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A simple ergometer for in vivo nuclear magnetic resonance (NMR) spectroscopy studies of human calf muscle. By MARIAPIA FRANCESCATO, VALENTINA CETTOLO and PIETRO E. DI PRAMPERO, *Dipartimento di Scienze e Tecnologie Biomediche, Università di Udine, Via Gervasutta 48, I-33100 Udine*

NMR spectroscopy is a powerful technique to assess the metabolic status of muscles during exercise. However, specific amagnetic ergometers are required whose characteristics depend mainly on the muscle groups involved (Wilson *et al.* 1988; Quistorff *et al.* 1990; Gonzalez de Suso *et al.* 1993; Ryschon *et al.* 1995; Takahashio *et al.* 1995; Yoshida *et al.* 1996). Our aim was to build an ergometer that: (1) fitted easily into a standard 1.5 T Magnetom SP 4000 system (Siemens, Erlangen, Germany), (2) induced calf muscle exercises of different intensities, (3) allowed the monitoring of the work performed.

The ergometer consists of a wooden frame on which the subject lies supine. The exercise consists of repeated rhythmic plantar flexions of the feet, which act on two independent pedals, free to rotate on teflon axles. A potentiometer is mounted on each axle in order to record continuously the pedal's rotation. The heel end of the pedal is connected, by means of a nylon cable, to a wooden block free to move along a guide carved in the main frame. The other end of the block is connected to a rigid structure by means of elastic cords which constitute the resistance against which the muscles work. A force transducer records the force applied to stretch the elastic cords. The work intensity can be varied by changing: (1) the number and/or resistive force of the elastic cords, (2) the maximal rotation of the pedals, (3) the frequency of contraction.

The potentiometers are non-ferromagnetic and their calibration signals acquired inside or outside the magnet were not significantly different (p NS, n 32 for each pedal, analysis of covariance). On the contrary, the force transducers contain ferro-magnetic materials, and their base-line signals differ significantly ($p < 0.001$, n 224). Nevertheless, their sensitivity, as given by the delta output per unit delta force, was not statistically different inside or outside the magnet (p NS, n 224).

To test the system, six healthy females (age 24-31 years; mean body mass 65.0 (SE 5.9) kg) exercised twice on the new ergometer for 6 min; the corresponding mechanical powers were 10.9 and 15.7 W. Measurements included O_2 consumption (VO_2) at rest (5 min), and during the last 3 min of exercise (steady state; SS). Simultaneously, a series of NMR spectra were acquired at rest and at SS. All data were analysed according to standard procedures. The results show that: (1) VO_2 amounted to 0.21 (SE 0.02) l/min at rest and to 0.48 (SE 0.04) l/min and 0.59 (SE 0.04) l/min during the two SS conditions, respectively, (2) the ratio of phosphocreatine (PCr) to the sum of PCr and inorganic phosphate (Pi) decreased from 0.89 (SE 0.01) at rest to 0.71 (SE 0.02) and 0.60 (SE 0.03), and (3) the ratio of PCr to the sum of PCr and Pi decreased linearly with increasing VO_2 (r 0.76, $p < 0.001$, n 17).

In summary, the characteristics of the described ergometer are: (1) the muscle groups contributing substantially to mechanical work performance are the *triceps surae*, (2) the two lower limbs can be investigated independently, (3) force and velocity of contraction, thus mechanical work, can be monitored by commercial transducers.

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Creatine kinase reaction studied in human skeletal muscle at rest and during exercise by the saturation transfer method. By MARC FRANCAUX[1], JEAN-FRANCOIS GOUDEMANT[2], ROGER DEMEURE[2] and XAVIER STURBOIS[1], [1]*Institut d'Education Physique et de Réadaptation and* [2]*Unité de Résonance Magnétique Biomédicale, Université catholique de Louvain, Louvain-la-Neuve, Belgium*

The first role attributed to the creatine kinase (EC 2.7.3.2; CK) reaction is energy buffering, whereas another possible function is the energy transfer from ATP production sites (mitochondria) to ATP consumption sites (myofibrils and ion channels) (Bessman & Geiger, 1981). This hypothesis has been corroborated in cardiac muscle cells (Bittl & Ingwall, 1985) by showing a coupling between the flux through the CK reaction and ATP synthesis-consumption rate. In the present study the saturation transfer method (Koretsky *et al.* 1986) was used at rest and during exercise to estimate the rate of CK reaction in the direction of ATP synthesis (V_{for}), in order to verify whether V_{for} increases with exercise intensity in human skeletal muscle.

Six healthy male volunteers (22 to 28 years old) were placed in a supine position in a Bruker Biospec NMR spectrometer working at 4.7 T and 81 MHz for ^{31}P, equipped with a 50 mm surface coil placed under the calf muscle. The subjects were submitted to four levels of exercise intensity which consisted of 15 min of plantar flexion-extensions of the ankle (0.5 Hz) against weights of 2.5, 5, 7.5 and 10 kg respectively. The exercise bouts were interspersed by a recovery period of at least 24 h.

^{31}P signals were acquired after pseudo-90° pulse (130 μs), repetition time 8s, number of scans 12. Quantification of peak areas was performed in the time domain by means of a VARPRO algorithm. Concentrations were calculated from a non-irradiated spectrum, considering an [ATP] of 8.2 mM at rest. The pseudo-first order rate constant (k_{for}) of P exchange between phosphocreatine (PCr) and [γ-P]ATP was measured by a saturation transfer method. Saturation pulses were applied on the [γ-P]ATP peak. The saturation was achieved by a DANTE pulse sequence consisting of a train of short pulses (10 μs) separated by a 490 μs delay. Spectra were recorded after different times of saturation (t). k_{for} was estimated by fitting the area of the non-irradiated exchanging resonance (PCr peak) against t, according to an exponential function. V_{for} was calculated by multiplying k_{for} by [PCr].

The main results are presented in the Table.

Workload	Rest		2.5 kg		5 kg		7.5 kg		10 kg	
	Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE
[Pi] (mM)	4.5	0.2	5.2	0.4	6.3	0.4	8.1	2.2	8.9	1.8
[PCr] (mM)	28.5	0.9	28.7	1.5	26.9	1.2	26.0	2.0	21.9	1.5*
[ATP] (mM)	8.2		9.1	0.5	8.7	0.6	8.2	0.8	7.9	0.7
[ADP] (μM)	27	3	29	4	35	6	33	4	48	6*
pH_i	7.04	0.01	7.04	0.01	7.05	0.01	7.02	0.02	7.02	0.04
k_{for} (1/s)	0.44	0.03	0.41	0.02	0.49	0.01	0.47	0.05	0.38	0.04
V_{for} (mM/s)	12.4	0.9	11.8	1.0	13.1	0.6	12.1	1.5	8.4	1.4*

Mean values were significantly different from those of rest, * $P < 0.05$

As expected, a [PCr] decrease was observed during exercise, matched by an increase in the Pi content, [ATP] remaining stable. The exercise protocol did not change the intracellular pH. k_{for} was not modified with exercise in comparison to rest. V_{for} remained identical at the three lowest exercise levels and decreased at the highest exercise intensity.

This decrease of V_{for} argues against the hypothesis of a functional coupling between ATP synthesis-consumption rate and CK activity which would lead to a rise in V_{for} with increasing aerobic metabolism, as observed in cardiac muscle (Bittl & Ingwall, 1986). The decrease in the flux recorded during exercise (–32 %) is not sufficient to reduce the function of energy buffering of the enzyme since its velocity remains well above the fluxes of ATPases, known to be in the range of 0.5-1.6 mM/s (Shoubridge *et al.* 1984). *This protocol was approved by the committee on the ethical use of human subjects of the Université catholique de Louvain.*

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Phosphorus-31 nuclear magnetic resonance spectroscopy (^{31}P-MRS) and near infrared spectroscopy (NIRS) detected oxidative capacity of human gastrocnemius muscles. By MASAO MIZUNO[1], TAKAFUMI HAMAOKA[2], TAKUYA OSADA[2], AIVARAS RATKEVICIUS[1], TOSHIHITO KATSUMURA[2] and BJØRN QUISTORFF[1], [1]*Copenhagen Muscle Research Centre, Panum NMR-Centre, University of Copenhagen, Denmark and* [2]*Department of Preventive Medicine and Public Health, Tokyo Medical University, Japan*

Interindividual variation in muscle energy metabolism during exercise and recovery detected by ^{31}P-MRS reflects the metabolic difference between slow-twitch (ST) and fast-twitch (FT) fibres (Mizuno *et al.* 1994*a,b*). In the present study the rate of muscle deoxygenation at the initiation of a rhythmic plantar flexion exercise, as evaluated by NIRS, and the rate of phosphocreatine (PCr) resynthesis during recovery, as determined by ^{31}P-MRS, were related to the oxidative capacity of the gastrocnemius muscles as expressed by the number of capillaries per fibre (capillaries:fibre) determined histochemically and the activity of the mitochondrial enzyme citrate synthase (EC 4.1.3.7; CS) in muscle biopsies obtained from ten untrained male subjects (23-26 years, 172-194 cm, 62-82 kg).

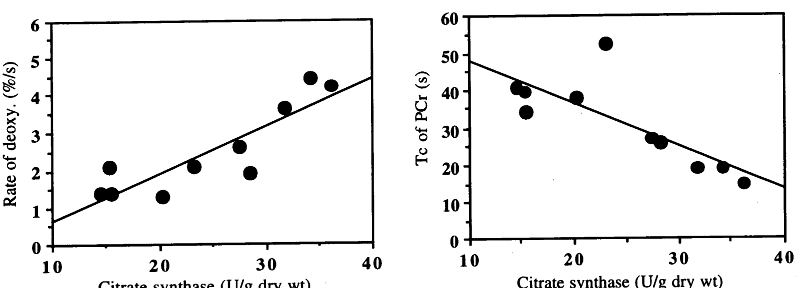

Fig. The activity of the mitochondrial enzyme citrate synthase correlates to the rate of muscle deoxygenation at initiation of exercise (Rate of deoxy.: left panel) and to time constant (Tc: right panel) of PCr resynthesis during recovery.

The proportion of ST fibres, capillaries:fibre ratio and CS activity were 54(SD21) %, 2.4(SD 0.7) and 24.7(SD8.1) µmol/min per g dry weight, respectively. The rate of muscle deoxygenation upon the initiation of exercise (0-30 s) relative to the maximal deoxygenation observed during resting arterial occlusion using a pressure cuff (2.4(SD1.0) %/s) was positively correlated with the capillaries:fibre ratio (r 0.871, P<0.01) and CS activity (r 0.874, P<0.001) (Fig.). At the termination of exercise the reduction of PCr was 50(SD10) % of the resting value, and muscle pH decreased slightly from 7.04(SD0.05) at rest to 6.96(SD0.08) (P<0.05). The time constant (Tc) of PCr resynthesis during recovery was inversely correlated with the capillaries:fibre ratio (r -0.680, P<0.05) and CS activity (r -0.789, P<0.01) (Fig.). No such correlation was observed with the Tc of muscle reoxygenation during recovery.

These results suggest that both the initial rate of muscle deoxygenation at the initiation of exercise and the rate of PCr resynthesis during recovery are closely related to the oxidative capacity of human skeletal muscle.

This study was approved by the Central Scientific Committee of Denmark (C-1995-21).

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Quantitation of human liver and muscle volume and glycogen concentration during exercise and recovery. By ANNA CASEY[1]†, ROB MANN[2], KATIE BINGHAM[2], JOHN FOX[1], PETER G. MORRIS[2], IAN A. MACDONALD[1] and PAUL L. GREENHAFF[1], [1]*School of Biomedical Sciences, University of Nottingham Medical School, Nottingham NG7 2UH, and* [2]*Magnetic Resonance Centre, Department of Physics, University of Nottingham, Nottingham NG7 2RD, UK*

No studies have simultaneously quantified human liver and muscle glycogen content during exercise and recovery. The purpose of the present experiment, which had ethics committee approval, was to investigate the effects of prolonged exercise and subsequent carbohydrate (CHO) ingestion on tissue volume and glycogen resynthesis measured simultaneously in human liver and skeletal muscle (quadriceps femoris). Six healthy, well trained and familiarized males cycled at 70 % maximal O_2 uptake (VO_2max) for 90 (SE 10) min, on two separate occasions. Subjects were then administered 1g CHO / kg body mass, given orally in the form of glucose (18.5 g / l, n 6), or an equivalent volume of placebo (control, n 5). Over the following 4 h, liver and muscle volume and glycogen resynthesis were measured by magnetic resonance imaging and ^{13}C magnetic resonance spectroscopy respectively. Liver spectra and volume measurements were obtained pre and post exercise, within the first 30 min after CHO ingestion and hourly thereafter. Muscle spectra and volume measurements were obtained from 30 to 60 min and hourly thereafter. Values are means with their standard errors.

