Fat Oxidation After Acipimox-Induced Reduction in Plasma Nonesterified Fatty Acids During Exercise at 0°C and 20°C

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The main aim of this study was to investigate if whole body fat oxidation, after acipimox administration, during submaximal exercise in the cold, is different from that at temperate environments. Seven healthy recreationally active male subjects cycled at 70% $\dot{V}O_{2peak}$ for 60 minutes; once at 0°C and once at 20°C. To exclude availability, and therefore oxidation of plasma-derived nonesterified fatty acids (NEFA), 90 minutes before each cycling bout, subjects ingested 250 mg of the antilipolytic drug, acipimox. Blood and expired gas measurements were obtained at rest, immediately before exercise, and at 15, 30, 45, and 60 minutes of exercise. In both trials, after the ingestion of acipimox, plasma NEFA concentrations fell dramatically and immediately before and during exercise were lower than 0.05 mmol · L^{-1} in both trials. Pre-exercise and exercise values of glycerol, glucose, triacylglycerol (TG), and rectal temperature (T_{re}) were not different between the 0°C and 20°C trials. During exercise at 0°C, skin temperature (T_{sk}) was significantly reduced from pre-exercise values ($P < .05$) and at all time points was significantly lower than during exercise at 20°C. Muscle temperature did not differ between trials but in both trials was lower ($P < .05$) at 1 cm depth than at 3 cm and 2 cm. Gross energy expenditure of cycling (0°C trial, 3.6 ± 0.1 MJ; 20°C trial, 3.6 ± 0.1 MJ), the oxidation rates of carbohydrate (0°C, 32.4 ± 0.5 KJ · min^{-1}; 20°C, 32.6 ± 0.7 KJ · min^{-1}) and fat (0°C, 24.6 ± 1.2 KJ · min^{-1}; 20°C, 23.0 ± 1.8 KJ · min^{-1}), and the proportion of energy derived from fat (0°C, 45 ± 1 %; 20°C, 40 ± 4%) and carbohydrate (0°C, 55 ± 1%; 20°C, 58 ± 3%) were not different between the 2 trials. In conclusion, after acipimox administration, whole body fat oxidation during exercise, designed to avoid adjustment of core temperature or thermogenesis, is not different at 0°C compared with 20°C. This allows the inference that during submaximal exercise, cold has no effect on the utilization of intramuscular TG (IMTG).

THE IMPORTANCE of lipid as a substrate for energy expenditure during exercise is well documented. However, some investigations in a cold air environment have reported that, during submaximal exercise as short as 40 to 60 minutes, the contribution of fat to whole body energy expenditure is reduced.[1,2] In addition, this occurs in spite of maintenance of core and active muscle temperature.[2] The mechanisms responsible for the reduced utilization of fat under these conditions remain a matter of debate.

During most exercise situations, the contribution of fat towards exercise energy expenditure is a function of the plasma concentration of nonesterifed fatty acids (NEFA).[3,4] However, in the cold an uncoupling between availability and oxidation of plasma NEFA has been noted at rest[5] and during exercise.[6] This suggests that one of the mechanisms involved in the reduced oxidation of fat seen during exercise in the cold involves reduced utilization of plasma NEFA. The utilization of other potential sources of fat such as intramuscular triacylglycerol (IMTG) and circulating plasma triacylglycerol (TG) lipoproteins, may also be influenced by the cold whilst exercising, but this possibility has not been explored.

Administration of acipimox, a nicotinic acid analog that is a powerful inhibitor of lipolysis in adipose tissue,[7] leads to the complete suppression of the exercise-induced increase in plasma concentration of NEFA[8-16] through its action on hormone-sensitive lipase (HSL).[17] It is therefore likely, that after administration of acipimox, there is a removal of fat oxidation obtained from plasma NEFA. Of the other sources of lipid, very-low-density lipoproteins, the principle source of TG-rich lipoproteins in the fasted state, contributes little to exercise energy metabolism[18,19] and is further reduced by acipimox administration.[20] On the contrary, evidence suggests that utilization of IMTG, a major contributor of non–plasma-derived NEFA during low- and moderate-intensity aerobic exercise,[21] is increased following acipimox administration.[22] Thus, investigation of total fat oxidation during exercise under conditions of acipimox-induced reduction in plasma NEFA could serve as useful model to provide an insight on the effect of cold on utilization IMTG.

