Study No. T-6295.31; ST77
AN INVESTIGATION OF THE MECHANISM OF TOXICITY IN ADULT RATS EXPOSED TO HIGH LEVELS OF PERFLUOROOCTANESULFONATE (PFOS).

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Study Objective

The objective of this study was to investigate the hypothesis that Perfluorooctanesulfonate (PFOS) causes damage to muscle mitochondria, possibly leading to rhabdomyolysis and lactic acidosis at toxic exposure levels. This may explain the relatively rapid onset of toxicity and precipitous decline seen in prior rodent and primate toxicity studies as well as provide insight in to the mechanism by which PFOS decreases viability in the neonatal rat and mouse.

Methods Summary

This study was performed in the 3M Strategic Toxicology Laboratory under a defined protocol (1, 2) and classified as a "Class B Study" as explained in TOX SOP 0950, Strategic Toxicology Lab GLP Program Procedure (3). All in-life procedures were approved by the 3M Laboratory Animal Review Committee (3M LARC) and are detailed in animal usage application 2001-0182 (4). Details of the study methods can be found in the study protocol and protocol amendment (1, 2).

Briefly, adult male rats (12 per dose group) were administered either 400 mg/kg PFOS or vehicle (2% tween 80) via oral gavage on day zero of the study. The dose level of 400 mg/kg was chosen as it has been demonstrated to cause toxicity in previous rodent studies and, because this study was designed to investigate the mechanism of toxicity, a toxic dose was required. In the original study design, 6 rats per dose group were to be euthanized 24-hours post-dose and the remaining 6 rats per dose group were to be euthanized 48-hours post dose. However, a decision was made to humanely euthanize all animals at 24-hours post-dose due to signs of pain and distress (hunching and extreme lethargy) and severe diarrhea in treated animals. All animals were euthanized via CO2 asphyxiation. Plasma, liver, and kidneys were collected from each animal. In addition, urine samples from 6 rats per dose group were collected approximated 24-hours after dosing.

Plasma lactate/pyruvate ratios were examined as a marker of mitochondrial dysfunction. Urinary myoglobin, plasma myoglobin , and plasma creatine kinase (CK) were examined as markers of muscle damage. Due to limited sample size, a decision was made to eliminate the clinical chemistry (cholesterol, blood urea nitrogen (BUN), and potassium) and PFOS analysis from the original study design. No analysis of liver or kidneys was performed.

All results were compared between treated and control animals and analyzed to determine statistical differences using the students T-test.

Results

As expected, weight loss was observed in all animals, regardless of treatment, following dosing. Treated animals, however, lost significantly more weight than control animals ($P \leq 0.01$) and exhibited signs of diarrhea (Table 1 and Figure 1). Liver weight was significantly increased in treated rats ($P \leq 0.01$) (Table 1 and Figure 2). All values for plasma and urinary myoglobin were below the detection limit of 1ug/L and 1 mg/L, respectively (Table 1). Plasma lactate (measured as lactic acid) was significantly lower in treated animals than in control animals ($P \leq 0.01$)

(Table 1 and Figure 3), while no significant difference was observed in plasma pyruvate levels (Table 1 and Figure 4). Due to the decreased plasma lactate levels observed in treated animals, the plasma lactate/pyruvate ratio was significantly lower ($P \leq 0.01$) in treated animals (Table 1 and Figure 5). Plasma CK levels were significantly increased in treated animals as compared to control animals ($P \leq 0.01$) (Table 1 and Figure 6).

Discussion

The diagnosis of rhabdomyolysis is made primarily from measurement of circulating CK. A rise in CK is usually observed 2-12 hours after muscle injury with peak concentrations occurring within 1-3 days and a decline after 3-5 days of injury. Serum myoglobin usually increases before a rise in CK and drops more rapidly than does the concentration of CK. Myoglobinuria is also expected with rhabdomyolysis. The role of myoglobin is to store and carry oxygen and thus maintain the ability of the muscles to contract. If the concentration of myoglobin in the urine is high, the urine may take on a red-brown or cola colored appearance (6).

