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Smaller muscle ATP reduction in women than in men by repeated bouts of sprint exercise

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Esbjörnsson-Liljedahl, Mona, Kristina Bodin, and Eva Jansson. Smaller muscle ATP reduction in women than in men by repeated bouts of sprint exercise. *J Appl Physiol* 93: 1075–1083, 2002. First published May 10, 2002; 10.1152/japplphysiol.00732.1999.—It was hypothesized that the reduction of high-energy phosphates in muscle after repeated sprints is smaller in women than in men. Fifteen healthy and physically active women and men with an average age of 25 yr (range of 19–42 yr) performed three 30-s cycle sprints (Wingate test) with 20 min of rest between sprints. Repeated blood and muscle samples were obtained. Freeze-dried pooled muscle fibers of types I and II were analyzed for high-energy phosphates and their breakdown products and for glycogen. Accumulation of plasma ATP breakdown products, plasma catecholamines, and blood lactate, as well as glycogen reduction in type I fibers, was all lower in women than in men during sprint exercise. Repeated sprints induced smaller reduction of ATP and smaller accumulation of IMP and inosine in women than in men in type II muscle fibers, with no gender differences in changes of ATP and its breakdown products during the bouts of exercise themselves. This indicates that the smaller ATP reduction in women than in men during repeated sprints was created during recovery periods between the sprint exercises and that women possess a faster recovery of ATP via reamination of IMP during these recovery periods.

Wingate test; muscle biopsy; single fibers

RECOVERY OF FORCE AFTER HEAVY resistance exercise or explosive strength loading has been shown to be faster in women than in men (12, 15, 21). Also, recovery of power, between three repeated 30-s cycle sprints, was found to be faster in women. We observed that recovery of peak power output was complete in women, but not in men, at the onset of the second and third sprint (42 men and 39 women, $P < 0.01$ for gender difference; Esbjörnsson-Liljedahl and Jansson, unpublished observations).

The mechanisms behind a gender-related difference in recovery of force or power are not known. However, there is strong evidence that muscle fatigue (reduction in force or speed) is related to a net breakdown of high-energy phosphates and accumulation of breakdown products, such as inorganic phosphate and ADP (e.g., Refs. 1, 8). Thus the faster recovery of force or power in women could be explained by gender-related differences in the exercise-induced breakdown of high-energy phosphates. It is important to emphasize that such a difference may develop during the exercise bout itself and/or during recovery after exercise.

Recently, we found that the decrease of high-energy phosphates or the accumulation of related breakdown products in type I and type II muscle fibers during one bout of sprint exercise did not differ between women and men (10). Neither the reduction in muscle glycogen nor the accumulation of muscle lactate differed between the genders in type II fibers. The glycogen reduction in the type I fibers was, however, smaller in women than in men and was the only gender-related difference related to energy metabolism that was detected (10).

The metabolic changes during repeated bouts of sprint exercise or during recovery after sprint exercise have not been previously investigated from a gender perspective. Therefore, the present study was undertaken to analyze metabolic changes in muscle and blood during repeated bouts of sprint exercise, including during both exercise and recovery periods, in both women and men.

It was hypothesized that the reduction of high-energy phosphates in muscle after repeated bouts of sprint exercise is smaller in women than in men.

METHODS

Subjects

Seven men and eight women (students at a college for sports and recreation instructors) volunteered for the study. None of the subjects was at an elite or competitive athletic level. However, subjects did participate in leisure-time sports (e.g., various ball games and jogging for the men and mainly calisthenics, aerobics, and jogging for the women). All subjects took part in the same theoretical and practical classes (physical exercise). A questionnaire was used to estimate the physical activity level during leisure time. The subjects answered nine different questions from which an activity index (minimum value of 5.5 and maximum value of 20.5) was

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calculated (19). As estimated by this questionnaire, the physical activity level did not differ between the genders (Table 1). Anthropometric data for the subjects are given in Table 1. Fat-free body mass was estimated from skinfold measurements (triceps, biceps, and subscapula; Ref. 9).

All experiments were performed in the morning after an overnight fast. The subjects were asked not to perform any heavy exercise during the 24-h period preceding the experiment. All female subjects were on oral contraceptives, and the experiments were performed between *day 21* and the last day of the menstrual cycle. The subjects were fully informed about the procedures and potential risks of the experiment before giving their consent to participate. The study was approved by the Ethics Committee of Karolinska Institutet.

Experimental Protocol

The experimental protocol is shown in Fig. 1. After a familiarization period, conducted at least 24 h before the experiment, subjects performed three 30-s cycle sprints (Wingate test; Ref. 4) on a mechanically braked cycle ergometer (Cardionics, Bredäng, Sweden) with 20 min of rest between sprints. The subjects were instructed to pedal as fast as possible with an individual braking load set at 0.075 kp (kilopound)/kg body wt. A sensor-microprocessor assembly counted flywheel revolutions. The flywheel progression per pedal revolution was 6 m. The average power for 5-s periods was automatically printed. Peak power (i.e., the highest 5-s power) and mean power (the average power during the 30-s duration) were calculated for each of the three 30-s cycle sprints.

