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Moderate-intensity endurance exercise prevents short-term starvation-induced intramyocellular lipid accumulation but not insulin resistance

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ABSTRACT

Exercise has the potential to alleviate the resistance to insulin-mediated glucose uptake precipitated by elevated circulating free fatty acids (FFAs) in conditions such as obesity, lipid infusion, and starvation. In this study, 6 lean healthy men underwent two 3-day periods of starvation with either no exercise or daily endurance exercise (80 min d^{-1} at 50% maximal rate of oxygen consumption) and a 3-day mixed diet without exercise. Insulin sensitivity was determined by intravenous glucose tolerance test, and intramyocellular lipid (IMCL) concentration was measured by ^{1}H magnetic resonance spectroscopy. In both starvation conditions, fasting plasma FFAs were significantly elevated, whereas plasma glucose and whole-body insulin sensitivity were significantly reduced. Vastus lateralis IMCL to water ratio was significantly elevated after starvation without exercise compared with that after starvation with exercise or that after mixed diet. Intramyocellular lipid to water ratio was not different between starvation with exercise and mixed diet. In healthy lean men, exercise during starvation prevents the accumulation of IMCL yet does not affect the starvation-induced changes in FFAs and insulin sensitivity. Unlike during lipid infusion or obesity-induced insulin resistance, exercise cannot overcome the reduction in insulin action caused by starvation. We propose that carbohydrate availability is a key modulator of the combined effects of exercise and circulating FFAs on insulin sensitivity.

1. Introduction

A sedentary lifestyle is associated with insulin resistance and its comorbidities [1]; and conversely, regular physical activity ensures whole-body insulin sensitivity (Si) [2]. The latter appears to be due primarily to repeated short-term effects of exercise on promoting insulin-mediated glucose disposal rather than some chronic, long-term adaptation [3]. Even in

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athletes undergoing chronic endurance training, a few days of inactivity drastically reduces Si [4,5]. However, in both healthy sedentary people [1] and endurance-trained people who have undergone a few days of inactivity [6], a single exercise session restores Si to near trained levels.

Measures of whole-body Si are also reduced in situations where blood plasma free fatty acids (FFAs) are elevated such as obesity [7]; intravenous lipid infusion [8,9]; low-carbohydrate, high-fat diet [10,11]; or starvation [10,12,13]. The combination of inactivity and an acute increase in plasma FFAs leads, within a few hours, to accretion of intramyocellular lipid (IMCL) and a simultaneous and proportional reduction in Si [14]. However, the effect of exercise superimposed upon elevated FFAs is unclear.

Two studies have directly examined this situation in humans. Schenk et al [8,15] reported that prior exercise (90 minutes at 65% of Vo_{2peak}) prevented the decrease in Si that otherwise occurs with an overnight lipid infusion, implying that exercise is able to either compensate for or directly prevent a diet-induced decrease in Si. Conversely, Sparti and Decombaz [11] reported that prior exercise (30 minutes at 80% of estimated maximum heart rate followed by 30 minutes of resistance exercise then ~10 intervals of 2 minutes at 90% of maximum heart rate) did not mitigate the insulin resistance observed following a subsequent 36-hour very low-carbohydrate, high-fat diet. The dissonance between these studies may be due to different means of elevating FFAs; but regardless, a better understanding of how exercise and elevated FFAs interact to affect Si would be useful. In this context, the effect of superimposing physical activity on starvation in physically fit individuals is to date unknown.

Although Si has historically been the primary focus of research into glycemic control, glucose effectiveness (Sg) (ie, the ability of blood glucose to promote its own disposal and suppress hepatic glucose output) is also an important component of glucose tolerance [16], especially in patients with impaired insulin secretion. Like Si, Sg is decreased by physical inactivity [17,18] and is increased after a single exercise bout [19]. However, the effect of exercise on Sg in a state of elevated FFA and reduced Si has not been examined.

The aim of this study was to test the hypothesis that exercise during starvation would prevent the changes in Si, Sg, plasma FFA, and IMCL normally observed during starvation without exercise. To this end, we exposed lean, physically fit men to 2 approximately 3-day periods of starvation with different levels of total energy expenditure, as well as approximately 3 days of standardized moderate-carbohydrate diet.