Table Liver volume (L volume; litres) and glycogen concentration (L glycogen; mmol/l), and muscle volume (M volume; litres) and glycogen concentration (M glycogen; mmol/l), pre- (rest) and post-exercise (PE) at 70 % VO_2max, and during recovery (R1-R4)

	Rest		PE		R1		R2		R3		R4	
	Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE
Control												
L volume	1.87	0.10	1.80	0.09	1.75	0.10	1.72*	0.12	1.69	0.14	1.63*	0.15
L glycogen	375	61	191**	52	130**	12	155**	11	135**	16	151**	18
M volume	4.86	0.27	5.17	0.43	5.05	0.40	5.10	0.45	4.98	0.42	5.04	0.42
M glycogen	166	35	70**	18	58**	7	69**	10	64**	3	68**	8
Glucose												
L volume	1.89	0.14	1.82	0.14	1.76**	0.14	1.79	0.10	1.74**	0.11	1.77**	0.13
L glycogen	397	84	177**	43	213**	44	211**	49	242**	51	228**	49
M volume	5.06	0.26	5.07	0.25	5.03	0.27	4.99	0.24	4.95	0.28	4.99	0.29
M glycogen	153	39	60**	12	100**	46	85**	36	93**	38	92**	38

Mean values were significantly different from those at rest: * P < 0.05, ** $P \leq$ 0.01 (ANOVA, Newman Keuls).

Liver volume was lower during the recovery period following exercise when compared with resting values, and appeared to fall further in the absence of exogenous CHO (Table). This may be partly a consequence of a fall in liver glycogen concentration, which followed a similar pattern. Glucose administration arrested the decline in both liver glycogen concentration and liver volume. However, a significant relationship between liver glycogen concentration and liver volume was not found in either condition. Changes in muscle volume were not found. In conclusion, changes in liver volume should be accounted for during submaximal exercise and recovery. This research was supported by SmithKline Beecham.

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Magnetic resonance spectroscopy: a useful tool to investigate the relationship between delayed onset muscle soreness and muscle metabolism. By MONICA ZEHNDER[1], MIRJAM MÜLLI[1], RETO BUCHLI[2] AND URS BOUTELLIER[1], [1]*Exercise Physiology, Swiss Federal Institute of Technology, 8057 Zurich, Switzerland and* [2]*Paul Scherrer Institute, 5232 Villigen, Switzerland*

The aim of the present study was to show the relationship between delayed onset muscle soreness (DOMS), muscle glycogen (Gly) resynthesis, high-energy phosphates ATP and phosphocreatine (PCr), and their decay-product inorganic phosphate (Pi) of the *M. gastrocnemius* and *M. soleus* after vigorous exercise using ^{13}C- and ^{31}P-magnetic resonance spectroscopy (MRS). To indicate the severity of DOMS, the perceived state of soreness on a scale from 0 to 10 was measured.

Seventeen healthy trained athletes were randomly divided into two groups. After a Gly-reducing exercise, which consisted of 90 m sprints with 30 s breaks in between until exhaustion followed by a 1 h run on a treadmill at 65% V_{max}, one group additionally performed an eccentric toe-raise exercise (DOMS group). During the 48 h which followed the exercise, all subjects consumed the food provided which contained 10.0 g carbohydrates, 0.8 g fat and 1.0 g protein/kg body mass per d. Measurements were performed before, and immediately, 3, 15, 24 and 48 h after exercise. The experimental protocol was approved by the University of Zurich Ethic's Committee of Physiology and Pharmacology.

During the endurance exercise both groups decreased their Gly by the same amount from a mean value of 146 to 71 mmol/kg wet weight (ww) (-51%). In the following 3 h of recovery, Gly of the DOMS group decreased further to 60 mmol/kg ww (-59%). After 48 h of recovery, the Gly-concentration of the DOMS group was 116 mmol/kg ww which was still below the resting level (-21%) whereas the CONTROL group was recovered after 24 h (Fig. 1). During the endurance exercise the CONTROL group decreased their Pi from 3.0 to 2.6 mmol/kg ww (-13%) and showed in the following recovery-time resting concentrations. The DOMS group decreased their Pi during the toe-raise exercise from 3.2 to 2.2 mmol/kg ww (-31%) whereas during the following recovery it increased above the resting level to 4.4 mmol/kg ww (+38%) (Fig. 1). The ATP resting concentrations of the DOMS group was 5.8 mmol/kg ww and decreased after the toe-raise exercise to 5.2 mmol/kg ww (-10%) and 48 h was not sufficient for complete recovery, while in the CONTROL group the resting concentration of 5.5 mmol/kg ww did not change. The mean PCr resting concentrations of both groups were 23 mmol/kg ww and there was no change during the experimental time. In the DOMS group the perceived soreness increased above the resting situation after 15 h and remained at that level, while it did not change in the CONTROL group.

In conclusion, the Gly-repletion process was slowed down by about a factor of 3 due to DOMS. The reduced Gly-repletion, the elevated Pi concentrations, and the perceived state of soreness in the DOMS group indicate damaged muscle fibres and inflammation process after eccentric exercise.

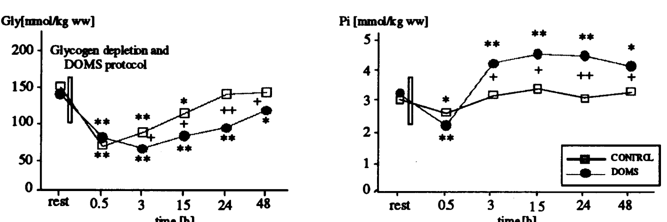
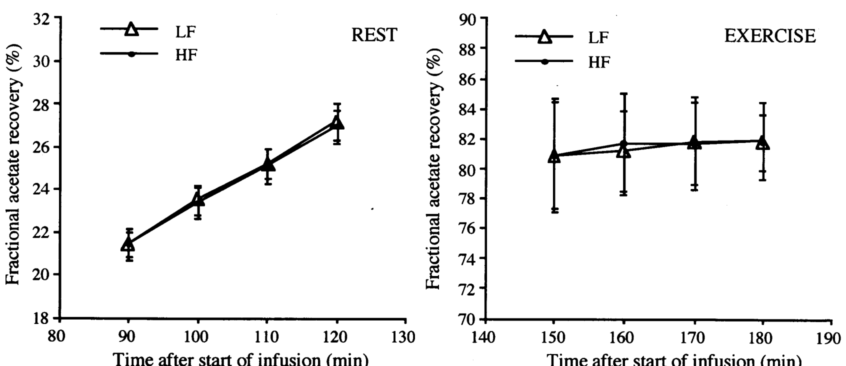


Fig. 1. Gly and Pi concentrations during the experimental protocol. * means significant difference from rest. + means significant difference between DOMS and CONTROL group. * + P< 0.05, ** ++ P < 0.001.

The effect of diet composition on acetate recovery factor during rest and exercise. By PATRICK SCHRAUWEN, ANTON J. M. WAGENMAKERS, WOUTER D. VAN MARKEN LICHTENBELT, WIM H. M. SARIS and KLAAS R. WESTERTERP, *Nutrition Research Institute NUTRIM, Maastricht University, Maastricht, The Netherlands*

The validity of estimations of plasma fatty acid oxidation using ^{13}C and ^{14}C tracers has often been questioned. Recently we showed that the [1,2-^{13}C] acetate recovery factor can be used for correction of the rate of [U-^{13}C] palmitate oxidation in infusion studies of 2 h in resting conditions (Schrauwen et al. 1998). However, we also showed that the intra-subject and inter-subject coefficient of variation of the acetate recovery factor was 4.0 (SE 1.5) % and 8.3 (se 0.6)% respectively, indicating that it had to be determined in each individual. Since stable isotope techniques are an elegant tool for studying dietary effects on the different compartments of fat oxidation, in the present study we examined the effect of extremes in diet composition on the acetate recovery factor. Seven healthy non-obese males (age 23 (SE 2) years; height 1.85 (SE 0.03) m; weight 70.4 (SE 2.3) kg; % body fat 13 (SE 1)) consumed either a low-fat diet (LF, 30 % energy as fat, 55 % energy as carbohydrate, 15 % energy as protein) for 4 d or a high-fat diet (HF, 60 % energy as fat, 25 % energy as carbohydrate, 15 % energy as protein) for 5 d. After an overnight fast, an intravenous infusion of [1,2-^{13}C] acetate (0.0645 μmol/min per kg) was started and continued for 2 h at rest. After this, acetate infusion rate was doubled and subjects started exercising on a cycle ergometer for 1 h at 50 % of the maximal aerobic workload. Breath samples were collected in vacutainer tubes and the $^{13}CO_2$ enrichment was measured by GC-isotope ratio mass spectrometry (Finnigan; MAT 252; Bremen; Germany). VCO_2 was determined by indirect calorimetry (Oxycon ß; Mijnhardt; Bunnik; The Netherlands). Total $^{13}CO_2$ excretion in the breath was calculated by multiplying VCO_2 with the $^{13}CO_2$ enrichment. The fractional acetate recovery was calculated as: total $^{13}CO_2$ excretion/(2xinfusion rate) x 100 %. The study was approved by the ethical committee of the Maastricht University.

Acetate recovery gradually increased at rest both after the LF diet and after the HF diet between t=90 and t=120 (Fig. left). During exercise, a plateau in acetate recovery was present at about 81 % after both the LF and HF diets (Fig. right). There were no significant differences in acetate recovery between the diets.



In conclusion, the results from the present study show that the acetate recovery factor is not influenced by diet composition. Furthermore, we again show that, at rest, only up to 26 % of infused acetate is recovered, indicating the importance of this recovery factor for correcting palmitate oxidation rates. During exercise the acetate recovery factor plateaus within 30 minutes and not using the correction factor during exercise only underestimates palmitate oxidation by about 20 %.

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Evidence for glycerol uptake by subcutaneous adipose tissue. By JAS S. SAMRA, MO L. CLARK, SANDY M. HUMPHREYS, PETER A. BANNISTER, LUCINDA K.M. SUMMERS, IAN A. MACDONALD and KEITH N. FRAYN, *Oxford Lipid Metabolism Group, Radcliffe Infirmary, Oxford OX2 6HE*

The assumption that glycerol utilization in peripheral tissues is negligible forms the basis of many isotopic studies in which the rate of appearance of glycerol is used as an estimate of whole-body lipolysis. However, several observations challenge this asssumption. For instance, Kurpad *et al.* (1994) infused [^{2}H]glycerol and found greater dilution across subcutaneous adipose tissue *in vivo* than expected from measured glycerol release. Their tracer infusion was continued for only 2 h, raising the possibility of incomplete equilibration. We have now infused [$^2H_5$]glycerol into sixteen normal subjects for 7 h after an overnight fast and measured arterialized and adipose-tissue venous glycerol concentrations and enrichments over the last 6 h. If equilibration is a problem with short infusions then the discrepancy between expected and measured adipose-venous enrichments should decrease with time. The protocol for the studies and details of [$^2H_5$]glycerol infusion and measurement of enrichment in plasma are given by Samra *et al.* (1996, 1998). The expected venous glycerol enrichment was calculated as arterialized enrichment multiplied by arterial glycerol concentration/adipose-venous glycerol concentration (Kurpad *et al.* 1994). The studies were approved by the local research ethics committee and all subjects gave informed consent.

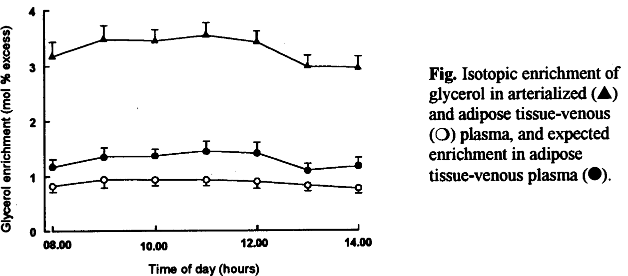

Fig. Isotopic enrichment of glycerol in arterialized (▲) and adipose tissue-venous (○) plasma, and expected enrichment in adipose tissue-venous plasma (●).

The Figure shows that the measured adipose-venous enrichment was consistently lower than the expected (*P*=0.026 by repeated-measures ANOVA), implying uptake of glycerol. The discrepancy did not decrease with time (*P*=0.4). In contrast, the mass balance was consistently a net output (mean arterialized glycerol 85 (SE 11) µmol/l; mean adipose-venous glycerol 227 (SE 21) µmol/l). Fractional extraction of labelled glycerol was 33 (SE 6) %, representing utilization at a rate 32 (SE 8) % of the net release rate. We investigated whether apparent glycerol uptake arose because we measured mass concentrations in whole blood, but enrichments in plasma. In 120 samples from six healthy subjects glycerol was measured in both plasma and whole blood. There was a close relationship between them, which was not different in arterialized (*n* 60) and adipose-venous (*n* 60) samples. We conclude that glycerol uptake by subcutaneous adipose tissue may be significant, although other data show no such utilization (Coppack *et al.* 1999). Some methodological issues therefore remain to be investigated.

