Ammonia and Lactate Response to Leg Press Work at 5 and 15 RM

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Reference Data

ABSTRACT
Plasma ammonia (NH$_3$) and lactate (La) concentrations were measured before and after shorter and longer (5-RM at 15-20 sec vs. 15-RM at 40 sec) leg press tasks. Ten young men experienced in resistance training took part in both conditions of the investigation. NH$_3$ and La samples were taken pre, 5 min after the warm-up (W-up), and at Sets 1 and 3 of each condition. The 3 sets were each separated by a 6-min rest period. NH$_3$ had a significant interaction between condition and measurement occasion. Plasma NH$_3$ levels were not significantly increased at any measurements in the 5-RM (repetitions max) condition. At 15-RM the NH$_3$ levels were significantly greater after Set 3 than at the Pre and W-up measurements. Also, the concentration of NH$_3$ following Set 3 was greater at 15-RM than at 5-RM. These data support the hypothesis that the purine nucleotide cycle was activated more at 15-RM than at 5-RM. La levels were significantly greater after Sets 1 and 3 for the 15-RM than the 5-RM condition. Furthermore, plasma La concentrations increased progressively and significantly at every measurement for both conditions (Pre < W-up < Set 1 < Set 3). Changes in plasma NH$_3$ and La concentrations did not parallel one another in either condition.

Key Words: purine nucleotide cycle, anaerobic metabolism, resistance exercise

Introduction
During resistance activities there is typically a rapid turnover of adenosine triphosphate (ATP) (1, 3, 15). Anaerobic activity cannot be sustained indefinitely when ATP resynthesis is less than its degradation (12). This imbalance between ATP utilization and synthesis is evidenced by the ATP-to-adenosine diphosphate ratio (ATP:ADP) at rest (e.g., ~8.4) being markedly greater than after fatiguing isometric work (e.g., ~4.9) (12). One metabolic process that partially offsets this decrease in the ATP : ADP ratio is the myokinase (MK) catalysis of two ADPs to form ATP and adenosine monophosphate (AMP), as shown in Reaction 1:

\[ \text{ADP + ADP} \rightleftharpoons \text{ATP + AMP} \]

Reaction 1 exists in equilibrium, however, and an accumulation of AMP can see it move toward the left and not the right. Such a leftward shift would remove valuable ATP from the system and further reduce the ATP : ADP ratio. To maintain the desired direction of Reaction 1, and thus help sustain high intensity work for longer (19), AMP is deaminated to inosine monophosphate (IMP) and NH$_3$ via the purine nucleotide cycle (PNC) (4, 21), as shown in Reaction 2:

\[ \text{AMP + H}_2\text{O} \rightleftharpoons \text{IMP + NH}_3 \]

This reaction, which is catalyzed by adenylate deaminase, produces a significant reduction in the total adenine nucleotide pool (12, 21). Most of these adenine nucleotides are not lost indefinitely, as the anabolic element of the PNC sees the reconstitution of AMP following exercise (4, 17).

Schlick et al. (23) reported that both intensity and duration of sprinting appeared to influence the level of plasma NH$_3$. That is, the PNC was not activated until there was rapid cycling of ATP and a marked depletion of anaerobic substrate (18, 20, 22). There is some debate as to whether it is the depletion of creatine phosphate (CP) and/or glycogen reserves that are critical to PNC activation. Sahlin and Broberg (20) suggested PNC activation would not occur without a 40% reduction in CP reserves. Norman et al.'s (18) data implicated significant glycogen depletion in PNC activation during submaximal exercise. The role played by the depletion of each anaerobic substrate has been partially obscured by differential concentrations of adenylate deaminase in various fiber types (FTb > FTa > ST) (4, 16), and differential patterns of glycogen depletion during activities of varying intensity (1, 2).

An understanding of the metabolism associated with various repetition structures used in bouts of resistance activity may allow strength and conditioning specialists, coaches, and sport scientists to better tailor resistance activity prescriptions to meet each individual's needs.