The aim of this study was to investigate if whole body fat oxidation, after acipimox administration, during 60 minutes of cycling exercise in the cold at 70% $\dot{V}O_{2max,}$ to avoid adjustment of core temperature or thermogenesis, is different from that at temperate environments. More specifically, we aimed to test the hypothesis that during this type of exercise cold exposure has no impact on the oxidation of IMTG.

MATERIALS AND METHODS

Subjects

Subjects were 7 healthy men with the following physical characteristics (mean ± SD): age, 24.1 ± 8 years; body mass index, 22.8 ± 0.9 kg · m^{-2}; body fat level, 13.0% ± 0.5%; peak oxygen uptake ($\dot{V}O_{2peak}$) 55.1 ± 4.7 mL · kg^{-1} · min^{-1}.

The University of Strathclyde Ethics Committee approved all procedures and subjects provided written consent. All subjects were nonsmokers and none used any medication. The subjects were recreationally active.

Study Design

Subjects underwent 2 trials, with an interval of 5 to 7 days between trials. For each trial subjects reported to the laboratory, between 9 and

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Submitted October 23, 2003; accepted March 25, 2004.

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0026-0495/04/5309-0004$30.00/0
doi:10.1016/j.metabol.2004.03.016

10 AM, in a fasted state. Subjects cycled at 70% $\dot{V}O_{2peak}$, with an air velocity of 0.35 m · s^{-1} for 60 minutes. At 0°C an insulated hood covered the flywheel to ensure that the temperature at this point was maintained at approximately 20°C and therefore did not alter the workload. Four subjects were exposed to 0°C before 20°C and the remaining 3 the converse. Ninety minutes before cycling they ingested acipimox. In the 24-hour period prior to each trial, subjects abstained from alcohol, caffeine, and vigorous physical activity. To minimize differences in metabolic heterogeneity, subjects weighed and recorded all the food and drink they consumed during the 48 hours leading up to the first experimental trial, and were asked to replicate this prior to the subsequent trial. To ensure euhydration subjects consumed 1 L of water the night before and 0.5 L two hours prior to each experiment.

Preliminary Exercise Tests

Prior to the main experimental trials, 2 preliminary tests were conducted. In the first test, $\dot{V}O_{2peak}$ was determined. Subjects performed a continuous incremental cycling test to volitional exhaustion on a mechanically braked cycle ergometer (Monarch 864, Varburg, Sweden) at a room temperature of 20°C. Expired air was collected and analyzed via an on-line system (Oxycon Gamma, Mijnhardt, Holland). A pilot study conducted on 6 subjects revealed that $\dot{V}O_{2peak}$ was not affected by ambient temperatures ranging from −10°C to 20°C. In the second test, subjects completed a familiarization trial in an environmental chamber at 20°C at a workload corresponding to 70% $\dot{V}O_{2peak}$, which confirmed the workload for the main experimental trials.

Main Exercise Trial

On arrival at the laboratory, subjects were weighed nude and a rectal thermistor was inserted 12 cm beyond the anal sphincter (Grant Instruments, Cambridge, UK). During all trials subjects wore the same shorts, socks, and shoes. An antecubital vein of either the right or left arm was cannulated for the collection of blood samples. Subjects then rested in a seated position for 30 minutes in a thermoneutral room during which time skin thermistors and heart rate (HR) monitors were put in place. A resting blood sample was drawn and expired gas collected to assess metabolic homogeneity prior to exercise between conditions. Following the collection of resting samples subjects ingested a 250-mg capsule of acipimox (Olbetam; Farmitalia Carlo Erba, Milton Keynes, UK). Ninety minutes after acipimox ingestion, pre-exercise blood samples and expired gas were collected, and measurements of HR, skin temperatures (T_{sk}), and rectal temperature (T_{re}) were made. Subjects then entered the climatic chamber (SANYO Gallenkamp, Loughborough, UK) and commenced cycling immediately. Every 15 minutes during exercise a blood sample was drawn, expired air collected, and HR, T_{sk}, T_{re}, and ratings of perceived exertion (RPE)[23] were recorded. Immediately post-exercise, muscle temperature (T_m) of vastus lateralis was recorded.

