During Exercise in the Cold Increased Availability of Plasma Nonesterified Fatty Acids Does Not Affect the Pattern of Substrate Oxidation

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Exercise in the cold was investigated to establish if the relative contribution of fat to energy expenditure is affected by the increased availability of circulating nonesterified fatty acids (NEFA). Seven men after an overnight fast cycled at approximately 70% of peak oxygen uptake for 60 minutes at an ambient temperature of 0.0°C ± 0.1°C. Fifteen minutes prior to exercise and then throughout the exercise, subjects were infused with either heparin (heparin) or saline (control). Immediately before exercise NEFA concentration (control, 0.27 ± 0.04 mmol · L^{-1}; heparin 1.09 ± 0.13 mmol · l^{-1}) was significantly higher ($P < .05$) in the heparin trial. Pre-exercise concentration of plasma triacylglycerol (TG), blood glycerol, glucose, oxygen consumption ($\dot{V}O_2$) and respiratory exchange ratio (RER) were not significantly different between heparin and control trials. During exercise, plasma NEFA and blood glycerol concentrations were significantly higher ($P < .05$) in the heparin trial, and levels of plasma TG and glucose were not different between trials. Over the exercise period rectal temperature, mean skin temperature, $\dot{V}O_2$, RER, and heart rate (HR) were not different between the 2 trials. Gross energy expenditure of cycling (control, 3.3 ± 0.1 MJ; heparin 3.3 ± 0.1 MJ), the oxidation rates of fat (control, 0.67 ± 0.05 g · min^{-1}; heparin, 0.71 ± 0.06 g · min^{-1}) and carbohydrate (CHO) (control, 1.68 ± 0.04 g · min^{-1}; heparin, 1.62 ± 0.17 g · min^{-1}) and the proportion of energy derived from fat (control, 43 ± 4%; heparin trial, 44 ± 9%) and CHO (control, 57 ± 4%; heparin trial, 56 ± 4%) were not different between the 2 trials. These findings suggest that despite increased availability of plasma NEFA, the pattern of substrate oxidation during exercise in cold temperatures does not change. This implies that uncoupling between the availability and oxidation of plasma NEFA may be a mechanism involved in the reduced oxidation of fat seen during cold exposure. Further research is needed on the utilization of intramuscular TG and circulating plasma TG-rich lipoproteins in the cold.

THE EXACT mechanisms that dictate the relative contribution of fat and carbohydrate (CHO) towards energy metabolism at a given exercise intensity are still not elucidated.[1,2] Several studies have reported a significant increase in total fat oxidation with elevation of nonesterified fatty acids (NEFA) concentration during submaximal exercise conducted at ambient temperatures (for review see Hawley[3]). In contrast, the only study to investigate the effect of elevated NEFA on metabolism during exercise below 20°C is that of Pitsiladis et al[4] in which subjects exercised to exhaustion at 10°C with and without elevation of plasma NEFA. In this study, the investigators conclude that the total amount of fat and CHO oxidized over the first 90 minutes of exercise was not influenced by NEFA availability, although there was a tendency for the amount of CHO oxidized to be lower and fat to be higher in the fat trial. In addition, in the fat trial, the respiratory exchange ratio (RER) was significantly lowered at 30 minutes of exercise compared with the 15-minute time point, whereas in the CHO trial, this reduction in RER was evident only after 90 minutes of exercise. This suggests a possible increase in the dependency on fat oxidation in the elevated NEFA trial.

Subjects in the study of Pitsiladis et al[4] exercised at 10°C. Evidence indicates, however, that alterations in substrate utilization during exercise at 10°C is not different from that at 20°C when exercising at submaximal[5-7] and low[8] intensities and only becomes evident at temperatures ≤ 0°C.[7] This implies that clear effects of cold on the relationship between elevated NEFA and fat oxidation may be evident only at these lower temperatures. In the study by Layden et al,[7] there was no significant difference in plasma NEFA concentrations between trials, yet fat oxidation was significantly lower during exercise in the cold, suggesting that one of the mechanisms involved may be related to the dissociation between the availability and oxidation of plasma NEFA. Therefore, to check the hypothesis that exercising in a cold environment causes the uncoupling between the availability of NEFA and oxidation of fat during exercise, a study was designed in which exercise bouts were conducted in the cold with and without an elevation of NEFA.

