Academic Sciences Asian Journal of Pharmaceutical and Clinical Research
Vol 6, Issue 1, 2013 ISSN - 0974-2441

Research Article

THE ANTI-ANDROGENIC EFFECT OF CONTINUOUS INTAKE OF MICROWAVE EXPOSED FOOD ON SWISS ALBINO MICE

PRIYANKA RAGHUVANSHI, PRIYANKA MATHUR

The IIS University,Jaipur,Email:priyanka.raghuvanshi@gmail.com, pmathur1278@yahoo.com

Received: 15 October 2012, Revised and Accepted:7 December 2012

ABSTRACT

Microwaves are electromagnetic waves with wavelengths ranging from as long as one meter to as short as one millimeter. Microwaves also have other subtle (athermal) effects, but the heating effect is the best understood. The microwave oven cooks food using 2.45 GHz microwaves .In this study male albino mice were fed with the fixed amount of food exposed to the microwave radiation for 10 minutes at 320°C. The food was given as their normal dietary intake for 2 week, 3 week, 4 week, 4 week recovery to 3 groups namely experimental, control and sham. It was observed that the level of cholesterol showed significant increase in experimental and sham group as compared to the control group but the increase in sham is comparatively lesser than experimental and simultaneously the level of testosterone showed the significant decline in the same pattern .The decline in testosterone may be because of increased amount of cholesterol as cholesterol is known to be a precursor in androgen synthesis in the testis, its enhanced level can be co-related with the inhibition of synthesis of testosterone leading to infertility.

Keywords: Microwave cooked food, Cholesterol, Testosterone.

INTRODUCTION

The quality of life in terms of income, spending and lifestyle has improved with economic development. [1] Every cooking method can destroy vitamins and other nutrients in food. The factors that determine the extent are how long the food is cooked, how much liquid is used and the cooking temperature. Microwave ovens heat food through a process of creating molecular friction, but this same molecular friction quickly destroys the delicate molecules of vitamins and phytonutrients (plant medicines) naturally found in foods [2].

Histological studies [3] with microwaved broccoli and carrots have revealed that the molecular structures of nutrients are deformed by high-frequency reversal of polarity. The microwaves-induced reversal of the polarity causes the cells in the nutrients to become destructively polarized, possibly allowing for the creation of free radicals [3].

Watanabe *et al*[4] reported that microwave heating caused appreciable loss (30–40%) of vitamin B_{12} due to the degradation of vitamin B_{12} molecule by microwave heating. the conversion of vitamin B_{12} to the inactive vitamin B_{12} degradation products occurs in foods during microwave heating. Scandinavian study of the cooking of asparagus spears found that microwaving caused a reduction in vitamin C [5].George *et al* [6] showed that microwaves cause a higher degree of "protein unfolding" than conventional heating.

The restriction of nutrient intake or deficiency of particular nutrients in experimental animals delays sexual maturity and causes rapid regressive changes in male accessory organs. Therefore, successful reproduction requires complete provisions of macro- and micronutrients, including vitamins, fatty acids and proteins [7]. Available evidence supports the hypothesis that nutritional factors can alter hormonal metabolism. Vertebrate male reproductive endocrine systems commonly comprise hypothalamus, anterior pituitary gland and testes, which form the hypothalamic-pituitary-gonadal (HPG) system. A number of studies [8-13] have shown that specific constituents (eg, protein and fat) may act directly on the anterior pituitary or other glandular organs and modify their response to demands imposed by nutritional factors. The endocrine system is sensitive to nutritional status. The hypothalamus reacts to poor nutrition by reduced secretion of hormones which normally stimulate the release of pituitary hormones essential for fertility of male by secretion of hormones which inhibit the release of other hormones controlling growth. The secretion of hormones by the pituitary, ovaries and liver is also directly inhibited by different degrees of nutritional inadequacy on varying timescales. The hypothalamic-pituitary system is inhibited by caloric restriction, and by deficiencies of vitamins such as pyridoxine and folic acid, and by deficiencies of minerals such as zinc and magnesium.[14]

Testosterone is a steroid hormone secreted from the Leydig cells of the testes. Previous studies have demonstrated that steroid hormone concentrations are subject to dietary regulation [15-17]. Individuals consuming a diet containing ~20% fat compared with a diet containing ~40% fat[18-20] have significantly lower concentrations of Testosterone. Also, replacement of dietary carbohydrate with protein has been shown to decrease Testosterone concentrations [15]. These studies indicate that the energy supplied by the different macronutrients has a significant influence on Testosterone concentrations. Both the amount and composition of the energy-providing macronutrients may modify Testosterone concentrations.[21] The raw material for testosterone biosynthesis in the testis is cholesterol. The role of cholesterol differs in the two compartments of the testis. In the interstitial tissue, cholesterol is necessary for the synthesis of testosterone, whereas in the seminiferous tubules, membrane cholesterol content in developing germ cells will influence the gametes' fertility.[22]Testosterone deficiency causes impairment of sperm production.[23]