Table 1. *Anthropometric, morphological, and power output data in 7 men and 8 women*

	Men	*P*	Women
Anthropometric data			
Age, yr	25(19–42)	NS	23(21–24)
Body mass, kg	75(69–101)	<0.05	63(54–70)
Fat-free mass, kg	63(51–82)	<0.0001	46(36–54)
Height, cm	175(168–184)	<0.01	164(157–171)
Activity index	17(13–18)	NS	16(14–19)
Fiber type, %			
Type I	58 ± 6	NS	56 ± 10
Type IIA	28 ± 5	NS	29 ± 9
Type IIB	13 ± 7	NS	15 ± 8
Type IIC	1 ± 1	NS	0 ± 1
Relative Area of fiber type, %			
Type I	57 ± 8	NS	60 ± 12
Type IIA	31 ± 7	NS	29 ± 11
Type IIB	12 ± 7	NS	11 ± 8
Fiber area, μm²			
Type I	4,874 ± 991	=0.09	4,102 ± 621
Type IIA	5,442 ± 644	<0.0001	3,728 ± 538
Type IIB	4,698 ± 773	<0.001	3,002 ± 536
Weighted mean fiber area (I + IIA + IIB)	4,982 ± 766	<0.01	3,860 ± 553
Power output, W			
Peak power			
Sprint 1	837 ± 76	<0.0001	587 ± 68
Sprint 2	825 ± 92	<0.0001	581 ± 65
Sprint 3	771 ± 90	<0.0001	567 ± 75
Mean power			
Sprint 1	644 ± 46	<0.0001	461 ± 48
Sprint 2	656 ± 66	<0.0001	446 ± 39
Sprint 3	622 ± 65	<0.0001	433 ± 40

Values represent mean (range) or ±SD. NS, not significant. *P* values denote the level of significance between men and women (*t*-test).

An indwelling catheter was inserted into an antecubital vein ~20 min before exercise. With the subject in the supine position, 2 ml of blood were sampled ~2 min before each sprint exercise and subsequently at 3, 6, and 9 min after each 30-s sprint exercise. In total, five skeletal muscle biopsies per subject were obtained from the vastus lateralis muscle with the percutaneous biopsy needle technique (5). From the same leg, and with two incisions applied, three biopsies were obtained before, immediately after *sprint 1*, and before *sprint 2*. The last two biopsies were obtained before and immediately after *sprint 3* from the other leg, with one incision applied. The biopsies were obtained within ~10 s after the cessation of exercise, frozen within a further 5 s in isopentane precooled with liquid nitrogen, and stored at −70°C until later analysis.

Histochemical and Morphological Analyses

The biopsy taken before the first bout of exercise was mounted in an embedding medium and analyzed histochemically for the fiber types I, IIA, IIB, and IIC with a myofibrillar ATPase stain (see Ref. 28). Approximately 400 fibers per biopsy were counted. The relative number of the different fiber types (type I%, type IIA%, type IIB%, and type IIC%) was calculated. A cross-sectional fiber area was analyzed morphologically by planimetry from an NADH-dehydrogenase stain (27) in 20 fibers of fiber types I, IIA, and IIB. The relative areas of the different fiber types (fiber-type area) were also calculated. In addition, mean type I, IIA, and IIB fiber areas and weighted mean fiber area of all fibers combined were calculated. For more information about the histochemical and morphological analyses, see Jansson and Hedberg (19).

Single Muscle Fiber Preparation Procedures and Analyses

After histochemical analysis of the biopsy taken before the first sprint, the remainder of this and the four subsequent biopsies were freeze-dried and ~100 single fiber fragments were dissected from each biopsy. These were classified histochemically as fiber type I or type II (11) and thereafter divided into separate pools of fiber type I and type II. The mean (range) weight of the analyzed pools was 35 (13–50) μg. The fiber pools used for establishing content of ATP, ADP, inosine monophosphate (IMP), inosine, hypoxanthine, xanthine, uric acid, and phosphocreatine (PCr) were extracted in perchloric acid (10) and thereafter analyzed by an HPLC technique (26). Muscle xanthine and uric acid were not detectable either at rest or after exercise (detection limit was 0.05 mmol/kg dry muscle). The detection limit of AMP was 0.2 mmol/kg dry muscle. Because the levels of AMP are of this magnitude in skeletal muscle, it was not possible to measure AMP content. The contribution of AMP to changes in TAN pool is, however, very small, and the calculation of the TAN pool was based on the sum of ATP and ADP.

The fiber pools used for establishing glycogen content were enzymatically degraded to glucose (14) and analyzed by a fluorometric enzymatic method (23).

Blood and Plasma Preparation and Analyses

Blood samples for plasma analyses were centrifuged immediately after collection. The supernatant (plasma) for determination of ammonia was analyzed directly by flow injec-

