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Influence of squalene feeding on plasma leptin, testosterone & blood pressure in rats

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***Background & objectives*: Obesity, hyperlipidaemia and hypercholesterolaemia are known risk factors in the pathogenesis of hypertension. Squalene has been shown to reduce serum cholesterol and triglycerides in dogs although its therapeutic use in high BP and obese patients has not been established. This study evaluates the effect of feeding high doses of squalene on plasma leptin, glucose, testosterone, blood pressure (BP) and body fat in rats.**

***Methods*: Wistar rats (male, 22 days old) were randomly divided into two groups receiving either regular control diet or a squalene-containing diet. After feeding squalene for 4 wk, 10 rats each from the squalene and control groups were sacrificed and blood samples were collected for measurement of leptin, cholesterol, triglycerides and testosterone. Blood pressure was monitored weekly.**

***Results*: Following squalene feeding, BP and body weight gain were lower in the squalene group. BP was significantly lower from 47 days of age in squalene fed group compared to controls. The levels of plasma leptin, glucose, cholesterol and triglycerides were significantly lower in squalene fed rats than those from the control group at 51 and 75 days of age. However, testicular weights (only 75 days) and testosterone levels were significantly higher in rats from the squalene group than those from the control group at days 51 and 75.**

***Interpretation & conclusions*: Our results suggested that squalene may counteract the increase in body fat, BP and levels of plasma leptin, glucose, cholesterol and triglycerides. These effects of squalene may be further explored as a likely new approach for clinical management of high BP and obesity.**

Key words Blood pressure - leptin - squalene - testosterone

Prevention, treatment and management of hypertension and its complications have drawn attention of physicians and scientists. Obesity, hyperlipidaemia, and hypercholesterolaemia are among the most important risk factors in the pathogenesis of hypertension. Clinical study has demonstrated elevated plasma leptin levels in patients with essential hypertension and an overt positive correlation between plasma leptin levels and blood pressure (BP), independent of body adiposity in both normotensive and hypertensive individuals[1].

Obesity is typically associated with resistance to leptin[2]. Leptin, a 16-kDa protein, is secreted by white adipose tissue primarily involved in the regulation of food intake and energy expenditure. Leptin receptors are widely distributed in adipose tissue, heart, kidney, spleen, liver, pancreatic island and testicular tissues[3]. Plasma leptin concentration is proportional to the amount of adipose tissue and is markedly increased in obese individuals. Leptin is shown to be involved in cardiovascular complications of obesity and hypertension[4]. High leptin levels are often observed in human obesity and are implicated in obesity-related hypertension[5]. Hypertension is often associated with high leptin levels, similar to those found in ageing adults[5]. Hyperleptinaemia was found to be a crucial risk factor for elevated blood pressure in the elderly, especially in men[6]. A positive correlation has been shown between the leptin levels and blood pressure in young individuals based on logistic analysis[7].

Squalene is abundant in deep-sea fishes, especially sharks. Amaranth seed oil, olive oil and palm oil are the major plant sources of squalene[8]. Due to its high bioactivity, squalene has been widely used in health and cosmetic care. Squalene possesses a wide spectrum of biological functions including prevention of cell deterioration, anti-senescence, and improved immunity and sexual function[9,10]. Data from dog studies did not reveal any appreciable toxicity from either short (14 days) or long (3 months) squalene treatment, despite a relatively high dose (1200 mg/kg/day, orally)[11,12]. Therefore, squalene is deemed to be a safe and pure natural product. Squalene may be useful in the treatment of dietary hypercholesterolaemia[13]. Squalene elicits its beneficial effects through a reduction of blood levels of cholesterol and triglycerides[9,14], which in turn improve the sensitivity of the body to leptin[15]. Our earlier study indicated that feeding with squalene could reduce the levels of serum leptin and improve the reproductive performance in boars[19]. We studied the effect of squalene on BP, blood levels of leptin, glucose, cholesterol, triglycerides in rats fed with squalene-rich diet.