Measurements

Expired gas was collected into a Douglas bag over a 1-minute period and immediately analyzed for O_2 and CO_2 concentrations (Servomex 1440, Crowborough, UK). The expired gas volume was determined using a dry gas meter (Harvard, Kent, UK), and O_2 and CO_2 production were calculated using the Haldane transformation. Energy expenditure and substrate oxidation during cycling were estimated from O_2 uptake and CO_2 production using indirect calorimetry, neglecting protein oxidation.[24]

Skin temperatures were measured at 8 sites using surface thermistors (Grant Instruments) attached with a single layer of waterproof tape and $\bar{T}_{sk}$ was calculated with the following equation[25]: $\bar{T}_{sk} = 0.07 \times T_{forehead} + 0.175 \times T_{right\ scapula} + 0.175 \times T_{left\ upper\ chest} + 0.07 \times T_{right\ upper\ arm} + 0.07 \times T_{left\ forearm} + 0.05 \times T_{left\ hand} + 0.19 \times T_{right\ anterior\ thigh} + 0.2 \times T_{left\ calf}$.

All thermistors were calibrated (±0.1°C) against a certified reference mercury thermometer (Zeal, London, UK). Temperatures were recorded using a portable data logger (1206 Series Squirrel, Grant Instruments).

Muscle temperature was recorded using a muscle needle probe (Ellab, Roedovre, Denmark), inserted into the vastus lateralis while subjects remained seated on the bicycle. Muscle temperature measurements were recorded at 3, 2, and 1 cm from the skin surface, as during exercise in the cold there is a variation of muscle temperature at different depths.[26] The coefficient of variation for the measurement of muscle temperature, using data from 5 subjects who completed exercise at 0°C on 2 occasions, without any intervention, was calculated to be 0.8%, 1.9%, and 7.0% at 3 cm, 2 cm, and 1 cm depth, respectively.

Analytical Methods

Hemoglobin (cyanmethemoglobin method) and hematocrit (microcapillary technique) were measured for estimation of changes in plasma volume.[27] Blood samples were dispensed into precooled K-EDTA monovettes. Duplicate 100-μL aliquots from each blood sample were deproteinized in 0.4 mmol/L perchloric acid for the measurement of glycerol[28] and glucose (glucose oxidase method, Boehringer Mannheim GmbH Diagnostica, Berkshire, UK). The remaining blood was immediately centrifuged at 4,000 rpm at 4°C for 10 minutes. The plasma was then frozen at −20°C until analyses of NEFA concentration (enzymatic colorimetric method, Boehringer Mannheim GmbH Diagnostica) and TG (INFINITY Triglyceride Reageant, Sigma Diagnostics, Dorset, UK). Samples from both trials were treated in the same way.

Statistical Analysis

Results are shown as means ± SEM. Differences in resting and pre-exercise values were compared by paired *t* test. Responses during the exercise period were compared by 2-factor (trial by time) repeated-measures analysis of variance (ANOVA) with a Tukey post hoc test used to locate the differences. The level of significance was set at $P < .05$.

RESULTS

Plasma volume was similar before each cycling (difference, 0.8% ± 0.8%), and changes during exercise were not significantly different (0°C, 3.1% ± 0.9%; 20°C, 4.9% ± 0.7%). Plasma concentrations of metabolites therefore were not adjusted.

Resting plasma NEFA concentrations were not significantly different between 0°C and 20°C trials (0°C trial, 0.35 ± 0.04 mmol · L^{-1}; 20°C trial, 0.32 ± 0.05 mmol · L^{-1}). Nor was there a difference in resting TG (0°C trial, 0.73 ± 0.10 mmol · L^{-1}; 20°C trial, 0.77 ± 0.11 mmol · L^{-1}). The main side effect of acipimox, flushing, was observed transiently in 4 subjects, but this disappeared quickly and skin colour returned to normal prior to cycling. Following acipimox ingestion, plasma NEFA levels were significantly reduced from resting values in both trials ($P < .05$) and remained low throughout exercise with no significant differences being observed between trials (Fig 1). Pre-exercise values of glycerol (0°C trial, 28 ± 6 μmoL · L^{-1}; 20°C trial, 33 ± 6 μmoL · L^{-1}), glucose (0°C trial, 5.3 ± 0.01 mmol · L^{-1}; 20°C trial, 5.3 ± 0.01 mmol · L^{-1}), TG (0°C trial, 0.69 ± 0.13 mmol · L^{-1}; 20°C trial, 0.77 ± 0.13 mmol · L^{-1}), T_{re} (0°C trial, 36.6 ± 0.1°C; 20°C trial, 36.6 ± 0.1°C), and $\bar{T}_{sk}$ (0°C trial, 32.2 ± 0.2°C; 20°C trial, 32.3 ± 0.3°C) were not significantly different between the 2 trials. During exercise,