In the current study, significant elevations in plasma CK were observed in treated animals. This effect has also been observed in previously performed PFOS and ammonium perfluorooctanoate (APFO) primate studies. Despite this evidence of muscle damage, elevations of urinary or serum myoglobin could not be observed because all measured values in treated and control animals were below the limit of detection. Possible explanations are that, due to limited sample size, it was not possible to accurately measure myoglobin levels or that a rat-specific assay is necessary. The assay used in the current study was not specific for the rat, but developed for use with human samples. In addition, the sample collection may have occurred after an initial rise and fall in circulating serum myoglobin levels. However, the urine of treated rats in the current study did not take on a red-brown color as would be expected with increased levels of urinary myoglobin.

Blood lactate/pyruvate ratios are widely used as a noninvasive test for the detection of mitochondrial toxicity (7). Despite previous observations (see study background in the protocol) suggesting that PFOS may cause mitochondrial toxicity, the lactate/pyruvate ratios measured in this study do not support this hypothesis. In addition, the decreased lactate levels observed in this study do not support the hypothesis that PFOS treatment leads to lactic acidosis. Despite this, it is obvious that irritation/stress occurs in PFOS treated animals that cause elevations in circulating levels of CK. It is possible that the animals in this study had not yet progressed to frank rhabdomyolysis, and that if sampled at a later time point, would have shown increased lactate and lactate/pyruvate ratios as expected.

Conclusions

The results of this study show that PFOS causes an increase in plasma CK levels prior to overt moribundity. This supports the hypothesis that muscle damage occurs in PFOS treated rats. Because neither urinary nor plasma myoglobin could be detected in control or treated samples, no conclusions can be drawn and further studies are required to investigate these endpoints as well as to explain the increased CK levels observed in this and other PFOS and APFO studies. Future studies should, if possible, utilize a rat-specific assay for urinary and serum myoglobin. Although expected increases in lactate and lactate/pyruvate ratios were not observed, further

studies are required to investigate these endpoints and to determine if PFOS causes damage to muscle mitochondria, possibly leading to rhabdomyolysis and lactic acidosis at toxic exposure levels. It is possible that the animals in this study had not yet progressed to frank rhabdomyolysis. It may be more appropriate to investigate these endpoints by dosing at lower levels over a period of days to reach the same cumulative dose; thus allowing for the progression to a state of overt rhabdomyolysis.

List of Tables and Figures:

Table 1: Raw Data											
Animal number 13XX	dose group (mg/kg PFOS)	Body Weight day 0 (dosing)	Body Weight day 1 (24 Hr Post Dose)	Body Weight Change	Liver weight (g)	Urinary Myoglobin (mg/L)	plasma lactate (mmol/L)	plasma pyruvate (mmol/L)	lactate / pyruvate	plasma creatine kinase (U/L)	plasma myoglobin (ug/L)
65	0	248	232	-16	9.5	NA	6.1	0.02	305	129	<1
66	0	240	231	-9	9.3	NA	6.7	0.02	335	110	<1
67	0	220	203	-17	8.0	NA	6.5	0.02	325	88	<1
68	0	227	221	-6	9.3	NA	8.1	0.03	270	132	<1
69	0	233	216	-17	8.6	NA	5.8	0.02	290	100	<1
70	0	222	207	-15	8.0	NA	5.3	0.01	530	127	<1
71	0	230	207	-23	9.4	<1	4.3	0.02	215	84	<1
72	0	253	229	-24	7.8	<1	6.0	0.04	150	102	<1
73	0	241	218	-23	9.0	<1	7.4	0.02	370	103	<1
74	0	236	211	-25	9.0	<1	7.7	0.02	385	110	<1
75	0	236	212	-24	9.0	<1	6.3	0.04	158	86	<1
76	0	219	199	-20	8.2	<1	NA	NA	NA	77	<1
77	400	249	223	-26	9.7	NA	5.9	0.02	295	109	<1
78	400	242	218	-24	10.1	NA	3.7	0.02	185	NA	NA
79	400	240	213	-27	9.5	NA	4.4	0.02	220	154	<1
80	400	239	214	-25	9.4	NA	3.5	0.04	88	329	NA
81	400	242	217	-25	8.7	NA	4.3	0.02	215	77	<1
82	400	232	210	-22	10.0	NA	5.7	0.02	285	391	<1
83	400	250	222	-28	9.2	<1	1.4	0.02	70	264	<1
84	400	220	192	-28	8.7	<1	5.3	0.03	177	NA	NA
85	400	242	215	-27	9.5	<1	7.0	0.03	233	1112	<1
86	400	213	190	-23	8.7	<1	6.2	0.02	310	NA	NA
87	400	246	217	-29	10.2	<1	5.5	0.03	183	NA	NA
88	400	230	206	-24	8.6	<1	NA	NA	NA	NA	NA

