which may lead to cell death (Al-Robai *et al*., 1993).

Pramyothin *et al*. (2004) reported that (+) UA (0.15-6 µM) possessed uncoupling activity in isolated rat liver mitochondria. It stimulated cell respiration by mitochondria respiring with glutamate plus malate or succsinate as substrates by activated ATPase activity. Increasing the concentration of (+) UA (>6 µM) exhibited loss of respiratory control and ATP synthesis.

Higher doses of UA (>2 µM) were reported by Pramyothin *et al*. (2004) to attack lipids on cell membranes and membrane like structures such as mitochondria and endoplasmic reticulum and to stimulate lipid peroxidation, disturb Ca^{2+} homeostasis, and result in cell death. The damage to cell membrane integrity causes the release of cellular hepatospecific enzymes, mainly the transaminase (aspartate aminotransferase and alanine aminotransferase). The same authors used isolated rat liver mitochondria to demonstrate the direct effect of (+) UA on mitochondrial function. (+) UA caused maximal stimulation of both state 4 respiration (three- to seven-folds, depending on substrates used) and ATPase activity (seven-fold). This uncoupling effect of oxidative phosphorylation is dose-dependent and is similar to results reported in mouse liver mitochondria by Abo-Khatwa *et al*. (1996). Loss of respiratory control appears after maximal stimulation.

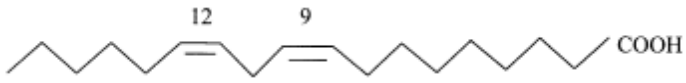
Lauterwein *et al*. (1995) reported that antimicrobial effects of UA could be attributed to its effects on oxidative phosphorylation interfering with trans-membrane ion gradients and

mitochondrial function. Low concentrations of UA (0.1 µg/ml, or approximately 0.3 µM) stimulated oxygen consumption by the mitochondria-containing fungus *Saccharomyces cerevisiae*, while concentrations above 100 µM inhibited oxygen consumption (Cardarelli *et al*., 1997).

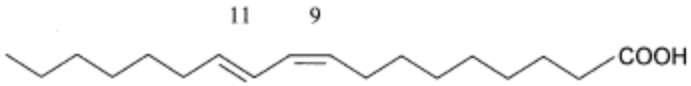
Kumar and Muller (1999) suggested that UA does affect intact cells in culture and may be cytotoxic. Single doses of 5-20 mg/kg appear to be tolerated in animals (Krishna and Venkataramana, 1992).

1.3.1 Conjugated linoleic acid (CLA)

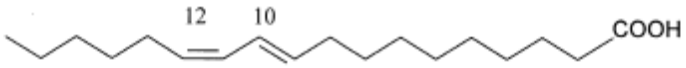
Conjugated linoleic acid (CLA; $C_{18}H_{32}O_2$) is a term given to a group of positional and geometric isomers of the omega-6 essential fatty acid linoleic acid (9, 12-*cis*, *cis*-octadecadienoic acid, LA). The biochemical nomenclature for linoleic acid designates this fatty acid as an 18 carbon ("octa-deca") fatty acid containing two double bonds (Kelly, 2001). The conjugated double bonds occur at carbon atoms 10 and 12 or 9 and 11, with the possible *cis* and *trans* combinations (Fig. 1.5). Although the conjugation of double bonds occurs as part of free radical-mediated oxidation of linoleic acid, CLA is a true isomer of linoleic acid in that it does not possess additional oxygen (MacDonald, 2000).



Linoleic acid (18:2)



cis-9, *trans*-11 CLA



trans-10, *cis*-12 CLA

Fig. 1.5 Structure of linoleic acid, *cis*-9, *trans*-11 CLA and *trans*-10, *cis* 12 CLA. (Evans *et al*., 2002a).

