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Original paper

# Adaptations to skeletal muscle with endurance exercise training in the acutely fed versus overnight-fasted state

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We would like to dedicate this paper to the memory of our friend, colleague and co-author, Dr Hans Edge, who was tragically killed on the 25th March 2010 in Auckland whilst riding his bicycle home from work.

**Abstract**

Minimising carbohydrate (CHO) status in the peri-training period may accelerate the training adaptations normally observed. The aim of this study was to compare adaptations to endurance training undertaken in the acutely CHO fed and overnight-fasted states. Eight female and six male untrained, healthy participants: aged 26.6 ± 5.8 years (mean ± SD); height 174.7 ± 7.6 cm; weight 75.3 ± 11.4 kg; $VO_{2max}$ 3.48 ± 0.67 l/min; were randomly divided into two training groups and undertook four weeks of five days per week endurance cycle ergometer training in either the overnight-fasted (FAST) or acutely fed (FED) state. FAST training had no effect on RER or plasma glucose, lactate and FFA concentrations during subsequent submaximal exercise. Training-induced changes in *Vastus lateralis* citrate synthase (CS) and 3-hydroxy-CoA dehydrogenase (HAD) activities were not different between training groups ($P = 0.655$ and 0.549, respectively), but when the effect of gender was considered, men responded better to FAST and women responded better to FED. The FAST group showed a significantly greater training-induced increase in $VO_{2max}$ and resting muscle glycogen concentration than FED ($P = 0.014$ and $P = 0.047$ respectively), but there was no gender interaction. In conclusion, these results suggest that (a) meal ingestion prior to daily exercise can modify some of the exercise training-induced adaptations normally seen with endurance training compared to when daily exercise is undertaken in the overnight-fasted state; and (b) the extent of these adaptations in skeletal muscle differ slightly between men and women.


*Keywords:* Carbohydrate; Citrate synthase; Skeletal muscle glycogen; RER; Cycling; Gender

## 1. Introduction

Carbohydrate (CHO) ingestion both before *and* during prolonged exercise can improve endurance performance. The weight of research evidence on this topic has seen organisations such as the American College of Sports Medicine and the American Dietetic Association recommend the timing and dose of CHO intake before, during and after exercise.[1] These recommendations have been extrapolated to training by athletes and advisors alike, to encourage CHO intake in the peri-training period.[2] Consequently, there are now many carbohydrate-based sports nutrition supplements which are available and encouraged for use during training, yet there is little scientific evidence that these improve or accelerate the training response.

To ensure the most potent training stimulus, it has been argued that many training sessions should be conducted in the least performance-enhancing situation.[3] Therefore recommendations aimed at ensuring optimal performance in a single bout of exercise, such as maximising CHO intake and absorption, may not be advantageous during training. Using reverse logic, exercise training when carbohydrate status is

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reduced, such as in the overnight-fasted state, should maximise the training stimulus and promote more rapid adaption.

To investigate how endurance training adaptations may be modulated by training in an overnight-fasted state, De Bock et al.[4] and Nybo et al.[5] have trained previously untrained participants for six and eight weeks, respectively, in fed versus overnight fasting conditions. Overnight-fasted training was associated with increased skeletal muscle fatty acid binding protein and 3-hydroxy-CoA dehydrogenase (HAD) activities, though in the Nybo study this did not result in a significantly increased rate of lipid oxidation during submaximal exercise. Interestingly, the overnight fasted-trained groups in both studies exhibited higher levels of resting muscle glycogen concentrations, suggesting that training when circulating CHO status is low stimulates the capacity to accrue muscle glycogen in recovery.

Both the aforementioned studies have recruited only males and the muscle metabolic response to an acute bout of exercise and endurance training appears to differ slightly between men and women, particularly in terms of lipid oxidation and intramuscular substrates.[6,7] To date, no one has yet tested the effect of fed versus overnight-fasted endurance training in women.

The primary aim of this study was conducted to test the hypothesis that endurance training undertaken in the overnight-fasted state would encourage greater changes muscle oxidative capacity and resting glycogen concentrations when compared with training undertaken in the acutely fed state. A secondary aim was to observe whether men and women responded differently to this training diet intervention.

## 2. Methods

Eight healthy untrained females ($24.6 \pm 6.1$ years (mean $\pm$ SD), $21.4 \pm 4.4\%$ body fat, $VO_{2peak}$ $3.01 \pm 0.33$ l/min) and six untrained males ($29.3 \pm 4.5$ years, $15.2 \pm 3.5\%$, $4.12 \pm 0.44$ l/min) agreed to participate in the study as approved by the University Human Ethics Committee. All provided written informed consent before participation.

Before any laboratory testing participants completed a four-day weighed food diary including one weekend day. The diary was analysed using specialised dietary analysis software (Foodworks®, Xyris, Brisbane).

