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THE INFLUENCE OF FAT DIET, WINE AND ETHANOL ON CHANGES IN SKELETAL MUSCLES IN WISTAR RATS*

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Consumption of fat diet causes deposition of fat into skeletal muscles and alcohol increases fat deposition. This study determined effects of fat diet, wine and ethanol on changes of skeletal muscles of rats. It was found that the group of rats fed with fat diet and wine had the lowest daily nutrients energy intake, the lowest average muscle weight but had the highest percentage of fat in skeletal muscles and the highest burning value. Average weights of skeletal muscles of rats consuming alcohol (wine or ethanol) were significantly lower than average weights of skeletal muscles of rats not consuming alcohol in case of fat diet. Results of chemical analysis show that chronic alcohol consumption increases fat deposition in skeletal muscles and increases their burning value in case of fat diet. No difference between effects of ethanol or wine on changes of skeletal muscles was observed.

Keywords: skeletal muscles, alcohols, wines, dietary fats, muscle damage

Chronic ethanol intake causes damage to different groups of muscles, e.g. heart muscle, muscles of the gastrointestinal tract and skeletal muscles. Alcohol directly affects skeletal muscles, which is expressed as acute or chronic myopathy. Skeletal muscle myopathy occurs in one to two-thirds of all chronic alcohol misusers, according to TROUNCE and co-workers (1987) and PREEDY and co-workers (1994a). In 1822 James Jackson, professor of medicine at Harvard University, described consequences of chronic spirit consumption as neuritis and disease weakness of muscles. He described the state after six months of abstinence to be irreversible. Diseases of skeletal muscles can be expressed in different forms such as endocrine, metabolic and nutrition disorders (RUBIN, 1979; MARTIN et al., 1982; SLAVIN et al., 1983; PREEDY et al., 1993; 1994a; b; ROMERO et al., 1994; FERNÁNDEZ-SOLÁ et al., 1996). Electron microscopic experiments by MARTIN and co-workers (1982) have demonstrated lipid accumulation between myofibrils and adjacent to the sarcolemma.

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Ethanol overdose is frequently associated with malabsorbtion, which is a consequence of nutrient intake and decreased metabolism, absorption, transport, deposition and excretion of different nutrients under direct or indirect effects of ethanol (WINDHAM et al., 1983; GRUCHOW et al., 1985; HILLERS & MASSEY, 1985; HALSTED & KEEN, 1990; BARWELL et al., 1991; LEO & LIEBER, 1999). Studies have shown that average values of daily intake of different nutrients such as proteins, fat, carbohydrates in alcoholic and non-alcoholic groups were similar (HALSTED & KEEN, 1990). Body weight of alcoholics does not depend on daily intake of nutrients, it depends on malabsorption and maldigestion of nutrients. Ethanol inhibits albumin synthesis, distracts release of proteins from the liver, diminishes gluconeogenesis and impairs the usage of vitamins. Ethanol can prevent transformation of thiamine into its active form. In chronic alcohol consumption, losses of nitrogen by faeces and losses of zinc, calcium, magnesium and phosphate by urine were observed by MORGAN (1982) and GUMASTE and co-workers (1991). Studies have shown vitamin deficiency in alcoholics with liver diseases. Folate deficiency expressed by megaloblastic anaemia and macrocytosis of intestinal epithelia was also noticeable, based on results of COOPER and co-workers (1997) and BRIDGES and co-workers (1999). PREEDY and co-workers (1993) found vitamin D deficiency in alcoholics, which was expressed as myopathy.

1. Material and methods

1.1. Animals

Experimental animals: Male Wistar rats, 10 weeks old (Medical Experimental Centre, Ljubljana). Animals were divided into five groups (29 animals in each), depending on dieting regimen (Table 1). Each group comprised of 29 animals. Prior to our study all animals were weighed, and then weight was controlled every four weeks. Animals were kept in animal rooms at 20–23 °C and 40–70% relative humidity with natural light regimen. After six months, animals were euthanised by CO_2, weighed, hind limb skeletal muscles were removed and weighed and then frozen until further analysis.

1.2. Dieting regimen

Animals were fed with pelleted diet M-K-02 (Biotechnical Faculty, Homec). Fat diet was prepared by addition of pork fat to the pelleted diet. Rats in groups 2 and 5 drank diluted wine (Teran, Kras-Slovenia) in 8 v/w% concentration, and rats in group 4 drank diluted ethanol in the same concentration as wine. Food and liquid consumption was measured daily. Animals were weighed every four weeks. Average food and liquid consumption per one rat was calculated using appropriate statistical parameters (Table 1 and Table 2).