1.3.2 Sources of CLA

Lipids from ruminant animals (beef, lamb, and dairy) contain much higher levels of CLA than lipids from non-ruminants. CLA concentrations in dairy products typically range from 2.9 to 8.92 mg/g fat, of which the 9-*cis*, 11-*trans* isomer makes up to 73-93% of the total CLA (MacDonald, 2000). CLA is produced in rumens of ruminant animals by the fermentative bacteria, *Butyrovibrio fibrisolvens*, which isomerize linoleic acid into CLA.

Non-animal sources, such as vegetable oils and margarines, contain little CLA. CLA concentrations in fats from non-ruminants and vegetable oils typically range from 0.6 to 0.9 mg/g fat (MacDonald, 2000). However, the CLA content in ruminant meat products vary depending on animal species, tissue, and diet (Evans *et al*., 2002a).

1.3.3 Biological activities of CLA

CLA has been found to be an effective antioxidant. It was proved that it acts as an anticancer agent both *in vitro* and *in vivo*. CLA was inhibitory to cancer cell growth and inhibited the proliferation of human malignant melanoma and colorectal cancer cells (MacDonald, 2000; Pariza *et al*., 2000; Rainer and Heiss, 2004). Ip *et al*. (1991) and Whigham *et al*. (2000) reported that 1% CLA in the diet suppressed mammary carcinogenesis in rats. Moreover, it was indicated that dietary CLA has an ability to block both the local growth and systemic spread of human breast cancer via mechanisms independent of the host immune system (Visonneau *et al*., 1997; MacDonald, 2000).

Since the immune system is central to defense against cancer, it is possible that the anticancer activity of CLA may be mediated through enhanced immune function (MacDonald,

2000). Cytokines are hormone-like mediators produced by macrophages and other immune cells when they are stimulated and allow the host immune system to attack the invading pathogen (Pariza *et al*., 2000). However, these cytokines have an overall catabolic effect on non-lymphoid tissues (Whigham *et al*., 2000). Yang and Cook (2003) reported that dietary CLA decreased the macrophage tumor necrosis factor-ά (TNF-ά) production and modified the splenic lymphocytes (splenocyte) cytokines production.

Dietary CLA is an effective inhibitor of atherogenesis. Kritchevsky *et al.* (2000) found that even at levels as low as 0.1% of the diet, CLA inhibited atherogenesis in rabbits. At 1% of the diet, it will cause significant regression of atheromata. Similar results were reported by Lee *et al*. (1994).

Conjugated linoleic acid has a protective effect against bone loss. MacDonald (2000) found an increase in the percentage of ash in CLA fed chicks. This effect is presumed to be due to protection conferred by CLA on bone loss. Bone loss evoked by an increase in cytokines appeared to counter the act by CLA (Banu *et al*., 2006). Rodents that were fed butter had a greater trabecular bone formation (most important to prevent osteoporosis) than animals that were fed vegetable oil (MacDonald, 2000). Banu *et al*. (2006) studied the effects of CLA and exercise on bone mass in young male Balb/C mice. They found that weight loss caused by CLA and exercise was not associated with bone loss.

Dietary CLA normalizes impaired glucose tolerance and prevents the progression to hyperglycemia and diabetes in the young Zucker diabetic fatty *fa/fa* rat (Houseknecht *et al.*, 1998). However, Thrush *et al*. (2007) reported that CLA increases the skeletal muscle ceramide content and decreases insulin sensitivity in overweight, non-diabetic humans.

Stangl (2000) suggested that the CLA mixture is a modulator of lipid metabolism under conditions of enhanced fat store mobilization in rats. It exhibited a strong fat-to-lean partitioning

effect, reduced serum VLDL lipids, and redistributed tissue lipids in food restricted rats. Nagao *et al*. (2005) reported that dietary CLA alleviated nonalcoholic fatty liver disease in Zucker *fa/fa* rats.

