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

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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. 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 1.

Histological studies 2 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 B₁₂ due to the degradation of vitamin B₁₂ molecule by microwave heating, the conversion of vitamin B₁₂ to the inactive vitamin B₁₀ 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. George et al 5 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 6. 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 7−11 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 12.

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 13−17. 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 19. 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. 20. 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. 21. Testosterone deficiency causes impairment of sperm production. 22.

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 4 weeks and after that they were given normal mice fed for 4 weeks.

Cholesterol

The concentration of cholesterol is estimated by Lieberman and Burchard reaction method. 23

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

<table>
<thead>
<tr>
<th>Group</th>
<th>Autopsy interval</th>
<th>Concentration of Cholesterol (mg/gm)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>2 weeks</td>
<td>3 weeks</td>
</tr>
<tr>
<td>Group I (Control)</td>
<td>11.2±1.02</td>
<td>6.1±1.4</td>
</tr>
<tr>
<td>Group II (Sham)</td>
<td>16.4±1.47**</td>
<td>17.6±1.56*</td>
</tr>
<tr>
<td>Group III (Experimental)</td>
<td>19.6±2.04**</td>
<td>20.4±2.48*</td>
</tr>
</tbody>
</table>

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

<table>
<thead>
<tr>
<th>Group</th>
<th>Autopsy interval</th>
<th>Concentration of Testosterone (ng/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>2 weeks</td>
<td>3 weeks</td>
</tr>
<tr>
<td>Group I (Control)</td>
<td>5.86±0.35</td>
<td>6.7±0.25</td>
</tr>
<tr>
<td>Group II (Sham)</td>
<td>5.72±0.25*</td>
<td>6.4±0.087**</td>
</tr>
<tr>
<td>Group III (Experimental)</td>
<td>5.53 ±0.144**</td>
<td>5.7±0.656**</td>
</tr>
</tbody>
</table>

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 P450scc), 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.

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. 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 spermatogenesis 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 testis. This is conceivable 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 is impaired by high cholesterol levels 26. Optimal leydig cell function and testosterone secretion are known to be prerequisites for the normal activation of spermatogenesis.

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. 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 26. This leads to free cholesterol being transported to the inner mitochondrial membrane via the steroidogenic acute regulatory (STAR) protein. 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 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 25. 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 30.

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.

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