MATERIALS AND METHODS

Sexually mature male mice (*Mus musculus*) weighing between 25 to 30 g were randomly selected .They were housed separately in plastic cages under controlled condition of temperature and light. The animals were divided into 3 groups :Control, Sham and Experimental. The experimental mice were given food pellets (Hindustan Lever Pvt. Ltd.) heated in microwave at 320° C for 10 minutes. The sham group was given the normal food in low quantity whereas control was given normal food in sufficient amount. The experimental group was fed with fixed amount of microwave cooked mice pellets daily for 2 weeks (Experiment 1), 3 weeks (Experiment 2), 4 weeks(Experiment 3).The recovery group(Experiment 4) was given the microwave pellets for 4weeks and after that they were given normal mice fed for 4 weeks.

Cholesterol

The concentration of cholesterol is estimated by Liebermanan and Burchard reaction method.[24]

Testosterone

Total testosterone levels were analyzed in serum via radioimmunoassay (RIA).

RESULTS

Cholesterol accumulation in testis increases significantly with the increasing duration of food administration (microwaved) in experimental group as compared to sham and control. The recovery

group fails to recover. The difference within control group is attributed to increasing age of mice. (Table I)

Table I: Testicular cholesterol concentrations in Swiss Albino mice fed continuously with food exposed to microwave radiations

Group	Autopsy interval Concentration of Cholesterol(mg/gm)			
	2 weeks	3 weeks	4 weeks	4 weeks recovery
Group I (Control)	11.2±1.02	9.6±1.4	5.8±1.6	8±0.51
Group II (Sham)	16.4±1.47**	17.6±1.56*	12.8±2.8*	6±1.41**
Group III (Experimental)	19.6±2.04**	20.4±2.48*	18±3.69**	30.8±1.02*

Significance in relation to control,* p<0.05, ** p<0.01

The level of testosterone shows the complete reversal as compared to cholesterol. The testosterone levels declines significantly with increasing duration of food administration (microwaved) in experimental group as compared to sham and control. The recovery group shows significant recovery.

Table II: Peripheral blood testosterone profiles in Swiss Albino mice fed continuously with food exposed to microwave radiations

Group	Autopsy interval Concentration of Testosterone (ng/ml)			
	2 weeks	3 weeks	4 weeks	4 weeks recovery
Group I (Control)	5.86±0.503	6.7±0.625	6.83±0.517	5.8±0.967
Group II (Sham)	5.73±0.85**	6.4±0.379**	6.23±0.887**	5.73±1.68**
Group III (Experimental)	5.63 ±0.144**	5.7±0.656**	6.56±1.35**	5.2±0.264**

Significance in relation to control,* p<0.05, ** p<0.01

In our previous studies we found significant decline in the nutrient, of food exposed to microwave radiations, particularly Carbohydrate, Protein and Vitamin D.

DISCUSSION

One probable reason for decreased testosterone could be because of inhibition of pregnenolone production by the testis. This inhibition can only be caused by a reduction in the activity of the mitochondrial enzyme which converts cholesterol into pregnenolone (cytochrome $P450_{scc}$), and/or by an impairment in the multistep process by which luteinizing hormone (LH) stimulates the mobilization of cholesterol to this enzyme. The view is further supported by the accumulation of cholesterol in testis.[25]

Total cholesterol levels in the testes of mice increases. It has been established that there is a blood –testes or blood – male reproductive tract barrier for cholesterol [26, 27]. It is attributable to the intra - gonad alteration in lipid distribution; namely increased mobilization from the membrane of the cells within the testes and / or increased prostatic secretion of cholesterol into the seminal plasma. It is therefore likely that secretion and build-up of cholesterol in the testes is a biological event that is meant to protect spermatozoa from oxidative stress and damage. The presence of high level of cholesterol in the testes and prostate may be an indication of decreased androgen production by the testes. This is conceivably so because this hormone is produced by leydig cells (a group of cells that make up the testes) and the function of stimulated leydig cells are impaired by high cholesterol levels [26]. Optimal leydig cell function and testosterone secretion are known to be prerequisites for the normal activation of spermatogenesis. [28]