MATERIALS AND METHODS

Subjects

Subjects were 7 healthy men with the following physical characteristics (mean ± SD): age 26.3 ± 5.0 years; body mass index 23.5 ± 0.7 kg · m^{-2}; body fat 13.0 ± 0.5%; peak oxygen uptake ($\dot{V}O_2$peak) 52.5 ± 4.5 mL · kg · min^{-1}. All subjects were nonsmokers and did not use any medication. The subjects were recreationally active and participated in regular exercise at least 3 times per week. The University of Strathclyde Ethics Committee approved all procedures and subjects provided written consent.

Study Design

Each subject underwent 2 trials with an interval of 5 to 7 days between trials. On each occasion subjects reported to the laboratory, between 9 and 10 AM, not having consumed food for at least 12 hours and completed 60 minutes submaximal cycling (70% $\dot{V}O_2$peak), with an air velocity of 0.35 m · s^{-1}, at an ambient temperature of 0°C, while being infused with either heparin (heparin) or saline (control). Three subjects were randomly assigned to the heparin protocol first, while the remaining 4 subjects underwent the control trial first. In the 24-hour period prior to each trial, subjects abstained from alcohol, caffeine, and vigorous physical activity. To minimize differences in metabolic ho-

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mogeneity, 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 second trial. To ensure euhydration, subjects consumed 1 L water the night before and 0.5 L 2 hours prior to each experiment. Subjects wore the same shorts, shoes, and socks in all trials.

Preliminary Exercise Tests

Prior to the main experimental trials, 2 preliminary tests were conducted. In the first, $\dot{V}O_2$peak was determined. Subjects performed a continuous incremental cycling test to volitional exhaustion on a mechanically-braked cycle ergometer (Monark 864, Varberg, 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 $\dot{V}O_2$peak is not affected by ambient temperatures ranging from −10°C to 20°C. In the second, subjects completed a familiarization trial at a workload corresponding to 70% $\dot{V}O_2$peak, which confirmed the workload for the main experimental trials.

Main Exercise Trials

On arrival at the laboratory, subjects were weighed nude and inserted a rectal thermistor 12 cm beyond the anal sphincter (Grant Instruments, Cambridge, UK). Cannulae (Venflon 18G; BOC, Ohmeda, Sweden) were inserted into the antecubital forearm vein of each arm, one for blood sample collection and one for the infusion of either heparin or saline. Subjects then rested in a seated position for 30 minutes during which 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. After the collection of resting samples, subjects were infused with 500 U heparin bolus or saline. Fifteen minutes postheparin or saline infusion preexercise blood samples and expired gas were collected, and measurements of HR, mean skin temperature (T_{sk}), and rectal temperature (T_{re}) were performed. Subjects then entered the climatic chamber (SANYO Gallenkamp PLC, Loughborough, UK) and commenced cycling immediately. Throughout the exercise bout another 1,000 U heparin with 50 mL saline (heparin trial) or 50 mL saline (control) was administered via a constant infusion pump (Braun Melsungen AG, Melsungen, Germany). Every 15 minutes during exercise a blood sample was drawn, expired air collected, and HR, T_{sk}, and T_{re} were recorded. Immediately postexercise muscle temperature 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.[9]

Skin temperatures were measured at 8 sites using surface thermistors (Grant Instruments) attached with a single layer of waterproof tape and T_{sk} was calculated with the following equation[10]:

$$\bar{T}_{sk} = 0.07 \times T_{\text{forehead}} + 0.175 \times T_{\text{right scapula}} + 0.175 \times T_{\text{left upper chest}} + 0.07 \times T_{\text{right upper arm}} + 0.07 \times T_{\text{left forearm}} + 0.05 \times T_{\text{left hand}} + 0.19 \times T_{\text{right anterior thigh}} + 0.2 \times T_{\text{left calf}}$$

