



THE EFFECT OF ANTIOXIDANT TREATMENT ON BLOOD
LACTATE AND PYRUVATE CONCENTRATIONS IN
A RABBIT MODEL OF OBESITY

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Summary

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In the current study, the impact of dietary antioxidant supplementation on fasting blood lactate and pyruvate concentrations in a rabbit model of obesity was investigated. A total of 26 rabbits were randomly divided into 3 groups: castrated and obese animals (CO, n=7); castrated, obese and treated with antioxidants (Immunoprotect, n=7, CIm) and non-castrated, non-treated controls (NC, n=12). At the end of the 2-month treatment period blood lactate concentration, lactate to pyruvate and lactate to glucose ratio in CIm were significantly ($P<0.05$) lower than in NC and tended to be lower than in CO. No group differences in pyruvate and glucose concentrations were found. There was a significant negative ($r = -0.53$; $P<0.05$) correlation between lactate and glucose concentrations. On the other hand, lactate was correlated significantly positively with pyruvate ($r=0.72$; $P<0.01$), lactate to pyruvate ratio ($r=0.94$; $P<0.001$), lactate to glucose ratio ($r=0.75$; $P<0.001$) and pyruvate to glucose ratio ($r=0.81$; $P<0.001$). In conclusion, the decrease of blood lactate concentration in antioxidant treated rabbits could be considered as a mechanism for improvement of skeletal muscle insulin sensitivity. In addition, antioxidant treatment could be used as an alternative tool to counteract lactic acidosis in obesity and diabetes.

Key words: antioxidant supplementation, insulin resistance, lactate, obesity, pyruvate, rabbits

It is well known that in normal conditions the skeletal muscle is the major site of lactate production by conversion of pyruvate. However, increasing body of evi-

dences indicated that in obesity, adipose tissue is also a significant source of lactate release (Qvisth *et al.*, 2007; Crawford *et al.*, 2010).

Recently it has been found that lactate might have an insulin promoted glucose uptake effect in rats after physical activity and exercise (Hamamdžić *et al.*, 2008) and that increased lactate concentration induces insulin resistance in obesity and type 2 diabetes (Lovejoy *et al.*, 1992; Vettor *et al.*, 2000; Choi *et al.*, 2002). On the other hand, obesity has been described as a state of chronic oxidative stress characterised by increased plasma and tissue concentration of reactive oxygen species (ROS) and free radicals, which probably play an important pathogenic role in the axis of obesity, insulin resistance and type 2 diabetes (Valdecantos *et al.*, 2009; Lin *et al.*, 2012). Little is known about the effect of exogenous antioxidant treatment on blood lactate in obesity and its association with pyruvate and glucose concentrations. On the other hand, rabbits are increasingly used as an appropriate model to study various obesity-associated abnormalities such as metabolic syndrome, type 2 diabetes, cardiovascular diseases etc., as their lipid profile and metabolism is similar to those of humans (Zheng *et al.*, 2009; Wakar *et al.*, 2010; Georgiev *et al.*, 2011).

Therefore, this study was conducted to investigate the effect of antioxidant supplementation on fasting blood lactate and pyruvate concentrations in a rabbit model of obesity.

The experimental procedure was approved by the Commission of Ethics at the Faculty of Veterinary Medicine in Trakia University, Stara Zagora.

Twenty-six clinically healthy male, 2–2.5 months old, New Zealand white rabbits were used. The animals were housed in individual cages (80×60×40 cm) in a temperature-controlled room (20–22 °C).

The rabbits had free access to food and water. They were fed a commercially available standard chow diet. During the whole experimental period (2 months) the rabbits were determined to be healthy using routine examination, daily monitoring of behaviour, food and water intake and faeces consistency.

The rabbits were divided into 3 groups: castrated and obese animals (CO, n=7); castrated, obese and treated with antioxidants (CIm, n=7) and non-castrated, non-treated controls (NC, n=12). The castration of the rabbits was performed under general anaesthesia, as described (Georgiev *et al.*, 2009).

Immunoprotect is a nutritional supplement consisting of two components: vitamin E (10 mg equivalent to 15 IU) (Bieri & McKenna, 1981) and organic extract from citrus fruits peel (90 mg), which contains in high proportion d-limonene (Michaelakis *et al.*, 2009). Limonene is a monoterpene belonging to the group of isoprenoids. Immunoprotect was produced and gifted by Pharmaray, Sofia, Bulgaria in the form of gelatinous pearls. The rabbits from the CIm group received two pearls *per os* daily for 2 months.

