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Effects of Long-Term Boron Administrations on High-Energy Diet-Induced Obesity in Rabbits: NMR-Based Metabonomic Evaluation

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Abstract: The aim of this study is to provide insight into boron metabolism and to identify metabolic pathways which may explain the presumed increased susceptibility of livers. Boron was administrated in rabbits at three different doses and 96 h intervals for 7 months. Metabolomic profile based on NMR analysis was performed. The most pronounced findings were significant changes in alanine, methionine, pyruvate and creatine. Boron seems to be effective in the prevention of obesity and fatty liver. Metabolic end-points obtained by NMR can be easily assessed and interpreted alone or in combination each other and with classical biochemical parameters for better understanding obesity and boron and liver metabolism.

Key words: Obesity, fatty liver, boron, NMR, methionine, metabolism, creative

INTRODUCTION

Obesity poses one of the greatest public health challenges for the 21st century in the developed world. The prevalence of obesity has increased by 10-40% in most European countries over the last decade (Thande *et al*., 2008).

Non-alcoholic fatty liver disease is the clinical hepatic expression of metabolic syndrome and has become the most common cause of liver disease worldwide. Its prevalence is around 20-30% and with a rapid increase in the metabolic risk factors in the general population (Myers, 2009).

As in human medicine, fatty liver is a metabolic disease that affects up to 50% of dairy cows in early lactation. Fatty liver is associated with an increased incidence and duration and decreased treatment success of infectious and metabolic diseases such as mastitis, metritis and ketosis as well as decreased reproductive performance. These disorders continue to be a cause of economic loss to the dairy industry and an animal welfare concern (Mulligan and Doherty, 2008).

^{1}H NMR spectroscopy techniques are rather fast and straightforward to apply to all body fluids *in vitro* and also to various tissues *ex vivo* and *in vivo*. Approaches combining data on various biofluids and/or tissues of the same individuals (integrated matabonomics) are increasingly used to study systems level biochemistry (Ala-Korpela, 2008).

In the previous studies, boron altered the lipid profile when administered in dogs (Basoglu *et al*., 2000) boron might play a role on lipid metabolism, particularly serum triglycerides and VLDL secretion of the liver in dairy cows (Basoglu *et al*., 2002) boron seems to be effective on hepatic and visceral fat accumulation and high boron doses can be tolerated for a long time at 96 h intervals (Basoglu *et al*., 2010).

This study was designed to determinate the long term effects of boron at different doses on NMR based metabolic differences and to establish concentration ranges for different metabolite markers that are highly specific for obesity and fatty liver.

MATERIALS AND METHODS

Animals, diets and boron administration: The experimental design was approved by the Committee on Use of Animals in Research of Selcuk University, Faculty of Veterinary Medicine.

A total of 60 female New Zealand White rabbits, aged 8 months have been used as a material. The animals have been housed in cages of 5 animals in an air-conditioned room with controlled temperature (20-23°C) and automatic

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lighting and fed a low energy diet for 4 weeks after arrival. Next, the animals have been randomly divided into 5 groups. The average body weights of the groups were equal at the beginning of the experiment. One (control 1) of the first two groups was fed with a low energy diet (only alfalfa hay), another (control 2) only a high-energy diet (as pellet containing 2800 kcal kg^{-1} metabolizable energy). Remaining 3 groups (experimentals) were fed with high energy diet and boron (in solution of 1%) were given by oral gavage at the dose of 10, 30 and 50 mg kg^{-1}, respectively in boron compound (borax deca-hydrate, $Na_2B_4O_7.10H_2O$) every 96 h during the experiment (7 months). Some alfalfa hay was weekly added to the experimental groups diet for microbial balance. Boron administration was shamed by water in controls. The diet consumption and body weight-gain were measured monthly (Basoglu *et al*., 2010).

NMR analysis: ^{1}H, ^{31}P and ^{11}B NMR were performed on a Bruker 600 MHz DRX spectrometer. Serum and liver samples were prepared in previously mentioned method (Beckonert *et al*., 2007).

Statistical analysis: All data were presented as the mean±SEM. The data were evaluated by one way ANOVA-Duncan using the SPSS 13 program and the differences between the means assessed using Duncan's multiple-range test. Statistical significance was considered at $p<0.05$.

RESULTS AND DISCUSSION

Rabbits body and liver weights in control 2 and experimental groups were significantly heavier than control group 1 and also there was a decline in body weight of experimental group 3. Boron reached pick levels in blood at first hours following borax administration then it gradually decreased and was about the beginning level at the 96th h. It was observed that liver did not accumulate boron (Basoglu *et al*., 2010). There was a clear trend for higher creatine, pyruvate, methionine and alanine intensities in experimental groups at the end of experiment (Table 1). All rabbits also demonstrated no differences in ^{31}P and ^{11}B analysis.

Being obese or significantly overweight are major causes of poor health and health disparities in Europe. Their prevalence is increasing with rates doubling in some countries over recent decades. Weight gain is a result of an imbalance between food intake and physical activity but knowledge concerning underlying determinants is limited and information about obesity prevention interventions is scattered. A systematic and integrated approach is needed to better understand interventions and the factors determining obesity and to translate this knowledge into effective obesity prevention policy (Zhang *et al*., 2008).