We thank the Wellcome Trust for support.

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Samra JS, Clark ML, Humphreys SM, Macdonald IA & Frayn KN (1996) *American Journal of Physiology* **271,** E541-E546.

Glycerol exchange in human skeletal muscle at rest and during exercise. By MASSIMO SACCHETTI, GERRIT van HALL and BENGT SALTIN, *Copenhagen Muscle Research Center, Rigshospitalet, Denmark*

Glycerol is commonly used as an index of whole-body and regional lipolytic activity. A major assumption is that glycerol produced from triacylglycerol breakdown cannot be re-utilized in the cell and therefore must be released into the circulation. However, uptake of glycerol has been shown to occur at rest in the forearm (Elia *et al.*, 1993), which points towards the possibility that this substrate is metabolized in skeletal muscle. The aim of the present study was to investigate glycerol turnover in skeletal muscle at rest and during exercise by combining femoral arterio-venous differences and tracer dilution techniques.

Five subjects , aged 25 (SE 5) years, weight 78 (SE 4) kg , were studied in the post-absorptive state at rest and during 2 h of one-leg knee extensor exercise at 60 % of leg W_{max}. A primed (1.5 µmol/kg) constant (0.085 µmol/kg per min) infusion of D_5-glycerol was given through a catheter placed in an antecubital vein. Femoral and arterial glycerol concentration and enrichment (E_{glyc}) were measured before and after 90 and 120 min from the beginning of the D_5-glycerol infusion, as well as at 90, 105, and 120 min of exercise. Blood flow in the femoral artery was assessed by ultrasound doppler. Net glycerol exchange was calculated as the product of artrerio-venous glycerol concentration difference and leg blood flow. Glycerol uptake by the leg was calculated by considering the fractional extraction of D_5-glycerol, and glycerol release by subtracting the obtained value from the net exchange.

	Net glycerol release (µmol/min)		Arterial E_{glyc} (tracer:tracee)		Venous E_{glyc} (tracer:tracee)		Glycerol uptake (µmol/min per leg)		Glycerol release (µmol/min per leg)	
	Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE
Rest -30 min	-6	2	0.051	0.011	0.017*	0.002	4	1	10	3
Rest 0 min	-9	2	0.051	0.009	0.016*	0.002	7	1	16	4
Exercise 90 min	-97†	51	0.026	0.004	0.017*	0.002	114†	16	211†	52
Exercise 105 min	-91†	59	0.021	0.003	0.015*	0.002	116†	43	208†	78
Exercise 120 min	-86†	35	0.019	0.002	0.014*	0.002	121†	44	208†	66

* Significantly different from arterial E_{glyc}tracer:tracee ratio (P< 0.05).
† Significantly different compared to rest (P< 0.05).

At rest and during exercise a net release of glycerol was observed from the leg. However, from the tracer dilution on the arterial and venous side a simultaneous glycerol uptake and release was found. During exercise glycerol uptake and release were substantially increased.

In conclusion, the present data show that glycerol is taken up by the leg at rest and during exercise. These findings suggest that glycerol is metabolized in skeletal muscle and therefore cannot be used as a quantitative index of muscle triacylglycerol breakdown.

Elia, M., K. Khan, Calder G.,& Kurpad A. (1993). *Clinical Science* 84: 99-104

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Lactate production in response to glucose and triacylglycerol infusion at rest and during aerobic exercise in human subjects. By MARK ROEF[1], DIRK-JAN REIJNGOUD[3], HELMA STRAVER[2], RUUD BERGER[2] and KEES DE MEER[1], [1]*Department of Gastroenterology, and* [2]*Laboratory for Metabolic Diseases, University Children's Hospital, Utrecht, and* [3]*Laboratory for Metabolic Diseases, Department of Pediatrics, University Hospital Groningen, Groningen, The Netherlands*

In human subjects, effects of substrate availability on pyruvate and lactate production and disposal have not been studied extensively. The present study evaluated effects of infusion of isoenergetic amount of glucose (5 mg/kg) v. triacylglycerol (1.85 mg/kg) on plasma lactate concentration and whole-body (lactate+pyruvate) turnover in seven healthy volunteers at rest and during exercise (at 15 % of individual's Wmax). A primed constant infusion of [1-^{13}C]lactate was used to study rate of appearance (Ra) of (lactate+pyruvate), assuming a single common pool model for lactate and pyruvate (wolfe *et al.* 1988). Pyruvate oxidation rates were derived from indirect calorimetry. Non-oxidative (lactate+pyruvate) disposal (NOD) rates were calculated by subtraction of pyruvate oxidation from Ra (lactate+pyruvate) in steady-state experiments. The experimental protocol was approved by the Medical Ethical Committee of the hospital.

	Rest				Exercise			
	Glucose		Triacylglycerol		Glucose		Triacylglycerol	
	Mean	SE	Mean	SE	Mean	SE	Mean	SE
Plasma lactate (mmol/l)	1.2	0.1	0.6***	0.1	0.8	0.1	0.8	0.1
Glucose infusion (in pyruvate equivalents)	55.6		-		111.1		-	
Ra (lactate+pyruvate)	50.4	1.5	29.4***	2.2	59.8	4.6	46.7*	2.3
Pyruvate oxidation	26.2	3.0	15.4**	1.4	95.8	6.1	49.4***	2.9
Whole-body NOD rate	24.2	4.4	14.0*	1.6	-36.0	8.2	-2.7	4.0

Values in µmol/kg per min. Mean values were significantly different from those for glucose: *$P < 0.05$, **$P < 0.01$, ***$P < 0.001$.

Results are depicted in the Table. At rest, NOD rates as well as plasma lactate concentrations were higher with glucose loading compared with triacylglycerol loading. NOD rates correlated significantly with plasma lactate concentrations (R^2 0.72, $P < 0.01$). During low-intensity exercise, plasma lactate concentrations were not different between the two infusion conditions, while negative values were calculated for NOD.

We conclude that at rest the plasma lactate concentration in human subjects reflects whole-body NOD under different substrate conditions. During glucose infusion NOD is 40 % of external glucose infusion rate. During exercise, negative NOD rates suggest incomplete isotopic label exchange between lactate and pyruvate pools. The common pool model for lactate and pyruvate is applicable to human studies at rest, but not during low-intensity exercise.

Wolfe RR, Jahoor F & Miyoshi (1988) *American Journal of Physiology* **254**, E532-E535.

Measurement of [U-^{13}C]glucose enrichment by GC combustion (GC-C-) isotope ratio mass spectrometry (IRMS). Precision and reproducibility of laboratory standards and biological samples. By ANNEMIE P. GIJSEN, FRANK VAN DE VEGT, and ANTON J. M. WAGENMAKERS, *Nutrition and Toxicology Research Institute Maastricht, Stable Isotope Research Centre, Maastricht University, The Netherlands*

IRMS can be used to measure the ^{13}C-enrichment of metabolites at and slightly above the natural background enrichment. Modern IRMS equipment operates on line with a GC and a combustion interface (GC-C-IRMS), which generates CO_2 pulses which are transferred into a sector mass spectrometer for measurement of the $^{13}CO_2$:$^{12}CO_2$ ratio. Suggestions have been made by some tracer users that isotope fractionation may occur in the GC (especially when operated in split mode) and/or the combustion interface and may prevent accurate enrichment measurements of plasma metabolites. The aim of the present study was to investigate how precise and reproducible measurements of glucose enrichment are in laboratory standards (enriched with [U-^{13}C]glucose) and in plasma samples obtained from human subjects during continuous infusion of a [U-^{13}C]glucose tracer.

Enriched glucose standards were made by mixing accurately-measured volumes of an analytical solution of naturally enriched glucose (5.275 mM) with a solution (0.5565 mM) of [U-^{13}C]glucose (Cambridge Isotope Laboratories, 98.9 % enriched). In the human experiments a [U-^{13}C]glucose tracer was given as a continuous infusion via a calibrated IVAC 560 pump. Random blood samples with different enrichments were obtained by taking samples at various time points and heparinized plasma was prepared by centrifugation. Portions (250 µl) of the laboratory standards and of plasma were extracted first with methanol-chloroform (2.3:1, v/v) and then with chloroform-water (2:1, v/v). The clear water layer was dried overnight and the butylboronic acid-acetyl derivative was made according to standard procedures. The glucose derivative (1 µl) was injected in the GC (split ratio 1:15) and analysed by GC-C-IRMS (Finnigan MAT-252). Portions of the standards (5 µmol) were also dried into tin capsules and analysed via an elemental analyser (Carlo-Erba) system on line connected to the IRMS. The ^{13}C-enrichment was measured in delta per mil *v.* a laboratory CO_2 standard of known enrichment. The ^{13}C-enrichments were converted to tracer:tracee ratios (TTR) using standard equations and using a carbon-dilution factor of 6/16 for the butylboronic acid-acetyl derivative. TTR were also calculated from the mixed amounts of glucose tracer and naturally enriched glucose.

Table. TTR (x10^{-6}) of glucose standards analysed in six repeated measurements

Weighed standard	GC-combustion IRMS		Carlo-Erba IRMS	
	Mean	SD	Mean	SD
211	213	7	216	1
530	527	8	535	1
1066	1062	2	1079	0.6
2153	2154	3	2187	0.6
3263	3260	6	3306	1
5552	5555	5	5642	2

The Table shows that TTR do not differ for the weighed standards and the GC-C-IRMS measurements. Regression analysis shows a line with a slope of 1.001 and a correlation of 1.000. This indicates that no isotope fractionation occurs. Precision is better than 1 % and reproducibility is better than 3 % in all cases. The precision of GC-C-IRMS seems to be better even than that of direct combustion of pure glucose in the elemental analyser. The reproducibility of plasma glucose is in the same order of magnitude: TTR (x10^{-6}) of one background and two random plasma samples analysed in six repeated measurements were: 0 (SD 10); 292 (SD 6); 1615 (SD 6). We conclude that no fractionation occurs during GC-C-IRMS analysis and that the technique is suited to measure the [^{13}C]glucose enrichment with great precision and excellent reproducibility both in laboratory standards and biological samples.

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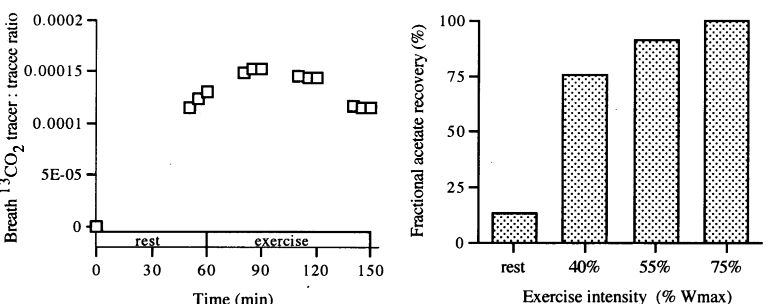
The effect of exercise intensity on breath $^{13}CO_2$ recovery during [1,2-^{13}C]acetate infusion. By LUC J.C. VAN LOON, PATRICK SCHRAUWEN and ANTON J.M. WAGENMAKERS, *Nutrition Research Institute NUTRIM, Maastricht University, Maastricht, The Netherlands*

Previous research has shown that during oxidation of a ^{13}C-palmitate tracer considerable amounts of tracer are incorporated in products of the TCA cycle. This could lead to a gross underestimation of plasma palmitate oxidation rates. Therefore, the [1,2-^{13}C]acetate recovery factor should be used to correct [U-^{13}C]palmitate oxidation rates at rest and during exercise (Sidossis *et al.* 1995; Schrauwen *et al.* 1998). Acetate recovery increases over time in resting conditions but plateaus after 30 min during exercise at a much higher value (Schrauwen *et al.*; parallel presentation at this meeting). In the present study we examined the effects of exercise intensity on the acetate recovery factor.

After an overnight fast eight cyclists (age 22 (SE 1) years; height 1.86 (SE 0.03) m; body weight 74.5 (SE 2.2) kg; maximal workload (Wmax) 413 (SE 9) W) were studied for 1 hr at rest during [1,2-^{13}C]acetate infusion (0.0736 µmol/min per kg). Thereafter infusion rates were doubled and subjects performed an exercise test consisting of three 30 min stages at a workload of 40, 55 and 75 % Wmax, respectively. Breath samples were collected in vacutainer tubes and $^{13}CO_2$ enrichment was measured by GC-isotope ratio mass spectrometry (Finnigan, MAT 252). Breath gases were analysed to determine VCO_2 (Oxycon β, Mijnhardt). We observed an increase in fractional acetate recovery as exercise intensity was increased (see Fig.).



We conclude that the acetate recovery factor increases with exercise intensity and reaches 100 % recovery at an exercise intensity of 75 % Wmax in highly trained subjects (VO_2 >3.9 l per min), which makes the correction for label fixation needless. Omission to apply the acetate correction factor leads to a gross underestimation of tracer oxidation rates under resting conditions, but has no impact during exercise at 75 % Wmax in highly trained athletes.