Figure 1: Body Weight Change (Day 0 - Day 1)

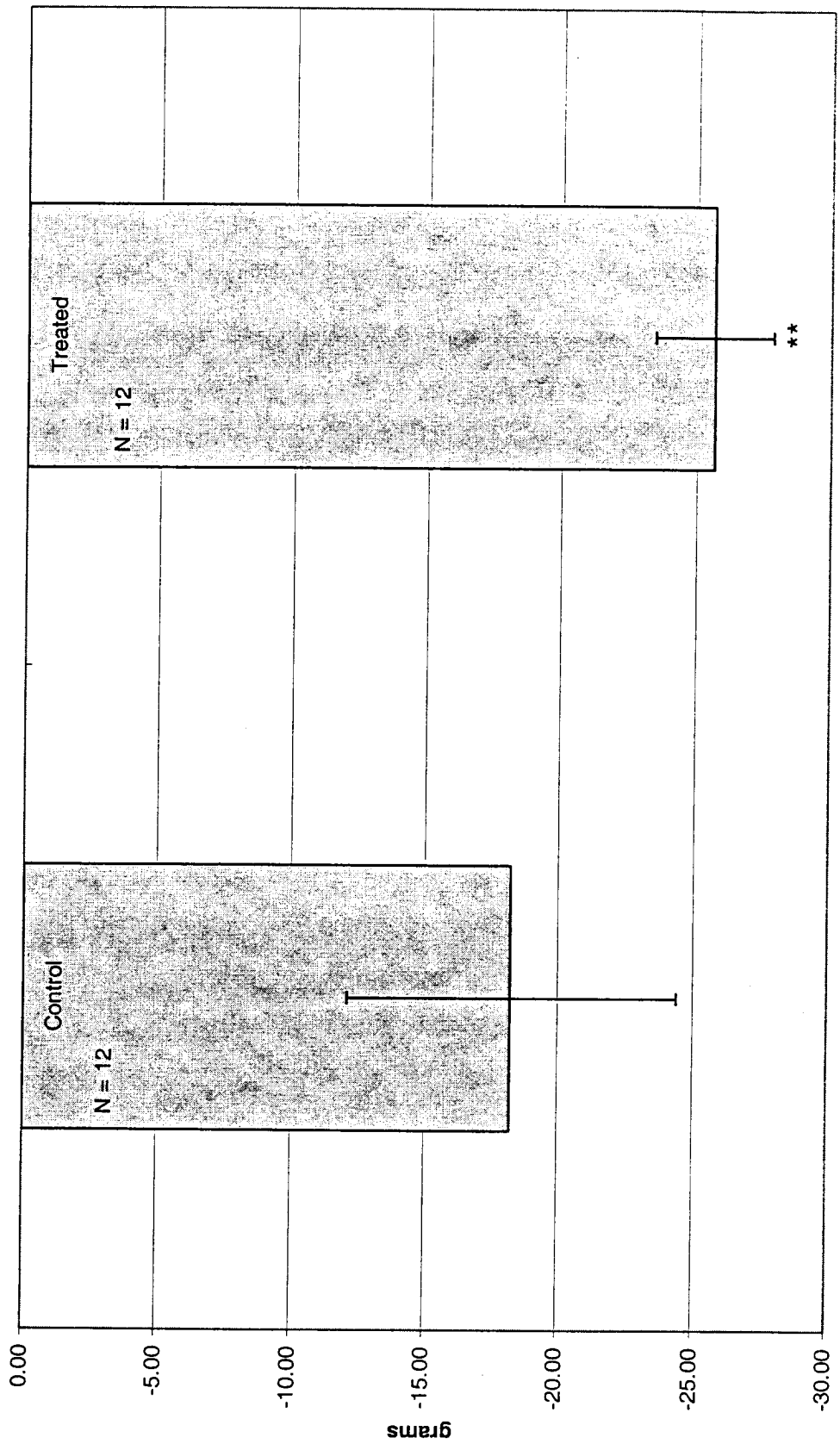