2. Methods

2.1. Subjects

Six healthy, physically fit men volunteered for this study. Participants' physical characteristics are given in Table 1. All participants reported regularly undertaking exercise for more than 1.5 hours daily, at least 5 days per week. Participants were informed of the study protocol and risks before providing their written consent. The study was approved by the University of Sydney human research ethics committee and conformed to the Declaration of Helsinki.

Table 1 – Participants' physical characteristics

Age (y)	38.8 ± 12.7
Body mass (kg)	72.9 ± 8.8
Body fat (%)	13.5 ± 2.1
RMR (mL O_2 min^{-1})	306 ± 35
VO_{2max} (mL kg^{-1} min^{-1})	75.5 ± 16.0

Values are mean ± standard deviation. VO_{2max} indicates maximal rate of oxygen consumption.

2.2. Preliminary testing

One week before participation in the first intervention, submaximal and maximal oxygen uptake tests were performed on an electronically braked cycle ergometer (Lode ergometer, Groningen, the Netherlands) as previously described [20]. External power output and VO_2 attained during the final minute of each submaximal workload and the maximal ramp were used to formulate regression equations from which workloads for the control exercise bout were derived. On a separate occasion, participants presented at the laboratory following a 12-hour overnight fast for measurement of resting metabolic rate (RMR) using respiratory gas analysis while lying supine after 30-minute rest. Body density was assessed via hydrodensitometry; and percentage body fat was then calculated using a 2-compartment model, as previously described by Brozek et al [21]. Underwater body weight measurements were corrected for measured residual lung volume using the methods of van der Ploeg et al [22].

2.3. Experimental protocol

All participants underwent 3 supervised dietary and exercise interventions in random order. A minimum 7-day washout was allowed after the mixed-diet intervention and 25 days after the starvation interventions. Each intervention period was of 67-hour duration and comprised a water-only starvation diet with no physical activity (inactivity + starvation), a water-only starvation diet with controlled physical activity (exercise + starvation), or a mixed control diet with no physical activity (inactivity + mixed diet). Beginning the day before initiation of the diet intervention (36 hours before initiation), subjects refrained from exercise and recorded all dietary intake. Before subsequent interventions, subjects again refrained from exercise and consumed the same diet as before the first intervention. This preconditioning period plus the intervention period resulted in 4.5 days (108 hours) of diet and exercise control before each testing session. There were 63 hours between the last exercise session and the intravenous glucose tolerance test (IVGTT) in the inactivity conditions and 15 hours between the last exercise session and the IVGTT in the exercise condition.

Upon initiation of the diet, participants ingested either a carbohydrate snack or water only, according to their allocation to mixed diet or a starvation intervention. The snack, a sports drink containing glucose and sucrose, provided 1 g carbohydrate per kilogram of body weight (100% energy from

carbohydrate) in the mixed diet, or water only in the starvation diets. In the mixed diet, participants ingested an evening meal 2 hours later. This meal contained 1.5 g carbohydrate per kilogram of body weight and supplied 50% of energy from carbohydrate, 35% of energy from fat, and 15% of energy from protein. Beginning the following morning and continuing for the remainder of the mixed dietary treatment (48 hours), participants received a diet that provided energy to match a daily expenditure of 1.5× RMR to maintain energy balance. Diets were designed to deliver 50% of energy from carbohydrate, 35% of energy from fat, and 15% of energy from protein. In the starvation conditions, participants continued a water-only diet until completion of the experimental treatment. In the inactivity + starvation condition, participants performed no unnecessary physical activity. The exercise + starvation condition was identical except that participants reported to the laboratory and performed a standardized exercise bout (50% of the power associated with maximal rate of oxygen consumption for 80 minutes) on the cycle ergometer at 5:00 PM on the second and third days (28 and 52 hours after initiation of starvation). Diet composition was quantified via Foodworks (Xyris Software, Melbourne, Australia) using the New Zealand–Standard database. In all dietary interventions, participants were instructed to maintain activities of daily living and avoid all forms of recreational exercise. Participants were based in the university environment, returning to their homes to sleep. The researchers maintained a minimum of daily contact with participants to ensure compliance with the dietary and exercise protocols.