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Sidossis L S , Coggan A R , Gastaldelli A & Wolfe R R (1995) *American Journal of Physiology* **269**, E649-E656.

Fat utilization during exercise at low and moderate intensity after a single fat- or carbohydrate-rich meal. By HEIDI HUBER[1], BEAT KNECHTLE[1], URS MÄDER[1], YVES SCHUTZ[2] and URS BOUTELLIER[1], [1]*Exercise Physiology, Federal Institute of Technology and University of Zurich, and* [2]*Institute of Physiology, University of Lausanne, Switzerland*

It is well etablished that fat oxidation rate is at maximal level during exercise of moderate intensity (Romijn et al., 1993), whereas a high fat diet increases the availability of lipid substrates (Jansson and Kaijser, 1982). Little is known about the effect of a single fat-rich meal on the availability and use of lipid substrates of the different stores. Constant infusions of stable-isotope tracers ([1-^{13}C]palmitate) and indirect calorimetry were used to evaluate the regulation of fat metabolism in relation to exercise intensity and pre-exercise meal. Eight endurance-trained subjects cycled four times for 60 min at 45 and 65 % of maximal oxygen consumption ($\dot{V}_{O_2,max}$) after an overnight fast. They performed each intensity either after consuming a single fat-rich meal (FAT) (1674 + 28 kJ·kg body mass; 70 % energy as fat, 15 % as carbohydrate) or after a carbohydrate-rich meal (CHO) (1674 + 28 kJ·kg body mass; 15 % energy as fat, 70 % as carbohydrate) 12 h before the trials. All trials were repeated with a constant [1-^{13}C]acetate infusion to determine the acetate recovery factor (Sidossis et al., 1995).

Intensity (% $\dot{V}_{O_2,max}$)/meal	45 %/FAT		45 %/CHO		65 %/FAT		65 %/CHO	
Values after 60 min of exercise time	Mean	SD	Mean	SD	Mean	SD	Mean	SD
Fat oxidation (J/kg per min)	358	74	323	75	357	67	301	119
Consumption of $\dot{V}_{O_2}$ (µmol/kg per min)	1545**	152	1531**	147	2098	210	2080	232
Production of $\dot{V}_{CO_2}$ (µmol/kg per min)	1298**	128	1311**	122	1856	197	1873	189
Production of $\dot{V}_{^{13}CO_2}$ (µmol/kg per min)	0.139*	0.017	0.132*	0.011	0.153	0.017	0.149	0.024
Oxidation of infused palmitate (%)	83*	6	79*	10	92	10	89	14
Acetate correction factor	0.87*	0.10	0.84*	0.13	0.97	0.13	0.93	0.14
Corrected oxidation of infused palmitate (%)	96	12	94	7	95	11	96	16
Plasma free fatty acid (mmol/l)	0.53	0.18	0.47	0.23	0.56	0.24	0.49	0.16
Glycerol (mmol/l)	0.29*†	0.04	0.24*	0.06	0.38†	0.08	0.32	0.07

$\dot{V}_{^{13}CO_2}$: enrichment above background measurement at rest. Oxidation of infused palmitate: $\dot{V}_{^{13}CO_2}$/infusion rate.
Mean values were significantly different from 65 % $\dot{V}_{O_2,max}$: *$P < 0.05$, **$P < 0.001$.
Mean values were significantly different from CHO: †$P < 0.05$.

The results show that energy expended from fat during exercise was not influenced by the composition of the pre-exercise meal and was similar at both intensities. Computed from the $\dot{V}_{^{13}CO_2}$, oxidation of infused [1-^{13}C]palmitate was decreased before acetate correction during the trials at 45 compared with 65 % $\dot{V}_{O_2,max}$. After acetate correction the percent of the infused [1-^{13}C]palmitate oxidised was similar. Glycerol concentrations were decreased during exercise at 45 compared with 65 % $\dot{V}_{O_2,max}$ and were elevated by the FAT meal compared with the CHO meal. The lower concentrations of plasma glycerol indicate a reduced lipolysis activity at 45 compared with 65 % $\dot{V}_{O_2,max}$ and this is contradictory to the similar fat utilization and the similar plasma FFA levels during the trials at both intensities.

These findings suggest that total fat oxidation rate is similar at either intensity and after either pre-exercise meal, although higher lipolysis activity is indicated during exercise at 65 % $\dot{V}_{O_2,max}$ and after a high-fat meal.

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Sidossis L S, Coggan A R, Gastaldelli A & Wolfe R R (1995) *American Journal of Physiology* **269**, E649-E656.

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The metabolism of [^{13}C]-linoleic acid in healthy men and women. By SUSANNE H.F. VERMUNT, RONALD P. MENSINK, ANTON J.M. WAGENMAKERS, MARIANNE M.G. SIMONIS and GERARD HORNSTRA, *Department of Human Biology, Maastricht University, PO Box 616, 6200 MD Maastricht, The Netherlands*

Conversion of dietary linoleic acid (18:2*n*-6) has mainly been examined *in vitro* and in animal models. We have now investigated *in vivo* the oxidation of [^{13}C]18:2*n*-6 and its conversion into long-chain polyunsaturates (LCP) in six healthy subjects. After an overnight fast, a blood and a breath sample were collected, after which a single dose of 45 mg uniformly labelled [^{13}C]18:2*n*-6 (Martek Biosciences Corporation, Columbia, MD, USA) dissolved in 8 g olive oil was given to the subjects. Other blood and breath samples were collected every 2 h during the first 12 h (non-fasted), and after 24, 48, 72, 96, 168 and 336 h (overnight fasted). During these 2 weeks, subjects consumed their habitual diets. ^{13}C enrichments in breath and plasma total lipids were analysed with GC-combustion-isotope ratio mass spectrometry (GC-C-IRMS), and absolute fatty acid compositions in plasma were analysed with a GC with flame ionization detection (GC-FID). Tracer tracee ratios (TTR) were calculated from ^{13}C enrichments, and absolute amounts of [^{13}C]-fatty acids (^{13}C-FA) were calculated from TTR and absolute fatty acid compositions. Values were averaged and are presented as means with their standard errors.

Fatty acid (*n* 6)	TTR (*10^{-5})		Maximal concentration in plasma total lipids (mg/L)		Maximal absolute amount of ^{13}C-FA (mg)	
	Mean	SE	Mean	SE	Mean	SE
18:2*n*-6	113.38	22.21	1065.6	61.5	3.51	0.78
18:3*n*-6	48.81	17.37	12.2	1.4	0.019	0.008
20:3*n*-6	18.28	5.77	52.5	3.6	0.028	0.011
20:4*n*-6	2.80	0.70	221.7	8.0	0.018	0.005

TTR of 18:2*n*-6 in plasma total lipids already started to increase during the first 2 h after intake of [^{13}C]18:2*n*-6. Maxima were obtained 8 - 12 h after intake of the tracer except for one subject, who reached a maximum after 48 h. Mean maximal TTR, as shown in the Table, was highest for 18:2*n*-6. Conversion of [^{13}C]18:2*n*-6 into [^{13}C]18:3*n*-6 started almost directly after the appearance of [^{13}C]18:2*n*-6 in plasma, and peak TTR of 18:3*n*-6 were reached after 8 - 48 h. After 168 h most of the [^{13}C]18:2*n*-6 and [^{13}C]18:3*n*-6 had disappeared. Because 20:3*n*-6 concentrations in plasma total lipids were higher than 18:3*n*-6 concentrations, maximal absolute amounts of [^{13}C]20:3*n*-6 were higher than those of [^{13}C]18:3*n*-6. Maximal TTR of 20:3*n*-6 were reached after 48 - 168 h. After 168 h, TTR had not returned to baseline yet. Time to peak TTR of 20:4*n*-6 varied between subjects and TTR were rather low. In addition, TTR of 22:4*n*-6 and 22:5*n*-6 were very low. Oxidation rates were significant in the first 12 h, but then fell rapidly. Peak TTR in breath were reached after 3 - 4 h. The mean proportion of [^{13}C]18:2*n*-6 recovered in breath after 12 h was about 15 %.

These findings suggest that a single bolus of 45 mg [^{13}C]18:2*n*-6 can be used to study the conversion of [^{13}C]18:2*n*-6 into its LCP. Only a small fraction of the tracer was found in plasma total lipids and as $^{13}CO_2$ in breath.

Increased *in vivo* liver nitric oxide production during endotoxin challenge in pigs. By MAAIKE J. BRUINS, PETER B. SOETERS and NICOLAAS E.P. DEUTZ, *Department of Surgery, Maastricht University, Maastricht, The Netherlands*

NO plays an important role in host defense and under conditions such as sepsis and inflammation, NO is produced in increased amounts by activation of the inducable NO synthase enzyme (NOS; EC 1.14.23). Arginine (ARG) is the precursor of NO, produced in various tissues during sepsis. Conversion of ARG by NOS yields stable citrulline (CIT) and unstable NO in equal amounts. Therefore, *in vivo* NO synthesis is estimated by the rate of conversion of the stable isotope $^{15}N_2$-ARG to ^{15}N-CIT.

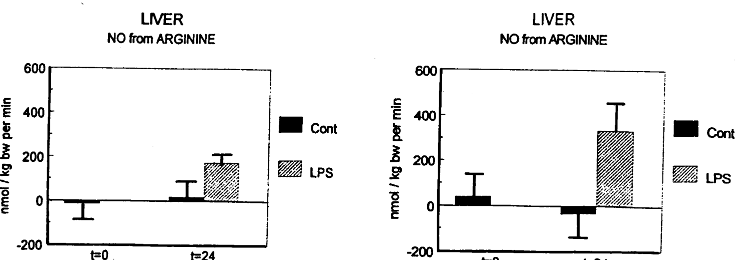
Conscious, healthy female pigs (Yorkshire x Dutch Landrace, about 20 kg body weight (BW), *n* 12) in which catheters were placed in the portal vein, the hepatic vein and the abdominal aorta, were studied two weeks after operation. ARG and CIT net balances (NB) across the portal drained viscera (PDV) and liver were calculated as the product of blood flow and arteriovenous concentration differences. Arteriovenous enrichment differences were used to calculate ARG and NO kinetics. After an overnight fast, as a model of sepsis, endotoxin (Lipopolysaccharide (LPS), E. Coli) was given intravenously at a rate of 3 μg per kg BW per h for 24 h. Plasma samples were obtained 24 h after initiation of LPS. The control group did not receive LPS. L-[*guanidino*-$^{15}N_2$]ARG was infused (0.1 μmol/kg bw) after an initial prime (0.1 μmol/kg bw per h) to calculate ARG disposal (Rd) and production (Ra) and production of NO across PDV and liver *in vivo*. Liquid chromatography-mass spectrometry was used to determine isotopic enrichments.

	ARG NB† (nmol/kg bw per min)		ARG Rd (nmol/kg bw per min)		ARG Ra (nmol/kg bw per min)	
	CON	LPS	CON	LPS	CON	LPS
PDV	152 ± 68	155 ± 55	333 ± 52	535 ± 143	486 ± 48	697 ± 118
Liver	-275 ± 78	-497* ± 77	636 ± 126	505 ± 109	377 ± 104	112 ± 66

Mean values were significantly different from control group: *$P<0.05$ (*t* test).
†Positive values indicate production, negative values indicate uptake.

ARG disposal and production in the PDV were higher in the LPS group compared with control group, however, NB was not different (Table). A small increase in NO production across PDV was found in the in LPS group and not in the control group (Figure). In liver, ARG net uptake was significantly increased by LPS infusion; this was caused by a decreased ARG production (Table). LPS significantly increased liver NO production (Figure). Across PDV, ARG net balance was not different between both groups (Table). Liver NO production in the LPS group was 65 % of ARG consumption.

In this study, using *in vivo* tracer techniques, we were able to study organ NO production during sepsis. The increased liver ARG uptake during endotoxemia is related to increased liver NO production.



