

The Effects of Added Drinking Water Nitrate on Plasma Leptin, Insulin and Thyroid Hormone Concentrations in Rats

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Abstract: The purpose of this study was to determine the effects of drinking nitrate on plasma leptin, insulin, thyroid hormones levels in rats. All rats were divided into one control and one treatment group ($n = 8$) and water with 150 mg L^{-1} sodium nitrate added was given to Group I during 8 weeks period. At the end of the experiment when plasma T4 level in the Group I was increased as compared with the control group, plasma T3 was decreased ($p < 0.05$). There were not found statistically significance in the other parameters in experiment group compare to control group. In conclusion, the present study indicates that nitrate intake in water influenced thyroid function but not affected the plasma leptin and insulin hormone concentrations which playing a key role in the energy metabolism.

Key words: Nitrate, leptin, insulin, thyroid hormones, rat, Turkey

INTRODUCTION

Accumulation of nitrates/nitrites in the environment, naturally found in soil, water and plants has significantly impacted by excessive agricultural practice, food processes and industrial development (Jordao *et al.*, 2002). The nitrates/nitrites incorporation of humans and animals take place via food, feed and drinking water and they are widely used especially as flavouring, fixing coloring and antimicrobial agents in food such as red meat, poultry and fish products (Chow and Hong, 2002). However, the contamination of drinking water consumed by humans and animals with nitrates/nitrites can be potentially hazardous with health risk. The main source of nitrate and nitrite in drinking water include nitrates/nitrites sewage from soil where urban and industrial waste water and nitrogen-based fertilizers are used. Value for nitrate in drinking water up to 50 mg L^{-1} is not a risk factor however, nitrate levels above 100 mg L^{-1} increases the risk. Nitrate concentrations of underground water are low ($0\text{-}18 \text{ mg L}^{-1}$) under normal conditions (Young and Morgan-Jones, 1980) and the World Health Organization (WHO) recommended that the quality of drinking water a maximally admissible nitrate concentration of 50 mg L^{-1} (WHO, 1993). Drinking water supply is provided by ground and underground water resources in most countries and the finding of nitrate and nitrites in drinking water accepted as an indicator of pollution have been reported to be increasing during the last few decade (Eskioçak *et al.*, 2005).

Nitrate and nitrite have been known to occur as a consequence of normal metabolism of nitric oxide in many cells such as macrophage, neutrophil and endothelial cells. Nitrate transported into the bloodstream does not create the initial problem but it can be reduced to nitrite by bacterial nitrate reductase in saliva and gastrointestinal system and then reabsorbed back into bloodstream, thereby intensifying the problem (Chow and Hong, 2002; Takahashi *et al.*, 1998). Excessive nitrite in the blood reduces Fe^{+2} to Fe^{+3} in the hemoglobin. Nitrite reacts with hemoglobin to form methaemoglobin. Methaemoglobin is unable to carry oxygen owing to its ferric form, resulting in cyanosis and anoxemia if the level of methaemoglobin becomes sufficiently high. The cellular functions terminate as a result of anoxia, the most important symptom of nitrate and nitrite toxicity (Takahashi *et al.*, 1998; Undersander *et al.*, 2007). In fact, Takahashi *et al.* (1998) reported that every 10% of methaemoglobin formation instead of oxyhemoglobin in the erythrocytes reduced oxygen consumption by 10.3%. Nitrate ingestion dosage dependently has been linked to impairment of thyroid function, interference with Vitamin A and E metabolism and decreased feed consumption (Jahreis *et al.*, 1986; Bruning-Fann and Kaneene, 1993). Accordingly, in addition to thyroid hormones, uptaken nitrate have been considered to affect leptin and insulin hormones responsible for energy metabolism. In fact, leptin is a hormone which regulates body weight by suppressing food intake and stimulating energy expenditure, leading more fat to be burned

(Delavaud *et al.*, 2002; Chilliard *et al.*, 2005). Leptin level in the circulation shows variations in body fat reserves (Wegner *et al.*, 2001; Maffei *et al.*, 1995).

In the present study, the aim was undertaken to determine the effects of intake of chronic nitrates in drinking water on leptin, insulin and thyroid hormone levels, playing a key role in the energy metabolism and some biochemical parameters (glucose, NOx, total protein and cholesterol).

MATERIALS AND METHODS

A total of 16 male Albino Sprague-Dawley Albino rats (3 months old) with body weights of 230-240 g were separated into one control and three treatment groups (n = 8 for all) for 8 weeks study period. During this period, control group was given without water any addition of nitrate. Water with 150 mg L⁻¹ sodium nitrate added was given to Group I. At the end of this study lasted 8 weeks, the blood samples were taken from all rats after the animals were fasted overnight and sacrificed by anesthetizing with a combination of ketamine (80 mg kg⁻¹ i.p.) and Xylazine HCl (Rompun® Bayer Ilac Sanayii, Istanbul; 10 mg kg⁻¹ i.p.). The plasma was prepared by centrifugation (3000×g, 10 min, +4°C) to measure the biochemical parameters.