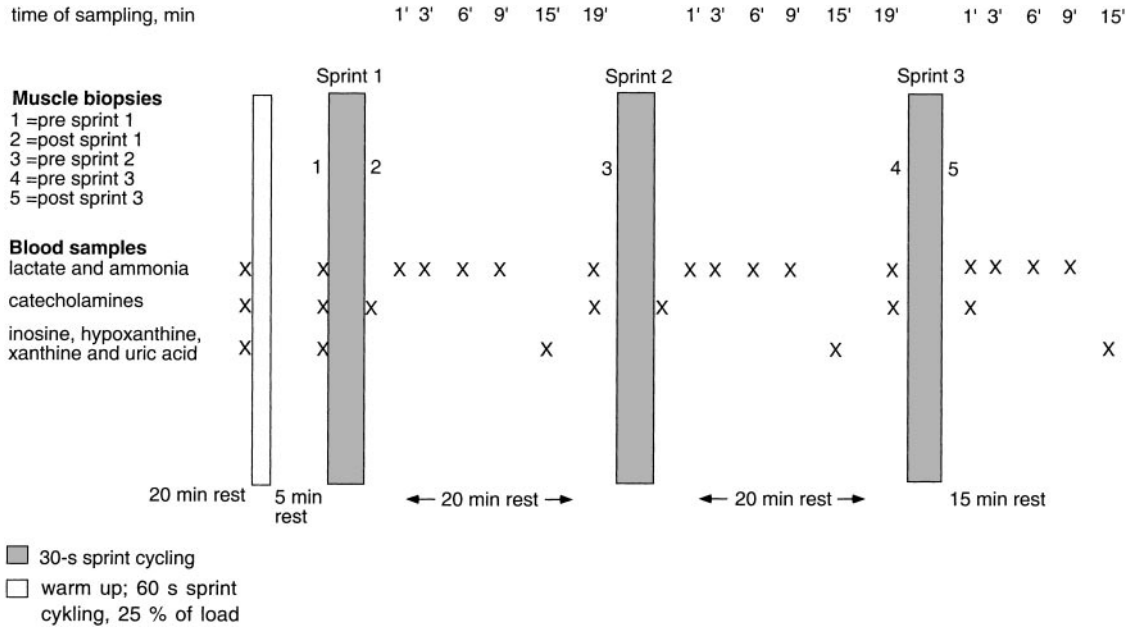

Fig. 1. Exercise and sample protocol for 8 women and 7 men.

tion analysis (30). Remaining plasma was stored at −70°C until later analysis for catecholamines by HPLC (17) and for inosine, hypoxanthine, xanthine, and uric acid in a neutralized perchloric acid extract of plasma by HPLC (26). Blood lactate was determined in neutralized perchloric acid extract of whole blood by a fluorometric enzymatic method (23).

Methodological Error

Methodological error (imprecision) for a single analysis of glycogen, ATP, ADP, IMP, inosine, PCr, and creatine was determined by analyzing extracts from two corresponding fiber pools in 15 subjects and was found to be between 5 and 8%. The imprecision for a single blood or plasma analysis was determined by analyzing two extracts from the same blood or plasma sample (lactate, inosine, and bases) or analyzing plasma twice (ammonia and catecholamines); this imprecision varied between 2 and 8%.

Statistics

Unless otherwise stated, values in the text are means ± SD. The P values were accepted as statistically significant at $P < 0.05$. For the blood and plasma variables, a two-factor, repeated-measure ANOVA (gender and time, see Figs. 2 and 3) was applied to test the difference in response to repeated bouts of exercise between men and women. Single muscle fiber data was analyzed by a two- or three-factor, repeated-measure ANOVA. The factors were "gender and time" or "gender, time, and sprint." The term gender refers to women and men. Time refers to the variable's response to one of the following: *1*) before *sprint 1* and after *sprint 3* (the whole exercise period), *2*) after *sprint 1* and before *sprint 2* (the recovery period), or *3*) before and after sprints, independently of whether it is *sprint 1* or *3*. Sprint refers to *sprint 1* or *3*. No post hoc testing followed the repeated-measure ANOVA. Student's *t*-tests were applied for paired observations or independent groups. When means are described in the text as being different from each other, the P value was less than 0.05 unless otherwise stated.

RESULTS

Power Output

Power output is shown in Table 1. The peak power in *sprints 2* and *3* was 98 and 92%, respectively (comparison between *sprints 1* and *3*, $P < 0.05$), of the peak power in *sprint 1* in the men. Corresponding values for the women were 99 and 97% and not significantly different from peak power in *sprint 1*. The mean power in *sprint 3* compared with *sprint 1* was significantly and similarly reduced (~5%) in both women and men.

Fiber Types and Sizes

There were no differences in the relative number or in the relative area of different fiber types or in cross-sectional area of type I fibers between women and men (see Table 1). The cross-sectional area of type IIA and IIB fibers was 31 and 36% smaller in women than in men.

Metabolites and Substrates in Blood and Plasma

Metabolite and substrate in blood and plasma results are shown in Figs. 2 and 3. Repeated sprint exercise induced increases of ammonia, inosine, hypoxanthine, xanthine, uric acid, epinephrine, and norepinephrine in plasma and of lactate in blood, which all were smaller in women compared with men. The greatest difference between the genders was in plasma ammonia: the exercise-induced increase was 80% smaller in the women than in the men, observed 9 min after *sprint 3*. The exercise-induced increase in inosine was ~60% smaller in women than in men 15 min after *sprint 3*. Plasma hypoxanthine and xanthine showed a gender-related difference similar to that of inosine. In contrast to the other plasma metabolites, values of uric

acid were different between women and men already at rest. The levels of uric acid were lower in women than in men throughout the three sprints, although the difference between the genders increased by the third bout of exercise ($P < 0.005$). The exercise-induced increase in plasma epinephrine was ~40% smaller in women than in men immediately after *sprint 3*. For norepinephrine, this gender difference was 50%. The exercise-induced increase in blood lactate was ~30% smaller in women than in men at 9 min after *sprint 3*.