Blood samples were collected from the jugular vein after at least 12 h overnight fasting. Lactate, pyruvate and glucose concentrations were measured in whole blood.

The glucose concentration was determined with a glucometer (Home Diagnostics, Inc., Ft. Lauderdale, Florida, USA) based on glucose oxidase method, using one drop of whole blood. Plasma concentrations of lactate and pyruvate were determined spectrophotometrically.

The statistical analyses were performed using Statistica v.7.1 for Windows (StatSoft Inc., USA). The descrip-

tive statistical tests, including the mean and standard error of the mean were calculated according to the standard methods. The ANOVA test was used to evaluate the effect of group on the concentrations of lactate, pyruvate, glucose and their ratios. When the effect of groups was significant, the differences between groups were determined by means of the LSD test of PostHoc procedure. Data for lactate, pyruvate, glucose concentrations and their ratios were subjected to correlation analysis. The significance of differences was set at $P < 0.05$.

The fasting lactate, pyruvate and glucose concentrations are shown in Fig. 1. The lactate concentration in CIm group was significantly ($P < 0.05$) lower than in control rabbits and tended to be lower ($P < 0.1$) than in CO animals, while there were no differences between CO and NC rabbits. No differences ($P > 0.05$) in the concentrations of pyruvate and glucose were found among groups (Fig. 1).

Similar to lactate concentration, lactate to pyruvate ratio in CIm group was significantly ($P < 0.05$) lower, compared to NC and tended to be lower ($P < 0.1$) than in CO (Table 1). Lactate to glucose ratio in CIm was significantly ($P < 0.05$) reduced in comparison with NC, while pyruvate to glucose ratio only tended to be decreased ($P < 0.1$) (Table 1).

There was a significant negative ($r = -0.53$; $P < 0.05$) correlation between basal lactate and glucose concentrations. On the other hand, lactate was correlated significantly positively with pyruvate ($r = 0.72$; $P < 0.01$), lactate to pyruvate ratio ($r = 0.94$; $P < 0.001$), lactate to glucose ratio ($r = 0.75$; $P < 0.001$) and pyruvate to glucose ratio ($r = 0.81$; $P < 0.001$).

The major finding in this study is that blood lactate concentration and lactate to pyruvate ratio in castrated rabbits treated

with Immunoprotect were lower than in castrated obese and non-castrated non-obese rabbits (controls) and that this was not accompanied with changes in blood pyruvate and glucose concentrations.

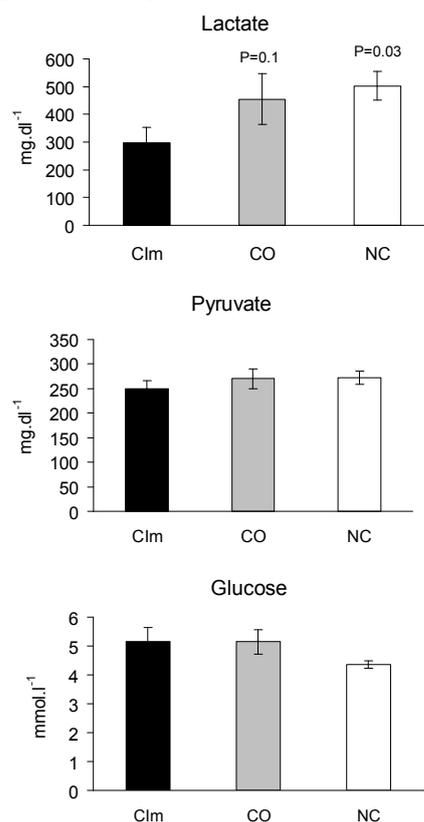


Fig. 1. Fasting blood lactate, pyruvate and glucose concentration (mean \pm SEM) in castrated obese rabbits treated with Immunoprotect (CIm, n=7), castrated obese (CO, n=7) and non-castrated non-obese (NC, n=12) rabbits compared to CIm.