One week later, participants attended the laboratory to measure their submaximal and peak oxygen utilisation ($VO_{2peak}$) on an electronically braked cycling ergometer (Lode Excalibur Sport- Netherlands). A relationship between steady-state cycling workload and $VO_2$ was developed for each participant as previously described.[8] During this and subsequent tests respiratory gases were collected and analysed via on 'on-line' gas analysis system (Exerstress®, Sydney University) which was calibrated with gases of known concentration and a 3 l calibration syringe prior to use. The individual's $VO_{2peak}$ was defined as the average of the highest four consecutive 10-s $VO_2$ recordings.

At least three days later, participants attended the laboratory after an overnight fast for a 75-min steady-state submaximal exercise test at a workload calculated to elicit 65% $VO_{2peak}$. One hour prior to exercise they consumed a light meal containing 1.5 g CHO/kg body weight to assist metabolic control. During exercise respiratory gases were collected for analysis at 5, 10, 25, 50 and 75 min. Each participant was then provided with a standard meal and a food diary for the next 48 h. Exactly 48 h later, participants again presented to the laboratory. During this visit a muscle sample was obtained using the needle biopsy technique[9] with suction applied to the needle using a 50 ml syringe. Approximately 80–200 mg of muscle tissue was removed, placed straight onto blotting paper (to absorb any blood), quickly divided thence immersed into liquid nitrogen. The divided portions were then placed in labelled cryovials and stored in liquid nitrogen at $-196\,^{\circ}C$ for subsequent analysis.

Three days later, participants attended the laboratory for the first training session. Training was undertaken at a workload calculated to elicit 65% $VO_{2peak}$, and was for four weeks, five mornings per week. This intensity of training has previously been employed to promote short-term changes in muscle oxidative capacity.[10] During the first training session, expired gases were collected for analysis and continual display during exercise (as described above), and the workload 'fine-tuned' to ensure that 65% of the pre-training $VO_{2peak}$ was reached by the 15 min time point. Training duration during the first week was 25 min, and this was increased by 25 min each week until 100 min during the fourth week. Water was available ad-libitum during training and hydration was encouraged.

Before beginning training, participants were randomly divided, in a balanced (for sex), factorial fashion (sex $\times$ treatment), into two groups of seven: training in the fed state (FED); or training in the overnight-fasted state (FAST).

Participants in FED were required to consume a standard cereal breakfast (1.5 g CHO/kg lean body) 60 min prior to each training session. During weeks three and four they were also given 30 g of maltodextrin in 750 ml water to be consumed during training. The FAST group trained after an overnight fast where water only was ingested during exercise, but consumed the same breakfast shortly *after* training so that timing of breakfast ingestion, rather than omission was the primary intervention.

During the final training week, participants were again required to complete a four-day, weighed record of their food intake over the same weekdays as the pre-training weighed record.

Participants attended the laboratory two days after the last training session for post-training submaximal/$VO_{2peak}$ testing; the same as the pre-training test. Two days later, participants again undertook a submaximal steady-state test exactly the same as the pre-training test, but the workload

was calculated to elicit 65% of post-training $VO_{2peak}$. After the test, food for the day was provided and the 48 h meal diary given to ensure equivalent metabolic control for the post-training biopsy as before training.

In preparation for enzyme activity determination, a measured amount of wet muscle (20–40 mg) was added to a homogenising buffer (containing 0.1 M $KH_2PO_4$ and 0.5 g/l BSA, pH 7.3), in a 1 mg to 50 μl ratio and homogenised. The activity of HAD was determined using the principles previously described.[11] Pre- and post-training muscle samples for each individual were performed on the same occasion using the same reagents. Total citrate synthase (CS) activity was determined from the same homogenate used for the HAD assay using methods described by Bergmeyer et al.[11]

To measure glycogen content, a small muscle sample was freeze-dried then thawed to room temperature surrounded by desiccant to remove contaminants (connective tissue, blood, fat) under binocular microscope. A portion of the cleaned muscle (0.5–1.5 mg) was hydrolysed in HCl. After neutralising with KOH, fluorometric analysis for free glucose concentration was performed after correction for a water blank, evaporative losses, and dilution.

Anthropometrical measures, muscle, $VO_{2peak}$ and food diary dependant variables were compared using three-way, repeated measures (pre- and post-training) ANOVA to investigate the influence of (a) training; (b) the intervention (i.e. FED versus FAST); and (c) gender. Data from the submaximal steady-state test were compared using four-way, repeated measures ANOVA. Power calculations were made for selected dependant variables. Significance was considered to be at or greater than the 95% level of confidence ($P \leq 0.05$). Statistical analyses were performed using SPSS Version 10.

## 3. Results

One FAST subject missed one 75 min training session, and another in FED missed one 100 min training session. One subject in FAST did one extra 100 min session, as their post-training performance test was postponed by two days. Otherwise, all participants completed the four-week (five days/week) training programme as prescribed.