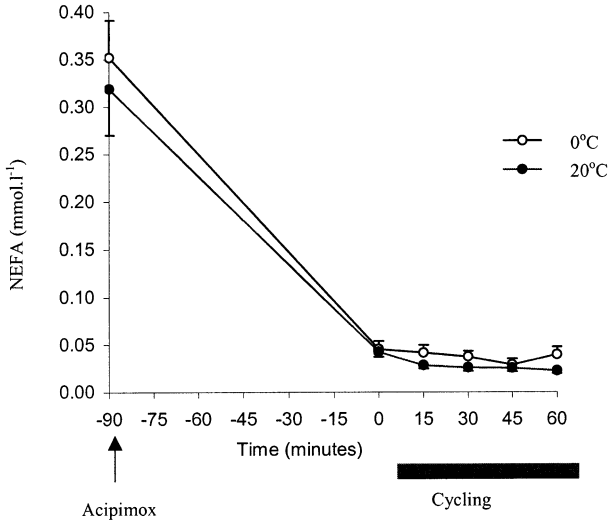

Fig 1. Plasma NEFA concentration before ingestion of 250 mg of acipimox (−90 minutes), immediately before (0 minutes), and during 60 minutes of submaximal cycle ergometer exercise conducted at 0°C and 20°C. Values are mean ± SEM.

concentration of glycerol, glucose, and TG, and values of T_{re} were not different between the 0°C and 20°C trials (Table 1). During exercise at 0°C, $\bar{T}_{sk}$ was significantly reduced from pre-exercise values ($P < .05$) at all time points and was significantly lower than during exercise at 20°C (Table 1). Muscle temperature did not differ between trials but in both trials the muscle temperature was less ($P < .05$) at 1cm depth than at 3 and 2 cm (Table 2).

Subjects exercised at a mean of 70.2% ± 1.6% $\dot{V}O_{2peak}$ at 0°C and 70.2% ± 2.2% $\dot{V}O_{2peak}$ at 20°C. Oxygen consumption, HR, and RPE were not significantly different during exercise at 0°C and 20°C (Table 1). Gross energy expenditure of cycling (0°C trial, 3.6 ± 0.1 MJ; 20°C trial, 3.6 ± 0.1 MJ) and exercise values of the RER (Table 1) were not significantly different between the 2 trials. This resulted in oxidation rates of carbohydrates and fat (Fig 2), and the proportion of energy derived from fat and carbohydrate (Fig 3) being similar in both conditions.

DISCUSSION

The major finding of the present study is that whole body fat oxidation, after acipimox administration during exercise, designed to avoid adjustment of core temperature or thermogenesis, is not different at 0°C compared with 20°C. This allows the inference that during exercise of this type, fat oxidation obtained from the combined contribution of IMTG and plasma TG lipoproteins is not influenced by cold. More specifically, taking into consideration the fact that very-low-density lipoproteins, the principal source of TG-rich lipoproteins in the fasted state, contributes little to energy metabolism[18,19] and is further reduced by acipimox administration,[20] the data of this study confirm the hypothesis that during submaximal exercise cold has no effect on the utilization of IMTG. These indirect findings contribute to the understanding of mechanisms responsible for the reduced utilization of fat seen during exercise in the cold[1,2] and allows the suggestion that the decrease in fat oxidation seen during exercise at lower ambient temperatures could not be explained by the effect of cold on the utilization of IMTG or plasma TG lipoproteins. Therefore, as suggested in our previous study,[6] the uncoupling between the availability and oxidation of plasma NEFA may be the only mechanism involved in the reduced oxidation of fat in the cold.