The purpose of this investigation was to determine whether plasma NH$_3$ levels were increased by two common resistance training routines: 3 sets of 5- or 15-RM.
Resistance activity is associated with rapid ATP cycling (1, 3). But it is not clear whether both 5-RM sets at 15-20 sec and 15-RM sets at 40 sec would be long enough to deplete anaerobic substrate and activate the PNC. This was suggested by MK activity (Reaction 1) increasing after training that utilized longer but not shorter (e.g., 2 × 30 sec vs. 10 × 6 sec) bouts of isokinetic work (6, 14). Consequently it was hypothesized that plasma NH$_3$ levels would be increased after 3 sets of 15-RM but not 3 sets of 5-RM leg press work. The second purpose of this investigation was to determine whether changes in plasma La and NH$_3$ levels were parallel.

Methods

Subjects
Ten active young men with a history of recreational resistance training volunteered to participate in this investigation. All completed both levels of the independent variable, that is, the 5- and 15-RM conditions. Subject characteristics (mean ± SEM) were: age 20.2 ± 0.5 yrs; Ht 179.5 ± 2.1 cm; Wt 79.1 ± 2.8 kg; 5-RM leg press strength, 255.0 ± 11.1 kg; 15-RM leg press strength, 214.0 ± 11.6 kg.

Participation was conditional on written informed consent. The experiment conformed with the Australian National Health and Medical Research Council guidelines and was approved by the Medical Research Ethics Committee of The University of Queensland. Testing and analyses were conducted in the gym and the Department of Human Movement Studies at The University of Queensland.

Design
This experiment involved 3 phases and 4 days of testing over a total of 17 days. The familiarization phase was followed at 5 daily intervals by pretesting and testing phases. The testing phase involved 2 measurement occasions 1 week apart and saw the subjects complete either 3 sets of 100% 5-RM or 100% 15-RM leg press work, as follows (mean ± SEM):

- Set 1 at 5-RM = 1,275.0 ± 55.4 kg
- Set 1 at 15-RM = 3,210.0 ± 173.3 kg
- Set 3 at 5-RM = 3,825.0 ± 166.3 kg
- Set 3 at 15-RM = 9,630.0 ± 519.8 kg

During the familiarization phase, the subjects were instructed in the 45° leg press task and undertook several sets of work approximating 5- and 15-RM loads. At pretest the 5- and 15-RM were determined. On each of the 2 testing days, capillary blood samples were drawn. The first sample was taken after subjects sat for 10 min upon arriving at the lab (Pre). The second sample was taken 5 min after the warm-up (W-up), followed 1 min later by 3 sets of leg press activity. Each set was separated by a 6-min recovery. Capillary blood was also drawn 5 min after Sets 1 and 3. Pilot work had revealed near-peak plasma NH$_3$ levels 5 min following leg press work. This was consistent with other anaerobic activities such as sprint running (23). Subject assignment to the 5- and 15-RM conditions was counterbalanced to minimize any order effect.

Procedures
The leg press task was completed on a 45° leg press machine (Calgym, model 172). Although the men were familiar with the leg press task, the following points were emphasized and monitored during all phases of the experiment: (a) Their feet were located in the center of the leg press plate, shoulder-width apart and with an out-toeing of no more than 30°. (b) The movement was rhythmic and even in that the eccentric phase was controlled, lasted approximately 2 sec, and there were no intra- or inter-repetition pauses. (c) The depth of leg flexion was constant and limited to 90°. (d) Finally, to minimize any confounding influence of upper body isometric contraction on NH$_3$ and/or La concentrations, arms were raised above the head with hands open and relaxed.