Metabolites and Substrates in Muscle

Metabolite and substrate results in muscle are shown in Tables 2 and 3. The statistical evaluation of the gender-related changes over time was mainly based on a three-factor ANOVA. The factors were gender (male and female), time (before and immediately after 30-s bout of sprint exercise), and sprint (*sprint 1* and *sprint 3*). Three types of interactions were identified: gender × time, gender × sprint, and sprint × time. No differences were observed between women and men at rest (before *sprint 1*) for ATP, ADP, TAN, PCr, or glycogen contents in type I or type II fibers. At the same point of time, IMP, inosine, and hypoxanthine were not detectable.

Gender × Time Interactions

No differences between women and men were detected for changes in ATP, ADP, TAN, IMP, PCr, or hypoxanthine contents during sprint exercise (no gender × time interactions in either type I or type II fibers). The exercise-induced reduction in glycogen was smaller in women than in men in type I fibers (gender × time, $P = 0.02$). No difference was found between women and men in glycogen reduction in type II fibers during sprint exercise.

Gender × Time × Sprint Interaction

Muscle inosine showed an accelerating difference over time: men developed a greater inosine content than woman in type II fibers (gender × time × sprint, $P < 0.001$). This gender difference appeared because of a gender × time interaction both during recovery and during the third bout of exercise.

Gender × Sprint Interactions

Independent of before or after exercise, ATP and TAN contents were higher and IMP and inosine lower in women than in men during *sprint 3* but not during *sprint 1* in type II fibers (gender × sprint, $P < 0.001$–0.01). A similar gender × sprint interaction was also seen in type I fibers but only for inosine ($P < 0.001$). Most likely, these gender-related differences were created during recovery periods because no gender-related differences in these metabolites could be detected during the bouts of exercise themselves. Support for a gender difference during recovery was found by evaluating the muscle metabolic change over the first 20-min period of recovery (post-*sprint 1* vs. pre-*sprint 2*). There were no significant differences between women

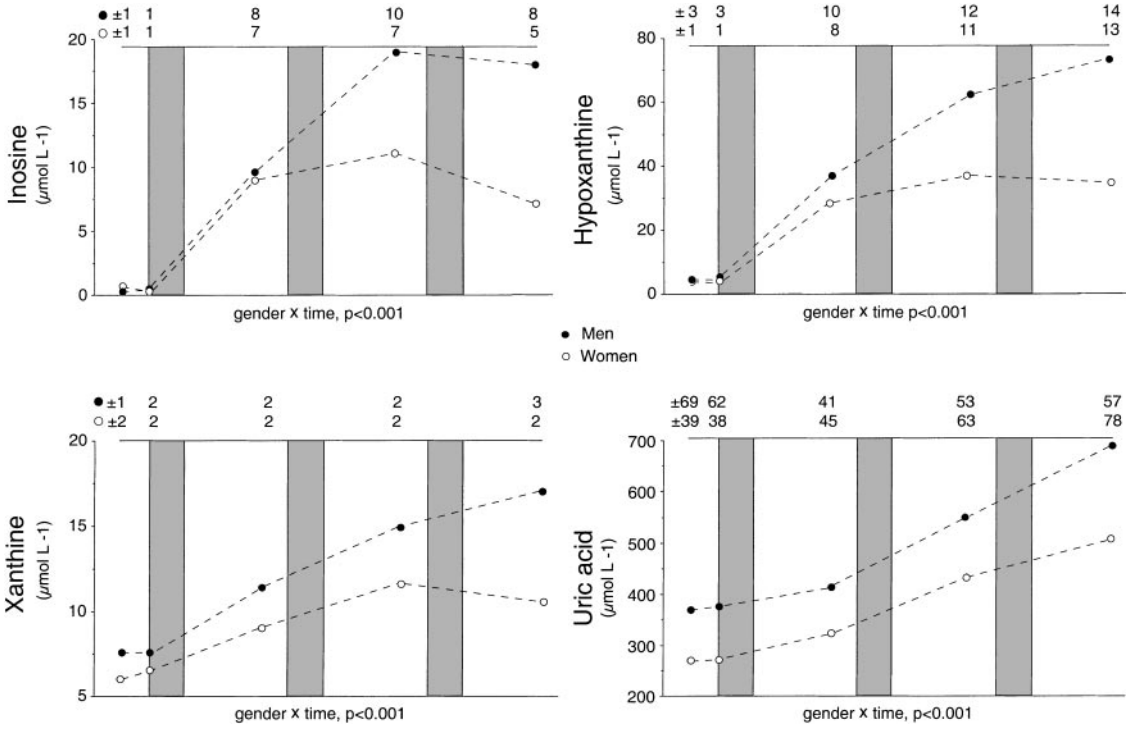

Fig. 2. Plasma inosine, hypoxanthine, xanthine, and uric acid concentration during repeated bouts of sprint exercise (see Fig. 1) in 8 women and 7 men. *P* value for the interaction term, gender × time, indicates the level of significance for the gender difference in changes in concentration of the different plasma variables. ± (*Top*) indicates SD.