It is well known that lactate metabolism is altered in obesity (Lovejoy *et al.*, 1992). A marked association between increasing obesity and type 2 diabetes in particular and fasting plasma lactate concentration has been reported (Vettor *et al.*, 2000; Qvisth *et al.*, 2007; Crawford *et al.*,

Table 1. Lactate to pyruvate, lactate to glucose and pyruvate to glucose ratios in castrated obese rabbits treated with Immunoprotect (CIm, n=7), castrated obese rabbits (CO, n=7) and non-castrated non-obese (NC, n=12) (mean ± SEM)

Parameters	Groups		
	CIm	CO	NC
Lactate/Pyruvate	1.17 ± 0.18 ^a	1.61 ± 0.25	1.81 ± 0.14 ^b
Lactate/Glucose	63.88 ± 14.64 ^a	97.24 ± 26.48	118.08 ± 14.62 ^b
Pyruvate/Glucose	51.45 ± 5.91 ^a	54.78 ± 6.71	63.56 ± 4.37 ^b

The different superscripts a, b in the same row indicate significant differences (P<0.05) between groups.

2010). As both conditions are closely related to insulin resistance, increased basal plasma lactate may deeply affect insulin action (Lovejoy *et al.*, 1992; Vetter *et al.*, 2000; Choi *et al.*, 2002).

The high concentration of lactate suppresses glycolysis, impairs insulin signal transduction pathway and glucose transport activity in muscle cells, and consequently causes insulin resistance in skeletal muscle (Vetter *et al.*, 2000; Choi *et al.*, 2002). Choi *et al.* (2002) demonstrated that the lactate effect on glycolysis appears to involve inhibition of two rate limiting enzymes – 6-phosphofructo-1 kinase and pyruvate dehydrogenase which cause impairment of insulin signalling. The same authors have also found that lactate did not affect insulin stimulated receptor, insulin receptor substrate-1 (IRS-1) and IRS-2 phosphorylation. At the same time IRS-1 and IRS-2-associated phosphoinositol 3-kinase activity is markedly decreased by lactate, leading to reduced insulin ability to translocate GLUT4 to the plasma membrane (Choi *et al.*, 2002). In case of tissue hypoxia, which accompanies obesity and type 2 diabetes, the pyruvate might be converted to lactate and this could be the reason of increased lactate concentration. We found

that there were no differences in blood lactate, pyruvate and glucose concentration between CO and NC groups. These results show that glycolysis and insulin signalling in obese rabbits were not yet affected, probably because of the short period of obesity (2 months), characterised by compensatory increase of insulin secretion. However, a significant negative correlation between lactate and glucose was found which is in agreement with the results of Metz *et al.* (2005). In contrast, a marked decrease in blood lactate concentration in castrated-obese rabbits treated with Immunoprotect was detected. The reduction of blood lactate levels could be due to the decreased lactate production from pyruvate or to the increased lactate to pyruvate conversion in liver. Previous results obtained in our laboratory indicated that exogenous antioxidant supplementation increased insulin sensitivity as shown by improved glucose tolerance, lipid profile, insulin sensitivity index and by decreased lipid content in liver (Georgiev *et al.*, 2009; 2011). On the other hand, there is accumulating evidence implicating insufficient oxidative capacity in insulin resistance (Crawford *et al.*, 2010). Taken together, these results show that an accelerated rate of mitochondrial oxida-

tive capacity and oxidative phosphorylation could be expected in antioxidant-treated rabbits. Therefore, the decreased blood lactate concentration after antioxidant supplementation is rather due to the reduced synthesis from pyruvate than to the higher lactate to pyruvate conversion which is confirmed by the lower lactate to pyruvate and lactate to glucose ratios. Besides, it has been reported that lactate is an energy substrate for oxidative oxidation in peripheral tissue, suggesting the presence of an intracellular shuttle where intracellularly produced lactate could be transported directly in the mitochondria for oxidation (Metz *et al.*, 2005; Qvisth *et al.*, 2007).

In conclusion, the decrease of blood lactate concentration in antioxidant-treated rabbits could be considered as a mechanism for improvement of skeletal muscle insulin sensitivity. The antioxidant treatment seemed a promising alternative tool to counteract lactic acidosis in obesity and diabetes. One limitation of this study is the short period of treatment and observation (2 months), therefore, long-term studies are needed for better understanding and reliable interpretation of observed effect of antioxidants on blood lactate concentration.

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