Chouinard *et al*. (1999) found that dietary supplementation of CLA increased the milk fat content of CLA, altered the milk fatty acid composition, and markedly reduced the content and yield of milk fat. Mosley *et al*. (2007) indicated that supplemental CLA consumption does not influence milk macronutrient contents in all healthy lactating women**.**

1.3.4 CLA toxicity and adverse effects

Although CLA supplementation has been shown to significantly reduce body fat and weight gain in different animal models, the concomitant enlargements of the liver and spleen have raised safety issues (Terpstra *et al*., 2002; Wang and Jones, 2004). The increased liver weight associated with CLA treatments is likely due to the liver lipid accumulation caused by increased delivery of fatty acids to the liver in response to CLA feeding since this has been found in a variety of dietary manipulations (Delany *et al*., 1999). Similarly, since CLA has been reported to modulate immune functions, perhaps through cytokines, it is also not surprising that spleen weight was affected (Delany and west, 2000).

Other negative effects of CLA feeding, particularly at high levels of CLA, are increased plasma insulin levels. This indicates that feeding high levels of CLA induces insulin resistance, possibly due to increased levels of free fatty acids (Delany and west, 2000; Rainer and Heiss, 2000; Larsen *et al*., 2003). Moreover, dietary CLA induces insulin resistance by reducing plasma leptin and increasing peroxidative stress (Wang and Jones, 2004). Similar findings were reported in obese men by Riserus *et al*. (2004).

However, several studies (Riserus *et al*., 2002; Moloney *et al*., 2004; Riserus *et al*., 2004) have shown that *trans*-10, *cis*-12 CLA supplementation induces an alteration of insulin sensitivity in humans. The inflamed lipoatrophic adipose tissue that was observed in CLA-treated mice provides a potential mechanism of insulin resistance that adds to concerns about the safety of dietary supplements containing *trans*-10, *cis*-12 CLA that are widely promoted as nonprescription antiobesity agents (Poirier *et al*., 2006).

In contrast, Scimeca (1998) found that dietary administration of CLA (1.5%) to male rats for nine months at levels substantially greater than the estimated human consumption indicated a lack of toxicity.

Adverse effects reported after CLA administration in eight of 60 overweight human subjects include gastrointestinal complaints and fatigue (Rainer and Heiss, 2000; Kelly, 2001).

1.3.5 Effect of CLA on body weight

Dietary CLA decreases adiposity in various animals and humans. Feeding CLA causes a reduction in body fat accumulation in male mice (AKR/J) at relatively low doses (0.5-1% CLA) and in as short as 2 weeks at the 1% CLA dose (Delany *et al*., 1999). In addition, MacDonald (2000) reported a more than 50% reduction in whole body fat in rats, mice, and chicks.

Moreover, an increase in body protein accumulation was found in rats, mice, and chicks that were fed CLA (Delany *et al*., 1999; MacDonald, 2000).

CLA improved the feed efficiency in mice, rats, and pigs (Chin *et al*., 1994). The improved feed efficiency suggested that either the metabolic rate was altered to conserve energy (unlikely) or CLA was affecting body composition (Cook and Pariza, 1998). On the other hand, some studies

reported that there is no effect of CLA on the food intake in mice and rats (Sugano *et al*., 1997; Delany *et al*., 1999).

Feeding Sprague-Dawley rats 0.25-0.5% (w/w) of a crude mixture of CLA isomers for 5 weeks reduced retroperitoneal and parametrial fat pad weights without affecting the growth rate or food intake (Azain *et al*., 2000).

Sugano and colleagues (1997) found that CLA had no effect on weight gain and lipids of serum and liver in rats. Similar results were reported by Scimeca (1998).

Dietary CLA reduced adiposity in lean but not obese Zucker rats (Sisk *et al*., 2001). Feeding CLA to broilers resulted in a substantial reduction in liver fat accumulation and promoted CLA incorporation into hepatic lipid pools (Badinga *et al*., 2003).

Kamphuis *et al*. (2003) reported that the regain of fat-free mass was favorable, was dose-dependent and affected by a 13-week consumption of 1.8 or 3.6 g CLA/day, and consequently increased the resting metabolic rate in overweight subjects. However it did not result in improved body weight maintenance after weight loss.