The order in which 5-RM and 15-RM were determined at pretest was randomized between subjects. While both repetitions maximum were determined on the 1 day, there were 10-min recovery intervals between all trials. The initial weight selected was 100% of body weight. Mass was increased by 40 kg on each effort until approximately 250% body mass or the subject encountered difficulty. At this point smaller load increments were made until the particular RM was obtained, usually within 5 lifts. As reported elsewhere, it was highly unlikely that the preceding lifts would have adversely affected the final RM value (24).

The warm-up prior to testing involved stretching and 3 sets of 5 or 15 leg press repetitions, respectively, for the 5- and 15-RM conditions. The intensity of the sets was 60, 70, and 80% of the RM load to be lifted in that session. A 3-min recovery followed the 1st and 2nd sets, and there was a 6-min recovery between the 3rd warm-up set and the 1st experimental trial.

Capillary blood was drawn from the fingertip. In all, 50 μl was collected in a heparinized capillary tube. Samples were immediately refrigerated (4°C). They were spun down (8,000 rpm for 5 min) within 60 min of collection in a Jouan centrifuge (Hema-C). Only the plasma portion of the sample was used in subsequent analyses. Two 10-μl plasma lots were placed on the NH$_3$ and La reagent slides, which were in turn fed into a Kodak Ecktachem DT analyzer (model DT60) for analysis. Calibration trials were conducted before and after every 3 trials.

Statistical Analysis
Means (±SEM) for plasma NH$_3$, and La were determined for the 5- and 15-RM conditions at Pre, W-up, and Sets 1 and 3 measurements. The NH$_3$ and La data were compared with 2 × 4 (5-RM/15-RM conditions × measurement occasion) ANOVA with repeated measures on both
factors. An alpha level of 0.05 was adopted. Paired t-tests were used in subsequent post hoc analyses because of the repeated nature of the measurements (13).

Results

The interaction between condition and measurement occasion for the NH₃ ANOVA was significant ($F = 2.98$, $p = 0.039$). In the 5-RM condition, NH₃ levels were not significantly increased at any measurement (Figure 1). But at 15-RM the NH₃ levels after the 3rd set were significantly greater than at Pre and W-up measurements (Figure 1). Furthermore, the difference in NH₃ level between the 5- and 15-RM conditions was significant following Set 3.

The interaction between condition and measurement occasion for La was statistically significant ($F = 22.53$, $p = 0.0001$). In both the 5- and 15-RM conditions there were significant increments in La from Pre, through W-up, through the 1st and 3rd sets (Pre < W-up < Set 1 < Set 3) (Figure 2). Furthermore, following both Sets 1 and 3, plasma lactate levels were significantly greater at 15-RM than at 5-RM.

Discussion

Plasma NH₃ levels of recreational resistance trainers were elevated by the longer (15-RM) but not the shorter (5-RM) sets of maximal leg press work (Figure 1). This was attributed to the 15-RM set structure reducing anaerobic substrate and pH enough to activate the purine nucleotide cycle. NH₃ levels were higher after the 3rd set than the 1st set. This may have been due to glycogen depletion and/or reductions in muscle pH increasing adenyate deaminase activity. The La data were consistent with such explanations (Figure 2). Significant increments in plasma La were evident at every measurement in both the 5- and 15-RM sets, reinforcing the notion that significant changes in plasma La and NH₃ levels are not necessarily parallel.

Plasma NH₃ levels following sprint running appear to be a function of the interaction between task intensity and duration (8, 20, 23). Of interest was whether these two preconditions were present during some or all patterns of resistance activity. One index of resistance task intensity is the level of FT fiber recruitment.

Two lines of evidence indicate that FT fibers are recruited during resistance activities similar to those in this investigation. First, preferential depletion of glycogen has been reported from FT fibers during moderate to high intensity resistance activity (5, 9). Second, FT fiber hypertrophy following a variety of resistance training programs is well documented (1). Both the 5- and 15-RM conditions would be expected to be intense enough to activate the PNC (1). Longer resistance tasks (e.g., 240 sec of isokinetic work) do not necessarily exhaust glycogen reserves (20% depletion) (25).