Training (main effect) increased both relative and absolute $VO_{2peak}$ ($P < 0.001$ and $P = 0.015$ respectively) in both groups. There was also a significant group × training interaction with FAST increasing their absolute $VO_{2peak}$ by 9.7%, whereas FED increased by 2.5% ($P = 0.014$, Table 1). There was no significant overall difference between groups ($P = 0.624$), indicating that both groups had the same $VO_{2peak}$ prior to training, but their response to the training differed significantly. No effect of gender or interaction between gender and the other independent variables was evident. Whilst peak power increased in both groups, there was a strong tendency for FAST to improve their peak power more than FED ($P = 0.051$). Maximum heart rate was significantly lower after training ($P = 0.004$), but there was no difference in this training response between groups.

Table 1
$VO_{2peak}$ pre- and post-training and *V. lateralis* biochemical analyses.

| Training group | Pre-training | Post-training | % Change |
|---|---|---|---|
| $VO_{2peak}$ (l/min) | | | |
| Fasted-trained ($n = 7$) | 3.52 ± 0.76 | 3.86 ± 0.86 | 9.7*,† |
| Fed-trained ($n = 7$) | 3.45 ± 0.64 | 3.53 ± 0.63 | 2.5 |
| HAD activity (μmol/g wet muscle/min) | | | |
| Fasted-trained ($n = 7$) | 8.08 ± 2.3 | 8.36 ± 2.5 | 3.5 |
| Fed-trained ($n = 7$) | 8.42 ± 1.3 | 9.19 ± 2.0 | 9.1 |
| CS activity (μmol/g wet muscle/min) | | | |
| Fasted-trained ($n = 7$) | 19.0 ± 4.2 | 22.4 ± 3.6* | 17.9 |
| Fed-trained ($n = 7$) | 20.9 ± 2.2 | 24.9 ± 4.1* | 19.1 |
| Muscle glycogen content (mmol/kg dry muscle) | | | |
| Fasted-trained ($n = 6$)# | 464 ± 121 | 718 ± 123*,† | 54.7 |
| Fed-trained ($n = 7$) | 545 ± 112 | 561 ± 181 | 2.9 |

Values are means for each training group ± SD.
* Denotes a significant difference between pre- and post-training values ($P < 0.05$).
† Denotes a significantly different ($P < 0.05$) change compared to fed-trained group.
# Insufficient biopsy sample to enable glycogen analysis in one subject meant that only 6 FAST group subjects could be analysed for glycogen content.

In both 75 min submaximal steady-state tests, RER significantly decreased with duration ($P < 0.001$), an effect that was attenuated with training in both groups ($P < 0.001$). However, RER of the groups during this exercise did not respond differently to training ($P = 0.603$), nor was there any significant influence of gender.

A training × group interaction was evident, such that training resulted in a significantly greater increase in glycogen levels for FAST than FED ($P = 0.047$; Table 1), though this was not influenced by gender ($P = 0.761$). In contrast, training significantly increased *Vastus lateralis* CS activity ($P < 0.001$), but this adaptive response was not significantly different between training groups ($P = 0.66$). However, there was a training × group × gender interaction which revealed that when gender was considered, CS activity in men responded better to FAST, whereas women responded better to FED ($P = 0.011$). Although HAD activity was unchanged by training ($P = 0.214$) with no difference between training group responses ($P = 0.640$), the training × group × gender interaction approached significance ($P = 0.063$). A training × gender interaction was apparent ($P = 0.043$) indicating that regardless of timing of meal intake around training, the HAD activity of female muscle generally increases to a greater extent in response to the same training stimulus compared to males (Fig. 1).

Two four-day, weighed food records (pre-training and final week-training) were completed by 13 of the 14 participants who completed the entire study. Average daily energy intake significantly increased during training ($P = 0.007$), but this increase was not different between training groups ($P = 0.15$). Both average daily absolute protein and CHO intake increased with training (84.3–110.4 g and 277–395 g

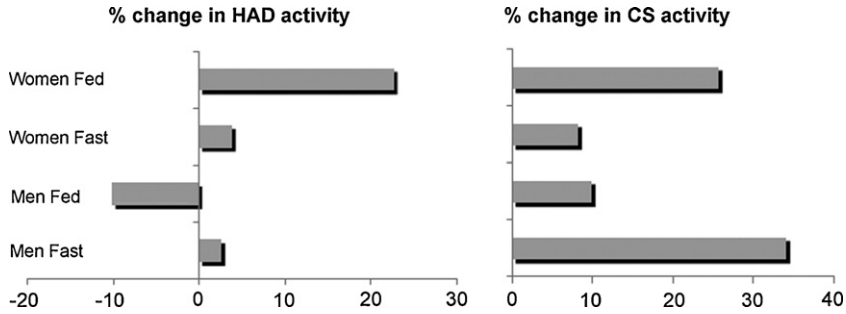


Fig. 1. Percentage change in HAD CS activity following training in men and women in each training group.

respectively), but this training response was not different between groups ($P = 0.17$, $P = 0.31$). Average daily fat intake was not influenced by training ($P = 0.42$), though the percentage of energy contributed by fat was decreased during training ($P = 0.03$). This latter effect was not different between training groups ($P = 0.89$).