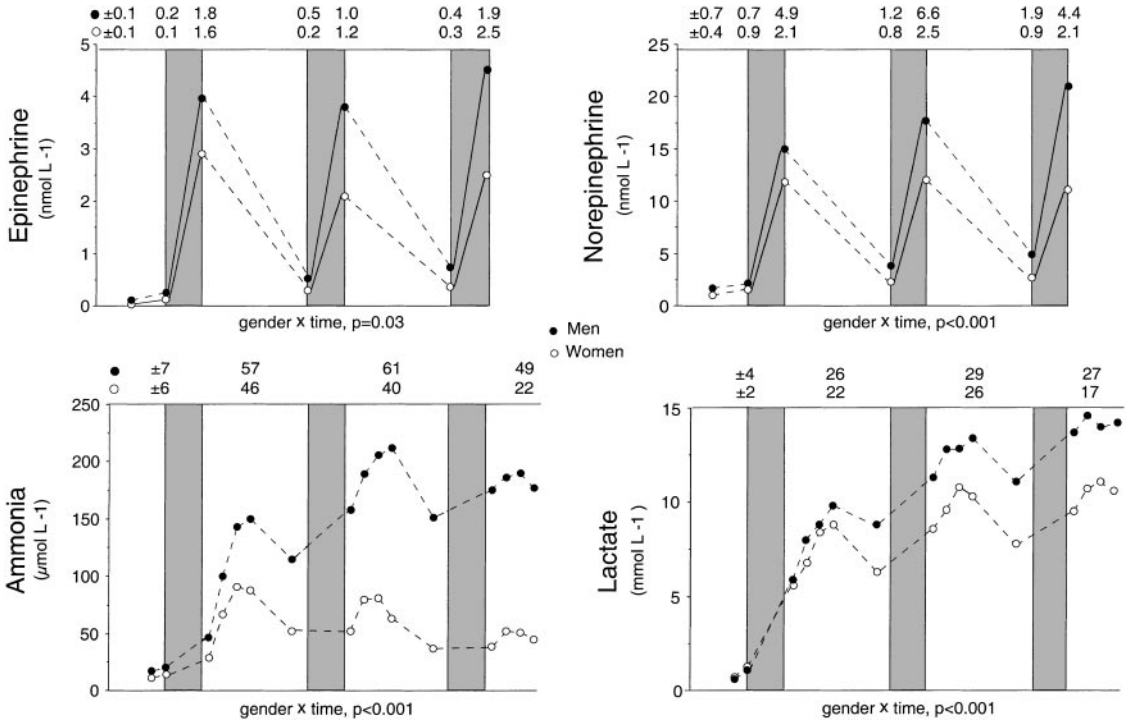

Fig. 3. Plasma ammonia, epinephrine, norepinephrine, and blood lactate concentration during repeated bouts of sprint exercise (see Fig. 1) in 8 women and 7 men. *P* value for the interaction term, gender × time, indicates the level of significance for the gender difference in changes in concentration of plasma variables and blood lactate. ± (*Top*) indicates SD.

and men directly after *sprint 1* for any of the studied metabolites or substrates. During the first 20-min period of recovery (post-*sprint 1* vs. pre-*sprint 2*), gender-related differences in metabolic or substrate changes were found. The decrease in IMP content was greater ($P < 0.04$) and the inosine content was smaller ($P < 0.04$) in women than in men in type II fibers. Furthermore, at the onset of *sprint 3* compared with before *sprint 1*, the ATP and TAN content was ~20% higher ($P < 0.03$–0.04) and the IMP content was 48% lower ($P < 0.03$) in women than in men in type II fibers. The inosine content was ~45% lower ($P < 0.02$) in women than in men in both fiber types. Again, inasmuch as no gender-related differences were found in the purine changes during the bouts of exercise themselves, these changes (pre-*sprint 3* vs. pre-*sprint 1*) are therefore suggested to indirectly reflect gender-related differences during recovery (rest) between the sprints.

Sprint × Time Interactions

The exercise-induced changes in ATP and TAN content were of similar magnitude and showed to be 60–65% smaller in both fiber types during *sprint 3* vs. for *sprint 1* (sprint × time, $P < 0.02$–0.001). The exercise-induced increase in IMP content was 75% smaller during *sprint 3* than for *sprint 1* in both fiber types (sprint × time, $P < 0.001$). The exercise-induced decrease in PCr in type II fibers content was ~35% greater during *sprint 3* than for *sprint 1* (sprint × time, $P < 0.001$), whereas no significant difference was found between sprints in PCr decrease in the type I fibers.

DISCUSSION

Major Findings

Possible differences between genders in the metabolic response to repeated bouts of maximal sprint exercise were investigated. Novel findings confirm the hypothesis that repeated sprint exercises including recovery periods induces smaller reduction of ATP and smaller accumulation of its breakdown products, IMP and inosine, in women than in men, especially in type II fibers. This was demonstrated without any gender-related differences in changes of ATP and its breakdown product inosine during the exercise bouts themselves. During recovery between the sprints, the decrease of IMP was greater and the subsequent inosine accumulation was smaller in women than in men. This indicates that the smaller ATP reduction in women than in men during repeated sprints was created during recovery periods between the sprint exercises and that women possess a faster recovery of ATP via reamination of IMP during these recovery periods.