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***In vivo* whole-body nitric oxide synthesis, determined by the conversion of [$^{15}N_2$]arginine to [^{15}N]citrulline, is not increased in acute endotoxin-treated mice.** By MARCELLA M. HALLEMEESCH, PETER B. SOETERS and NICOLAAS E. P. DEUTZ, *Department of Surgery, Maastricht University, The Netherlands*

NO is synthetized from arginine by the enzyme nitric oxide synthase. It has been suggested that the therapeutic modulation of nitric oxide production may be achieved by supplying arginine, or by inhibiting nitric oxide synthase. It is therefore desirable to gain a better understanding of whole body arginine metabolism, and its quantitative relationship with the arginine:NO pathway during disease. The most commonly used method to estimate whole-body production of the unstable molecule NO is plasma NO_2/NO_3 levels. However, direct estimation of the whole-body flux of NO by conversion of the stable isotope [$^{15}N_2$]arginine (Arg) to [^{15}N]citrulline (Cit) is preferable. Therefore, we studied whole-body flux of NO in an acute endotoxin mouse model, using a new stable isotope technique. Endotoxin (lipopolysaccharide *E.Coli*, 250 µg in 0.5 ml normal saline) was administered intraperitoneally to seven male Swiss mice. Eight control mice (CON) received 0.5 ml saline. At t=5 h, a primed constant infusion protocol of L-[*guanidino*-$^{15}N_2$]arginine and L-[*ureido*-$^{13}C,^{2}H_2$]citrulline was given (prime: 2 µmol [$^{15}N_2$]arginine, 0.4 µmol [$^{13}C,^{2}H_2$]citrulline, infusion: 10 nmol/ 10 g BW per min [$^{15}N_2$]arginine, 2.5 nmol/ 10 g BW per min [$^{13}C,^{2}H_2$]citrulline). Tracer steady-state was obtained after 20 min. Enrichments of arginine and citrulline were measured by liquid chromatography mass spectrometry. Plasma arginine (Q_{Arg}) and citrulline (Q_{Cit}) fluxes were calculated from the plasma isotope enrichment values of arginine (M+2) and citrulline (M+3) respectively, using steady state isotope dilution equations [#966]. Calculation of the rate of conversion of arginine to citrulline, via the NO synthesis reaction, was made as follows: $Q_{Arg \rightarrow Cit} = Q_{Cit} \times (E_{Cit}/E_{Arg}) \times [Q_{Arg}/(I_{Arg} + Q_{Arg})]$, where E_{Cit} and E_{Arg} are the respective plasma enrichments of citrulline (M+1) and arginine (M+2), and I_{Arg} is the rate of infusion of labeled arginine [#791].

In endotoxin-treated mice, plasma NO_3 was significantly increased. The plasma fluxes of arginine and citrulline were not increased. Also, the whole body NO production was not significantly different from that of control mice.

	Control		LPS	
	Mean	SE	Mean	SE
Plasma NO_3 (µM)	26	3	151*	20
Arginine flux (nmol/ 10 g BW per min)	60	5	68	7
Citrulline flux (nmol/ 10 g BW per min)	31	6	40	8
NO production (nmol/ 10 g BW per min)	3.6	0.7	6.6	2.4

* Significantly different from control, $P<0.01$ (Student's t test).

Although plasma NO_3 was significantly increased, 6 h after endotoxin injection in mice, we did not observe a significant increase in the directly estimated whole-body NO production. Our results cast doubt on the validity of using plasma NO_3 levels as a marker for whole-body NO production.

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The effect of drink composition on deuterium accumulation in able-bodied and spinal-cord-injured men. By D. BALL[1], J.B. LEIPER[2] and R.J. MAUGHAN[2], [1]*Exercise and Sport Science, Manchester Metropolitan University, Alsager ST7 2HL, and* [2] *Biomedical Sciences, University Medical School, Aberdeen AB25 2ZD*

It is unknown whether a low-level spinal cord lesion will affect the availability of ingested water when compared with able-bodied individuals. With local ethics committee approval, ten male subjects volunteered for the present study. Five subjects were able-bodied (AB) and five had a spinal cord injury (SC). The subjects' mean age and body mass were: AB, 21 (SD 2) years and 73.1 (SD 9.6) kg; SC, 29 (SD 9) years and 65.8 (SD 14.7) kg. On three separate occasions, 5 d apart, each subject reported to the laboratory following an overnight fast, and ingested 500 ml of one of three beverages: sugar-free flavoured water, an 80 g/l glucose-electrolyte (GE) solution, and a 160 g/l-GE solution. Deuterium oxide, a tracer for water, was added to each drink at a dose of 71.4 mg/kg body mass. Beverages were administered in randomized order. Arterialized-venous blood samples were taken before ingestion and at intervals up to 75 min after consumption. Blood samples were analysed for blood deuterium (^2H) content. Deuterium accumulation in the circulation was measured by i.r. spectrophotometry following vacuum distillation of the blood samples. Data were analysed by Kruskall-Wallis test and by post-hoc Mann Whitney test.

	Rate (ppm/min)		Cmax (ppm)		Tmax (min)	
	Median	Range	Median	Range	Median	Range
Group AB						
Water	11.8	7.5-27.3	236	225-273	20	10-30
80 g/l-GE	9.0	6.9-10.2	206	197-269	30	20-30
160 g/l-GE	3.8	2.2-4.1	183	166-227	60	45-75
Group SC						
Water	10.0	4.5-12.4	209*	175-238	20	20-30
80 g/l-GE	4.0*	3.4-9.8	180	109-223	30	20-45
160 g/l-GE	2.2	1.2-3.4	143*	96-163	60	45-60

Median values were significantly different from the corresponding values for group AB:* $P<0.05$.

The rate of ^2H accumulation (Rate) in the circulation was faster ($P<0.04$) in AB subjects in the 80 g/l-GE trial than SC subjects, and tended to be higher in the 160 g/l-GE trial ($P<0.06$). In the AB group the rate of ^2H accumulation was faster ($P<0.012$) with water than the 160 g/l-GE but not the 80 g/l-GE trial. The rate of ^2H accumulation in the 80 g/L-GE trial was, however, faster ($P<0.012$) than the 160 g/l-GE trial. In SC subjects the rate of ^2H accumulation was faster ($P<0.012$) with water compared with the 160 g/l-GE trial. The rate of ^2H accumulation in the 80 g/L-GE trial was not different from either water or the 160 g/l-GE trial. The maximum concentration (Cmax) of ^2H was higher in the AB subjects than the SC subjects in both the water ($P<0.047$) and the 160 g/l-GE trials ($P<0.009$) and tended to be higher in the 80 g/l-GE trial ($P<0.076$). In the AB group Cmax was lower in the 160 g/l-GE trial than both water ($P<0.021$) and the 80 g/l-GE trial ($P<0.012$), with no difference between water and the 80 g/l-GE trial. The Cmax of ^2H in SC subjects was higher during the water trial ($P<0.012$) than the 160 g/l-GE trial but not the 80 g/l-GE trial; no difference in Cmax was observed between the 80 - and 160 g/l-GE trials. The time to reach Cmax (Tmax) was shorter ($P<0.009$) after both water ingestion and the 80 g/l-GE solution than the 160 g/l-GE solution in both groups. Based on ^2H accumulation in this study, high glucose concentrations in beverages produce slower rates of water uptake compared with water, this could be a function of a slower rate of gastric emptying or intestinal absorption. This effect appears to be augmented by a low-level spinal cord lesion but the difference in ^2H accumulation between groups could also be a function of different circulating volumes.

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Exercise in healthy adult dogs increases plasma thiobarbituric acid reactive substances (TBARS), an indicator of oxidative stress. By RIMI. OBRA[1], E. JEAN. HARPER[1], JOSEPH. LUNEC [2], [1]*Waltham Centre For Pet Nutrition, Melton Mowbray, LE14 4RT, UK;* [2]*Division of Chemical Pathology, MRC Centre For Mechanisms in Human Toxicology, Leicester University, Leicester L11 9HN*

Plasma TBARS measured by HPLC with pre-coloumn derivatization is a well-documented marker of lipid peroxidation *in vivo*. The aim of the present study was to survey the effects of a bout of exercise in dogs (*Canis familiaris*) upon this index of oxidative stress. A group of fourteen dogs of mixed breed and age was maintained on a nutritionally complete commercial dry diet (Pedigree Chum Complete; Pedigree Masterfoods, Peterborough, Cambs, UK) for 3 months before and throughout the duration of this trial. The extent of lipid peroxidation immediately before to and following an acute 20 min bout of paddock exercise was estimated by determining the malondialdehyde (MDA) formed as TBARS. This was measured according to the method described by Bird & Draper (1984). The table shows the plasma MDA TBAR levels measured before and after exercise for each dog. The results revealed a significant increase (22 %) in plasma TBARS (paired *t* test $p<0.05$) following exercise 0.74 (SD 0.2) μM pre-, 0.92 (SD 0.2) μM post-exercise.

Breed	Age (years)	Sex	Plasma MDA TBARS pre-exercise (nmol/ml)	Plasma MDA TBARS post-exercise (nmol/ml)
Springer	5	F	0.37	0.85
Poodle	7	M	0.58	0.60
Poodle	4	M	0.34	0.49
Beagle	4	F	0.74	1.23
Beagle	3	F	0.87	1.18
Beagle	4	F	1.05	1.12
Beagle	3	F	0.91	1.17
Labrador	6	F	0.76	0.80
Labrador	3	M	0.65	1.15
Labrador	3	F	0.72	0.77
Beagle	4	F	1.44	0.87
Beagle	3	F	0.64	0.96
Beagle	3	F	0.88	1.17
Beagle	3	F	0.49	0.60

In order to audit for increases occurring as a direct result of concentrated blood volumes post-exercise, packed cell volumes and plasma albumen were measured. Results did not reveal a significant difference pre- and post-exercise ($P>0.05$) and therefore the observations from this study suggest that augmented lipid peroxidation *in vivo* occurs as a direct result of exercise-induced oxidative stress. Previous work suggests that the specific site of oxidative damage is the cellular membrane, where peroxyl radicals ($RO_2^{\cdot}$) proliferate in conditions of high oxidative stress.

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Does exercise induce oxidative stress in endurance-trained kidney and heart transplant recipients? By LILIANA JIMÉNEZ[1], GUILLAUME LEFÈVRE[2], RUDDY RICHARD[1], ALAIN DUVALLET[1], JACQUELINE ETIENNE[2] and MICHEL RIEU[1], [1]*Laboratoire de Physiologie des Adaptations de la Faculté de Médecine Cochin-Port-Royal, Paris, France and* [2]*Service de Biochimie, Hôpital Tenon, Paris, France*

The level of malondialdehyde (MDA), a marker of lipid peroxidation, was reported to be significantly elevated in sedentary kidney (Hussain *et al.* 1995) and heart (de Lorgeril *et al.* 1993) transplant recipients compared with healthy subjects, which suggests that these patients are prone to oxidative stress at rest. Although the benefits of exercise are well known, evidence indicates that strenuous physical exercise is associated with a rise in free-radical production and increase in lipid peroxidation, both of which can be harmful (Alessio & Goldfarb, 1988)

The purpose of the present study was twofold: to evaluate the activity of scavenger enzymes in subjects at rest, and to compare the effects of a symptom-limited (to exhaustion) exercise treadmill test on the plasma levels of MDA (Lefèvre *et al.* 1996) and vitamin E (Bieri *et al.* 1979). Our study population consisted of twenty endurance-trained subjects: six kidney (KTR) and seven heart (HTR) transplant recipients and seven healthy control subjects (HC). Twelve of thirteen transplant recipients were treated with azathioprine, eleven with cyclosporine and seven with azathioprine and cyclosporine combined. A significance level of $P<0.05$ was set throughout.

At rest the erythrocyte activity of superoxide dismutase (EC1.15.1.1) was higher in both transplant groups (KTR: 1023.3 ± 151.0 U.g Hb^{-1}; HTR: 1074.9 ± 165.8 U.g Hb^{-1}) than in HC (803.3 ± 167.1 U.g Hb^{-1}), and that of glutathione peroxidase (EC1.11.1.9) was lower in both transplant groups (KTR: 30.3 ± 5.7 U.g Hb^{-1}; HTR: 31.1 ± 8.1 U.g Hb^{-1}) than in HC (104.1 ± 26.3 U.g Hb^{-1}). No difference in MDA or vitamin E concentration was found between groups. At exhaustion, there was no change in MDA in any group. Exercise increased vitamin E levels in KTR (24.6 ± 7.1 to 35.9 ± 10.6 mmol.l^{-1}), in HTR (22.7 ± 6.9 to 27.8 ± 5.7 mmol.l^{-1}) and in HC (27.7 ± 5.3 to 34.4 ± 6.8 mmol.l^{-1}). In KTR and HC the higher vitamin E level may be explained by haemo-concentration (evidenced by a rise in the total plasma protein level 14.0 % and 20.4 % respectively) and/or lipid modification (increased plasma triacylglycerols, KTR: 1.3 ± 0.4 to 1.6 ± 0.4 mmol.l^{-1} and HC: 1.5 ± 0.5 to 1.9 ± 0.7 mmol.l^{-1}). In HTR, on the other hand, exercise did not affect mean plasma volume but induced an increase in total plasma cholesterol (4.0 ± 1.5 to 5.7 ± 1.6 mmol.l^{-1}).

Despite our use of derivative spectrophotometry assay to measure MDA, we found no rise in MDA level. The specificity of MDA as marker of lipid peroxidation has been questioned.

Our results indicate that: (1) although there is a difference in scavenger enzyme activity between resting transplant recipients and healthy control subjects at rest, regular physical exercise presents no oxidative stress risk for our transplanted patients; (2) exercise does, however, raise plasma vitamin E in KTR, in HC and in most HTR.