Material & Methods

Male Wistar rats (22 days old), procured from Hubei Academy of Sciences of Prevention Medicine Wuhan, China and, were randomly assigned to either control or squalene groups with access to a basic diet. Control rats (n=20) received a diet without squalene and the squalene group (n=20) was provided with high squalene diet (Healthy Nature Resource Inc, USA) at a dose of 1000 mg/kg orally[16] for 4 wk. All animals were housed in a temperature-controlled environment (22°C) with a 12:12 h light dark cycle and free access to diet (procured from Institute of Prevention Medicine of Hubei province) and tap water. Tail arterial BP (Multi-Physiological-Signal Collection System-Processor, RM-6240BD/CD/B/C/BDJ, Chengdu instrument factory China) and body weight were monitored every week. When the rats were 51 days old (day 0 after the withdrawal of squalene) and 75 days old (day 24 after the withdrawal of squalene), 10 rats of the squalene group and 10 rats of the control group were sacrificed. Blood samples were collected from tail veins between 10:00 h and 12:00 h under non fasting conditions. All serum samples were stored at -80°C until analysis. The concentrations of plasma leptin and serum testosterone were determined using commercially available radioimmunoassay kits for rat (intra- and inter-batch coefficients of variation were ≤10 and ≤15 per cent, respectively, for leptin and testosterone, provided by Atom High Technique Ltd. Beijing, China). Blood chemistry was measured at the laboratory of Animal Biochemistry of Huazhong Agricultural University. Blood glucose, cholesterol, triglycerides were determined by enzymatic methods[17] (Zhongsheng Ltd., Beijing, China) using an automatic blood analyzer (Hitachi 747 auto-analyzer, Tokyo, Japan). The epididymal and retroperitoneal fat and testicles of each rat were weighed. This study was done in the experimental animal center of Huazhong Agricultural University and the protocol was approved by Institutional Animal Ethics Committee.

Statistical analysis: Student's t-test was used to determine the difference between the groups. SPSS software 12.5 for windows (SPSS Inc., Chicago, Ill., USA) was used for data analysis. $P<0.05$ was considered statistically significant.

Results

Body weight, food consumption and the mean BP were similar between the squalene and control groups prior to the start of the study. After squalene feeding for 4 wk, there was no significant difference observed between the squalene and control groups in food intake but BP and body weight gain were lower in the squalene group rats than those of the control group. The rats from the squalene group displayed significantly lower BP from 47 days ($P<0.01$, Fig.), plasma leptin ($P<0.05$), blood glucose ($P<0.05$),

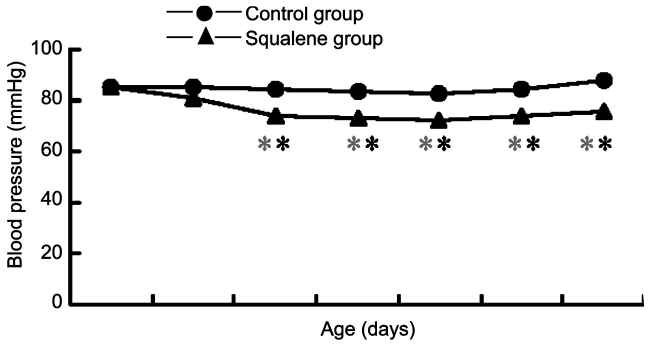

Fig. Weekly tail arterial blood pressure of rats (mmHg). ** $P<0.01$ compared to control group.

cholesterol ($P<0.05$) and triglycerides ($P<0.05$, Table I), body weight at 75 days ($P<0.05$) and body fat at 75 days ($P<0.05$, Table II), compared with rats from the control group**.** The testicular weight (only 75 days) and testosterone levels were significantly higher ($P<0.001$) (Tables I & II) in the squalene fed group.

Discussion

Our study suggested that a diet high in squalene content might sensitize the body to leptin, which may play a subtle but significant role in the loss of body weight, fat and reduced levels of blood pressure, leptin, cholesterol and triglycerides. The reduced blood pressure was maintained at lower levels for 3 wk or longer even after cessation of squalene feeding. These effects of squalene may suggest a likely new approach for clinical management of high BP and obesity. Squalene feeding is also beneficial for metabolic defect and diabetes[18].