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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, Roedoure, 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. 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

The hemoglobin (cyanmethemoglobin method) and hematocrit (microcapillary technique) were measured for estimation of changes in plasma volume.[11] 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

Table 1. Plasma and Blood Concentrations, T_{re}, T_{sk}, $\dot{V}O_2$, RER, and rate of CHO and Fat Oxidation at Rest (preinfusion) and Immediately Before Exercise (postinfusion) in the Control and Heparin Condition

	Rest		Pre-exercise	
	Control	Heparin	Control	Heparin
NEFA (mmol · L^{-1})	0.29 ± 0.04	0.25 ± 0.03	0.27 ± 0.04	1.09 ± 0.13*†
TG (mmol · L^{-1})	0.71 ± 0.10	0.73 ± 0.09	0.66 ± 0.12	0.65 ± 0.08†
Glycerol (mmol · L^{-1})	—	—	0.026 ± 0.003	0.035 ± 0.007
Glucose (mmol · L^{-1})	—	—	5.4 ± 0.1	5.4 ± 0.1
T_{re} (°C)	—	—	36.8 ± 0.1	36.8 ± 0.1
T_{sk} (°C)	—	—	32.1 ± 0.2	31.5 ± 0.2
$\dot{V}O_2$ (L · min^{-1})	0.34 ± 0.03	0.34 ± 0.01	0.34 ± 0.02	0.35 ± 0.01
RER	0.78 ± 0.03	0.79 ± 0.03	0.79 ± 0.03	0.78 ± 0.02
Fat oxid (g · min^{-1})	0.13 ± 0.02	0.11 ± 0.02	0.11 ± 0.02	0.11 ± 0.33
CHO oxid (g · min^{-1})	0.11 ± 0.05	0.16 ± 0.05	0.13 ± 0.05	0.15 ± 0.06

NOTE. Values are mean ± SEM (n = 7 subjects).

Abbreviations: T_{re}, rectal temperature; T_{sk}, mean skin temperature; $\dot{V}O_2$, oxygen consumption; RER, respiratory exchange rate; CHO oxid, carbohydrate oxidation.

*Significantly different ($P < .05$) from pre-exercise control.

†Significantly different ($P < .05$) from rest heparin.