The raw material for testosterone biosynthesis in the testis is cholesterol. Androgen synthesis and, by extension, sperm production are controlled by a feedback loop involving the testes, hypothalamus and pituitary gland. The Leydig cells are believed to be the primary cell in the testis capable of synthesizing testosterone from the cholesterol substrate [29]. This process is dependent on LH, released from the pituitary under the stimulus of the hypothalamic gonadotrophin-releasing hormone (GnRH) **.** LH binds to its receptor on the surface of Leydig cells **,** activating the receptor and causing an increase in intracellular cAMP, via adenylate cyclase [30-32]. This leads to free cholesterol being transported to the inner mitochondrial membrane via the steroidogenic acute regulatory (STAR) protein [33,34].Within the mitochondria, cholesterol is converted into 5-pregnenolone by P450scc (cholesterol side-chain cleavage enzyme, also known as CYP11A), which is encoded by a gene at 15q23-q24. From 5-pregnenolone, testosterone biosynthesis proceeds by one of two pathways: via 17-hydroxypregnenolone, dehydroepiandrosterone (DHEA), and [5]-androstenediol (the main pathway) or via progesterone, 17-hydroxyprogesterone, and androstenedione. Inhibition of the cholesterol ester hydrolase induces an increase in esterified cholesterol levels in whole mouse testis extracts that is accompanied with a decrease in serum testosterone levels [35]. Cholesterol precursors are derived from the blood in the form of circulating lipoproteins; from stores of free cholesterol inside the Leydig cell itself; or synthesised *de novo* from acetate [36].

Regulation of testicular steroid and cholesterol homeostasis is critical to the fertility of mammals. The accumulation of cholesterol and low testosterone points towards the disruption of the normal steroid and cholesterol homeostatic mechanisms within the testis. This may occur due to disruption of T-mediated feedback regulation within the HPG axis [37]

The study suggests microwave exposed food continuous feeding cause's recoverable changes in testosterone level. The result of the study is applicable to intake of microwave food only.

REFERENCES

1. Pandey M , Verma R K and Saraf SA. Nutraceuticals: new era of medicine and health. Asian J Pharm Clin Res.2010; 3(1):11-15
2. Mike Adams . Microwave Ovens Destroy the Nutritional Value of Your Food. NewsTarget/Truth Publishing, August 6, 2007 a href = http://www.NewsTarget.com/ 021966.html" > Microwave ovens destroy the nutritional value of your food
3. Schrumpf E and Charley H. Texture of broccoli and carrots cooked by microwave energy. J. Food Science 1975; 4(O): 1025-29
4. Watanabe F, Abe K, Fujita T *et al* . Effects of Microwave Heating on the Loss of Vitamin B 12 in Foods. J. Agric. Food Chem. 1998; 46:206–210
5. Kidmose U and Kaack K. Changes in texture and nutritional quality of green asparagus spears (*Asparagus officinalis* L.) during microwave blanching and cryogenic freezing. Acta Agric. Scand. 1999*;* B(49): 110–116.
6. George DF, Bilek MM, and McKenzie DR. Non-thermal effects in the microwave induced unfolding of proteins observed by chaperone binding. Bioelectromagnetics 2008; 29(4):324-30
7. Cheah Y and Yang W .Functions of essential nutrition for high quality spermatogenesis. Advances in Bioscience and Biotechnology, 2011; 2:182-197
8. Hill P, Chan PC, Cohen LA, Wynder EL and Kuno K. Diet and endocrine-related cancer. Cancer l977; 39:1820-6.
9. Hill PB and Wynder EL. Effect of a vegetarian diet and dexamethasone on plasma prolactin, testosterone,and dehydroepiandrosterone in men and women. Cancer Lett l979;7:273-82.