Numerous clinical and experimental studies have shown elevated plasma leptin in subjects with essential hypertension, representing a significantly positive correlation between leptin and BP independent of body adiposity in both normotensive and hypertensive individuals[1,19]. On the other hand, low levels of testosterone in men are associated with increased blood pressure[20]. Similar to cholesterol and triglyceride lowering agents, a reduction of plasma leptin levels has been postulated as a rational strategy for therapy of hypertension[21]. Our data revealed that squalene significantly decreased the levels of leptin, cholesterol and triglycerides in rats. It is not known whether squalene-induced hypoleptinaemic effects are a direct effect of squalene or indirectly mediated through triglycerides. Low triglyceride levels have been demonstrated to enhance the body sensitivity to leptin[15]. Although an explanation for the squalene-induced increase in testicular weight and testosterone levels is not available, it is possible that leptin exerted its effects through the hypothalamic-pituitary-testicular axis. Coupling of leptin to leptin receptors has been shown to turn on a number of physiological functions including the reproductive system[22,23]. Leptin can promote genital system development and sex maturity, maintain gestation and increase litter size[22,23]. There is an overt negative correlation between serum

Table I. Concentrations of leptin, glucose, cholesterol, triglycerides, testosterone in rats

Days	Group	Leptin (ng/ml)	Glucose (mmol/l)	Cholesterol (mmol/l)	TG (mmol/l)	Testosterone (ng/dl)
51	CG	0.203 ± 0.020	7.951 ± 0.443	1.150 ± 0.106	2.176 ± 0.152	576.17 ± 37.501
	SG	0.156 ± 0.013*	6.681 ± 0.301*	0.847 ± 0.032*	1.817 ± 0.055*	943.78 ± 72.35**
75	CG	0.208 ± 0.019	7.721 ± 0.432	1.161 ± 0.115	2.188 ± 0.141	599.28 ± 49.577
	SG	0.161 ± 0.012*	6.451 ± 0.290*	0.858 ± 0.041*	1.839 ± 0.044*	971.79 ± 72.35**

CG, Control group; SG, squalene group; TG, triglycerides
*$P<0.05$,**$P<0.001$ compared to control group; Values are mean ± SD

Table II. Effects of squalene on testicle and wet weight of fat pads (% b. wt) in rats

Days	Group	Testicle (%)	Fat (%)	Body weight (g)
51	CG	0.982 ± 0.039	2.954 ± 0.099	215.76 ± 22.369
	SG	0.971 ± 0.062	2.453 ± 0.084	206.475 ± 21.047
75	CG	0.872 ± 0.036	1.94 ± 0.143	330.767 ± 29.755
	SG	1.013 ± 0.019**	1.577 ± 0.069*	302.121 ± 18.849*

CG, Control group; SG, squalene group; Testicle (%), testicle weight (g)/body weight (g)
Fat (%), fat weight (g) /body weight (g); *$P<0.05$; **; $P<0.001$ compared to control group
Values are mean ± SD

leptin levels and testosterone levels in rats[3,24]. This is consistent with our observation of reduced plasma leptin levels and increased testicular weight associated with enhanced serum testosterone levels following squalene treatment. Leptin has a positive effect on reproductive performance and its functions are dependent on the combination with leptin receptors. So we can assume that squalene may activate leptin to combine with transmembrane receptors and reduce obesity and hypertension.

Most obese individuals exhibit leptin resistance, high leptin levels and a high incidence of hypertension[2,25]. Data from this study support clinical application of squalene and its products in the management of obesity, hypertension and associated metabolic disorders.

In conclusion, our results indicated that feeding with squalene may counteract the increase in body fat, BP and levels of plasma leptin, glucose, cholesterol, triglycerides, and may increase the testicular weight and testosterone levels. Further studies are warranted to better elucidate the precise mechanism of leptin action and the leptin signaling cascade following squalene exposure.

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