Poulos *et al*. (2001) suggested that the response to the CLA treatment may depend on the sex and age of the animal as well as the duration of feeding. These effects may be a result of the direct or indirect action by androgens and/or estrogens or a result of growth during different phases. Kloss *et al*. (2005) reported that CLA was more beneficial for controlling blood lipids and adiposity when supplemented to a diet rich in saturated and unsaturated fat.

Reductions in linoleic acid concentrations made mice more sensitive to CLA-induced body fat loss only when arachidonic acid concentrations were also reduced. If a metabolite of CLA causes mice to lose body fat, then a diet with lowered linoleic acid concentrations should allow a greater loss of body fat because CLA has greater metabolism when there is reduced competition

for the desaturase and elongase enzymes. Decreased arachidonic acid concentrations could also allow CLA-induced loss of body fat to be greater by allowing greater use of CLA (Hargrave *et al*., 2004).

Supplementation with CLA in healthy, overweight, and obese adults decreases body fat mass (BFM) in specific regions (the legs) and is well tolerated (Gaullier *et al*., 2007). Terpstra (2001) indicated that the body fat lowering effect of CLA is considerably lower in humans than in mice. Given at a dose of 3.2 g/d, CLA produces a modest loss in body fat in humans. Whigham *et al*. (2007) found that CLA has a beneficial effect on human body composition. Although this effect is modest, it could be important if accumulated over time, especially in an environment where continuous, gradual weight gain is the norm in the adult population.

1.3.6 CLA isomers and adipogenesis

Studies reported that the most effective members in affecting body weight and composition were *cis*-9, *trans*-11 CLA and *trans*-10, *cis*-12 CLA (Kelly , 2001; Evans *et al*., 2002a). It was suggested not only that CLA affects many different metabolic pathways, but that individual isomers of CLA act differently (Pariza *et al*., 2000; Whigham *et al*., 2000).

De Deckere *et al*. (1999) concluded that *trans*-10, *cis*-12 CLA appeared to be the physiologically active CLA isomer and that the natural isomer, *cis*-9, *trans*-11 CLA, present in dairy products and meat of ruminants had little or no effect on lipid metabolism in hamsters.

Riserus *et al*. (2004) reported that a CLA preparation containing the purified *cis*-9, *trans*-11 CLA isomer increased insulin resistance and lipid peroxidation compared with placebo in obese men. Moreover, *cis*-9, *trans*-11 CLA has no antiobesity effects, and this is in accord with the evidence in mice (Pariza *et al*., 2001). On the other hand, *trans*-10, *cis*-12 CLA has been found to be an antiadipogenic isomer (Wang and Jones, 2004).

Gavino *et al*. (2000) concluded that, under the experimental conditions of short-term feeding, *cis*-9, *trans*-11, thought to be the active compound in CLA, does not produce the same effect as the isomer mixture.

1.3.7 CLA mode of action on obesity

Several studies have suggested that CLA alters lipid metabolism (antiobesity) by several mechanisms.

The effect of CLA on adipogenesis and the TG content are multifarious, ranging from deceased preadipocyte proliferation (Evans *et al*., 2002) to adipocyte differentiation and metabolism (Wang and Jones, 2004). Similarly, Satory *et al*. (1999) reported that CLA inhibited proliferation and promoted *de novo* lipogenesis and lipid filling in 3T3-L1 preadipocytes, suggesting that CLA may reduce overall fat accumulation in growing animals by inhibiting stromal vascular preadipocyte hyperplasia.

Nutritional supplementation with *trans*-10, *cis*-12 CLA directly induces inflammatory gene expression in adipocytes (in white adipose tissue) and also promotes macrophage infiltration into adipose tissue, a local inflammatory state that contributes to insulin resistance (Poirier *et al*., 2006).