The extent of CrP depletion, however, appears to be influenced by the organization and modality of resistance activity (6, 10, 12). For example, isokinetic training involving prolonged efforts at $2 \times 30$ sec increased MK activity (Reaction 1) while shorter work bouts at $10 \times 6$ sec did not (6). This suggests that bouts of resistance activity longer than approximately 30 sec would activate the PNC. Consistent with this was the finding that IMP concentrations were significantly elevated following three 30-sec bouts of isokinetic work at 3.12 rad sec⁻¹ (11).

Our data were consistent with the notion that the appearance of plasma NH₃ after resistance activity is a function of the interaction between exercise intensity and duration. That is, in the 15-RM condition it is likely
there was sufficient CrP utilization and acidosis to activate the PNC and thus increase plasma NH\textsubscript{3} levels. In contrast, the 5-RM condition did not last long enough to activate the PNC. Further research is required to determine (a) whether the PNC is significantly activated for repetitions maximum between 5- and 15-RM, and (b) whether NH\textsubscript{3} response to an acute bout of resistance activity is influenced by factors such as training or diet.

NH\textsubscript{3} levels were greater following the 3rd set than the 1st set. Sprint running produced a similar effect (23). One explanation is that intramuscular pH was lower in the 3rd set and that this new metabolic environment enhanced adenylate deaminase activity (Reaction 2). The optimal pH for adenylate deaminase activity is 6.2 (4). The La data would suggest that by the 3rd set of leg press activity the pH would have been lower than in the earlier stages of the experiment. Clearly this explanation requires verification.

The significant increments in La levels for the 5- and 15-RM conditions were due to glycogenolysis (15). Glycogenolysis associated with resistance activities can cause significant glycogen depletion (5, 9). The La levels were greater at 15-RM. This may be partly explained by the longer duration and greater mass-set load at 15-RM than at 5-RM. In addition, the increased AMP production at 15-RM as a result of Reaction 1 may have also stimulated greater glycogenolysis (4).

In both conditions, La levels were greater after the 3rd than after the 1st set. This effect had been noted following sprint and resistance exercise (7, 23) and may again be partly explained by more glycolytic work being required to complete 3 sets than 1 set. Furthermore, the warm-up caused significant increments in La for both conditions. In absolute terms, however, the increments were small and similar in magnitude for both conditions. Thus the warm-up was not thought to cause the differences in La or NH\textsubscript{3} responses between both conditions. Finally, increments in plasma La preceded increases in NH\textsubscript{3}, as has been reported previously for submaximal and supramaximal exercise (23).

In summary, plasma NH\textsubscript{3} was increased by 1 and 3 sets of 15-RM leg presses. This was thought to be due to PNC activation following significant anaerobic substrate utilization, causing reductions in pH. Conversely, insufficient anaerobic substrate depletion was thought to be the reason for little change in NH\textsubscript{3} in response to 5-RM work. Plasma La response for the 5- and 15-RM conditions did not parallel changes to NH\textsubscript{3}. Finally, the plasma NH\textsubscript{3} and La concentrations at any instant were a function of their preexisting levels, production, translocation from the muscle, elimination, and/or utilization. Consequently, the interpretation of elevated plasma NH\textsubscript{3} and La levels was problematic, beyond stating that related metabolic events were occurring or had recently occurred. Therefore we would encourage further research, preferably intramuscular research, to test our conclusions.

**Practical Application**

Resistance training is used in a variety of ways by athletes to increase strength, power, and size. Understanding the acute responses and chronic adaptations to various forms of resistance training will see its benefits maximized. Purely at a metabolic level, it appears that 15-RM sets activate the purine nucleotide cycle. Consequently, RM loads of longer duration (15-RM) may be appropriate for athletes training for skills that involve significant activation of the PNC, while RM loads of shorter duration (5-RM) may be best for skills involving no significant activation of the PNC.

**References**


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