Figure 2: Liver Weight

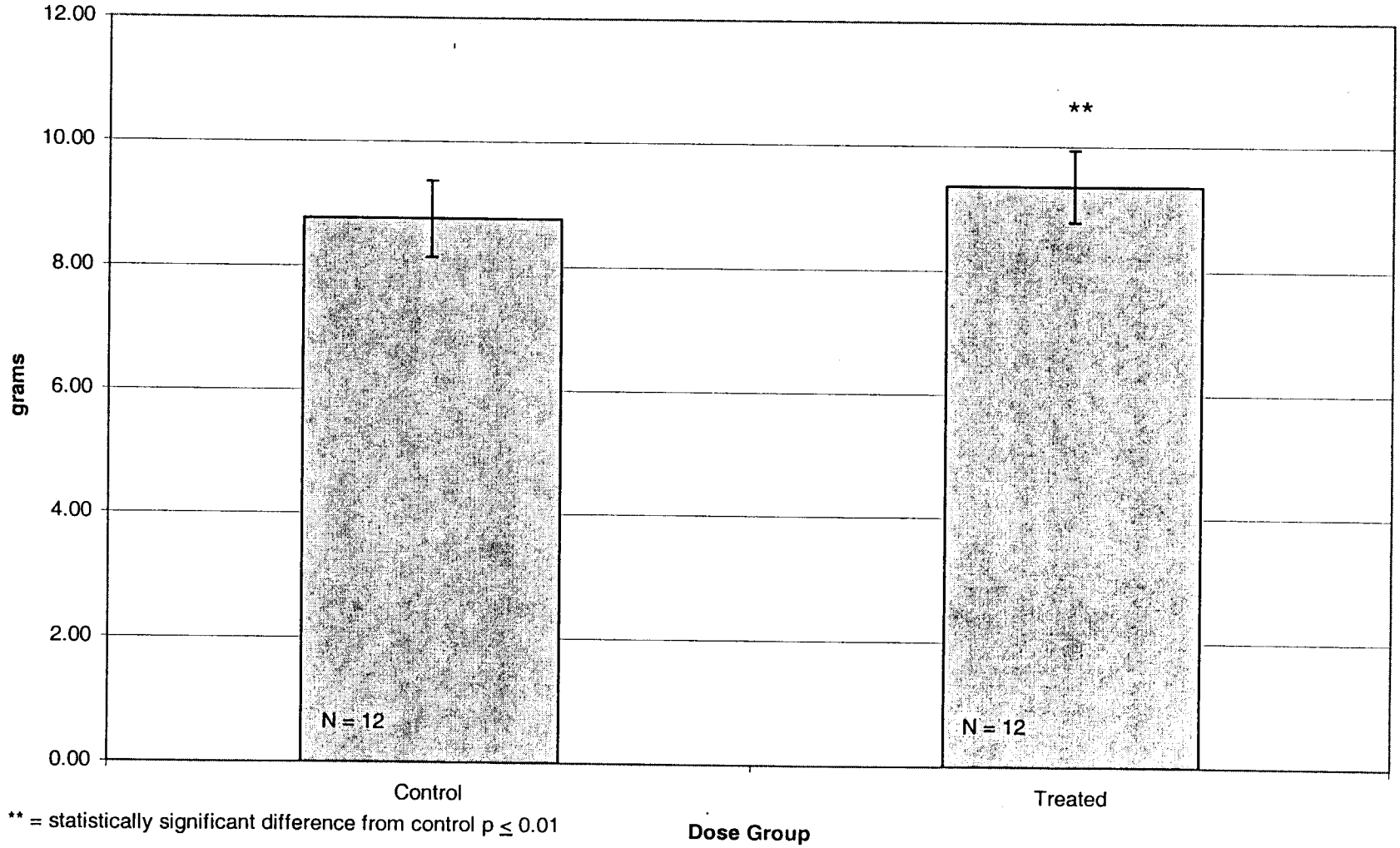


