results

2023-02-20

## Results

At baseline, there were no significant differences between training with glucose or placebo in muscle mass, isometric or isokinetic peak torque, total session volume, training intensity, total RNA content, or rRNA expression (Tab 3). Further, on pairwise consecutive days, there were no significant differences in mean macro nutrient (calories, carbohydrate, fat or protein) intake, or mean change in macro nutrient ingestion (Tab 4).

Glucose ingestion before and after RT led to significant increases in plasma levels of glucose and c-peptide compared to RT with placebo, before, during and after the session (Fig 1B, 120min: 38 ± 4% [*p* = 0.00000], 135min: 31 ± 4% [*p* = 0.00000], 150min: 32 ± 4% [*p* = 0.00000], Fig 1C, 120min: 95 ± 10% [*p* = 0.00000], 150min: 87 ± 10% [*p* = 0.00000]), measured during the final RT session. At 2hrs post-exercise, RT with glucose led to significantly lower blood glucose levels compared to RT with placebo (1B, 270min: -8 ± 4% [*p* = 0.03]. There were no significant difference in change of skeletal muscle strength, at time points post 2 RT sessions, post 4 RT sessions, 30min after 6th session, 2hrs after 6th session and 23hrs after 6th session (*p* > 0.05). After 5 RT sessions (Fig 1D, Post 5RT), there was a significant difference in strength between glucose and placebo, where placebo decreased strength 7% more than glucose (*p* = 0.04).

![](data:application/pdf;base64,)

**Figure 1:** A) An overview of the experimental design with 12 days of concomitant dietary intervention and resistance training (RT), preceded by 7 days involving familiarization. Between days -7 and -1, participants were familiarized to the RT exercises via 1RM leg press and knee extension testing, and to the strength tests via Humac Norm dynamometer (days -7 and -5). Before baseline testing, the participants were randomly allocated to exercise one leg with GLU (glucose) and the other with PLA (placebo), in a unilateral, alternating fashion. Further, non-dominant/dominant + GLU/PLA, and onset with GLU or PLA was also randomized, i.e. the figure illustrates an example where the participant was randomized to start RT with GLU. Biopsies were taken from m. vastus lateralis at baseline (Day 1 leg 1, Day 2 leg 2), and after five RT sessions (Day 11 leg 1, Day 12 leg 2). Three participants dropped out of the intervention, either due to reported muscular discomforts of heavy RT, or sickness in their family. B and C) Changes in plasma glucose (mmol/L) and c-peptide levels (pmol/L) during post testing (Day 11 leg 1, Day 12 leg 2). Glucose levels in blood was measured via finger draws 120 (-120), 90 (-90), 30 (-30) minutes and immediately before RT (0), during RT (15), immediately after (30) and 2hrs after RT (120). C-peptide levels were measured simultaneously to these finger draws, except for 90min before and during RT. Finger draws and venous blood were sampled at baseline (Day 1) and post (Day 11 leg 1, Day 12 leg 2). D) Changes in muscular performance/recovery measured as isometric and isokinetic peak torque (60 and 240 d/s) via Humac Norm Dynamometer, conducted at baseline (Day -1), during the intervention (Day 4 and 8 leg 1, Day 5 and 9 leg 2), after five RT sessions/before the 6th session (Day 11 leg 1, Day 12 leg 2), as well as 30min, 2hrs and 23hrs after the 6th RT session (Day 11/12 leg 1, Day 12/13 leg 2). The index was calculated by normalizing peak torque of each separate to the highest peak torque at each respective speed, and then summarised and used in change score calculations.

Values are presented as changes in estimated marginal means ± 95% CI. \* = *p* < 0.05 between groups. Glucose n = 13, placebo n = 13.

### Markers of ribosome biogenesis

Resistance training with glucose did not induce a higher accumulation of total RNA (fig 2A, *p* = 0.5) or rRNA (fig 2B, 47S: *p* = 0.502, 18S: *p* = 0.585, 28S: *p* = 0.74, 5.8S: *p* = 0.935, 5S: *p* = 0.79) compared to RT with placebo, measured as mean change from baseline to post. From baseline to post-intervention, there was a mean increase in levels of total RNA by 26 and 22% after RT with glucose and placebo, respectively (*p* < 0.05, compared to baseline). A robust accumulation was also observed in ribosomal RNA expression in both RT with glucose and placebo, with mean increases between 34-43% (GLU) and 33-41% (PLAC) (*p* < 0.05, compared to baseline) in the four rRNA’s. The expression of in the 47S pre-rRNA increased by 37 and 59% with glucose and placebo respectively, where only RT with placebo increased significantly from baseline to post (fig 45, *p* = 0.04).

FIG2 HERE

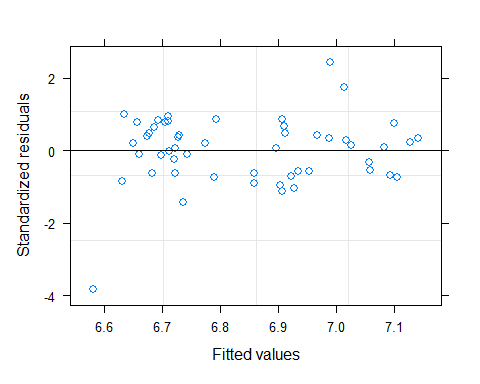
###### **Figure 2:** Changes in total RNA and ribosomal RNA. A) Total RNA, B) 47S pre-rRNA, 18S rRNA, 28S rRNA, 5.8S rRNA, 5S rRNA. Baseline = Day 1 leg 1/ Day 2 leg 2, Post = Day 11 leg 1, Day 12 leg 2. Total RNA and rRNA were analyzed in duplicates, with two duplicates per biopsy (two biopsies per time point), and normalized to ng x mg wet muscle weight for total RNA and external reference gene (Lambda) for rRNA. Total RNA and rRNA changes were calculated as log-fold change score per mg wet muscle weight. Mean change scores of the duplicates were calculated before modelling and transformed to the log-scale. Values are estimated marginal means fold change per leg per supplement ± 95% CI. Glucose n = 13, placebo n = 13.