CLA supplementation reduces adipose tissue by increasing apoptosis of adipocytes and develops lipodystrophy in mice (Tsuboyama-Kasaoka *et al*., 2000; Miner *et al*., 2001). In addition, feeding CLA to C57BL/6J female mice resulted in increases of the TNF-ά mRNA level in white adipose tissue but decreases in skeletal muscle (Tsuboyama-Kasaoka *et al*., 2000). Data from the above studies collectively suggest that CLA may differentially affect the concentrations of TNF-ά in the serum and adipose tissue (Wang and Jones, 2004).

Hargrave *et al*. (2002) indicated that CLA *trans*-10, *cis*-12, but not CLA *trans*-9, *cis*-11, can induce both body fat loss and adipocyte apoptosis in mice. Azain *et al*. (2000) and Wang and Jones (2004) found that the reduction in fat mass in rats that were fed CLA can be accounted for by a reduction in the cell size rather than a change in the cell number.

CLA decreased lipid synthesis and increased lipolysis, energy expenditure, and fatty acid oxidation (metabolic rate). For example, Delany and West (2000) have shown that CLA (a mixed isomer preparation) at a concentration of 0.5-1% (w/w) in both low-fat and high-fat diets has profound metabolic effects in mice that result in an increase in energy expenditure, a shift in the fuel mix burned, and a decrease of body fat content. They reported that CLA stimulating lipolysis during the night, which would provide more fatty acids for oxidation. The same result was found by Terpstra *et al*. (2002). Increased fat oxidation is involved in the decreased TG content and fat accumulation, but decreased *de novo* fat biosynthesis is not (West *et al*., 1998; Delany and west, 2000; Evans *et al*., 2002a; Wang and Jones, 2004).

Xu *et al*. (2003) suggested that a short-term intake of CLA inhibits lipoprotein lipase and glucose metabolism but does not enhance lipolysis in mouse adipose tissue.

West *et al*. (1998) and Miner *et al*. (2001) indicated that dietary CLA reduced the food intake. In contrast, DeLany *et al*. (1999) reported that CLA decreased fat accumulation and increased protein accumulation without any major effects on the food intake in mice.

Although animal and cell culture experiments seem to clearly support an isomer-specific role for CLA in preventing or reducing adiposity, it is too early to predict the extent to which CLA supplements will be useful in humans.

At present, it can be suggested that the above effects of CLA on adipogenesis and lipid metabolism in animals are dependent on the isomer type, dose, duration of supplementation, metabolic status, and species of the subject. Specifically, the *trans*-10, *cis*-12 isomer seems to be the isomer responsible for CLA's antiobesity effects in animals and in preadipocytes from both animals and humans (Evans *et al.*, 2000; Brown *et al*., 2001a; Brown *et al*., 2001b; Evans *et al*., 2001; Evans *et al*., 2002b; Hargrave *et al*., 2002; Wang and Jones, 2004).

Health food supplements containing UA have been promoted for use in weight reduction with little scientific support. Human clinical toxicology of UA was based on a case series of developed fulminant hepatic failure (Favreau *et al*., 2002; Durazo *et al*., 2004) and induced acute hepatitis in a family (Hsu *et al*., 2005) upon consumption of UA-containing products. Lichen intoxication with degenerative myopathy, developed paresis, and even death of Elk *Cervus elaphus* were reported by Cook *et al.* (2007) in the Red Rim Wildlife Habitat Management Area (RRWHMA), Wyoming, USA.

The present study was designed to evaluate the effect of UA, as a natural product, on both the body weight and hepatocytes in adult lean male Sprague-Dawley rats. Plasma level of UA

treated-rats, following oral administration of single UA dose (500 mg/kg), is determined using high-performance liquid chromatographic (HPLC) method. Possible adverse effects of UA on liver function and structure are examined. In addition, the changes in serum levels of insulin, leptin, and glutathione are recorded. The same parameters are applied for the CLA fed animal group. Morphometric and histological features of perirenal adipose tissue are also studied.