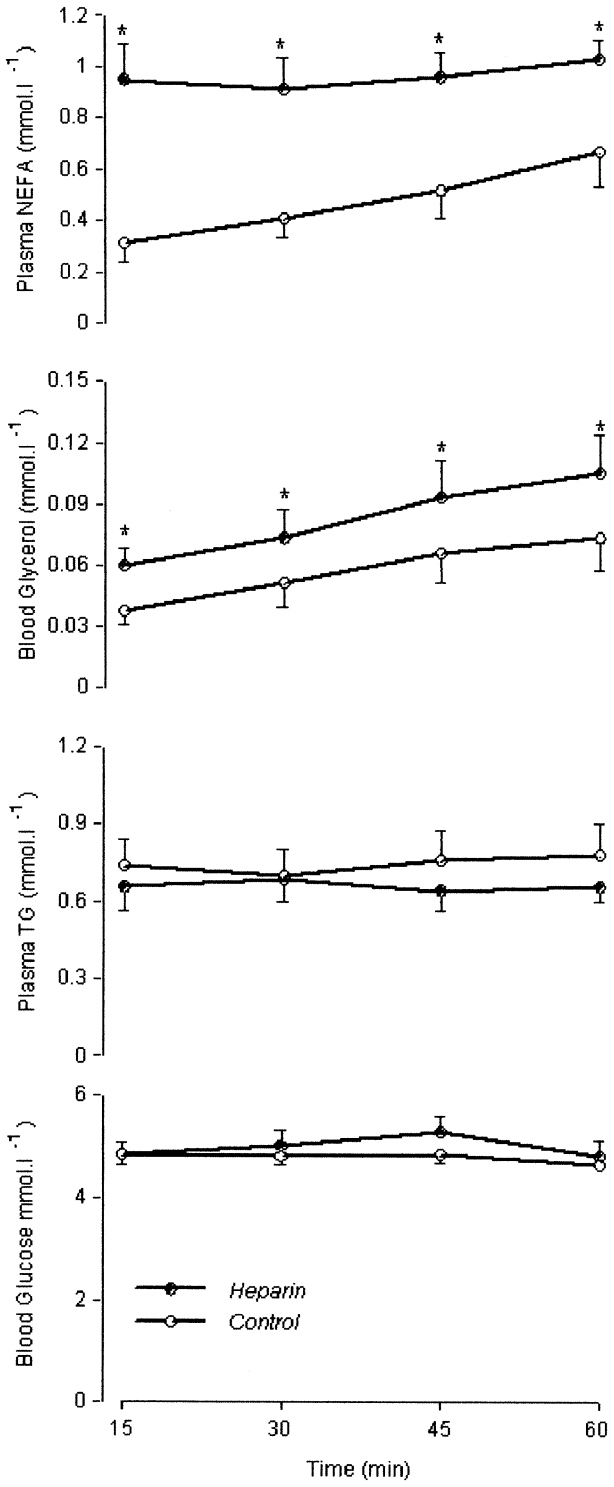

Fig 1. Plasma NEFA, blood glycerol, TG, and glucose concentration during 60 minutes of submaximal exercise at 0°C with infusion of saline (control) and heparin (heparin). Values are mean ± SEM (n = 7). *Significantly different ($P < .05$) from control.

of glycerol[12] and glucose (glucose oxidase method, Roche Diagnostics, Mannheim, Germany). 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 triacylglyerol (TG) (INFINITY; Triglyceride Reageant, Sigma Diagnostics, Dorset, UK). To prevent in vitro lipolysis after heparin infusion, whole blood was treated with 30 μL 0.2 mol · L^{-1} ethylene glycol-bis (β-aminoethyl ether)-N,N,N′,N′-tetraacetic acid (EGTA). Samples from the heparin and control trial were treated in the same way.

Table 2. T_{re}, $\bar{T}_{sk}$, $\dot{V}O_2$, HR, and RER During 60 Minutes of Submaximal Exercise at 0°C in the Control and Heparin Condition

	15 Minutes	30 Minutes	45 Minutes	60 Minutes
T_{re} (°C)				
C	37.1 ± 0.1	37.6 ± 0.1	37.9 ± 0.1	38.1 ± 0.1
H	37.1 ± 0.1	38.7 ± 0.1	37.9 ± 0.1	38.1 ± 0.1
$\bar{T}_{sk}$ (°C)				
C	22.0 ± 0.5	21.7 ± 0.5	21.6 ± 0.6	21.2 ± 0.6
H	21.2 ± 0.5	21.1 ± 0.3	20.8 ± 0.4	20.7 ± 0.3
$\dot{V}O_2$ (L · min^{-1})				
C	2.62 ± 0.08	2.72 ± 0.07	2.80 ± 0.08	2.86 ± 0.10
H	2.65 ± 0.08	2.72 ± 0.08	2.80 ± 0.08	2.82 ± 0.10
HR (b · min^{-1})				
C	134 ± 5	139 ± 5	143 ± 5	146 ± 5
H	134 ± 5	136 ± 6	141 ± 5	145 ± 5
RER				
C	0.88 ± 0.02	0.87 ± 0.02	0.85 ± 0.01	0.85 ± 0.02
H	0.88 ± 0.01	0.86 ± 0.02	0.84 ± 0.01	0.85 ± 0.02

NOTE. Values are mean ± SEM (n = 7 subjects).
Abbreviations: HR, heart rate; C, control; H, heparin.

Statistical Analysis

Results are shown as mean ± 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

Resting concentrations of plasma NEFA and TG were not significantly different between the heparin and control trials (Table 1). Heparin infusion increased pre-exercise plasma NEFA levels ($P < .05$) and reduced pre-exercise concentration of plasma TG ($P < .05$) (Table 1). Immediately before cycling, the concentration of plasma NEFA was significantly higher in the heparin trial ($P < .05$), and the concentrations of plasma TG, blood glycerol, and glucose were not significantly different between the control and heparin trials (Table 1). During exercise, plasma NEFA and blood glycerol concentrations were significantly higher in the heparin trial, and levels of plasma TG and glucose were not different between trials (Fig 1).