The comparison of fat oxidation obtained from IMTG during submaximal cycling at 0°C and 20°C was based on the following assumptions about the action of acipimox. First, acipimox is a powerful inhibitor of lipolysis in adipose but not in muscle tissue. The inhibition of lipolysis in adipose tissue through the observation of significantly reduced plasma NEFA throughout exercise conducted after oral acipimox administration has been shown in numerous studies[8-16] and is further supported by the current study. A lack of inhibition of HSL in muscle is supported by the fact that the pharmacological sites of action of nicotinic acid are largely restricted to adipose tissue and spleen.[29] In addition to this, it has

Table 1. Heart Rate, Rating of Perceived Exertion, Respiratory Exchange Ratio, Rectal Temperature, Mean Skin Temperature, Plasma Triacylglycerol, and Blood Glucose and Glycerol During 60-Minute Cycling at 70% $\dot{V}O_{2peak}$ at 0°C and 20°C

	15 min		30 min		45 min		60 min	
	0°C	20°C	0°C	20°C	0°C	20°C	0°C	20°C
$\dot{V}O_2$ (L · min^{-1})	2.74 ± 0.10	2.67 ± 0.08	2.80 ± 0.12	2.85 ± 0.08†	2.91 ± 0.10*	2.93 ± 0.08†	3.00 ± 0.11*	2.99 ± 0.09†
HR (beats · min^{-1})	139 ± 4	142 ± 5	143 ± 6	151 ± 6†	149 ± 6*	154 ± 6†	154 ± 6*	160 ± 7†
RPE	12 ± 0	12 ± 0	13 ± 0	13.1 ± 0	13 ± 1*	14.0 ± 1†	15 ± 1*	15 ± 1†
RER	0.88 ± 0.01	0.90 ± 0.02	0.86 ± 0.00	0.87 ± 0.01	0.86 ± 0.01	0.86 ± 0.01	0.86 ± 0.01	0.87 ± 0.01
T_{re} (°C)	37.1 ± 0.1	37.0 ± 0.1	37.7 ± 0.1	37.5 ± 0.1	38.0 ± 0.1	37.6 ± 0.3	38.1 ± 0.1	37.7 ± 0.3
$\bar{T}_{sk}$ (°C)	22.7 ± 0.2	30.0 ± 0.8‡	22.1 ± 0.2	30.8 ± 0.7‡	22.1 ± 0.3	30.9 ± 0.7‡	22.1 ± 0.3	30.7 ± 0.7‡
TG (mmol · L^{-1})	0.74 ± 0.16	0.62 ± 0.07	0.69 ± 0.13	0.67 ± 0.10	0.63 ± 0.10	0.67 ± 0.08	0.66 ± 0.16	0.67 ± 0.11
Glucose (mmol · L^{-1})	4.8 ± 0.02	5.0 ± 0.02	4.7 ± 0.02	5.1 ± 0.02	4.6 ± 0.02	4.9 ± 0.02	4.4 ± 0.02	4.7 ± 0.01
Glycerol (μmol · L^{-1})	39 ± 4	36 ± 5	37 ± 5	39 ± 7	36 ± 5	33 ± 7	37 ± 4	38 ± 9

NOTE. Values are mean ± SEM.

Abbreviations: $\dot{V}O_2$, oxygen consumption; HR, heart rate; RPE, rating of perceived exertion; RER, respiratory exchange ratio; T_{re}, rectal temperature; $\bar{T}_{sk}$, mean skin temperature; TG, triacylglycerol.

*Significantly different ($P < .05$) from value at 15 minutes in 0°C.

†Significantly different ($P < .05$) from value at 15 minutes in 20°C trial.

‡Significantly different ($P < .05$) from value at corresponding time point in 0°C trial.

Table 2. Muscle Temperature Immediately After 60-Minute Cycling at 70% $\dot{V}O_{2peak}$ at 0°C and 20°C

	Depth		
	3 cm	2 cm	1 cm
0°C	38.8 ± 0.1	38.3 ± 0.2	36.0 ± 0.6*
20°C	38.8 ± 0.1	38.3 ± 0.3	36.6 ± 0.4*

NOTE. Values are means ± SEM.
*Significantly different ($P < .05$) from 3 and 2 cm depth.