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Determination of hydroxylated derivatives of antipyrine in plasma with HPLC-Tandem-Mass Spectrophotometry. By STEFAN A.J. COOLEN[1], ERWIN P. MEIJER[2], MARK van LIESHOUT[1], WIM H.M. SARIS[2] and FRED A. HUF[1], 1 *Eindhoven University of Technology, Laboratory of Instrumental Analysis, P. O. Box 513, 5600 MB Eindhoven, The Netherlands, and* 2 *Department of Human Biology, Maastricht University, P. O. Box 616, 6200 MD Maastricht, The Netherlands*

Monitoring the amount of exercise-induced oxidative damage caused by free radicals is a major challenge in free radical and exercise research. Measurement of oxidative damage via trapping by exogenous markers is currently under investigation (Coudray *et al.* 1995). In the present study antipyrine was used as a marker for free radical damage. Two advantages of using antipyrine are the well-known metabolic pathway (Hartleb 1991) and the high reaction constant rate with hydroxyl radicals in the order of 10^{10} litres/mol per s (Forni *et al.* 1988). However, in *in vivo* experiments the concentrations of hydroxylated derivatives of antipyrine in plasma are very low and difficult to detect. In the present study a method was developed to determine antipyrine and its hydroxylated derivatives in plasma by means of reversed-phase HPLC combined with a mass spectrometer (RP-HPLC-MS). The sample pre-treatment consisted of a C18 solid phase extraction (SPE) in order to wash out the salts and proteins. In order to estimate the retention behaviour of antipyrine and its hydroxylated derivatives in the SPE procedure, the k values were determined on a C18 HPLC column, using buffers of different compositions and different pH. Capillary zone electrophoresis was used to control the washout of Na^+ ions and proteins. Washing twice with a volume of 0.5 ml water or buffer as washing solution resulted in the complete removal of NaCl and plasma proteins. If water was used as the washing solution, it resulted in the loss of the ortho-hydroxyantipyrine free-radical product. The SPE resulted in an almost complete washout of the proteins and NaCl. Recoveries of antipyrine and its hydroxylated products were 90 % (S.D. 5 %) in water and 100 % (S.D. 5 %) in plasma respectively. The HPLC procedure was optimised for detection with a mass spectrometer. Atmospheric pressure chemical ionisation was used as the introduction of the components into the mass spectrometer. The mass spectrometer was operated in the multiple reaction mode which resulted in the best signal/noise ratio. For the different hydroxylated antipyrines, two target ions (m/z=120, m/z=104) were used and optimised. As a result of this procedure a detection limit of 6 pg for antipyrine was obtained. The error in the analysis was less than 5 %. In conclusion, the above findings indicate that it is possible to measure the hydroxylated derivatives of antipyrine even at very low concentration with HPLC-MS.

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Antipyrine as a marker for exercise-induced oxidative stress in untrained young adults. By ERWIN P. MEIJER[1], STEFAN A.J. COOLEN[2], WIM H.M. SARIS[1] and FRED A. HUF[2], [1] *Department of Human Biology, Maastricht University, POBox 616, 6200 MD, and* [2] *Eindhoven University of Technology, Laboratory of Instrumental Analysis, P.O.Box 513, 5600 MB Eindhoven, The Netherlands*

The *in vivo* measurement of highly reactive free radicals in humans is difficult. Measurement of oxidative damage via trapping by exogenous markers is preferred to measurement of endogenous markers. In the present study antipyrine was used as an exogenous marker for oxidative damage. Three advantages of using antipyrine are the well-known metabolic pathway, the low clearance rate of the drug, and the high reaction constant rate with hydroxyl radicals which is in the order of 10^{10} liters/mol per s. Recently it was shown that ^{60}Co γ-radiolysis of antipyrine resulted in the formation of three specific hydroxylated derivatives of antipyrine, namely ortho-, meta- and para-hydroxyantipyrine (Coolen *et al.* 1997). The ortho- and meta-hydroxylated isomers are different from the enzymic metabolites of antipyrine formed in man. In this study, four untrained young adults (age 23 (SD) 3 years; body mass 75.5 (SD) 16.2 kg; VO_2max 3.45 (SD) 0.56 liters/min) were administered orally 10 mg antipyrine per kg body mass. At 1 h after administration they started to cycle for 2 h at 50% of their maximal power output (130 (SD) 23 W), which was determined during an incremental exercise test. Blood samples (10 ml) were obtained before administration, at 1 h after administration and every 10 minutes during exercise. The protocol was approved by the Ethics Committee of Maastricht University. Antipyrine and the hydroxylated derivatives were measured in blood plasma by means of reversed-phase HPLC combined with mass spectrometry (MS). Sample introduction in the MS was performed with an atmospheric pressure chemical ionization chamber (APCI) and the MS was operated in the multiple reaction mode (MRM). The sample pretreatment consisted of a C18 solid phase extraction (SPE) in order to wash out salts and proteins.

In all four subjects, plasma samples before administration of antipyrine showed no interfering peaks at the retention times of ortho- and meta-hydroxyantipyrine. During and immediately after exercise two specific peaks of phenolic derivatives of antipyrine at the retention times of ortho- and meta-hydroxyantipyrine were shown. Eight days later, the subjects carried out the same protocol without exercise to control for time effect and antipyrine metabolism at rest, however, data is not yet available.

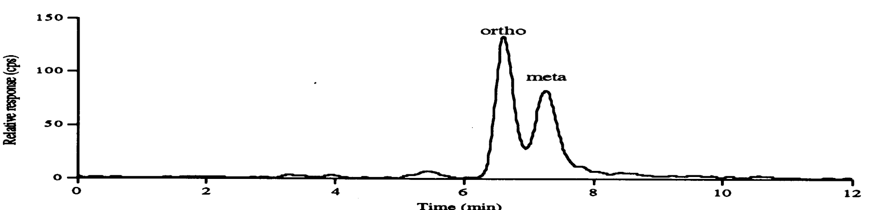

Fig. HPLC-APCI-MRM-MS chromatogram of a plasma sample of one subject immediately after two hours cycling.

The findings indicate that antipyrine seems to be a potential marker for exercise-induced oxidative stress. Cycling of moderate intensity for 2 h already resulted in exercise-induced oxidative stress as shown by the formation of two specific phenolic derivatives of antipyrine.

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Biological applications of electron paramagnetic resonance spectroscopy: methods and limitations By T. ASHTON[1], S. K. JACKSON[2], I. S. YOUNG[3], B. DAVIES[1], E. JONES[4], J. R. PETERS[5] and C. C. ROWLANDS[6], [1]*School of Applied Sciences, University of Glamorgan, Pontypridd CF37 IDL,* [2]*Department of Medical Microbiology, University of Wales College of Medicine, Cardiff CF4 4XN,* [3]*Department of Clinical Biochemistry, Queens University, Belfast BT12 6BJ,* [4]*University of Wales, Institute Cardiff, Cardiff CF3 7XR,* [5]*Department of Medicine, University Hospital of Wales, Cardiff CF4 4XW and* [6]*Department of Chemistry, University of Wales College Cardiff, Cardiff CF1 3TB*

Free radicals are in the main short-lived paramagnetic species whose detection is limited by their reactivity and low steady-state concentration. Electron paramagnetic resonance (EPR) spectroscopy is the most sensitive, specific and direct method of detecting free-radical species. We have used both spin-trapping (α-phenylbutyl-tert-nitrone) and non spin-trapping (ascorbyl radical) EPR methods to detect changes in free radical concentration in the plasma of healthy human volunteers (*n* 10) pre- and post-exercise. Additionally we examined the effect of ascorbic acid supplementation on exercise-induced free-radical production. We observed significant increases in the level of the ascorbyl radical (arbitrary units) from 0.02 (SE 0.001) pre-exercise to 0.03 (SE 0.002) post-exercise (P=0.04). This appears to be the first report of an increase in the concentration of the ascorbyl radical in human plasma post-exercise. We also observed increases in the spin-trapped radicals (arbitrary units) from 0.05 (SE 0.02) pre-exercise to 0.19 (SE 0.03) post-exercise (P=0.002), which are tentatively identified as being secondary alkoxyl radicals. Oral supplementation with 1000 mg ascorbic acid resulted in an attenuation of the production of alkoxyl radicals but not of the ascorbyl radical. Results for alkoxyl radicals (arbitrary units) were 0.02 (SE 0.01) pre-exercise *v.* 0.04 ±0.02 post-exercise (NS). Results for ascorbyl radical concentration (arbitrary units) were 0.04 (SE 0.01) pre-exercise *v.* 0.06 (SE 0.02) post-exercise (P=0.01). Changes in ascorbyl radical concentration have been suggested as a useful index of oxidative stress (Buettner & Jurkiewicz, 1993). The apparent increase in the concentration of the ascorbyl radical following ascorbic acid supplementation and exercise may indicate increased oxidative stress. However, we suggest that increases in both basal and post-exercise levels of the ascorbyl radical following supplementation reflect increased availability and hence increased one-electron oxidation of plasma ascorbate leading to the formation of the ascorbyl radical, and not necessarily increased oxidative stress. Thus, the usefulness of the ascorbyl free radical as a marker of *in vivo* oxidative stress in supplemented systems is limited.

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Regulation of non-esterified fatty acid release from human subcutaneous adipose tissue after an overnight fast. By SANDY M. HUMPHREYS, SIMON W. COPPACK, JAS S. SAMRA, LUCINDA K.M. SUMMERS, MO L. CLARK and KEITH N. FRAYN, *Oxford Lipid Metabolism Group, Radcliffe Infirmary, Oxford OX2 6HE*

Non-esterified fatty acid (NEFA) release from adipose tissue *in vivo* involves the intracellular action of hormone-sensitive lipase (HSL, *EC* 3.1.1.3) on stored triacylglycerol (TG) as well as the action of lipoprotein lipase (LPL, *EC* 3.1.1.34), bound to capillary endothelium, on the circulating TG-rich lipoprotein particles. The proportion of fatty acids re-esterified in the tissue may also regulate NEFA release. The relative contributions of these components vary with nutritional state (Frayn *et al.* 1995; Samra *et al.* 1996). Here we have analysed data from a large number of studies in lean people after an overnight fast using the arteriovenous (A-V) difference technique to measure net substrate uptake or release across abdominal subcutaneous adipose tissue. Seventy-three subjects were studied on between one and seven occasions each; mean values within each person were used for further analysis. A-V differences for glucose, glycerol and NEFA were measured in all subjects, and those for TG in sixty subjects. A-V differences for NEFA and TG were converted to whole-blood values. Results are expressed as mean (95 % CI) except for derived data which are given as medians. Some data on smaller numbers of subjects were published previously in abstract form (Samra *et al.* 1995). Studies were approved by the local ethics research committee and informed consent was obtained.

The mean veno-arterial (V-A) difference for glycerol was 137 (95 % CI 123, 151) μmol/l; one third of that for NEFA was 139 (95 % CI 125, 153) μmol/l. Thus, the percentage of fatty acids re-esterified, based on the ratio glycerol : NEFA release, was not significantly different from zero (median –1 %). The mean fractional extraction of TG was 7.4 (95% CI 6.1, 8.8) % and the A-V difference 41 (95 % CI 33, 49) μmol/l, potentially accounting for (median) 26 % of the NEFA release, similar to the value we have derived previously from smaller numbers of subjects (Samra *et al.* 1996). Across all subjects, the V-A differences for glycerol and NEFA and the A-V difference for TG were highly correlated (*r* 0.56 - 0.87, *P*<0.001 in each case; see Fig.). These correlations did not appear to arise spuriously from differences in adipose tissue blood flow, since there was a negative correlation between the V-A difference for NEFA release and the A-V difference for glucose uptake (*r* -0.33, *P*<0.01).

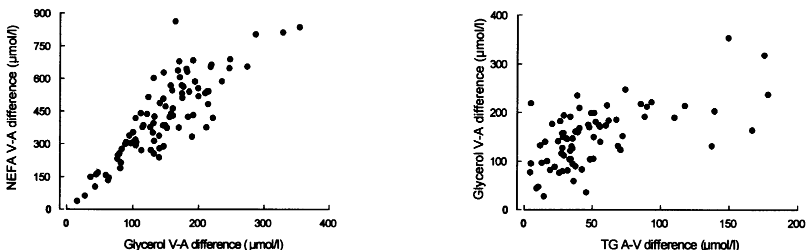

Fig. Interrelationships between arterio-venous (A-V) and veno-arterial (V-A) differences for NEFA, glycerol and TG.

These data suggest that both HSL and LPL contribute to NEFA release after an overnight fast. In contrast, re-esterification is negligible and hence does not regulate NEFA release in this situation.