2.4. Determination of IMCL content

After 65 hours of each diet, vastus lateralis proton magnetic resonance (^{1}H-MRS) spectra were obtained as previously described [10]. Spectral data were postprocessed by magnetic resonance user interface software (jMRUI version 3.0, EU Project). The vastus lateralis IMCL content was determined by the ratio of the methylene (–[CH2]n) resonance from IMCL at 1.3 ppm and intracellular water [23]. A 10-resonance model was used to determine IMCL concentration as we have detailed previously [10]. Muscle water signal amplitudes were measured from the non–water-suppressed spectrum using Hankel-Lanczos squares singular values decomposition. The ^{1}H-MRS processing was performed by an experimenter who was blinded to treatment allocation.

2.5. Intravenous glucose tolerance test

Following determination of IMCL content (15 hours after the last exercise session in the exercise + starvation condition), participants reported to the laboratory where glucose tolerance was assessed by frequently sampled IVGTT without modification by insulin infusion as previously described [10,24]. Intravenous rather than oral glucose tolerance test was chosen to avoid potential confounding effects of diet (including starvation [25]) on gastric emptying and subsequent glucose tolerance [26]. The IVGTTs were undertaken after a 12-hour overnight fast (or 67-hour fast in the starvation intervention). Additional blood samples were collected at 15, 30, 60, and 120 minutes for determination of plasma FFA concentrations.

2.6. Blood sampling

Before the IVGTT, 3 mL of venous blood was sampled by syringe, transferred into EDTA, placed on ice, and then centrifuged at 2000 *g* for 8 minutes within 30 minutes of collection. Plasma was decanted off and stored at –80°C for later analysis of FFA concentration. An additional 2 mL of venous blood was drawn into a lithium heparin-pelleted syringe. This sample was mixed and rested on ice for approximately 5 minutes, after which 1.3 mL was transferred into blood tubes and centrifuged at 2000 *g* for 8 minutes, and the plasma was frozen (–80°C) for later determination of plasma insulin concentration. Residual blood remained on ice in the lithium heparin-pelleted syringe for later determination of plasma glucose concentration. Blood for glucose, insulin, and FFA measurement was sampled according to these methods during the ensuing IVGTT according to the sampling schedule outlined previously [10,24].

2.7. Analytical procedures and calculations

Plasma glucose concentration was measured by autoanalyzer (EML 105; Radiometer, Copenhagen, Denmark). The plasma concentration of FFAs was determined using a Wako NEFA C test kit (WAKO Chemical, Richmond, VA) scaled for use in a microplate (Bio-Rad, Hercules, CA). All measurements were made in duplicate, and the mean was reported. Insulin sensitivity and Sg were determined via the minimal model analysis of the plasma glucose and insulin response to the IVGTT [24] using MINMOD Millennium (version 6.02; MinMod, University of Southern California, Los Angeles, CA).

2.8. Analysis

Differences in nutritional intake, all basal measures (plasma glucose, insulin, FFA, and ratio of IMCL to water) and differences in Si and Sg between conditions were compared by 1-way repeated-measures analysis of variance (ANOVA). Plasma glucose, insulin, and FFA concentrations during IVGTT were compared by 2-way repeated-measures ANOVA for investigation of treatment and time (diet-time) interactions. Where statistical significance was found, the Tukey honestly significant difference test was used to determine where the difference occurred. Statistical significance was accepted at $P < .05$. All values are expressed as mean ± standard deviation.

3. Results

3.1. Diet and exercise

During the mixed diet, subjects had an average daily energy intake of 12 299 kJ (49.5% ± 0.7% from carbohydrate, 34.3% ± 1.2% from fat, and 15.8% ± 0.9% from protein). Subjects consumed no energy during the starvation conditions. All subjects were able to complete the required exercise, although

2 subjects had to stop for a brief rest after 70 minutes during the last exercise session in the exercise + starvation condition.