## 4. Discussion

The main findings of the present study were that: training in an overnight-fasted state enhances storage of muscle glycogen compared to training in the fed state; skeletal muscle of men and women respond differently in terms of oxidative activity to training in the fed and overnight-fasted state; and peak $VO_2$ and peak power improved more when training in the fasted state compared to the fed state.

Despite following the same training regimen, FAST had higher levels of muscle glycogen than FED (Table 1). Indeed, there was little change (3%) in muscle glycogen stores in FED, but a large increase (55%) in the FAST. We cannot completely rule out the possibility that other factors affected this adaptation e.g. timing of the muscle biopsy. The final muscle biopsy was not taken until ~96 h after the final training session. Between the last training session and the biopsy, participants completed an incremental $VO_{2peak}$ test and a 75 min cycle at 65% $VO_{2peak}$ which could affect muscle glycogen stores. However, these conditions were the same for both groups, and the percentage of fat and CHO utilisation during the exercise bouts were not different. Additionally, participants were provided some food and asked to replicate food diaries reported during the equivalent pre-training period. Therefore, it is highly likely that the differences in glycogen stores between groups reflect the training intervention and not exercise timing or pre-biopsy diet. Indeed, differences may have been even greater between groups immediately after the completion of the training period, with the present results suggesting that this adaptation (elevated glycogen stores) could last for days, despite cessation of this (fasted) training practise. Importantly, our findings correspond to that of De Bock et al.[4] confirming that training whilst circulating CHO levels are low increases the capacity to accrue glycogen in the trained muscles. Our results also follow those of Hansen et al.,[12] who show that 10 weeks of training with lower glycogen levels results in greater elevation of resting muscle glycogen.

Although we report no differences in CS or HAD activity between training groups, there was a main effect of training on CS activity, suggesting that oxidative capacity of the muscle developed similarly. However, when gender was taken into account, we show that fasted training stimulates greater increases in CS activity in men than women. In support of this, Hansen et al.,[12] reported changes in CS activity for their "train low glycogen" but not "train high glycogen" group of men following 10 weeks of training. To date no other published studies have testing these training diet interventions in women, so a novel finding of the present study is that the muscles of women respond better to fed training.

Gender differences in adaptation may be related to differences in fuel utilisation and/or turnover of high energy phosphates during endurance exercise. Compared to females, males appear to rely less on intramyocellular triglyceride (IMTG) as fuel[13,14] and have a markedly greater activity of AMP kinase (AMPK) following endurance exercise (90 min at 60% $VO_{2peak}$).[15] As well as a "fuel sensor", AMPK has a role in mitochondrial biogenesis and therefore, gender-based differences in activation of this kinase may have contributed to the differing adaptations in CS reported in the present study. Furthermore, previous studies have shown that fuel selection also affects adaptation of fuel regulating enzymes.[16,17] Hence the greater increase in HAD activity by females may be due to a greater reliance up IMTG. Thus, previously reported gender differences during acute exercise may explain the differing training-induced muscle adaptations in the present study, however further work is required.

An unexpected finding of the present study was that training-induced increments in $VO_{2peak}$ were significantly greater when training was undertaken in the overnight-fasted state. Others have observed a similar effect on $VO_{2max}$ of increasing lipid availability during training, but with a short-term high fat versus high CHO diet[18,19] and explained their observations as either an increased cardiac output due to an increased sympathetic stimulation during exercise[19] or a greater oxidative capacity in the fat adapted muscle.[18] In contrast, De Bock et al. observed no improvement by overnight-fasted training on changes in $VO_{2max}$. Whilst interesting, we are unable to explain our ($VO_{2peak}$) observation

within the context of our measurements, and as such, they should be treated with caution until confirmed by further research.

## 5. Conclusions

Endurance exercise regularly undertaken in the overnight fasted state may expedite some of the biochemical adaptations normally seen following training. However, the extent of these adaptations in skeletal muscle differs slightly between men and women.

## 6. Practical Implications

- Endurance training in the overnight-fasted state confers no advantage in promoting lipid utilisation during subsequent endurance exercise of the same relative intensity;
- However, overnight-fasted training may increase the oxidative activity of the trained muscle in men to a greater extent than women;
- Training in the postprandial state may alter the effect of training on $VO_{2max}$.

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