been demonstrated that the control of IMTG hydrolysis is not solely related to the level of HSL activation[30] and that the limited inhibitory action of acipimox on HSL in muscle may be confounded by muscle contraction.[31,32] Thus, after administration of acipimox, utilization of IMTG, the main contributor of non–plasma-derived fatty acids, should remain significant. This is in line with the fact that during moderate intensity exercise conducted under the action of this drug, utilization of IMTG is increased rather than diminished.[22]

The second assumption of the methodological approach was that the contribution of TG-rich lipoproteins would be minimal over the exercise period. This can be partially defended through the observation that in this study over the whole period of exercise, plasma TG concentration remained stable and was not significantly different from pre-exercise values. At the same time, it is known that after an acute morning dose of acipimox, synthesis of very-low-density lipoprotein is nearly completely halted for several hours.[20] When combined together these 2 facts suggest that during exercise, conducted after acipimox administration, uptake and therefore oxidation of fatty acids derived from TG-rich lipoproteins is negligible.

Evidence exists to suggest that the velocity of the enzymes involved in energy pathways during submaximal exercise, may be associated with muscle temperature.[33,34] This is not surprising given that the Q_{10} (the fold increase in reaction rate for a 10°C rise in temperature) commonly found for enzyme-mediated reactions is 2.0 to 3.0.[35] Thus, it might have been expected that if muscle temperature had been lower at 0°C than at 20°C a direct Q_{10} effect on IMTG breakdown could have occurred. The finding that muscle temperature of the vastus lateralis, recorded at 3, 2, and 1 cm from the skin surface immediately post-exercise was not different between temperate and cold environment trials, perhaps could explain the integrity of the IMTG utilization process at 0°C.

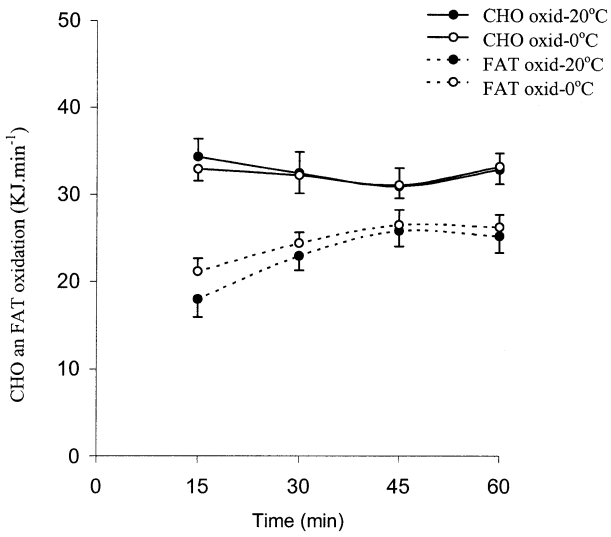

Fig 2. Rates of carbohydrate (CHO oxid) and fat oxidation (Fat oxid) during 60 minutes of submaximal cycle ergometer exercise conducted at 0°C and 20°C. Exercise was conducted 90 minutes after ingestion of 250 mg of acipimox. Values are mean ± SEM.

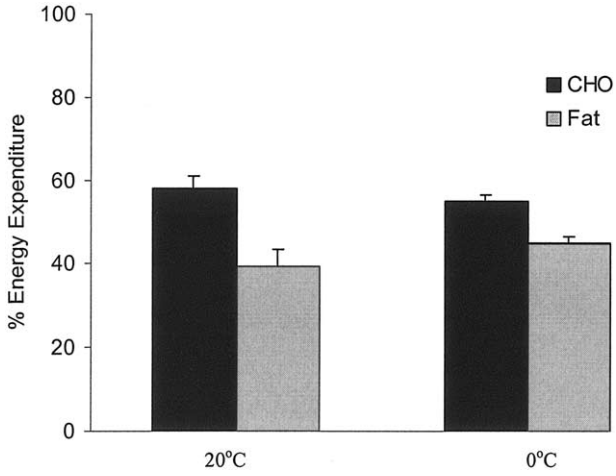

Fig 3. Relative contribution of carbohydrate (CHO) and fat to whole body substrate oxidation during 60 minutes of submaximal cycle ergometer exercise conducted at 0°C and 20°C. Exercise was conducted 90 minutes after ingestion of 250 mg of acipimox. Values are mean ± SEM.