Table 3. Muscle Temperature of Vastus Lateralis at 3, 2, and 1 cm From the Skin Surface Immediately Postexercise in the Control and Heparin Condition

	3 cm	2 cm	1 cm
Heparin	38.7 ± 0.1	37.8 ± 0.2	34.4 ± 0.7*†
Control	38.7 ± 0.1	37.7 ± 0.4	34.0 ± 1.0*†

NOTE. Values are mean ± SEM (n = 7 subjects).
*Significantly different ($P < .05$) from 3 cm.
†Significantly different ($P < .05$) from 2 cm.

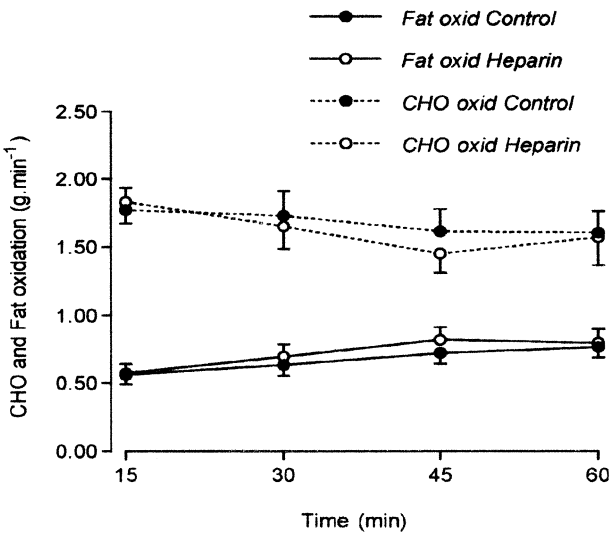

Fig 2. Calculated rate of CHO oxidation (CHO oxid) and Fat oxidation (Fat oxid) during 60 minutes of submaximal exercise at 0°C with infusion of saline (control) and heparin (heparin). Values are mean ± SEM (n = 7).

Immediately before cycling (Table 1) and during cycling (Table 2) T_r and T_{sk} were not significantly different between the control and heparin trials. At all time points of the exercise period, T_{sk}, but not T_r was significantly lower in comparison with the pre-exercise value ($P < .05$). Muscle temperature immediately post-exercise was not different between trials at any depth of measurement (Table 3).

Resting and pre-exercise values of $\dot{V}O_2$ and RER were not different between trials (Table 1). Subjects exercised at a mean of 68.3 ± 1.9% $\dot{V}O_{2peak}$ in the control condition and 68.3% ± 1.6% $\dot{V}O_{2peak}$ after heparin. During cycling $\dot{V}O_2$, HR, and RER were not different between the control and heparin trials (Table 2). Gross energy expenditure of cycling (control, 3.3 ± 0.1 MJ; heparin 3.3 ± 0.1 MJ), the oxidation rates of fat (control, 0.67 ± 0.05 g · min^{-1}; heparin, 0.71 ± 0.06 g · min^{-1}), CHO (control, 1.68 ± 0.04 g · min^{-1}; heparin, 1.62 ± 0.17 g · min^{-1}) (Fig 2), and the proportion of energy derived from fat (control, 43 ± 4%; heparin, 44 ± 9%) and CHO (control, 57 ± 4%; heparin, 56 ± 4%) were not significantly different between the 2 trials (Fig 3).

DISCUSSION

The major finding of the present study is that during submaximal cycle exercise at 0°C total fat oxidation is not affected by elevated plasma NEFA concentration, which implies that during exercise at 0°C the regulatory pathways controlling energy substrate selection are different from those in temperate environments. At temperatures around 20°C, the uptake and subsequent oxidation of NEFA is concentration dependent and therefore an increased availability during exercise causes an increase in whole body fat oxidation (for review, see Hawley[3]).