Figure 3: Plasma Lactate

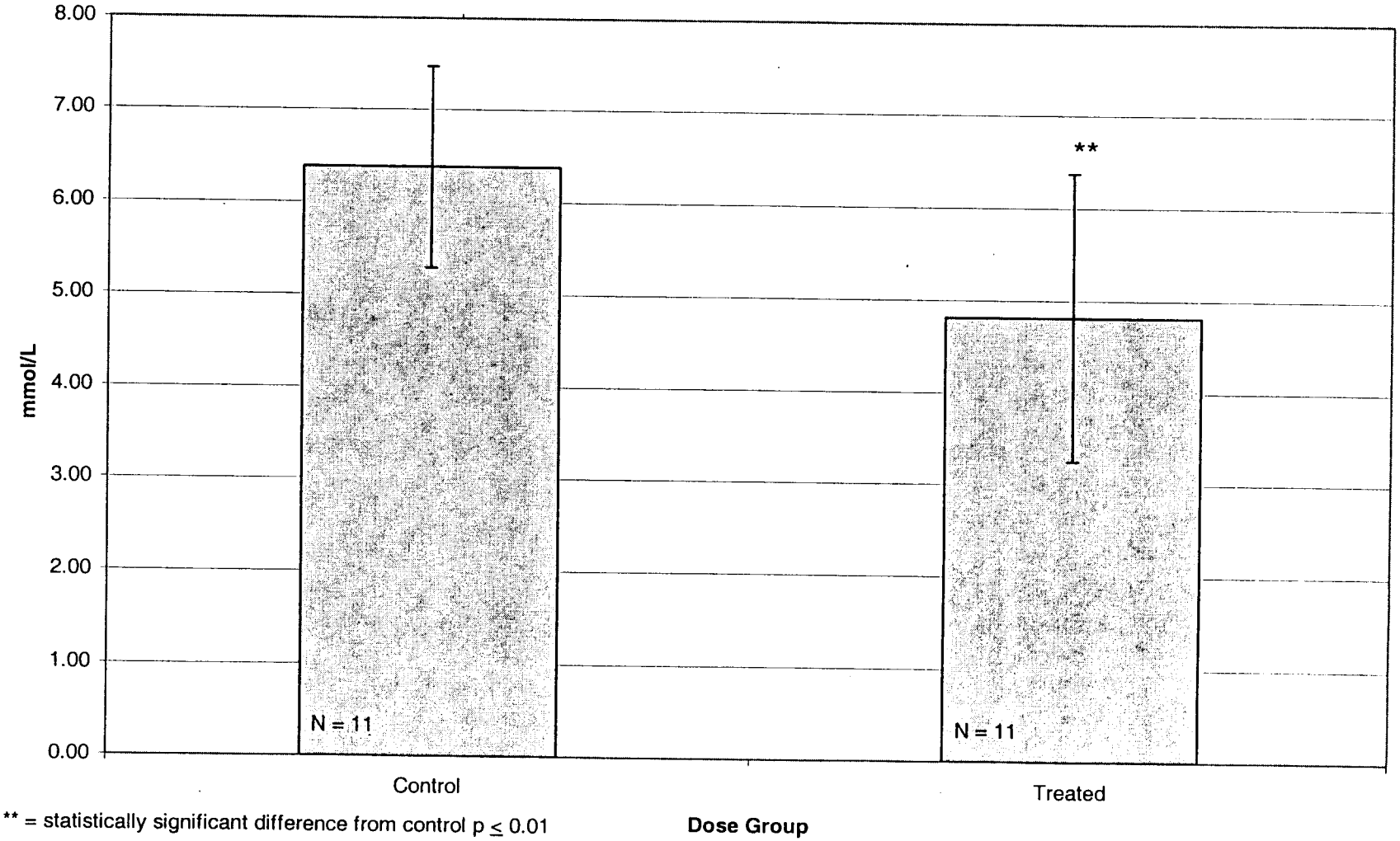


Figure 4: Plasma Pyruvate