In this study, during exercise at both 0°C and 20°C, fat oxidation accounted for approximately 40% of the total energy, which was consistent with the low average RER values seen over the whole period of exercise. This high value of fat oxidation following acipimox may not only reflect the fact that subjects exercised at 70% $\dot{V}O_{2peak}$, the intensity that elicits the maximum fat oxidation,[36] but also indicates that in the subjects of this study, the rate of IMTG utilization, the major contributor of fat after acipimox administration, was high. Indeed, based on the evidence that during exercise at 65% $\dot{V}O_{2max}$, at temperate environments, endurance-trained individuals show a 60% contribution from lipid sources[3] with approximately half being derived from IMTG,[3,37] the proportion of energy obtained from fat under conditions of this experiment would be expected not to exceed 30%. However, the high rate of fat oxidation is in line with the possibility of increased activity of muscle HSL under circumstances of reduced availability of plasma NEFA[38] and the fact that after administration of acipimox IMTG oxidation is upregulated to compensate for the reduction in plasma NEFA oxidation.[21]

In conclusion, whole body fat oxidation during cycling exercise, following acipimox ingestion, was not different at 0°C compared with 20°C. This indirectly suggests that in the cold, utilization of IMTG is not different from that at ambient temperatures. Future studies, employing muscle biopsy, isotope tracer, or ^{1}H-magnetic resonance spectroscopy, could be used to support this hypothesis, although these methods too have inherent errors.