Muscle Purines

There was no detectable accumulation of inosine or of the "downstream" metabolites (hypoxanthine, xanthine, or uric acid) during the exercise bouts in either type I or type II fibers. This was shown, despite the extremely high accumulation of IMP, during sprint exercise in both fiber types and indicates that the formation of inosine by the enzyme 5′ nucleotidase is a slow process. However, at rest between the bouts of

Table 2. *Purines and substrates in type I muscle fiber pools in 8 women and 7 men before and after sprint 1, before sprint 2, and before and after sprint 3*

	Women					Men					3-Factor ANOVA (g, t, s)	2-Factor ANOVA (g, t)	
	pre 1	post 1	pre2	pre 3	post 3	pre 1	post 1	pre 2	pre 3	post 3	pre 1, post 1, pre 3, post 3	post 1, pre 2	pre 1, pre 3
ATP	22.1	18.4	19.7	19.7	18.7	22.9	19.5	20.0	20.0	18.2	s × t: 0.01		
	±2.1	±1.9	±2.3	±1.8	±2.2	±1.5	±3.1	±1.9	±2.4	±3.8			
%	100	17	11	11	15	100	15	12	12	20			
ADP	3.1	3.4	3.2	3.1	3.5	3.1	3.5	3.2	3.1	3.4			
	±0	±1	±0	±0	±0	±1	±1	±1	±1	±1			
TAN	25.2	21.9	22.9	22.9	22.2	26.0	23.0	23.2	23.1	21.7	s × t: 0.02		
	±2.1	±2.2	±2.3	±1.9	±2.2	±1.7	±3.0	±1.7	±2.5	±3.9			
%	100	13	9	9	12	100	11	11	11	16			
IMP	0.0	5.6	1.0	0.3	1.4	0.0	4.0	1.0	0.6	2.1	s × t: 0.001	g × t: 0.08	
	±0	±2	±1	±0	±1	±0	±3	±1	±1	±2			
Inosine	0.00	0.03	0.31	0.33	0.28	0.00	0.04	0.55	0.63	0.74	g × s: 0.001		g × t: 0.01
	±0.0	±0.1	±0.2	±0.2	±0.1	±0.0	±0.1	±0.2	±0.1	±0.2			
HPX	0.00	0.00	0.18	0.24	0.19	0.00	0.00	0.20	0.23	0.27			
	±0.0	±0.0	±0.1	±0.2	±0.1	±0.0	±0.0	±0.0	±0.0	±0.1			
PCr	76	35	82	77	26	71	24	73	77	24			
	±10	±14	±10	±15	±14	±12	±12	±9	±8	±13			
%	100	54	−8	−1	66	100	66	−3	−8	66			
Glycogen	406	331	379	346	302	438	295	352	290	205	g × s: 0.01		g × t: 0.03
	±83	±47	±100	±95	±107	±114	±50	±45	±64	±71	g × t: 0.02		
%	100	18	6	15	25	100	32	19	34	53			

Values are means ± SD (in mmol/kg dry wt). The percent values denote the relative decrease at each point of time compared with before *sprint 1* (pre 1). The negative values denote an increase. TAN = ATP + ADP; PCr, phosphocreatine; HPX, hypoxanthine. ANOVA factors: g= gender, t = time [before (pre) and immediately (post) after the 30-s sprints] and s = *sprint 1* or *3*. Numbers shown after factors give statistical level of interaction (P ≤). Number after pre and post indicates sprint number.

exercise, the content of inosine increased in both fiber types.

Only a few studies have measured changes in inosine in human skeletal muscle during high-intensity exercise and the subsequent recovery (29, 31). In agreement with our findings, these studies showed that inosine increased mainly during recovery after exercise. The increase in inosine content found over the whole period of repeated sprint exercise was smaller in women than in men in both fiber types. In addition, the increase in inosine content during "recovery," i.e., from directly after *sprint 1* to before *sprint 2*, was significantly smaller in women than in men. This, together with the observation that no significant changes in inosine during exercise itself were observed, suggests that the gender-related difference in inosine content found over the whole exercise period seemed to be mainly generated during the recovery period between

Table 3. *Purines and substrates in type II muscle fiber pools in 8 women and 7 men before and after sprint 1, before sprint 2, and before and after sprint 3*