## # A tibble: 52 × 4  
## # Groups: subject, time [26]  
## subject time supplement mean.sign  
## <chr> <fct> <fct> <dbl>  
## 1 101 pre PLACEBO 0.835  
## 2 101 pre GLUCOSE 0.595  
## 3 101 post PLACEBO 0.936  
## 4 101 post GLUCOSE 0.725  
## 5 102 pre PLACEBO 0.880  
## 6 102 pre GLUCOSE 0.857  
## 7 102 post PLACEBO 1.38   
## 8 102 post GLUCOSE 1.20   
## 9 103 pre PLACEBO 0.801  
## 10 103 pre GLUCOSE 0.584  
## # … with 42 more rows

## # A tibble: 52 × 4  
## # Groups: subject, time [26]  
## subject time supplement mean.rna  
## <chr> <fct> <chr> <dbl>  
## 1 101 pre GLUCOSE 733.  
## 2 101 pre PLACEBO 918.  
## 3 101 post GLUCOSE 1100.  
## 4 101 post PLACEBO 947.  
## 5 102 pre GLUCOSE 867.  
## 6 102 pre PLACEBO 768.  
## 7 102 post GLUCOSE 853.  
## 8 102 post PLACEBO 880.  
## 9 103 pre GLUCOSE 795.  
## 10 103 pre PLACEBO 761.  
## # … with 42 more rows

## # A tibble: 52 × 6  
## # Groups: subject, time [26]  
## subject time supplement mean.sign mean.rna sd.ubf  
## <chr> <fct> <chr> <dbl> <dbl> <dbl>  
## 1 101 pre PLACEBO 0.835 918. -3.38  
## 2 101 pre GLUCOSE 0.595 733. -3.62  
## 3 101 post PLACEBO 0.936 947. -4.65  
## 4 101 post GLUCOSE 0.725 1100. -4.86  
## 5 102 pre PLACEBO 0.880 768. -51.6   
## 6 102 pre GLUCOSE 0.857 867. -51.6   
## 7 102 post PLACEBO 1.38 880. -8.73  
## 8 102 post GLUCOSE 1.20 853. -8.91  
## 9 103 pre PLACEBO 0.801 761. -3.70  
## 10 103 pre GLUCOSE 0.584 795. -3.92  
## # … with 42 more rows

## Linear mixed-effects model fit by REML  
## Data: joined.dat   
## AIC BIC logLik  
## -26.77728 -17.31817 18.38864  
##   
## Random effects:  
## Formula: ~1 | subject  
## (Intercept) Residual  
## StdDev: 0.06546126 0.1400949  
##   
## Fixed effects: log(mean.rna) ~ mean.sign + time   
## Value Std.Error DF t-value p-value  
## (Intercept) 6.608430 0.04431637 37 149.11937 0.0000  
## mean.sign 0.111284 0.03576816 37 3.11126 0.0036  
## timepost 0.212577 0.04545818 37 4.67633 0.0000  
## Correlation:   
## (Intr) mn.sgn  
## mean.sign -0.669   
## timepost -0.027 -0.519  
##   
## Standardized Within-Group Residuals:  
## Min Q1 Med Q3 Max   
## -3.8202441 -0.6174280 0.1148365 0.5236676 2.4291075   
##   
## Number of Observations: 52  
## Number of Groups: 13



Interestingly, RT with placebo led to significantly higher change in levels of rpS6 and UBF protein compared to RT with glucose, with no difference in levels of c-Myc (Fig 3A rpS6: *p* = 0.029, 3C UBF: *p* = 0.031, 3B c-Myc: *p* = 0.063). Levels of rpS6 change by 40% in placebo and 7% in glucose (Fig 3A), UBF levels changed by 68% in placebo and 26% in glucose (Fig 3C), and c-Myc levels changed by 110% in placebo and 59% in glucose (Fig 3B).

FIG3 HERE

###### **Figure 3:** Changes in levels of protein. A) Linear relationship between total RNA (ng x mg) and UBF levels (SD units), with time added as a covariate in the model.

(We must add time as a covariate, as both total RNA and UBF are expected to increase with “time” in this intervention, due to RT. Therefore, adding time as a covariate allows us to model the relationship between RNA and UBF, without variations of time interfering)

1. Representative total protein stain blot, both duplicates from one participant (115). C) Fold change in UBF, c-Myc and rpS6 (AU) + representative antibody stains of the respective proteins. Baseline = Day 1 leg 1, Day 2 leg 2, post = Day 11 leg 1, Day 12 leg 2. GLU = glucose, PLA = placebo. Protein samples were analyzed in two duplicates per biopsy per time point, loaded on separate gels in an inverted order, e.g. from gel 3 to 6, as shown by the duplicates (1 and 2). Change in protein levels were calculated as log-fold change scores normalized by pools (pool of all protein samples per gel). Mean change scores of the duplicates were calculated before modelling and transformed to the log-scale. Values are reverse transformed estimated marginal means fold change per leg per supplement ± 95% CI. \* = significant between groups statistic, glucose compared to placebo. Glucose n = 13, placebo n = 13.

Further, we observed a linear relationship between levels of UBF and accumulation of total RNA, where …

FIG4 HERE

######**Figure 4:** Relationship between levels of total RNA and UBF. Total RNA was normalized by wet muscle weight, and UBF levels were normalized by pools and total protein per lane. Values are presented as log-transformed means.