Table 1. Food regime for rats

Group number	Rat group	Dieting regimen
1	Control group	Control (pelleted diet with 10% fat EV[a] + tap water)
2	Fat diet + tap water	Fat diet (32% fat EV) + tap water
3	Standard diet + wine	Standard pelleted diet (10% fat EV) + wine (8v/w%)
4	Standard diet + ethanol	Standard pelleted diet (10% fat EV) + ethanol (8v/w%)
5	Fat diet + wine	Fat diet (32% fat EV) + wine (8v/w%)

[a] Energy value.

Table 2. Daily consumed energy value, fat and alcohol in tested meals

Rat group	Consumed food (g/day/rat)	Energy intake (kcal/day/rat)	Energy intake (kJ/day/rat)	Energy from fat (%)	Energy from alcohol (%)	Consumed alcohol (g/day/rat)
1 Control group	28.03	92.40	386.25	10.00	0.00	0
2 Fat diet + tap water	21.81	85.67	358.12	32.00	0.00	0
3 Standard diet + wine	23.21	95.08	397.46	8.04	16.26	2.21
4 Standard diet + ethanol	24.40	100.74	421.10	7.99	16.79	2.85
5 Fat diet + wine	17.22	81.43	340.38	26.92	14.02	1.63

1.3. Analytical methods

Burning values of samples were determined by adiabatic calorimeter (IKA-Calorimeter system, C4000A), where burning of certain amount of the component in the burning bomb at high oxygen surplus was performed.

Proteins were determined by Kjeldahl method. As a conversion factor, 6.25 was used to calculate protein content from nitrogen, according to PLESTENJAK and GOLOB (1993). Fats were determined by Soxhlet method (PLESTENJAK & GOLOB, 1993).

The calculation of all three parameters was based on the wet weight.

1.4. Statistics

Statistical analysis of the results was carried out using the Statgraphics Plus 4.0 package. The statistical characteristic of differences between the arithmetic means among groups of laboratory rats has been checked with the analysis of variance (ANOVA) and Duncan test for the following:

– amount of consumed food and liquid
– weight of skeletal muscles
– content of fats and proteins in skeletal muscles of rats
– burning value of skeletal muscles of rats.

2. Results and discussion

Weights of hind limb skeletal muscles were calculated for 100 g of total body weight. ANOVA test showed statistically significant differences in skeletal muscle weights between test groups. Values in test group, receiving fat diet and wine were 32% lower than in the control group ($P<0.001$) and 28% lower than in groups 3 and 4. A significant decrease in skeletal muscle weight in groups 3 and 4 ($P<0.001$) compared to the control group was observed.

Figure 1 shows that the lowest average weight was in group 5 (6.06 g). There was a highly significant difference ($P<0.001$) compared to groups 3 (7.99 g) and 4 (7.77 g), respectively. There was no statistically significant difference between groups 3 and 4. These results show that there is no difference in effects of ethanol or wine. Control group had significantly lower skeletal muscle weight than group 2 ($P<0.05$).

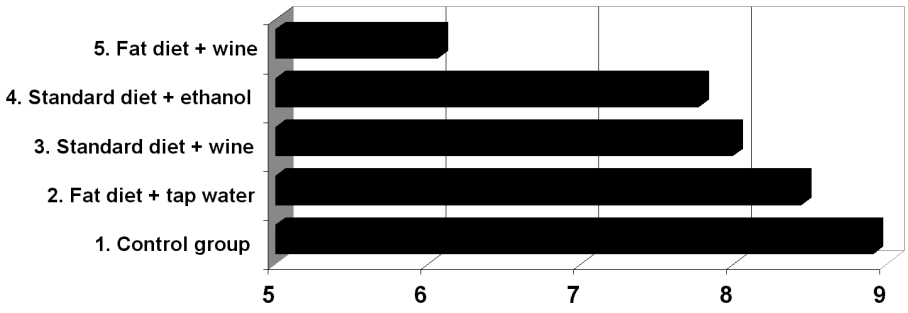

Fig. 1. Average rat hind limb skeletal muscle weights calculating per 100 g of body weight, g per 100 g

Results of skeletal muscle weights can be compared with clinical research data and studies on animal experimental models, which showed that atrophy in chronic alcohol myopathy is characterized by a 20% loss of the total skeletal muscle weight. Selectively diminished protein synthesis in type 2 muscle fibre is the primary cause of atrophy. Type 1 muscle fibres have greater antioxidative capacity (MARTIN et al., 1982; SLAVIN et al., 1983; TROUNCE et al., 1987; PREEDY et al., 1993; 1994b; FERNÁNDEZ-SOLÁ et al., 1996).