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Arterialized plasma leptin concentrations and adipose tissue leptin production following oral and intravenous administration of a lipid emulsion. By KEVIN EVANS, MO L. CLARK and KEITH N. FRAYN, *Oxford Lipid Metabolism Group, Radcliffe Infirmary, Oxford OX2 6HE*

Leptin is secreted by adipocytes and plays a role in the regulation of food intake. However, the regulation of leptin production by adipose tissue is unclear. Leptin mRNA expression increases within a few hours of stress, insulin administration or eating. Increased adipose tissue leptin production has been reported following a high-carbohydrate meal (Coppack *et al.* 1998) and a mixed meal (Astrup *et al.* 1997). We have investigated whether a pure fat load given intravenously or a high fat load given orally stimulates adipose tissue leptin production.

Six healthy volunteers were studied twice following an overnight fast. On one occasion they consumed a tomato soup containing 40 g triacylglycerol (Intralipid; Pharmacia Ltd, Milton Keynes, Bucks., UK) and 9.6 g carbohydrate, on the other occasion Intralipid was infused intravenously over 4 h to give the same fat load. Paired blood samples were obtained from an arterialized hand vein and a vein draining subcutaneous adipose tissue at baseline, and for 6 h following the soup or start of the infusion, and veno-arterial differences calculated. Leptin concentrations were measured in these samples by radioimmunoassay (Biogenesis, Poole, Dorset, UK). The study was approved by the Central Oxford Research Ethics Committee and all subjects gave informed consent.

Adipose tissue blood flow did not change during the study; veno-arterial differences have therefore been used, rather than true fluxes (Evans *et al.* 1999). Following both intravenous and oral fat loads arterialized plasma leptin concentrations decreased over 6 h ($P<0.001$ for time effect) with no difference between oral and intravenous fat loads. Similarly, adipose venous plasma leptin concentrations also decreased during the study ($P<0.001$ for time effect), again with no difference between oral and intravenous fat loads.

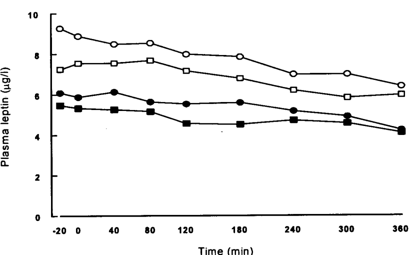

Fig. Arterialized (■●) and adipose venous (□○) plasma leptin concentrations following oral (■□) and intravenous (●○) fat loads.

A previous study by Coppack *et al.* (1998) showed a significant increase in arterial and adipose venous plasma leptin concentrations over 5 h after a high-carbohydrate meal, while leptin concentrations fell with continued fasting. The decreases in plasma leptin concentrations (by 31 % in arterial samples and 36 % in adipose venous samples) were similar to those observed in the present study (25 % in arterialized and 18 % in adipose venous concentrations following oral fat load and 31 % in both arterialized and adipose venous concentrations following intravenous fat infusion).

From our results we conclude that the increase in plasma leptin concentrations observed after meals is not simply a result of an energy load, but is in response to a signal that is not present following a fat load without carbohydrate. This is consistent with a previous report demonstrating carbohydrates having a higher satiety effect than fat (Lissner & Heitman, 1995).

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Measurement of glucose production during exercise in human subjects: comparison of the arteriovenous balance and isotopic tracer methods. By M. KJAER, R. BERGERON, L. SIMONSEN, J. BÜLOW and H. GALBO, *Sports Medicine Research Unit, Department of Clinical Physiology, Bispebjerg Hospital and Department of Physiology, Panum Institute, Copenhagen Muscle Research Centre, Denmark*

Two different methods are widely used to estimate hepatic glucose production, and studies in dogs have indicated that the arterio-hepatic venous balance technique and the tracer dilution method provide similar results during prolonged exercise (Wasserman et al. 1987). The present study which was approved by the ethical committee compared the arterio-hepatic balance technique (Rowell et al. 1964) and the tracer dilution method (Radziuk 1978) for estimation of hepatic glucose production during both moderate and heavy exercise in human subjects. Eight healthy, young males (25 (range 23-30) years) performed semi-supine cycling for 40 min at 50.4 (SE 1.5) $\%Vo_2max$ followed by 30 min at 69.0 (SE 2.2) $\%Vo_2max$. The splanchnic blood flow (SBF) was estimated by a primed (1000 µg), continuous (200 µg/min) infusion of indocyanine green , and net splanchnic glucose output was calculated as the product of SBF and arterio-hepatic venous blood glucose concentration differences. Glucose appearance rate (R_a) was determined by a primed (888 kBq), continuous (8.88 kBq/min) infusion of [3-^{3}H] glucose, and calculated using formulas for a modified single compartment in non-steady state, using individually calculated distribution space of our subjects (23.3 (SE 1.1) % body weight) asuming rapid changes to take place in 65 % of the distribution space.
SBF was 1.56 (SE 0.11) litres/min at rest, and decreased during exercise to 1.39 (SE 0.12) litres/min (50 % Vo_2max) and 0.87 (SE 0.19) litres/min (69 % Vo_2max). Arterio-hepatic venous glucose difference increased from 0.77 (SE 0.06) mmol/l at rest to 1.53 (SE 0.11) and 4.29 (SE 0.57) mmol/l during moderate and intense exercise respectively. The glucose specific activity was constant for the last 30 min preceding exercise, and decreased by 12 and 30 % during the two exercise loads respectively. Glucose production was similar whether determined by the arterio-hepatic venous balance technique or by the tracer dilution method, both at rest and during moderate (3.9 (SE 0.3) mg/kg/min (A-V) vs 3.7 (SE 0.6) (^{3}H)) and intense exercise (6.8 (SE 0.5) mg/kg/min (A-V) vs 6.0 (SE 0.4) ^{3}H)) ($P>0.05$). It is concluded that during exercise in human subjects, determination of glucose production can be performed equally well whether using a primed, constant infusion of radiolabelled ^{3}Hglucose together with the pool fraction model for non-steady state conditions to determine rate of glucose appearance, or the arterio-hepatic venous balance technique including blood flow determinations with infusion of indocyanine green to quantitate splanchnic glucose release.

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Splanchnic thermogenesis during a hyperinsulinaemic euglycaemic clamp. By LENE SIMONSEN and JENS BÜLOW, *Department of Clinical Physiology, Bispebjerg Hospital, University of Copenhagen, DK-2400 Copenhagen NV, Denmark*

Splanchnic thermogenesis in the resting and fasting subject accounts for 20-25 % of total energy expenditure. Ingestion of food increases the splanchnic blood flow and O_2 uptake to various degrees depending of the composition of the food. We have, in an earlier study, shown, that after an oral glucose load there is a late but substantial decrease in splanchnic O_2 uptake from the baseline values (Simonsen *et al.* 1995) and proposed that it could be due to inhibition of hepatic gluconeogenesis, an energy-requiring process.

In the present study we examined six healthy subjects (five males and one female) after an overnight fast. The study was approved by the Municipal Ethical Committee of Copenhagen. The mean age was 24.5 years and mean BMI 24.5 kg/m^2. Through the right femoral vein a catheter was advanced to a right-sided hepatic vein and another catheter was placed in an artery. Four determinations were made in the baseline period. Thereafter a primed/constant infusion of insulin was begun (4 mU/kg, 50 mU/m^2 per min). Arterial glucose concentrations were measured every 5 min during the insulin infusion, and an infusion of glucose in the femoral vein was adjusted accordingly, to keep the blood glucose concentration constant at the fasting control level. When a new steady state had been reached with respect to a constant glucose disposal rate, another four determinations were performed. Whole-body energy expenditure was measured by a ventilated-hood system. Splanchnic blood flow was measured by a primed/constant infusion of Indocyanine Green. The splanchnic metabolism and thermogenesis were calculated by arterio-venous differences of different metabolites and O_2 content multiplied by the blood flow. The results are the means of the four determinations in each period. The glucose disposal rate during the clamp was on average 7.1 (range 3.5-10.0) mg/kg per min. The results are shown in the Table.

	Fasting		Insulin clamp		*P* value
	Mean	SE	Mean	SE	(paired *t*-test)
Energy expenditure (kJ/min)	4.93	0.32	5.26	0.40	= 0.12
Respiratory quotient	0.81	0.02	0.90	0.02	< 0.02
Splanchnic plasma flow (ml/min)	853	38	954	43	< 0.02
Arterial insulin concentration (pmol/l)	32	5	590	30	< 0.01
Arterial glucose concentration (mmol/l)	5.26	0.15	5.03	0.10	= 0.14
Arterial FFA concentration (μmol/l)	656	38	25	12	< 0.01
Splanchnic oxygen uptake (mmol/min)	2.68	0.23	2.22	0.20	< 0.01
Splanchnic glucose output (mmol/min)	0.75	0.09	-0.32	0.11	< 0.01
Splanchnic FFA uptake (μmol/min)	190	20	10	6	< 0.01

FFA, free fatty acids.

The results show that despite an increase in splanchnic blood flow there was a significant decrease in splanchnic O_2 uptake during the hyperinsulinaemic euglycaemic clamp. The decrease in the splanchnic O_2 uptake can be fully explained by inhibition of the gluconeogenesis assuming that 50 % of the fasting splanchnic glucose output is generated by gluconeogenesis (Landau *et al.* 1996).

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Methodological modifications using the microdialysis technique in the peritendinous tissue during exercise. By H. LANGBERG, D. SKOVGAARD, J. BÜLOW and M. KJÆR, *Sports Medicine Research Unit, Copenhagen Muscle Research Centre and Department of Clinical Physiology Bispebjerg Hospital, Bispebjerg Bakke 23, 2400 Copenhagen, Denmark*

The microdialysis technique allows for *in vivo* determination of metabolic changes in the interstitial fluid both at rest and during physical exercise (Arner *et al.* 1990; Rosdahl *et al.* 1993). In an attempt to determine *in vivo* metabolism and inflammatory processes in relation to the Achilles tendon, microdialysis was performed in the peritendinous area immediately ventral to the Achilles tendon in human subjects (Langberg *et al.* 1999). However, it was found that only a fraction (approximately 10 %) of the perfusion fluid was recovered in the collected dialysate during muscular contraction of the triceps surae. An explanation for this could be a decrease in peritendinous tissue pressure during muscular contraction (Aratow *et al.* 1993), and to test this hypothesis the pressure in the peritendinous space ventrally to the human Achilles tendon was measured at rest and during graded workloads. Tissue pressure was determined just ventral to the Achilles tendon at 20, 40 and 50 mm proximal to the insertion of the tendon insertion using a Dialogue 2000 (Danica Biomedical), in eleven healthy, young individuals. The pressure was measured both at rest and during intermittent isometric calf muscle exercise at three different workloads (56, 112 and 168 Nm). The study was approved by The Ethical Commety of Copenhagen ((KF) 01-065/98). In all subjects a linear significant decrease in pressure was obtained with increasing torque ($P < 0.05$). Noo significant differences in pressure were obtained between the three areas measured ($P > 0.05$) indicating that the peritendinous space ventral to the Achilles tendon represents a uniform compartment.

	20 mm proximal to insertion		40 mm proximal to insertion		50 mm proximal to insertion	
	Mean (mmHg)	SEM (mmHg)	Mean (mmHg)	SEM (mmHg)	Mean (mmHg)	SEM (mmHg)
0 Nm	- 0.7	0.4	- 0.4	0.3	- 0.8	0.6
56 Nm	- 44.3 *	5.0	- 45.5	6.0 *	- 49.4	5.6 *
112 Nm	- 92.7 **	11.0	- 97.0	11.9 **	- 105.8	9.3 **
168 Nm	- 129.5 ***	11.0	- 134.7	11.9 ***	- 144.8	10.7 ***

* Significantly different from pressure at 0 Nm, ($P < 0.05$)
** Significantly different from pressure at 56 Nm, ($P < 0.05$)
*** Significantly different from pressure at 112 Nm, ($P < 0.05$)

Based on these observations, microdialysis was performed (CMA60; flow rate 1 μl/min), with a colloid osmotic active substance (Dextran-70, 0.1 g/ml) added to the perfusate aiming at counteracting the effect of the negative tissue pressure, during 30 min of intermittent static calf muscle exercise. Simultaneous microdialysis of the contracting muscle was carried out. The study was approved by The Ethical Commety of Copenhagen ((KF) 01-164/97).

	Recovered volume of dialysate (% of expected)			
	Connective tissue		Muscle	
	Mean	SEM	Mean	SEM
Rest	115	6	111	8
Exercise	100	4	118	7
Recovery	110	5	111	5

It is concluded that a marked uniform negative tissue pressure is generated in human peritendinous space around the Achilles tendon during contraction of the *m. triceps surea*. This requires modification of the microdialysis technique, when used in this area. However, with the addition of a colloid osmotic substance to the perfusate the dialysate volume could be fully recovered during contraction of the calf muscles.