	Women					Men					3-Factor ANOVA (g, t, s)	2-Factor ANOVA (g, t)	
	pre 1	post 1	pre 2	pre 3	post 3	pre 1	post 1	pre 2	pre 3	post 3	pre 1, post 1, pre 3, post 3	post 1, pre 2	pre 1, pre 3
ATP	22.1	10.6	17.6	15.5	11.4	23.5	10.6	16.3	12.9	8.8	g × s: 0.05,		g × t: 0.04
	±1.8	±2.9	±2.5	±3.3	±3.3	±1.8	±4.5	±3.6	±3.0	±2.5	s × t: 0.001		
%	100	52	28	29	48	100	55	31	45	62			
ADP	3.1	3.2	3.2	3.5	3.7	3.3	3.5	2.9	3.4	3.6			
	±0	±0	±0	±1	±1	±0	±0	±0	±0	±0			
TAN	25.2	13.9	20.8	19.0	15.0	26.8	14.1	19.2	16.3	12.4	g × s: 0.04,		
	±1.8	±3.0	±2.4	±3.5	±3.7	±1.6	±4.5	±3.6	±3.1	±2.8	s × t: 0.001		
%	100	44	17	24	40	100	47	28	39	53			
IMP	0.0	15.0	3.3	3.9	7.9	0.0	16.0	5.6	7.5	11.1	g × s: 0.04,	g × t: 0.04	g × t: 0.03
	±0	±3.6	±2	±3	±4	±0	±3.7	±3	±3	±2.3	s × t: 0.001		
Inosine	0.00	0.06	0.54	0.60	0.35	0.00	0.00	0.82	1.07	1.26	g × t × s: 0.01	g × t: 0.04	g × t: 0.02
	±0.0	±0.2	±0.3	±0.4	±0.4	±0.0	±0.0	±0.2	±0.3	±0.3			
HPX	0.00	0.01	0.17	0.25	0.22	0.00	0.00	0.17	0.30	0.30			
	±0.0	±0.0	±0.1	±0.2	±0.1	±0.0	±0.0	±0	±0.1	±0.1			
PCr	80	26	91	89	14	75	22	76	81	12	s × t: 0.001		
	±11	±9	±13	±13	±7	±18	±8	±9	±11	±7			
%	100	68	−14	−11	82	100	70	−1	−8	84			
Glycogen	499	356	393	350	229	444	326	337	275	149			
	±117	±94	±136	±104	±93	±75	±75	±79	±124	±80			
%	100	28	21	30	54	100	26	24	38	66			

Values are means ± SD (in mmol/kg dry wt). Numbers shown after ANOVA factors give statistical level of significance ($P \leq$). The percent values denote the relative decrease at each point of time as compared to before *sprint 1* (pre 1). The negative values denote an increase.

the bouts of exercise. It seems that the smaller increase in inosine content in the women during recovery was not caused by a gender-related difference in IMP accumulation during the bouts of sprint exercise themselves. On the assumption that the K_m or V_{max} for 5′ nucleotidase is similar in women and men, the finding of a smaller inosine accumulation during recovery in women supports the earlier suggestion that women reaminate IMP to ATP at a faster rate. A faster reamination of IMP will reduce the rate of IMP dephosphorylation to inosine by less substrate availability. The mechanism behind this putative ability of women to recover faster after sprint exercise is not known. However, one possibility is that the smaller cross-sectional muscle fiber areas and thereby shorter diffusion distances in the woman than in men will favor the recovery process in women.

In the present study, recovery of peak power output in the third compared with the first bout of sprint exercise was almost complete in women but not in men. In a larger unpublished material (Esbjörnsson-Liljedahl and Jansson, unpublished observations), women and men differed significantly in this respect (women reached 99% and men reached 95%, $P < 0.01$). Thus a faster functional recovery in women may be related to a faster recovery of purine nucleotides and thereby also a faster elimination of breakdown products of which an especially inorganic phosphate is known to be involved in the fatigue processes (1, 8).

The ATP reduction and the IMP accumulation were markedly depressed during the third compared with the first bout of sprint exercise. This is in agreement with Casey et al. (7), who demonstrated a lower ATP reduction during a second bout of sprint exercise compared with the first bout.

The increase in inosine after exercise was ~1.5- to 2-fold greater in type II than in type I fibers (interaction term; fiber type × time, $P < 0.001$). However, the exercise-induced increase in IMP was three- to fourfold greater in type II than in type I fibers. The fiber-type difference was thereby greater for IMP than for inosine. This means that the accumulation of inosine in relation to IMP was smaller in type II compared with type I fibers. Fiber-type differences in exercise-induced inosine accumulation have not been studied before in human muscle. However, muscle contraction-induced accumulation of inosine and IMP in relation to fiber types has earlier been studied in rat muscle (2). These authors showed that the relationship between the accumulation of IMP and inosine differed between the fiber types in a way similar to that in the present study. The smaller accumulation of inosine in relation to IMP in type II than in type I fibers could be related to the lower activity of 5′ nucleotidase in type II fibers (32).

Muscle Glycogen

The exercise-induced glycogen reduction was smaller in women than in men in type I fibers during both of the studied 30-s exercise periods. As for purines and PCr, no difference between genders was found for glycogen reduction in type II fibers, either during the first or the third bout of exercise. The findings of repeated bouts of sprint exercise confirm gender-related and fiber-type-specific differences in glycogen reduction after only one bout of sprint exercise noted in a previous study (10). In accordance with this, an earlier study demonstrated that lactate accumulation in mixed muscle was smaller in women than in men during high-intensity exercise (18). One explanation for the lower rate of glycogenolysis in type I fibers in women could be related to the smaller increase in plasma catecholamines during sprint exercise in women than in men, a finding also demonstrated by others (6, 13, 25). Muscle glycogenolysis is partly stimulated by a β-receptor-induced increase in cAMP (20), for example, and type I fibers may respond more to this stimulation than type II fibers (3), since type I fibers have a threefold higher density of β-receptors than type II fibers (24). This idea is supported by the finding that, during electrical stimulation of vastus lateralis, the glycogen depletion in type I fibers was more dependent on plasma epinephrine levels than it was in type II fibers (14).