Deficiency of certain nutrients can also lead to muscle damage. MARTIN and PETERS (1985) found that hypocalaemia or magnesium deficiency can cause damage of muscle tissue. Figure 2 and Table 3 show that fat food has lower amounts of proteins, carbohydrates, dietary fiber and minerals compared with the standard diet. Figure 2 and Table 3 show that group 5 consumed less protein than other groups and also less minerals. Lower consumption could induce chemical changes of skeletal muscles. We can conclude that fat diet causes fat deposition in muscles and alcohol increases the

process of deposition. In test groups fed a standard diet and wine or ethanol, respectively, there was no noticeable fat deposition, as the dietary intake of fat was too low (only 10%).

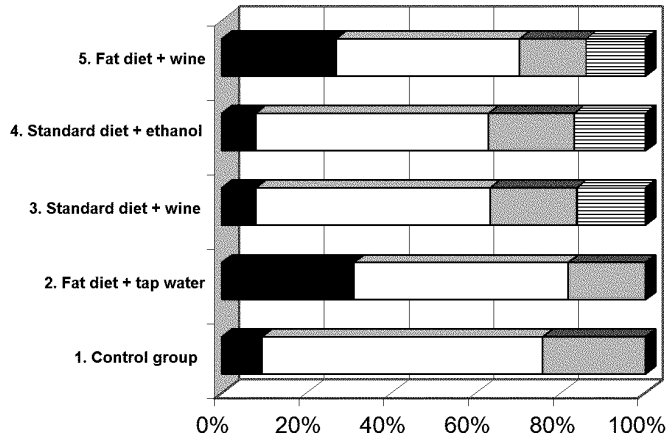

Fig. 2. Percentage of the distribution between the energy values of nutrients, %. ■: Fat; □: carbohydrate; ▨: protein; ▤: alcohol

Table 3. Composition of standard and fat diet

	Standard diet		Fat diet	
	KJ per 100 g	g per 100 g	KJ per 100 g	g per 100 g
Energy	1378		1642	
Protein	340	20.0	304	17.9
Fat	137	3.5	531	13.6
Carbohydrates	901	53.0	807	47.5
Calcium	1.2		1.07	
Phosphorus	1.0		0.9	
Sodium	0.03		0.026	
Dietary fiber	3.1		2.7	

Figure 3 shows that group 5 had the highest level of fat (7.41%) in skeletal muscles. If we compare test groups receiving standard diets and ethanol (3.98%) or wine (4.03%), there is a statistically significant difference ($P<0.001$). Statistical analysis showed significant differences ($P<0.001$) between the control group and groups 3 and 4, respectively. There was no significant difference in amounts of fat between groups 3 and 4. The protective (antioxidative) effect of wine was not proven.

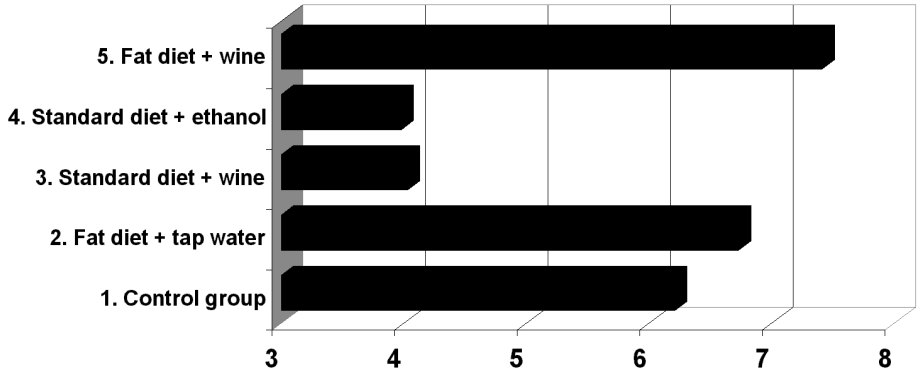

Fig. 3. Average values of fat content in rat skeletal muscles, g per 100 g wet sample

Amounts of fat in skeletal muscles was higher in group 5 (7.41%) than in group 2 (6.37%). We conclude that alcohol can accelerate the accumulation of fats in the muscles, but the difference is not statistically significant.