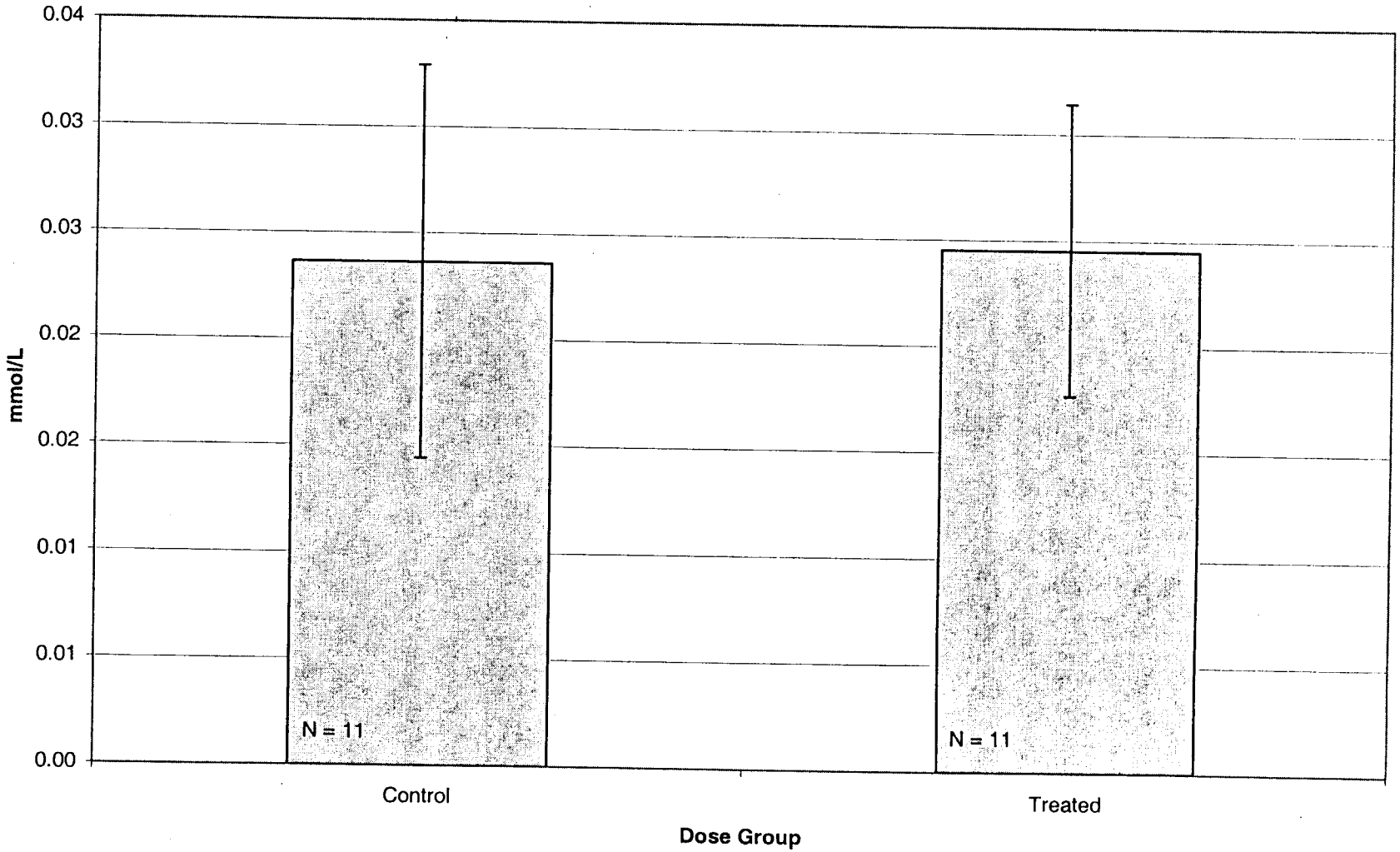


Figure 5: Plasma Lactate/Pyruvate