3.2. IMCL content

The ratio of vastus lateralis IMCL to water was significantly higher in the inactivity + starvation condition than in the mixed ($P = .0003$) or exercise + starvation conditions ($P = .009$, Table 2). The ratio of IMCL to water was not significantly different between the mixed and exercise + starvation conditions ($P = .10$). Intramyocellular lipid content demonstrated a significant within-subject correlation between the mixed and inactivity + starvation conditions ($r = 0.82$, $P = .008$), but there were no significant correlations between the exercise + starvation condition and either the mixed ($r = 0.57$, $P = .07$) or inactivity + starvation ($r = 0.6$, $P = .06$) conditions.

3.3. Basal plasma metabolite and insulin concentrations

Compared with either starvation condition, basal plasma glucose (inactivity, $P = .002$; exercise, $P = .001$) and insulin (inactivity, $P = .0003$; exercise, $P = .003$) concentrations were higher and FFA concentrations were lower (inactivity, $P < .0001$; exercise, $P < .0001$) after the mixed diet. There was no difference in basal glucose, insulin, or FFA concentrations between the starvation conditions (Table 2).

3.4. Plasma metabolite and insulin responses to IVGTT

In all 3 treatments, the plasma glucose concentration increased rapidly following the intravenous glucose infusion at the initiation of the IVGTT, reaching a peak between 3 and 14 minutes after initiation. Glucose concentration subsequently declined and was not different between treatments and not different from baseline by the last sample of the IVGTT at 180 minutes (Fig. 1A). There was a significant treatment-time interaction effect ($P = .039$) with mixed diet resulting in higher plasma glucose concentration compared with the starvation interventions at baseline and lower plasma glucose concentration compared with exercise + starvation from 40 to 100 minutes and compared with inactivity + starvation from 30 to 140 minutes (Fig. 1A).

Table 2 – Basal substrate and insulin concentrations and IVGTT results after 67 hours of dietary intervention

	Inactivity + mixed diet	Exercise + starvation	Inactivity + starvation
Plasma glucose (mmol L^{-1})	4.5 ± 0.3	3.4 ± 0.5 *	3.5 ± 0.3 *
Plasma insulin (pmol L^{-1})	19.8 ± 8.3	10.7 ± 6.5 *	8.5 ± 5.1 *
Plasma FFAs (μmol L^{-1})	379 ± 120	1188 ± 219 *	1180 ± 294 *
Vastus lateralis IMCL to water ratio ($\times 10^{-3}$)	13.6 ± 6.1	18.1 ± 5.8	25.6 ± 5.9 *,†
Si (L min^{-1} mU^{-1})	16.5 ± 6.8	6.0 ± 1.7 *	5.7 ± 1.5 *
Sg × 1000 (min^{-1})	4.4 ± 2.2	8.8 ± 3.0 *	6.4 ± 2.3

Values are means ± SD; n = 6 participants. Between-trials comparisons were performed using repeated-measures ANOVA.
* Significantly different vs inactivity + mixed diet ($P < .01$).
† Significantly different vs exercise + starvation ($P < .05$).

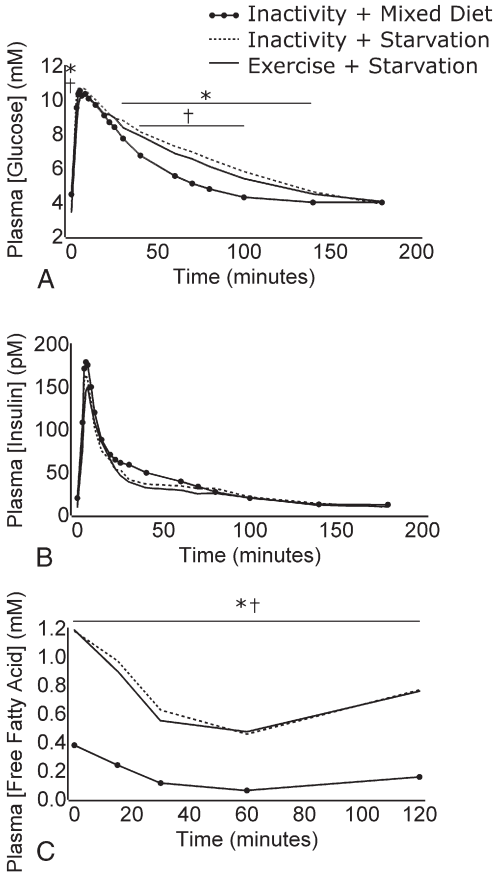

Fig. 1 – Effect of 67 hours of inactivity + mixed diet, inactivity + starvation, or exercise + starvation on (A) plasma glucose, (B) insulin, and (C) FFA concentrations during an IVGTT; n = 6 subjects. Between-trials comparisons were performed using repeated-measures ANOVA. *Significantly different inactivity + mixed diet vs inactivity + starvation ($P < .05$). † Significantly different inactivity + mixed diet vs exercise + starvation ($P < .05$).