Group 5 had the lowest daily energy intake of nutrients, the lowest average weight of skeletal muscles (calculated for 100 g of body weight), but had the highest level of fat in the muscles. We conclude that differentiation of skeletal muscles is also caused by the reduction of nutrient absorption caused by alcohol.

Figure 4 shows results of the burning values of rat skeletal muscles. The highest values are in group 5 (780.74 KJ/100 g) and are also statistically significantly different ($P<0.001$) from the control group and groups 3 and 4. Groups 3 (636.03 KJ/100 g) and 4 (639.14 KJ/100 g) had the lowest burning values. Comparison of burning values between the control group (714.91 KJ/100 g) and groups 3 and 4 shows highly significant differences ($P<0.001$). Fat deposition into skeletal muscles is lower at lower fat intake in spite of the same alcohol intake. Alcohol diminishes nutrients absorption in the intestine, which consequently causes changes in skeletal muscles (MARTIN et al., 1982; MORGAN, 1982; MARTIN & PETERS, 1985).

Figure 5 shows that the protein content is significantly lower ($P<0.05$) in group 5 (20.33%) compared with the control group (20.76%) as described previously in the literature by PREEDY and co-workers (1994a; b) and ESTRUCH and co-workers (1995). In groups 3 (20.94%) and 4 (21.15%), the amount of protein was higher than in the control group, which can be explained with the dehydration effect of alcohol (REINUS et al., 1989). The calculation was based on the wet weight. There was no statistically significant difference in the protein content between groups 3 and 4, which leads to a conclusion that protective effect (antioxidative effect) of wine was not proven.

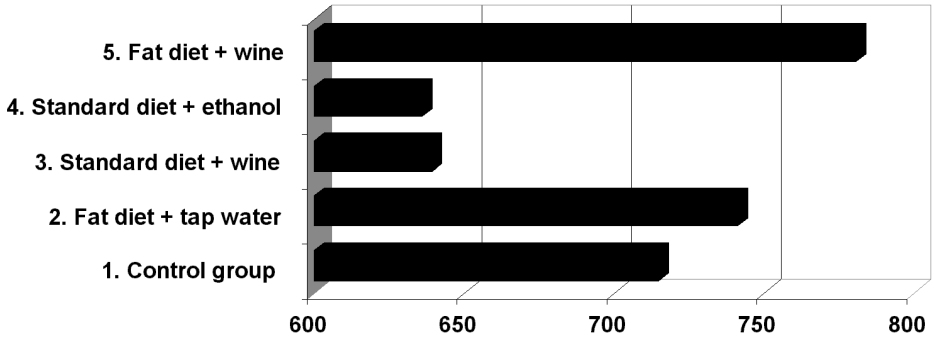

Fig. 4. Average burning values of rat skeletal muscles, kJ per 100 g of wet sample

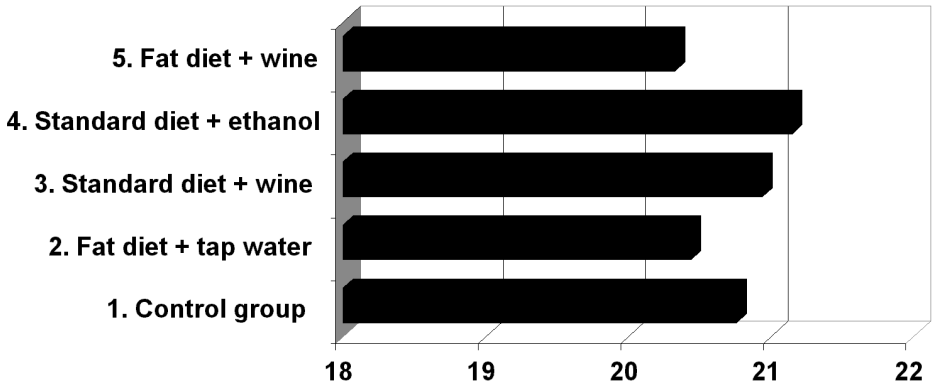

Fig. 5. Average values of protein content in rat skeletal muscles, g per 100 g of wet sample

3. Conclusions

In the group of rats fed fat diet (32% energy value) and wine higher amounts of fat in the skeletal muscles were found, which also had higher burning values compared to groups fed a standard pelleted diet (10% energy value) and wine or ethanol, respectively. We can conclude that the consumption of fat diet caused accumulation of fat in the muscles and alcohol stimulated that process. Poor protein supply was observed in group fed wine, and fat diet also contributes to lower growth of the muscle tissue and higher fat deposition. There was no significant difference between the wine and ethanol groups, regarding the effect on muscle changes.

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