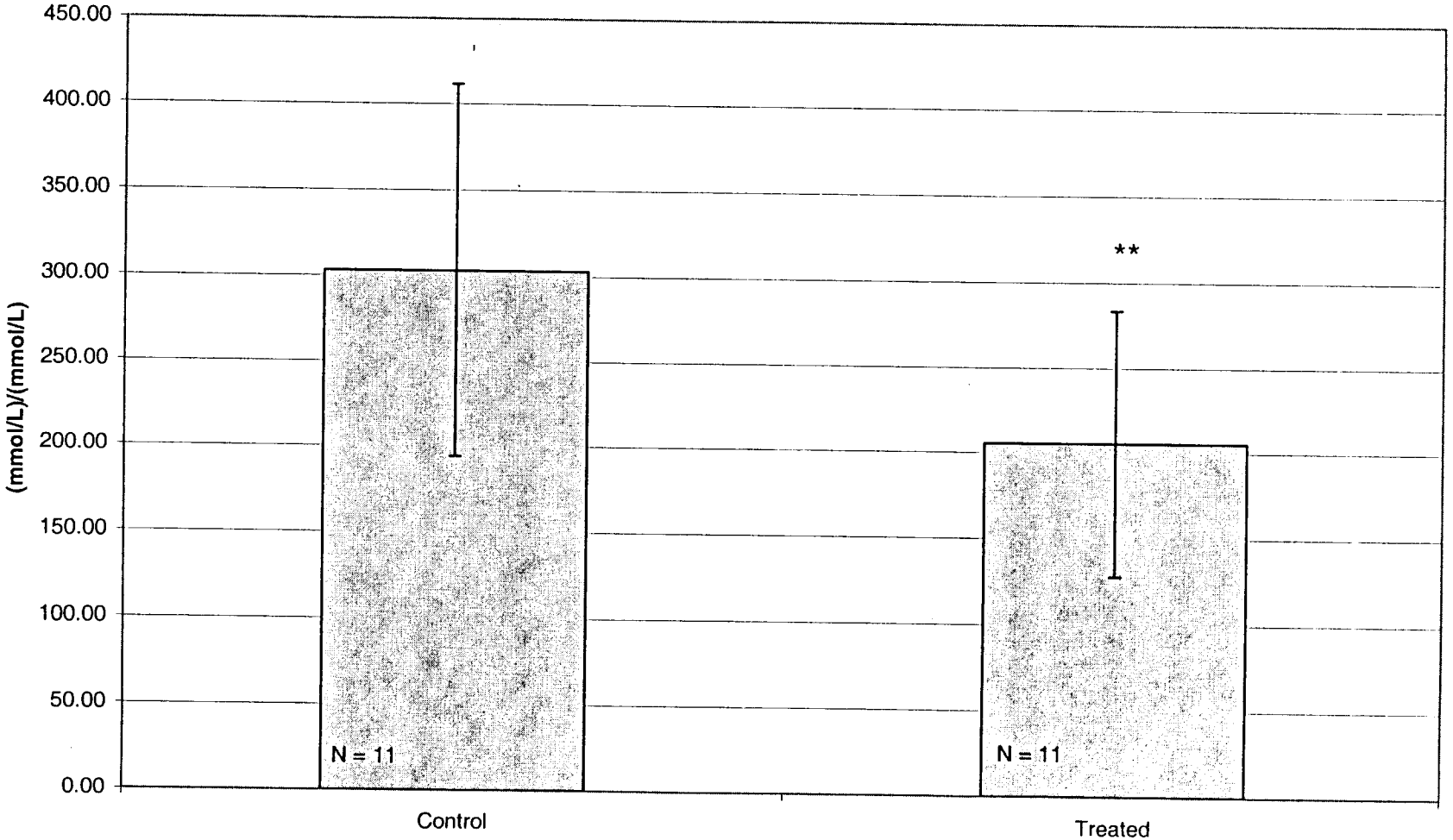
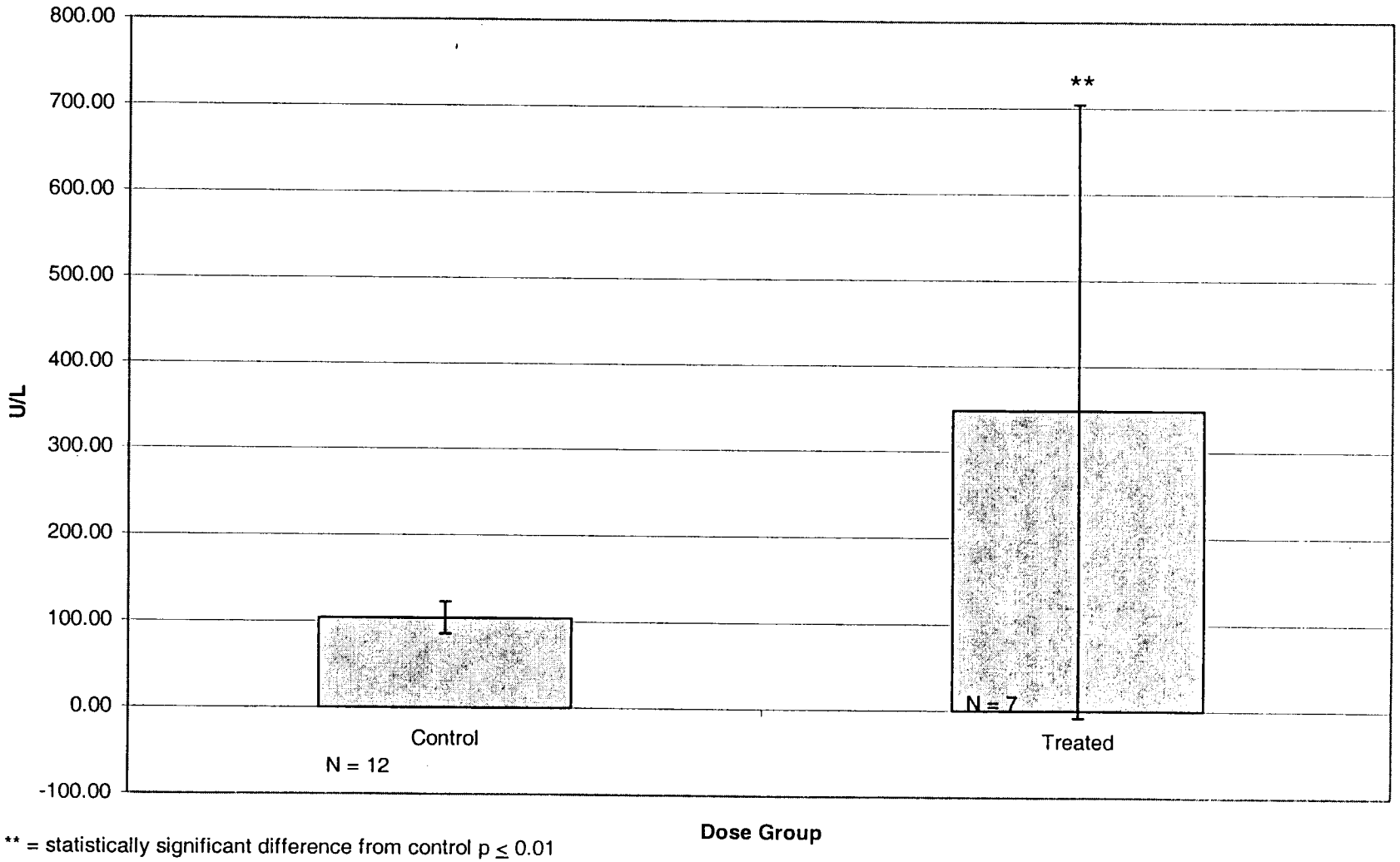