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In vivo comparison of three microdialysis probe types used in the monitoring of interstitial glycerol, glucose and lactate. By ROBERT C. HICKNER, *Human Performance Laboratory, Department of Exercise and Sports Science, and Department of Physiology, East Carolina University, Greenville, NC, USA*

The monitoring of metabolite concentrations in the interstitium of subcutaneous adipose tissue has been conducted with several probe types commonly used during microdialysis procedures. Previous research has indicated that relative efficiency (recovery), and the time course of response to physiological perturbations, of microdialysis can be dependent on the construction material used for the dialysis fibre (Hsiao *et al.* 1990; Tao & Hjorth, 1992). These previous studies were conducted *in vitro* or *in vivo* in brain tissue. There have been no studies of probe recovery and response time in peripheral tissues, e.g. fat and muscle, tissues commonly studied for metabolic research. We have therefore monitored interstitial glycerol, glucose, and lactate in subcutaneous adipose tissue under basal conditions and following ingestion of 75 g glucose (in 250 ml water) to compare the magnitude and time course of responses of three microdialysis probe types. Microdialysis probes used were the CMA/60 (30 mm polyamide membrane, 20 000 Da cutoff; CMA/Microdialysis AB, Stockholm, Sweden), CMA/20 (10 mm polycarbonate membrane, 20 000 Da cutoff; CMA/Microdialysis AB), and BAS LM-3 (30 mm polyacrilonitrile membrane, 30 000 Da cutoff; Bioanalytical Systems, West Lafayette, IN, USA). Probes (one of each type) were inserted (about 30 mm lateral to the umbilicus, about 10 mm apart; order of probe placement randomized) without local anaesthesia into the abdominal subcutaneous adipose tissue of six healthy young females. Probes were perfused with a Ringer solution, containing 1.8 MM-glucose, 5.0 MM-ethanol and 35 g dextran-70/l, at 0.3, 0.5, and 2.0 µl/min under basal conditions and at 2.0 µl/min following glucose ingestion. Following a 60 min equilibration period, basal samples (two per flow rate) were collected. The glucose beverage was then ingested, and six 10 min dialysate fractions followed by two 15 min fractions were collected in succession. Dialysate concentrations at the lowest perfusion flow rate (0.3 µl/min) for the BAS probes were 322.0(SE47.8) µM, 4.8(SE0.9) MM and 1.9(SE0.5) MM, for glycerol, glucose, and lactate, respectively. Corresponding values for the CMA/60 probes were 234.4(SE33.7) µM, 3.4(SE0.4) MM ($P<0.05$ *v.* BAS) and 3.3(SE0.7) MM. Corresponding values for the CMA/20 probes were 243.4(SE56.7) µM, 3.1(SE0.3) MM ($P<0.05$ *v.* BAS) and 1.5(SE0.4) MM. The higher dialysate glucose concentrations in the BAS than CMA probes were maintained at 0.5 and 2.0 µl/min. Temporal responses to the glucose challenge were not different between probe types. In response to glucose ingestion, the maximum decreases in dialysate glycerol concentrations (to about 65 % of basal) were similar in all probes. Dialysate glucose concentrations increased maximally to about 180 % of basal in the CMA/60 and BAS probes, but increased to only 150 % of basal ($P<0.05$ *v.* BAS) in the CMA/20 probes. Dialysate lactate concentration increased maximally to about 180 % of basal in the BAS and CMA/20 probes, but increased to only 110 % of basal ($P<0.05$ *v.* BAS) in the CMA/60 probes. There were no significant changes from basal in ethanol outflow:inflow ratio following glucose ingestion. In light of the differences noted, the composition of the dialysis fibre must be considered when interpreting microdialysis results.

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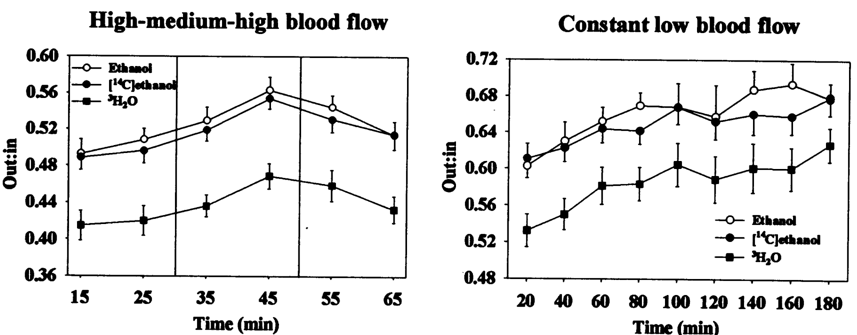
Estimation of rat muscle blood flow by microdialysis probes perfused with ethanol, [^{14}C]ethanol and $^{3}H_2O$. By B. STALLKNECHT, M. DONSMARK, L.H. ENEVOLDSEN, J.D. FLUCKEY AND H. GALBO, *Department of Medical Physiology, The Panum Institute, University of Copenhagen, and Copenhagen Muscle Research Centre, National University Hospital, Denmark*

We used the perfused rat hindquarter to evaluate if the microdialysis ethanol technique can be used to estimate qualitatively nutritive skeletal muscle blood flow. Four microdialysis probes were inserted in different hindlimb muscles in each of sixteen rats. Hindquarters were perfused at blood-flow rates ranging from 0 to 21 ml/100 g per min. The microdialysis probes were perfused at 2 µl/min with perfusate containing ethanol, [^{14}C]ethanol and $^{3}H_2O$.

Within and between experiments outflow:inflow ratios (o:i) generally varied inversely with blood flow. When a low flow or no flow was maintained in hindquarters, o:i first increased with time (for at least 60 min) and then levelled off. The long time constant impaired detection of rapid oscillations in blood flow, especially at low blood-flow rates. Contractions *per se* apparently decreased o:i independent of blood flow. Ethanol and [^{14}C]ethanol o:i did not differ. $^{3}H_2O$ o:i paralleled ethanol and [^{14}C]ethanol o:i, but was significantly lower.



In conclusion, differences in skeletal muscle blood flow can be detected by the microdialysis technique. However, the slow changes in o:i in particular at low blood-flow rates limit the usefulness of the technique for measuring dynamic changes in blood flow and caution must also be exerted during muscle contractions. $^{3}H_2O$ and [^{14}C]ethanol are good alternatives to ethanol in the determination of blood flow by microdialysis.

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Human muscle interstitial K$^+$ measured by microdialysis during rest and exercise. By SIMON GREEN and JENS BÜLOW, *Department of Clinical Physiology and Copenhagen Muscle Research Centre, Bispebjerg and Rigshospitalet University Hospitals, Copenhagen, Denmark*

It has been suggested that K$^+$ may play a role in muscle fatigue and pain during short-term exercise in addition to playing a role in the vascular tone (Saltin *et al.* 1981). K$^+$ concentrations in the range of 4-5 mmol/l at rest and about 9.5 mmol/l during exercise have been reported (Vyskocil *et al.* 1983) measured by ion-selective microelectrodes. However, in other studies this technique failed to produce accurate measurements of K$^+$ both during rest and exercise.

The aim of the present study was to develop the microdialysis technique enabling continuous measurements of interstitial K$^+$ in human muscle during rest and exercise. The microdialysis fibres were manufactured in the laboratory from commercially available fibres either Gambro GFS 16 or GFE 18 (Gambro, Lund, Sweden). Each probe consisted of a 40 mm dialysis fibre glued to afferent and efferent nylon tubes (id 0.5 mm od 0.6 mm; Portex, Hythe, Kent, UK). The probes were reinforced by a suture thread of 0.15 mm od (Ethicon; Johnson & Johnson, Sollentuna, Sweden) in order to improve the probe stability *in vivo*.

In order to have a reasonable time resolution, perfusion rates of 2-5 µl/min were applied. However, at these perfusion rates the relative recovery for K$^+$ *in vivo* is not 100 %. For estimation of the relative recovery of K$^+$, ^{201}Tl pertecnetate was applied as internal reference. ^{201}Tl was chosen because Tl$^+$ and K$^+$ have similar kinetics *in vivo* (Gehring & Hammond, 1967), and Tl$^+$ and K$^+$ have similar hydrated ion radii of 1.43 Å and 1.33 Å respectively.

Twelve probes were tested *in vitro* and four experimental subjects were examined *in vivo*. Each subject had two probes inserted into the gastrocnemius muscle of both legs. The study was approved by the medical ethical research committee of Copenhagen.

In vitro: when the probes were perfused at 10 µl/min a significant fluid loss of about 30 % was experienced, due to ultrafiltration. At lower perfusate fluid rates a linear relationship was observed between the K$^+$ uptake and the ^{201}Tl loss with the regression equation $y = 0.027 + 0.961x$ with both slope and y-intercept not being different from 1 and 0 respectively. The ^{201}Tl losses and K$^+$ recoveries ranged between 0.6 and 0.98. The level of agreement was, on average, not different from zero and was not systematically influenced by either perfusate or probe length.

In vivo: the average ^{201}Tl loss at a perfusate rate of 5µl/min was 0.42-0.45 and it remained stable during a 70 min rest period. Compared with this, exercise increased the loss significantly with increasing workload to 0.53 (SD 0.10) to 0.63 (SD 0.08) during the highest work load applied. Also, *in vivo* the recovery of K$^+$ was dependent on perfusate flow, and when 8 µl/min was applied drainage of interstitial K$^+$ was experienced. The resting K$^+$ concentration was measured to be in the range 3.9-4.3 mmol/l. During exercise the K$^+$ concentration increased to 6.9 (SD 0.4) mmol/l during the lowest force applied increasing further to 7.4 (SD 0.4) mmol/l during the highest forces.

In conclusion the findings of the present study demonstrate that K$^+$ can be accurately measured during rest and exercise using microdialysis and ^{201}Tl as an internal reference.

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Vyskocil F , Hnik F P , Rehfeldt H , Vejsada R & Ujec E (1983) *Pflugers Archive* **399,** 235-237.

The effect of endurance training on subcutaneous adipose tissue lipolysis in elderly women. By KAI H. W. LANGE[1], JEANNE LORENTSEN[1], JENS BÜLOW[2] and MICHAEL KJÆR[1], [1]*Sports Medicine Research Unit and Copenhagen Muscle Research Center, Bispebjerg Hospital, Denmark,* [2]*Department of Clinical Physiology, Bispebjerg Hospital, Denmark.*

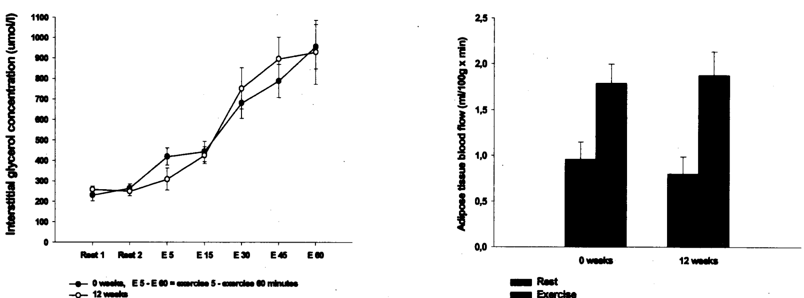
Endurance training results in increased fatty acid oxidation during exercise at a given absolute intensity, and it has been proposed, that lipolysis both at rest and during exercise should be enhanced as a result of endurance training. However, most studies addressing these issues have either been cross-sectional or have measured lipolysis on a whole-body level, rather than in local tissues. Also, since ageing is associated with both increased fat mass and inactivity, the aim of the present study was to investigate the effect of 12 weeks of endurance training in elderly individuals on lipolysis in local adipose tissue, both at rest and during exercise.

After ethical approval, seven healthy women (age 75 (SE 2) years; height 1.59 (SE 0.03) m; weight 59.5 (SE 2.5) kg; bodyfat 37.8 (SE 2.5) %) underwent a 12-week endurance training programme. At 0 and at 12 weeks abdominal subcutaneous adipose tissue lipolysis was measured by microdialysis at rest and during a subsequent 60 min exercise bout at 60 % of pretraining VO_2max. The microdialysis probes were calibrated using the internal reference technique and regional blood flow was measured simultaneously using the ^{133}Xe-washout technique. Also, body composition was determined by dual-energy x-ray absorptiometry.

Weeks	Weight (kg)		Bodyfat (%)		VO_2max (l/min)		HR-exercise (beats/min)		RER-exercise (beats/min)	
	Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE
0	59.5	2.5	37.8	2.5	1.32	0.09	123	5	0.81	0.02
12	58.7	2.3	36.2*	2.4	1.58*	0.09	113*	5	0.76*	0.02

Mean values were signigicantly different from those at 0 weeks: *$P < 0.05$

After 12 weeks of training, VO_2max was significantly increased, whereas HR and respiratory exchange ratio (RER) were significantly decreased during exercise, the latter indicating increased fatty acid oxidation.



Interstitial glycerol concentration in the subcutaneous abdominal fat tissue increased significantly from rest to exercise, but did not change in response to endurance training. Together with the findings of adipose tissue blood flow, the present data indicate that lipolysis in the subcutaneous adipose tissue increases with exercise in elderly people. However, endurance training does not enhance this response neither at rest, nor during exercise.

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