The finding of no increase in fat oxidation after increased plasma NEFA has been found at rest at 5°C[13] and during submaximal exercise at 10°C.[4] Close scrutiny of the findings of Pitsiladis et al,[4] however, indicate a tendency for the amount of CHO oxidized over the first 90 minutes of exercise to be lower ($P = .075$) and fat to be higher ($P = .096$) in the elevated NEFA trial. We hypothesized that this was because of the relatively moderate cold conditions and the fact that substrate utilization during submaximal exercise is different from that at 20°C only when temperatures are ≤ 0°C.[7] In the current study, values for total fat and CHO oxidation during the total exercise period of 60 minutes were very similar between the 2 trials ($P = .5$ for both total fat and CHO oxidation). Therefore, our study demonstrates that during exercise in the cold, the pattern of substrate utilization is not affected by increased availability of plasma fatty acids. Indirectly, this conclusion is supported by studies in cold water[14] where it was possible to substantially reduce the contribution of circulating NEFA, through the administration of nicotinic acid, without affecting the overall metabolic heat production. This finding implies that increased utilization of alternative lipid sources, such as intramuscular triacylglycerol (IMTG) and plasma TG-rich lipoproteins or CHO, compensate for the reduced circulating NEFA.

In the current study, during exercise with heparin infusion, an increase in blood glycerol was not totally matched by an elevation in NEFA, which may suggest that cellular uptake of NEFA and possibly fat oxidation obtained from plasma NEFA was increased in the heparin trial. This does not detract, however, from the conclusion that elevation in plasma NEFA did not cause an increase in fat oxidation. At the same time, this implies that reduced oxidation of fat seen during exercise in the cold may be related not only to the uncoupling between the availability of NEFA and the oxidation of fat, but to the effects of cold exposure on hydrolyses and oxidation of IMTG and circulating plasma TG-rich lipoproteins. Therefore, based on the fact that in the cold, fat oxidation obtained from circulating lipoproteins is minimal,[15] future research is needed to clarify the contribution of IMTG to energy turnover in the cold.

It is well established that the delivery and oxidation of NEFA increases with the duration of exercise.[16] Therefore, one of the concerns when drawing conclusions from this study, could be that a time-dependent increase in fat oxidation over a period of

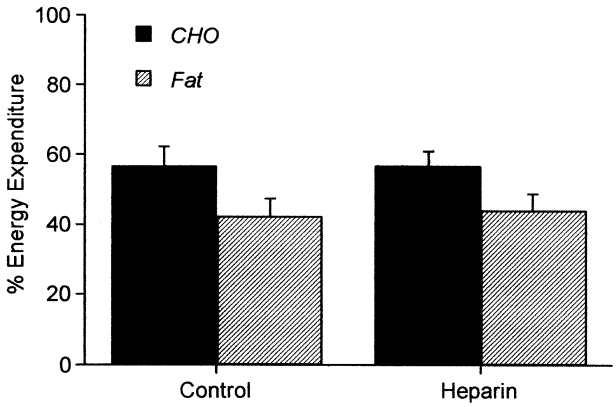

Fig 3. Percentage contribution of CHO and fat to gross energy expenditure during 60 minutes of submaximal exercise at ambient temperature of 0°C with infusion of saline (control) and heparin (heparin). Values are mean ± SEM (n = 7).

60 minutes was not significant enough to observe an increase in fat oxidation in response to elevated plasma NEFA. This however, is most unlikely, as other studies at a similar or higher intensity have noted increased fat oxidation with elevated NEFA within 60 minutes at temperate environmental conditions (for review, see Hawley[3]).

It might at first appear that the fasting values of NEFA in this study were low (0.25 to 0.29 mmol · L^{-1}) compared with values seen in most studies.[17-19] However, similar plasma NEFA values as in this study have been reported before.[20,21] These variations in resting NEFA are probably due to differences in the CHO and fat content of the diets[22,23] and training status.[24] During exercise, NEFA values increased, as expected, in the control trial, while remaining at a constantly high level in the heparin trial.