Figure 6: Plasma Creatine Kinase



** = statistically significant difference from control $p \leq 0.01$

References

1. 3M Medical Department, Corporate Toxicology Protocol for Study No. T-6295.31 ST-77.

2. An investigation of the mechanism of toxicity in adult rats exposed to high levels of Perfluorooctanesulfonate (PFOS), 2001.

3. 3M Medical Department, Corporate Toxicology Amendment #1 for Study No. T-6295.31 ST-77. An investigation of the mechanism of toxicity in adult rats exposed to high levels of Perfluorooctanesulfonate (PFOS), 2001.

4. 3M Medical Department, Corporate Toxicology Strategic Toxicology Laboratory Standard Operating Procedure (TOX SOP) No. 0950 – GLP Program Procedure, 1999.

5. 3M Lab Animal Review Committee Animal Usage Application No. 2001-0182, MECHANISTIC RESEARCH IN TIMED PREGNANT PREGNANT RATS AND MICE – AMENDMENT TO AUA # 2001-0182, 2001.

6. Omar M.A., Wilson J.P., and Cox T.S. (2001) Rhabdomyolysis and HMG-CoA reducatse inhibitors. Annals of Pharmacotherapy 35, 1096-1107.

7. De Pinieux G., Chariot P., Ammi-Said M., Louarn F., Lejonc J.L., Astier A., Jacotot B., and Gherardi R. (1996). Lipid-lowering drugs and mitochondrial function: Effects of HMG-CoA reductase inhibitors on serum ubiquinone and blood lactate/pyruvate ratio. Br J Clin Pharmacol 42: 333-337.

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