Wei *et al*. (2008) reviewed evidence that implicates mitochondrial dysfunction as a primary mechanism for development of NAFLD. Mitochondrial dysfunction may not only cause fat accumulation but also may lead to the generation of reactive oxygen species and cytokine production contributing to progression of NAFLD. Lipid peroxidation is linked with the excess generation of reactive oxygen species which may be contributed by the exogenous or the endogenous sources. Glutathione is a unique cellular tripeptide that plays a vital role in maintaining the oxidant/antioxidant balance in the tissue that is essential for normal cellular function. Severe depletion of glutathione is considered as the consequence or the cause of oxidative stress. Boron supplementation could replenish the depleted hepatic glutathione level. It should be noted that oxidative stress is often counteracted by glutathione, resulting in its depletion. Borax partly normalizes the liver and offsets the deleterious effects observed in fulminant hepatic failure by modulating the oxidative stress parameters (Pawa and Ali, 2006). In the present study a high level of lipid peroxidation associated with glutathione depletion was exhibited in group not receiving boron and lipid peroxidation inhibited significantly in experimental groups especially 2 (Basoglu *et al*., 2010).

Methionine metabolism may be in part responsible for the development of steatosis, induction of mitochondrial dysfunction and increased vulnerability of fatty livers to ischemia/reperfusion injury (Serkova *et al*., 2006). Boron is bioactive through affecting the formation or utilization of S-adenosylmethionine (Nielsen, 2009). Although, hepatic methionine could not be determinated in the present study, a trend towards decreased serum methionine was present in experimental groups. In fact, the inhibition of elevated methionine values in groups given boron as shown in Table 1, supports boron effectiveness on metionine metabolism.

Alanine plays a key role in glucose-alanine cycle between tissues and liver. In muscle and other tissues that degrade amino acids for fuel, amino groups are collected in the form of glutamate by transamination. Glutamate can then transfer its amino group through the action of alanine aminotransferase to pyruvate, a product of muscle glycolysis, forming alanine and alpha-ketoglutarate. The alanine formed is passed into the blood and transported to the liver. A reverse of the alanine aminotransferase reaction takes place in liver. Pyruvate regenerated forms glucose through

Table 1: ^{1}H NMR analysis in serum samples at the beginning and the end of the experiment