Plasma leptin and insulin concentration were determined by ELISA using a rat kit (Linco Research, Inc., St. Charles, USA). The manufacturer's suggested protocol was followed. Triiodothyronine (T3) (Code No.: DSL-10-3100S), Tetraiodothyronine (T4) (Code No.: DSL-10-3200), Free T3 (FT3) (Code No.: DSL-10-41100) and Free T4 (FT4) (Code No.: DSL-10-40100) concentrations were determined by specific ELISA-tests (DSL diagnostic systems laboratories, Inc., Texas, USA). Plasma NOx (nitrates + nitrites) was assayed by Colorimetric Method of Griess (Miranda *et al.*, 2001). Plasma glucose, total protein and cholesterol values were measured with commercially available assay kits (TECO Diagnostics, California, USA).

Statistical analysis was made with SPSS Statistical Software (SPSS for Windows; Standard Version 11.0). Comparisons between different groups were performed by one-way ANOVA if ANOVA revealed significant differences, the post-hoc comparisons were performed by Duncan's multiple range tests. The results were expressed as the Mean±Standard Errors (SEM). A value of p<0.05 was considered statistically significant.

RESULTS

All parameters are shown in Table 1. In the Group I, when plasma T4 level was increased as compared with the

Table 1: Plasma leptin, insulin, glucose, FT4, T4, FT3, T3, nitric oxide, total protein and cholesterol concentrations in all the groups

Parameters	Control group	Group I
Leptin (µg L ⁻¹)	3.65±0.24 ^{ab}	2.43±0.28 ^b
Insulin (µg L ⁻¹)	1.29±0.17 ^a	1.00±0.11 ^{ab}
Glucose (mmol L ⁻¹)	4.45±0.46 ^b	5.30±0.22 ^{ab}
FT4 (ng mL ⁻¹)	1.67±0.10	1.67±0.18
T4 (µg L ⁻¹)	55.80±2.00 ^b	65.80±4.50 ^a
FT3 (pg mL ⁻¹)	1.97±0.21	2.25±0.16
T3 (µg L ⁻¹)	0.89±0.10 ^a	0.67±0.04 ^b
Nitric oxide (µmol L ⁻¹)	11.87±1.60	12.45±1.16
T. protein (g L ⁻¹)	63.40±04.6	63.2±4.400
T. cholesterol (mmol L ⁻¹)	1.46±0.11	52.72±1.37

Results are expressed as means±SEM. T3: Total triiodothyronine; T4: Total thyroxine; FT3: Free Triiodothyronine; FT4: Free Thyroxine.

^{a-c}Different superscripts in the same row indicate significant differences (p<0.05)

control group, plasma T3 was decreased (p<0.05). The other parameters were not statistically significant difference in Group I compare to control group.

DISCUSSION

Zaki *et al.* (2004) reported that nitrate was induced a dose-dependent reduction of the body weight gain. This fall could be explained by a reduction of the water and food intake (Jahreis *et al.*, 1986) or by a growth slowing down induced by the low plasma T3 and T4 levels. In fact, T3 and leptin are important hormones to regulate the balance between energy intake and energy expenditure (Zabrocka *et al.*, 2006). Leptin decreases food intake and reduce body weight by decreasing NPY synthesis or by inhibiting NPY's action as an appetite stimulant (Trayhurn *et al.*, 1999; Schwartz and Seeley, 1997). In fact, Jahreis *et al.* (1986) suggested that a diet containing 3% KNO₃ led to a significant food intake depression at the end of 3 weeks and the weight gains of the animals of the nitrate group 38% lower at the end of 5 weeks. In the presented study, water and diet consumption of animals were not established however compared to the control group, the plasma leptin level in the group nitrate-fed rats was found lower than that of the controls. Although, this result was not statistically significant, it was decreased numerically on the other hand T3 was found statistically significantly lower. The results obtained indicate that nitrate inhibits food intake or T3 hormone synthesis. Actually, decrease in plasma leptin level is perceived as food deficiency and shows that organism takes precautions to protect its energy (Trayhurn *et al.*, 1999). In the presented study decrease in plasma leptin level were found to be consistent with the studies demonstrating that inadequate nutrition decreases leptin production and plasma leptin level in the adipose tissue (Blache *et al.*, 2000). As food intake decreases, plasma insulin level decreases as well and at the same time

decrease in leptin mRNA synthesis during fasting was suggested to originate from insulin release (Emilsson *et al.*, 1997; Trayhurn *et al.*, 1995). In the presented study similar to the leptin level, plasma insulin level also in nitrate given animals in comparison to that of the control group animals was found to decrease numerically but not statistical significance. There were many studies reporting increase in food intake and plasma insulin levels leads increase in body fat mass and accordingly increase in fat tissue leptin mRNA synthesis and plasma leptin level (Maffei *et al.*, 1995; Saladin *et al.*, 1995).