The estimated reduction of glycogen in mixed muscle during a 30-s sprint was ~20% smaller in women than in men in the present study (mixed glycogen data not shown). However, the mean power output per kilogram of fat-free body mass was not significantly different between the genders. Furthermore, there was no gender difference in net breakdown of ATP and PCr. Thus there seems to be a lack of ATP production in the women in relationship to the developed mean power if the amount of aerobically produced ATP is not greater in women than men during a 30-s sprint. In fact, Hill and Smith (16) measured oxygen uptake during a 30-s cycle sprint and found it to be relatively greater in women than in men.

Before the third bout of exercise, the glycogen level in type I fibers was decreased by ~15% in women, whereas in men glycogen was decreased by 35%, compared with levels observed before the first bout of sprint exercise (Tables 2 and 3). Most likely this can be explained by the smaller glycogen reduction seen in women compared with men during exercise bouts.

Plasma ATP Breakdown Products

The accumulation of ATP breakdown products in plasma was smaller in women than in men after sprint exercise. This gender-related difference was further accentuated by the repeated bouts of sprint exercise. This difference in the accumulation of ATP breakdown products does not seem to reflect differences between genders in the net ATP breakdown per unit muscle during sprint exercise, as measured either in type I or type II fibers. This conclusion is based on the difference between genders in accumulation of, for example, plasma ammonia and inosine after the series of sprints. This was seen despite any demonstrable difference between genders in accumulation of muscle IMP or inosine during each of the sprints in either type I or II fibers. A relationship between plasma ammonia

and muscle IMP accumulation is to be expected because ammonia and IMP are formed in equimolar amounts during net breakdown of ATP (22, 31). However, the difference between women and men in the sprint exercise-induced increase in plasma inosine could partly be related to their difference in accumulation of muscle inosine during the recovery periods between the sprints.

The lower plasma values of ammonia in women compared with men, despite a similar muscle accumulation of IMP after sprint exercise, suggest that women have a larger distribution volume for ammonia or a larger capacity to eliminate ammonia compared with men. However, knowledge regarding distribution volume of ammonia is lacking. The role of inactive muscle and fat tissue in ammonia metabolism and ammonia buffering requires further investigation.

Methodological Considerations

Physical activity. When attempting to identify gender-related differences in metabolic response during sprint exercise, it is important that the groups are comparable with respect to habitual physical activity. According to the questionnaire-based activity index calculated in the present study, the leisure-time physical activity was similar in men and women. Also, the physical activity during daytime was similar because they attended the same classes. Thus the subjects in the present study can be considered well matched with respect to physical activity. Furthermore, all subjects of both genders were on similar diets, as all meals were served at the boarding school.

Limitation of the biopsy procedure. To study a metabolic time course, multiple biopsies are needed. One problem with repeated muscle tissue sampling is that a preceding biopsy may affect the following by causing, for instance, bleedings, especially if the biopsies are obtained through the same incision. Thus, to avoid such interference, ideally the biopsies should be obtained from different incisions and different sites. However, the drawbacks of such a strategy are that the amount of local anesthesia and the number of incisions have to be increased. In turn, this elevates the risk of interference with the exercise-induced related changes, and furthermore the samples may represent different regions of the muscle possessing different metabolic and contractile properties. However, there are no methodological studies on this topic, and the chosen strategy in every single study is more or less based on the researcher's own experiences. In the present study, we took the following precautions to minimize the negative effects: In total, five biopsies were obtained from each subject. The first two (pre- and post-*sprint 1*) were obtained from the same leg and through the same incision but from different areas (the biopsy needle was directed slightly differently). The third biopsy was obtained through a second incision 4–5 cm distal to the first incision. The last two biopsies (pre- and post-*sprint 3*) were obtained from the other leg and through the same incision, as in the first leg. This strategy will minimize the amount of local anesthesia and the number of scars in that all three rest or recovery biopsies (1st, 3rd, and 4th) are obtained through separate incisions and are the first biopsy through each incision. Both exercise biopsies (2nd and 5th) were also obtained through separate incisions but as the second biopsy through each incision. To avoid damaged areas, the needle direction was slightly different from the first biopsy through the same incision. By this strategy, we think we have an optimal balance between the need for repeated biopsies and the need to minimize muscle damage and the risk to sample muscle tissue from different regions. To reach this, we decided not to take a biopsy directly after *sprint 2*. Supporting that we have minimized the methodological problems with repeated biopsy sampling is the finding of significant correlations for most of the metabolites between the two recovery biopsies (3rd and 4th biopsy) and also between the two exercise biopsies (2nd and 5th biopsy) (data are not shown).

In summary, repeated bouts of sprints with recovery periods in between induced a smaller reduction of ATP and smaller accumulation of IMP in women compared with in men. This gender-related difference was most prominent in type II muscle fibers and seemed to be mainly generated during the recovery periods between the bouts of exercise. It is suggested that recovery of ATP via the reamination of IMP during recovery might be faster in women than in men. Repeated sprint exercises also induced smaller reduction of glycogen, in type I fibers in women compared with men, a difference mainly generated during the exercise bouts. These described gender-related differences in acute metabolic response to repeated bouts of sprint exercise may contribute to the earlier demonstrated a faster functional recovery in women than in men after sprint exercise.

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