Metabolomics	Timing	Control 1	Control 2	Exp. 1	Exp. 2	Exp. 3	p-value
Formic acid	Beginning	0.68±0.120^{ab}	0.69±0.080^{ab}	0.52±0.030^{b}	1.00±0.150^{a}	0.91±0.250^{ab}	0.187
	End	0.65±0.070^{a}	0.70±0.100^{a}	0.59±0.090^{a}	0.64±0.040^{a}	0.59±0.040^{a}	0.815
Tyrosin	Beginning	6.68±0.420^{a}	6.45±0.430^{a}	5.94±0.180^{a}	5.86±0.470^{a}	6.96±0.680^{a}	0.406
	End	5.85±0.270^{a}	5.94±0.250^{a}	6.36±0.520^{a}	6.51±0.120^{a}	6.57±0.150^{a}	0.317
Histidin	Beginning	5.20±0.210^{a}	4.47±0.380^{ab}	4.00±0.200^{b}	3.73±0.380^{b}	4.62±0.320^{ab}	0.022
	End	4.66±0.590^{a}	5.23±0.530^{a}	4.80±0.660^{a}	4.74±0.570^{a}	5.52±0.490^{a}	0.797
Alphaglucose	Beginning	2.54±0.140^{b}	2.98±0.370^{ab}	3.71±0.440^{a}	4.00±0.330^{a}	3.90±0.340^{a}	0.021
	End	2.66±0.200^{a}	2.52±0.260^{a}	3.17±0.480^{a}	3.24±0.390^{a}	3.42±0.400^{a}	0.345
Betaglucose	Beginning	98.13±5.550^{b}	95.39±5.700^{b}	110.90±7.640^{ab}	112.93±7.480^{ab}	121.11±6.460^{a}	0.059
	End	100.12±5.790^{c}	124.67±5.730^{ab}	105.70±6.330^{bc}	116.23±3.420^{abc}	126.24±9.100^{a}	0.024
Lactate 1	Beginning	47.86±8.330^{a}	61.10±5.440^{a}	65.46±12.90^{a}	88.13±17.93^{a}	77.63±14.90^{a}	0.238
	End	52.20±4.430^{b}	58.40±3.320^{ab}	82.11±17.09^{a}	71.77±7.420^{ab}	68.58±5.270^{ab}	0.180
GPC	Beginning	215.06±12.12^{b}	216.93±15.23^{b}	229.92±13.62^{ab}	239.34±13.04^{ab}	264.58±15.27^{a}	0.111
	End	217.51±10.12^{b}	315.00±17.03^{a}	295.82±42.28^{a}	272.86±5.600^{ab}	303.82±27.61^{a}	0.062
Creatine	Beginning	60.28±1.330^{ab}	58.54±3.320^{ab}	56.66±1.400^{b}	57.37±2.350^{ab}	63.62±0.990^{a}	0.156
	End	57.45±1.280^{b}	57.36±2.450^{b}	65.68±2.410^{a}	65.45±2.100^{a}	66.40±1.760^{a}	0.003
Citrate	Beginning	33.89±2.730^{a}	28.11±3.050^{ab}	31.59±2.180^{ab}	23.10±3.850^{b}	35.69±3.140^{a}	0.053
	End	34.64±2.320^{a}	25.85±2.120^{a}	33.77±5.300^{a}	33.92±1.930^{a}	34.01±3.880^{a}	0.330
Glutamine	Beginning	53.22±2.910^{a}	53.54±1.760^{a}	53.05±1.340^{a}	52.37±4.010^{a}	56.67±2.000^{a}	0.792
	End	55.77±1.480^{a}	59.15±2.630^{a}	59.62±4.200^{a}	57.86±2.350^{a}	58.36±0.880^{a}	0.850
Pyruvate	Beginning	29.29±1.500^{a}	28.56±1.130^{a}	28.86±1.470^{a}	31.55±1.740^{a}	32.90±1.500^{a}	0.196
	End	30.80±0.710^{b}	34.09±0.930^{ab}	36.65±2.910^{a}	36.97±1.620^{a}	37.26±0.930^{a}	0.042
Methionine	Beginning	326.39±14.60^{a}	334.21±27.05^{a}	312.74±13.52^{a}	365.27±40.26^{a}	354.87±22.91^{a}	0.604
	End	320.32±5.520^{b}	418.15±19.16^{a}	399.41±16.86^{a}	383.11±12.77^{a}	399.96±14.50^{a}	0.001
Acetate	Beginning	28.72±1.660^{ab}	23.34±1.570^{b}	25.40±3.380^{ab}	33.55±1.860^{a}	31.61±5.400^{ab}	0.158
	End	28.51±2.760^{a}	25.87±1.360^{a}	30.16±6.130^{a}	27.95±1.270^{a}	29.38±1.380^{a}	0.899
Alanine	Beginning	53.38±3.330^{a}	60.20±4.850^{a}	59.92±3.660^{a}	67.45±8.650^{a}	69.55±4.290^{a}	0.188
	End	48.07±2.410^{b}	56.17±3.640^{ab}	66.18±7.110^{a}	63.04±4.570^{a}	62.72±2.840^{a}	0.053
Lactate 2	Beginning	154.41±26.35^{a}	198.71±23.21^{a}	215.96±51.22^{a}	300.87±80.55^{a}	256.38±63.20^{a}	0.383
	End	151.19±17.06^{ab}	196.22±15.64^{ab}	204.69±2.150^{ab}	231.45±31.31^{a}	214.69±23.34^{ab}	0.148
Hydroxybutyrate	Beginning	34.02±2.600^{a}	29.74±2.810^{a}	35.58±5.330^{a}	38.02±3.560^{a}	32.22±2.380^{a}	0.529
	End	38.02±3.250^{a}	37.96±1.580^{a}	47.57±6.880^{a}	48.24±2.310^{a}	48.40±2.840^{a}	0.109
Valine	Beginning	27.73±1.570^{a}	28.84±3.680^{a}	27.20±1.810^{a}	31.63±3.040^{a}	32.16±3.130^{a}	0.619
	End	27.20±1.350^{a}	23.18±1.520^{b}	25.95±1.110^{ab}	25.89±0.810^{ab}	26.71±1.340^{ab}	0.215
Lipids	Beginning	265.62±7.300^{a}	272.96±38.45^{a}	279.33±22.51^{a}	292.39±57.91^{a}	297.75±38.50^{a}	0.970
	End	202.00±9.070^{b}	375.60±34.86^{a}	339.05±15.97^{a}	322.83±26.03^{a}	324.30±33.64^{a}	0.001

Means with different supercripts within one row differ signifiantly ($p<0.05$)

gluconeogenesis which returns to muscle through the circulation system (http://wikipedia.org/wiki/creatine). In the present study the increased alanine and pyruvate associated with decreased glucose level showed that boron seems to be effective on the Kreb's and glucose-alanine cycles. Increase in alanine may also be associated with increase in gluconeogenesis.

Creatine by way of conversion to and from phosphocreatine is present and functions in all vertebrates as well as some invertebrates in conjunction with the enzyme creatine kinase. The presence of this energy buffer system keeps the ATP/ADP ratio high at subcellular places where ATP is needed which ensures that the free energy of ATP remains high and minimizes the loss of adenosine nucleotides which would cause cellular dysfunction. Alteration in ATP/ADP ratio indicates a mitochondrial dysfunction (http://wikipedia.org/wiki/creatine, Serviddio *et al*., 2008).

CONCLUSION

In the present study, increases of creatine have been observed in groups given boron. This may be considered as the consequence or the cause of improving mitochondrial function.

Metabolic end-points obtained by NMR can be easily assessed and interpreted alone or in combination each other and with classical biochemical parameters for better understanding obesity and boron and liver metabolism.

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