10. Hill P, Garbaczewski L, Helman P, Huskisson J,*et al*. Diet, lifestyle, and menstrual activity. Am J am Nutr l980; 33:l 192-8.
11. Goldin BR, Adlercreutz H, Gorbach SL, *et al*.Estrogen excretion patterns and plasma levels in vegetarian and omnivorous women. N Engl J Med l982; 307: 1542-7.
12. Carlson HE, Wasser HL, Levin SR and Wilkins JN. Prolactin stimulation by meals is related to protein content. J Am Endocrinol Metab 1983; 57:334-8.
13. Hill P. Garbaczewski L, Haley N and Wynder EL. Diet and follicular development. Am J Can Nutr 1984; 39:771-7.
14. Wynn MA. Effects of Nutrition on Reproductive Capability.Nutrition and Health January 1983; 1(3):165-178
15. Anderson KE, Rosner W, Khan MS, New MI, *et al*. Diet-hormone interactions: protein/carbohydrate ratio alters reciprocally the plasma levels of testosterone and cortisol and their respective binding globulins in man. Life Sci.1987; 40:1761–1768.
16. Bishop TD, Meikle WA, Slattery ML, *et al*. The effect of nutritional factors on sex hormone levels in male twins. Genet. Epidemiol.1988; 5:43–59.
17. Raben A, Kiens B, Ritchter EA, *et al* . Serum sex hormones and endurance performance after a lacto-ovo vegetarian and a mixed diet. Med. Sci. Sports Exercise 1992; 24:1290–1297.
18. Goldin BR, Woods MN, Spiegelman DL, *et al*. The effect of dietary fat and fiber on serum estrogen concentrations in premenopausal women under controlled dietary conditions. Cancer 1994; 74:1125–1131.
19. Reed MJ, Cheng RW, Simmonds M, *et al*. Dietary lipids: an additional regulator of plasma levels of sex hormone binding globulin. J. Clin. Endocrinol. Metab.1987;64: 1083–1085.
20. Hämäläinen E, Aldercreutz H, Puska P and Pietinen P .Diet and serum sex hormones in healthy men. J. Steroid Biochem.1984; 20: 459-464
21. Volek JS, Kraemer WJ, Bush JA, *et al*. Testosterone and cortisol in relationship to dietary nutrients and resistance exercise. Journal of Applied Physiology 1997; 82(1): 49-54
22. Kabbaj O, Yoon SR, Holm C , *et al*. Relationship of the Hormone-Sensitive Lipase-Mediated Modulation of Cholesterol Metabolism in Individual Compartments of the Testis to Serum Pituitary Hormone and Testosterone Concentrations in a Seasonal Breeder, the Mink (*Mustela vison*). Biology of Reproduction 2003;68: 3722-734
23. Desai A, Shirode AR, Mittal B and Kadam VJ. (2011).Assisted Reproductive Technology (ART): Combating Infertility. Asian J Pharm Clin Res.2011;4(1):18-21
24. Liebermann NC and Burchard,H. Estimation of total cholesterol .J.Biol Chem 1952;195:357
25. Robert W,Moore, Colin R. *et al* . 2,3,7,8-Tetrachlorodibenzo-*p*-dioxin inhibits steroidogenesis in the rat testis by inhibiting the mobilization of cholesterol to cytochrome P450.Toxicology and Applied Pharmacology 1991; 109(1):85-97
26. Shimamoto K and Sofikitis N . Effect of hypercholesterolemia on testicular function and sperm physiology. Yonago Acta. Medica.1998; 41: 23-29.
27. Wong EW, Mruk DD and Cheng CY. Biology and regulation of ectoplasmic specialization, an atypical adherens junction type, in the testis. Biochim Biophys Acta 2008; 1778:692–708.
28. Hikim AS, Swerdloff RS and Wang C. The Testes. In: Endocrinology, Basic and Clinical Principles. Shlomo Melmed and Corn Michael P (eds). Humana Press, New York,2005: pp 405-408.
29. Cooke BA, De Jong FH, Van der Molen HJ and Rommerts FF. Endogenous testosterone concentrations in rat testis interstitial tissue and seminiferous tubules during in vitro incubation. Nat New Biol 1972; 237:255–256.
30. Dufau ML. Endocrine regulation and communicating functions of the Leydig cell. Annu.Rev.Physiol 1988; 50: 483-508.
31. Cooke, BA, Choi MC, Dirami G. *et al* .Control of steroidogenesis in Leydig cells. J. Steroid. Biochem. Mol. Biol. 1992;43: 445–449.
32. Huhtaniemi I and Toppari J . Endocrine, paracrine and autocrine regulation of testicular steroidogenesis.Adv.Exp.Med.Biol1995;377: 33-54.
33. Clark BJ, Wells J, King SR and Stocco DM . The purification, cloning, and expression of a novel luteinizing hormoneinduced mitochondrial protein in MA-10 mouse Leydig tumor cells. Characterization of the steroidogenic acute regulatory protein (StAR). J.Biol Chem. 1994;269 :28314-28322.
34. Stocco DM and Clark BJ . Role of the steroidogenic acute regulatory protein (StAR) in steroidogenesis. Biochem.Pharmacol. 1996; 51: 197-205.
35. Bartke A, Musto N, Caldwell BV and Behrman HR. Effects of a cholesterol esterase inhibitor and of prostaglandin $F_{2\alpha}$ on testis cholesterol and on plasma testosterone in mice. Prostaglandins 1973;3:97-104
36. Payne AH and Youngblood GL (1995) Regulation of expression of steroidogenic enzymes in Leydig cells. Biol Reprod. 1995; 52: 217-225.
37. Stephen ME, Agarwal N, Qian K, *et al* . Hormonal Regulation of Testicular Steroid and Cholesterol Homeostasis. Molecular Endocrinology 2008; 22(3): 623-635