The studies performed demonstrate that high doses via drinking water (Wijngaarden *et al.*, 1953) and 1% of dietary nitrate inhibited competitively uptake of iodine by the thyroid gland (Bloomfield *et al.*, 1961). Nitrate, like thiocyanate and perchlorate anions is a goitrogenic substance clearly inhibiting uptake of iodine by thyroid glands (Jahreis *et al.*, 1986). Active iodine transport into thyrocytes is catalysed by the transmembrane Na^+/I^- transport protein and nitrate prevents iodine from the bond with this transport protein. Thereby, this process intrathyroidal iodine decreases (Jahreis *et al.*, 1986; Eskiocak *et al.*, 2005). Diet containing inhibited food intake in the piglets at the end of 3 weeks and decreased body weight gain by 38% at the end of 5 weeks (Jahreis *et al.*, 1986). While 3% KNO_3 in the diet has been reported to decrease serum T3 and T4 levels in rats (Mukhopadhyay *et al.*, 2005) and pigs, thyroid volume in humans (Jahreis *et al.*, 1986) has been reported to increase due to nitrate level. Eskiocak *et al.* (2005) reported that although, thyroid hormones decrease with 50 mg L^{-1} nitrate, TT3 level and thyroid gland weight increases with 100 mg L^{-1} nitrate but T3, FT3, FT4, TSH levels and thyroidal radioiodine uptake is not affected. According to this, at the 100 mg L^{-1} nitrate level, these results have shown that the hormonal adaptation mechanism was reactivated and serum hormone levels normalised. In contrary, 250 and 500 mg L^{-1} nitrate in water have been reported lead to histomorphological changes in the thyroid glands of rats (Eskiocak *et al.*, 2005). In the presented study nitrate at a level of 150 mg L^{-1} increased the level of plasma T4 and significantly decreased T3 level. These findings can be obtained as a result of decrease in the 5 and 5'-deiodinase enzyme activity in the peripheral tissue. In fact, the conversion of T4 to T3 and T3 in extrathyroidal tissue occurs through type I 5 and 5'-deiodinase enzyme and the activity of this enzyme is affected by factors such as fasting, inadequate protein intake and decline in liver and kidney functions (Kelly, 2000). At the same time, the increase in T4 level in the mentioned group indicates that the receptors cannot be adequately occupied by T3 due

to reduction in T3 level and as a result of this T4 release may have increased due to TSH stimulation in the pituitary gland. Accordingly, the results of the study have been supported by the reports of Zaki *et al.* (2004), explaining that a dose of 150 mg L^{-1} nitrate does not change T4 but reduces T3.

There has been no difference with respect to the levels of NOx in the nitrate given group which indicates that nitrate and nitrite taken up exogenously may decrease the endogenous NOx production. Actually nitrate and nitrite are known as both a NOx oxidation product and a ready NOx source (Chow and Hong, 2002). Nitrate taken from the diet passes into the plasma after being absorbed through the stomach and the small intestines and approximately 25% of the nitrate taken from the diet is released into saliva. Nitrate in the saliva is quickly reduced to nitrite by the anaerobic bacteria in the saliva. Saliva is then sent into the stomach by swallowing and converted into nitric oxide in the acidic medium of the stomach (Duncan *et al.*, 1995). Nitric oxide is an important biological molecule in the transmission and defense of cellular signals in numerous mammal species. Nitric oxide causes lipid peroxidation in biological membranes due to production of reactive nitrogen types at high concentrations while displaying paracrin effect at low concentrations (Chow and Hong, 2002). Due to its short half-life, it is quite difficult to measure nitric oxide directly since it oxidizes during a short period. Consequently plasma nitric oxide is measured as total nitrate and nitrite level (NOx) (Chow and Hong, 2002). As compared with the control group, total cholesterol and protein levels have been found insignificant in Group I which have been found consistent with the reports suggesting that administration of 400 mg L^{-1} nitrate (Ogur *et al.*, 2005).

CONCLUSION

The results of this study demonstrate that taking chronic nitrate by drinking water inhibited of thyroid function but not affected the plasma leptin and insulin hormone concentrations which playing a key role in the energy metabolism.

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