There were 7 data points in which the plasma insulin concentration was less than the detectable limit of the assay (6 pmol L^{-1}), and a value of 5 pmol L^{-1} was assumed for these points. Plasma insulin concentration increased rapidly following the intravenous glucose infusion at the initiation of the IVGTT, reaching a peak between 3 and 8 minutes after initiation. Insulin concentration subsequently declined and was not different from baseline by 140 minutes (Fig. 1B). There was no significant treatment-time interaction effect on insulin concentration ($P = .18$, Fig. 1B).

In all treatments, plasma FFA concentration declined after glucose infusion, reaching a minimum at 60 minutes, after which time it began to rise again (Fig. 1C). After the starvation conditions, plasma FFA concentration was significantly greater ($P < .0001$) throughout the IVGTT compared with the mixed diet. There was also a significant treatment-time interaction ($P < .0001$) during the IVGTT, with plasma FFA concentration declining more rapidly after starvation

than after the mixed diet (Fig. 1C). There was no difference in FFA concentration between the starvation conditions.

3.5. *Minimal model analysis*

Values for Si and Sg after each of the interventions are presented in Table 2. Compared with that after mixed diet, Si determined by the minimal model was significantly lower after starvation with inactivity ($P = .005$) and starvation with exercise ($P = .007$). There was no difference in Si between the starvation conditions. Glucose effectiveness was significantly greater after the exercise + starvation condition than after the mixed diet ($P = .022$), but there was no significant difference between starvation conditions ($P = .15$) or between inactivity + starvation and mixed diet ($P = .38$).

4. Discussion

The primary finding of this study is that daily endurance exercise does not mitigate the insulin desensitizing effect of 3 days of starvation despite preventing the accumulation of vastus lateralis IMCL.

The exercise performed in the starvation condition was considerably less than the habitual training load of our endurance-trained subjects. Nevertheless, the subjects found it difficult to complete the last exercise session during starvation; and 2 subjects required a brief rest to be able to complete the exercise. Previous research where exercise was imposed on starvation has demonstrated a 32% reduction ($P = .025$) in time-to-failure in an incremental exercise test after 2 days and a 45% reduction ($P < .0001$) after 4 days [27]. These large decreases in physical capacity demonstrate the challenge of performing physical work during starvation and suggest that the dose used in the present study approached the limit of what is achievable in this state.

To increase Si above habitual levels in the very fit endurance-trained subjects used in our study would require a prodigious amount of exercise, more than would be physically possible during starvation. To avoid this methodological difficulty, we used an intervention of starvation + inactivity to reduce Si and compared the results to a habitual diet control (mixed diet + inactivity) and a habitual physical activity control (starvation + exercise). In other endurance-trained groups of subjects, cessation of training resulted in a 28% decline in Si after 38 hours [5] and a 35% decline after 60 hours [4] compared with 12 or 14 hours after the last exercise session. Even when Si was reduced by 50% after 10 days of inactivity, Si was restored to normal levels by a single exercise session approximating a typical training session for the athlete [6]. Thus, our inactivity interventions, which involved 63 hours between the last exercise session and the IVGTT, would normally have markedly reduced Si compared with our exercise intervention, which included 2 extra exercise sessions and only 15 hours between the last exercise session and the IVGTT. As such, the lack of difference in Si between starvation + inactivity and starvation + exercise must be due to the starvation overriding or masking any impact of inactivity.