RER values during exercise in the control condition of this study were at the lower end of values seen during moderate intensity exercise in untrained and recreationally active men (0.89 to 0.96)[25-29] and values of 0.87 to 0.92 reported in well trained athletes.[17,30,31] This is not surprising, as subjects of this study were moderately trained and prior to each exercise session undertook an overnight fast of at least 12 hours, which is also an effective means to increase fat oxidation.[19]

The reasons for the different effect of increased availability of plasma NEFA on exercise substrate selection between studies conducted at temperate and cold environments are not clear, but may be due to temperature-mediated changes at the level of musculature and include differences in the transport of NEFA across the sarcolemmal membrane. This suggestion is based on results from an in vitro study, which reports that at 0°C palmitate uptake by sarcolemmal vesicles containing cytoplasmic fatty acid protein is lower than at 20°C.[32] In the present study, the postexercise muscle temperature of 38.7°C was comparable to muscle temperatures obtained during exercise of similar intensity conducted at temperate conditions,[6,33] and even at the most superficial depth, was not lower than resting muscle temperatures of 34°C to 35°C.[6,33,34] Therefore, it is unlikely that transport of NEFA from the vascular to the cytosolic compartment within exercising muscle would operate differently at temperate and cold environments. However, approximately 25% of the whole body muscle mass contributes to cycling[35] and the remaining mass, represented in the whole body respiratory data, could have significantly dropped its temperature. Thus, in the cold, temperature-mediated changes in transport of NEFA within inactive muscle may have prevented the increase in fat utilization at whole body level following increased availability of plasma NEFA.

Another mechanism to account for the observed lack of increase in fat oxidation with increased availability of NEFA may involve cold-induced increase in glycolytic flux within the muscle. Indeed, it has been determined that an increase in glycolytic flux associated with high CHO availability may actively impair the entry of NEFA into the mitochondria for oxidation.[36,37] The suggested increase in glycolytic flux to the muscle may be due to several reasons, one of which may involve a cold-induced increase in hepatic glucose output through an increase in central blood volume associated with increased peripheral vasoconstriction. This could lead to an increased uptake of gluconeogenic precursors and to greater rates of gluconeogenesis (GNG) and subsequent hepatic glucose output. There is some support for enhanced GNG from exercise studies in the cold. Penner and Himms-Hagen[38] identified increased GNG with cold exposure, and elevated GNG has been used to explain the later onset of blood lactate (Bla) accumulation and lower absolute concentrations of Bla throughout exercise in the cold compared with a temperate environment.[39,40] Although evidence suggests that an increase in a single gluconeogenic precursor supply does not increase hepatic glucose release at rest[41,42] and during prolonged light- and moderate-intensity exercises,[43] these investigations were conducted at laboratory temperatures of approximately 20°C. During cold stress due to the change in the hormonal milieu, a link between gluconeogenic precursors and glucose output may exist. Our previous study[7] supports this notion because the decrease in fat oxidation at lower ambient temperatures was accompanied by an increase in total CHO oxidation; yet it is known that muscle glycogenolysis is reduced during exercise in the cold compared with 20°C.[6,33] Another reason for the suggested increase in glycolytic flux during exercise in cold may be related to changes in the molecular signalling mechanisms. These include changes to membrane transport capacity through translocation of GLUT4 from a designated intracellular storage compartment to the plasma membrane, intrinsic activity of GLUT4, and the action of adenosine monophosphate (AMP)-activated protein kinase,[44] which all are recognized to be involved in the regulation of glucose uptake during muscle contractions. These speculations, however, are not in accordance with the fact that the active muscle temperature was not affected by the cold in this study.

In conclusion, our data suggest that during exercise at 0°C the pattern of substrate utilization is not affected by increased availability of plasma NEFA. This implies that uncoupling between the availability and oxidation of plasma NEFA may be one of the mechanisms involved in the reduced oxidation of fat seen during exercise in cold. Further research is needed on utilization of intramuscular TG and circulating plasma TG-rich lipoproteins in the cold.

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