REFERENCES

1. Febbraio MA, Snow RJ, Stathis CG, et al: Blunting the rise in body temperature reduces muscle glycogenolysis during exercise in humans. Exp Physiol 81:685-693, 1996
2. Layden JD, Patterson MP, Nimmo MA: Effect of reduced ambient temperature on fat utilization during submaximal exercise. Med Sci Sports Exerc 34:774-779, 2002
3. Romijn JA, Coyle E, Sidossis LS, et al: Regulation of endogenous fat and carbohydrate metabolism in relation to exercise intensity and duration. Am J Physiol 265:E380-E391, 1993
4. Horowitz JF, Klein S: Lipid metabolism during endurance exercise. Am J Clin Nutr 72:558S-563S, 2000 (suppl 2)
5. Vallerand AL, Zamecnik J, Jones PJH, et al: Cold stress increases lipolysis, FFA, Ra and TG/FFA cycling in humans. Aviat Space Environ Med 70:42-50, 1999
6. Layden JD, Malkova D, Nimmo MA: During exercise in the cold increased availability of plasma NEFA does not affect the pattern of substrate oxidation. Metabolism 53:203-208, 2004
7. Fuccella LM, Goldaniga G, Lovisolo P: Inhibition of lipolysis by nicotinic acid and by acipimox. Clin Pharmacol Ther 28:790-795, 1980
8. Carlson LA, Havel RJ, Ekelund LG, et al: Effects of nicotinic acid on the turnover rate and oxidation of the free acids of plasma in man during exercise. Metabolism 12:837-845, 1963
9. Heath EM, Wilcox AR, Quinn CM, et al: Effects of nicotinic acid on respiratory exchange ratio and substrate levels during exercise. Med Sci Sports Exerc 25:1018-1023, 1993
10. Howlett KF, Spriet LL, Hargreaves M: Carbohydrate metabolism during exercise in females: effect of reduced fat availability. Metabolism 50:481-487, 2001
11. Murray R, Bartoli WP, Eddy DE, et al: Physiological and performance responses to nicotinic acid ingestion during exercise. Med Sci Sports Exerc 27:1057-1062, 1995
12. Van Baak MA, de Haan A, Saris WH, et al: Beta-adrenoceptor blockade and skeletal muscle energy metabolism during endurance exercise. J Appl Physiol 78:307-313, 1995
13. Malkova D, Hardman AE, Bowness RJ, et al: The reduction in postprandial lipemia after exercise is independent of the relative contributions of fat and carbohydrate to energy metabolism during exercise. Metabolism 48:245-251, 1999
14. Head A, Jakeman PM, Kendall MJ, et al: The impact of short course of three lipid lowering drugs on fat oxidation during exercise in healthy volunteers. Postgrad Med J 69:197-203, 1993
15. Stellingwerff T, Watt MJ, Heigenhauser GJ, et al: Effects of reduced free fatty acid availability on skeletal muscle PDH activation during aerobic exercise. Pyruvate dehydrogenase. Am J Physiol 284: E589-E596, 2003
16. Wang W, Basinger A, Neese RA, et al: Effects of nicotinic acid on fatty acid kinetics, fuel selection, and pathways of glucose production in women. Am J Physiol 279:E50-E59, 2000
17. Madsen J, Malchow-Moller A: Effects of glucose, insulin and nicotinic acid on adipose tissue blood flow in rats. Acta Physiol Scand 118:175-180, 1983
18. Kiens B, Lithell H: Lipoprotein metabolism influenced by training-induced changes in human skeletal muscle. J Clin Invest 83:558-564, 1989
19. Turcotte LP, Richter EA, Kiens B: Increased plasma FFA uptake and oxidation during prolonged exercise in trained vs. untrained humans. Am J Physiol 262:E791-E799, 1992
20. Wang W, Basinger A, Neese RA, et al: Effect of nicotinic acid administration on hepatic very low density lipoprotein-triglyceride production. Am J Physiol 43:E540-E547, 2001
21. Spriet LL: Regulation of skeletal muscle fat oxidation during exercise in humans. Med Sci Sports Exerc 34:1477-1484, 2002
22. Coyle EF, Jeukendrup AE, Wagenmakers AJM, et al: Intramuscular triglyceride oxidation during exercise acutely increases with reduced plasma FFA mobilisation and oxidation. FASEB J 10:A143, 1998 (abstr)
23. Borg GAV: Perceived exertion as an indicator of somatic stress. Scand J Med 2:92-98, 1970
24. Frayn KN: Calculations of substrate oxidation rates in vivo from gaseous exchange. J Appl Physiol 55:628-634, 1983
25. International Organization for Standardization: Evaluation of Thermal Strain by Physiological Measurements (Report No. ISO 9886: 1992{E}). Geneva, Switzerland, ISO, 1992
26. Sargeant AJ: Effect of muscle temperature on leg extension force and short-term power output in humans. Eur J Appl Physiol Occup Physiol 56:693-698, 1987
27. Dill DB, Costill DL: Calculation of percentage changes in volumes of blood, plasma, and red cells in dehydration. J Appl Physiol 37:247-248, 1974
28. Boobis LH, Maughan RJ: A simple one-step enzymatic fluorimetric method for the determination of glycerol in microliters of plasma. Clin Chim Acta 132:173-179, 1983
29. Lorenzen A, Stannek C, Lang H, et al: Characterization of a G protein-coupled receptor for nicotinic acid. Mol Pharmacol 59:349-357, 2001
30. Watt MJ, Heigenhauser GJ, Spriet LL: Effects of dynamic exercise intensity on the activation of hormone-sensitive lipase in human skeletal muscle. J Physiol 547:301-308, 2003
31. Van der Vusse GJ, Reneman RS: Lipid metabolism in muscle, in Loring A, Rowell G, Shepherd JT (eds): Handbook of Physiology, Exercise Regulation and Integration of Multiple Systems. Bethesda, MD, Am Phys Soc, 1996, pp 952-994
32. Langfort J, Ploug T, Ihlemann J, et al: Hormone-sensitive lipase (HSL) expression and regulation in skeletal muscle. Adv Exp Med Biol 441:219-228, 1998
33. Parkin JM, Carey MF, Zhao S, et al: Effect of ambient temperature on human skeletal muscle metabolism during fatiguing submaximal exercise. J Appl Physiol 86:902-908, 1999
34. Starkie RL, Hargreaves M, Lambert DL, et al: Effect of temperature on muscle metabolism during submaximal exercise in humans. Exp Physiol 84:775-784, 1999
35. Florkin M, Stolz EH: Comprehensive Biochemistry. New York, NY, Elsevier, 1968
36. Achten J, Gleeson M, Jeukendrup AE: Determination of the exercise intensity that elicits maximal fat oxidation. Med Sci Sports Exerc 34:92-97, 2002
37. Helge JW, Watt PW, Richter EA, et al: Fat utilization during exercise: Adaptation to a fat-rich diet increases utilization of plasma fatty acids and very low density lipoprotein-triacylglycerol in humans. J Physiol 537:1009-1020, 2001
38. Jepson CA, Yeaman SJ: Inhibition of hormone-sensitive lipase by intermediary lipid metabolites. FEBS Lett 28:197-200, 1992