The results of the current study are consistent with previous research in which FFAs were elevated by low-carbohydrate diet [11], but are at odds with the ability of exercise to maintain Si in the face of plasma FFA concentrations elevated by lipid infusion [8,15]. A key difference between these 2 conditions is a difference in carbohydrate status brought about by the methods of raising FFAs. In low-carbohydrate diet and starvation, the increase in FFAs is accompanied by a reduction in circulating glucose availability; but with lipid/heparin infusion, it is not. This difference may suggest that some minimum level of carbohydrate status is required to enable exercise to increase whole-body Si. Taking a teleological perspective, such an interaction between exercise and carbohydrate status would be prudent, as an increase in Si with exercise when carbohydrate status is low could produce dangerous hypoglycemia.

The energy expended because of additional physical activity in the exercise condition is equivalent to the metabolizable energy of approximately 250 g of fat. Assuming an average 18 kg of active muscle mass recruited by the subjects in our study during cycling [28,29], the difference in total IMCL content between exercise and inactivity conditions is approximately 115 g. Thus, the extra oxidation of lipid to meet the energy requirements of exercise could account for the difference in IMCL stores between starvation + exercise and starvation + inactivity despite both conditions having similarly elevated plasma FFA concentration and similarly reduced Si [30]. This mechanism is also consistent with the elevation of IMCL stores despite exercise when FFAs are elevated by lipid infusion [8,15], as the greater carbohydrate availability and plasma insulin concentration in this situation would be expected to increase carbohydrate oxidation, decrease FFA oxidation, and increase FFA esterification [31]. However, differences in oxidation alone cannot explain the ability of exercise to maintain Si in the face of lipid infusion [8,15] but not starvation or low-carbohydrate diet [11]. A possible explanation for this effect is an alteration in the rate of FFA absorption by muscle cells. Membrane-bound fatty acid transport protein content increases in response to various physiological stimuli including insulin [32], muscle contraction [33], and starvation [34]. If myocellular fatty acid absorption capability is increased by low carbohydrate availability, then there would be a greater intramyocellular FFA load in starvation compared with lipid infusion despite a similar plasma FFA concentration.

The difference in exogenous fat intake in the current study and that of Schenk et al [8,15] is not likely to be responsible for the difference in the observed effects of exercise on Si. When carbohydrate status is low, as in the current study, circulating FFA will be elevated either from high exogenous fat intake [8-10] or from increased adipose tissue lipolysis [10,13]. Thus, skeletal muscle cells will be exposed to the same interstitial milieu regardless of dietary fat intake.

The exercise + starvation condition in the current study resulted in very high Sg. As Si is reduced in both starvation conditions, it seems paradoxical that Sg should be increased. However, it is likely that Sg is not quantitatively important at the low plasma glucose concentrations during the fast (Table 2). Increased Sg has important implications for patients with type 2 diabetes mellitus, as increases in Sg have the potential to improve glycemic control even when insulin secretion is absent. Furthermore, increased Sg provides a convenient

mechanism for very rapid initiation of muscle and liver glucose uptake and suppression of hepatic glucose production in response to an increase in plasma glucose.

Increased Sg during starvation could be mediated by reduced muscle glycogen concentration [3]. Whereas liver glycogen is substantially depleted after an overnight fast [35], muscle glycogen stores remain near full capacity after a mixed diet, are somewhat reduced after 3 days of inactivity + starvation, and are further reduced after exercise + starvation [36]. This order parallels the Sg values we observed.

The primary measure of Si and Sg used in this study was the IVGTT with minimal model analysis developed by Bergman et al [37]. The minimal model has good correlation with the hyperinsulinemic-euglycemic clamp [38] and low coefficient of variation [39]. Furthermore, the IVGTT will not be affected by alterations in glucose absorption by the gut, as might be expected after a 72-hour fast [25]. The minimal model has been shown to systematically overestimate Sg, with the degree of overestimation increasing with increasing insulin response during the first 20 minutes of the IVGTT. As such, caution should be used when comparing Sg results between groups with differing insulin response [40]. However, in the present study, there were no between-treatment differences in insulin response during the first 20 minutes of the IVGTT (Fig. 1); so the overestimation should remain consistent, and comparison between treatments is valid.

In summary, 3 days of starvation causes increased IMCL and circulating FFAs and decreased Si. The current study shows that exercise during starvation prevents the accumulation of IMCL and increases Sg yet does not affect the changes in Si or plasma FFA concentration. We propose that some minimum level of whole-body carbohydrate status is required to enable exercise to affect Si.

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