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SHORT COMMUNICATIONS

Metformin does not increase energy expenditure of brown fat

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Abstract—The antihyperglycaemic agent metformin (dimethylbiguanide) increases metabolic energy expenditure. The possibility that this might involve increased fuel utilization by brown adipose tissue (BAT) was studied. Basal and noradrenaline-stimulated (10^{-6} mol/L) oxygen consumption by isolated brown adipocytes from normal and streptozotocin diabetic mice was unaffected by incubation with metformin (10^{-4} mol/L) for 30 min. Basal and insulin-stimulated (10^{-8} and 10^{-6} mol/L) aerobic and anaerobic glucose metabolism was also unaffected during 2 hr incubations of BAT with metformin (10^{-4} mol/L). Treatment of normal mice with metformin (250 mg/kg/day) for 7 days did not significantly alter the amount or cytochrome oxidase activity of BAT, and a preliminary semi-quantitative estimation of mitochondrial uncoupling protein showed no apparent change. The results suggest that metformin does not increase energy expenditure of brown adipose tissue.

Metformin (dimethylbiguanide*) is a blood glucose-lowering agent used in the treatment of non-insulin dependent diabetes mellitus [1]. Its therapeutic effect is achieved without raising insulin concentrations, and it appears to reduce insulin resistance. Unlike the other major blood glucose-lowering agents, namely sulphonylureas and insulin, metformin does not cause weight gain [2]. Indeed, metformin may produce a small decrease in body weight during the initial months of treatment [3]. This cannot be attributed to a chronic decrease in food consumption or increased physical activity [4, 5], suggesting that the drug increases metabolic energy expenditure.

Brown adipose tissue (BAT) makes only a very small contribution to basal metabolic rate in adult humans, but stimulation of energy expenditure by this tissue may be sufficient to affect body weight [6]. This study investigates whether metformin affects energy expenditure by BAT of normal and streptozotocin (STZ) diabetic mice.

Materials and Methods

Chemicals and animals. STZ, crystalline bovine insulin, collagenase (type II), radioimmunoassay grade bovine serum albumin (fraction V), noradrenaline bitartrate, ferrocytochrome C, and HEPES were from the Sigma Chemical Co. (Poole, U.K.). D-[U-^{14}C]Glucose (270 mCi/mmol) was from Amersham International (Amersham, U.K.), lactate assay reagents were from BCL (Lewes, U.K.), glucose assay reagents were from Beckman Instruments Inc. (Galway, Ireland) and metformin hydrochloride (batch 2452) was from Lipha Pharmaceuticals (West Drayton, UK). Other reagents were from BDH, (Poole, U.K.). Adult male MF1 mice weighing 20–30 g were housed and maintained as previously [7]. Diabetes was induced by intraperitoneal injection of STZ (120 mg/kg in citrate buffer pH 4.5) after an overnight fast. Mice with a modest degree of hyperglycaemia (non-fasted plasma glucose concentration of 15–30 mmol/L) were accepted as diabetic. Glucose was measured by an automated glucose oxidase procedure [8].

Cellular respiration. Oxygen consumption was measured using a suspension of collagenase-isolated interscapular brown adipocytes [9]. The cells were prepared and maintained in Krebs–Ringer bicarbonate buffer containing 10 mmol/L glucose and 30 mg/mL fatty acid-free bovine serum albumin, saturated with 95% O_2:5% CO_2, pH 7.4, and stirred gently. Cells were preincubated for 30 min without or with 10^{-4} mol/L metformin. Basal oxygen consumption and the maximum response to noradrenaline (10^{-6} mol/L) were then measured within 10 min at 37° in an identical medium using a Clarke-type oxygen electrode (Chandos, Stockport, U.K.) in a chamber (capacity 1.6 mL) containing approximately 3×10^6 cells/mL.

Glucose metabolism. Glucose oxidation was measured by $^{14}CO_2$ production from [U-^{14}C]glucose and anaerobic glucose metabolism was assessed by lactate production as previously [7]. Slices (approximately 20 mg) of interscapular BAT were incubated for 2 hr at 37° in pregassed (95% O_2:5% CO_2) Krebs–Ringer bicarbonate buffer containing 10 mmol/L glucose, 1 μCi/mL [U-^{14}C]glucose, 20 mg/mL fatty acid-free bovine serum albumin either without or with metformin (10^{-4} mol/L) and/or insulin (10^{-8} and 10^{-6} mol/L). $^{14}CO_2$ was trapped on filters impregnated with NaOH at the end of the incubation. Lactate accumulated in the medium was measured by a lactate dehydrogenase method [10].

Cytochrome oxidase and uncoupling protein. Normal mice were treated for 7 days with metformin (250 mg/kg/day) or placebo in the drinking water. Body weight, food and fluid intake were monitored [5]. After 7 days mice were killed by cervical dislocation, and the interscapular BAT was excised, weighed and homogenized (10 mg/mL) in 0.25 mol/L sucrose buffered with 1 mmol/L HEPES. Cytochrome oxidase activity was determined spectrophotometrically [11]. One hundred microlitres 0.1 mmol/L potassium phosphate buffer, pH 7.0, 830 μL water and 70 μL 1% (w/v) ferrocytochrome C were mixed in a cuvette at 25°. Ten microlitres of diluted homogenate (2 mg/mL) was added, and the decrease in absorbance at 550 nm was measured at 10 sec intervals for 2 min. The initial rate of reaction was determined from a tangent extrapolated back to zero time. Uncoupling protein was assessed semi-quantitatively by immunoassay using mitochondria prepared from the homogenate of BAT. The procedure, which has been described elsewhere [12], was kindly performed by Professor P. Trayhurn, Rowett Research Institute, Aberdeen.

Metformin dosage and concentration. The concentration of metformin used for *in vitro* studies, namely 10^{-4} mol/L is slightly above the normal maximum plasma concentration (approx. 3×10^{-5} mol/L) achieved clinically after oral consumption of a therapeutic dosage of the drug. A similar plasma concentration of metformin is achieved in mice after administration of the drug in the drinking water at the concentration used in the present study, namely 250 mg/kg/day [13].

* Abbreviations: metformin, dimethylbiguanide; BAT, brown adipose tissue; STZ, streptozotocin; HEPES, hydroxyethylpiperazine-ethanesulphonic acid.

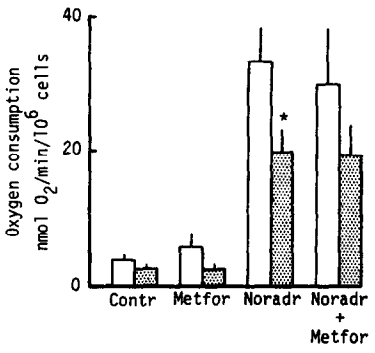

Fig. 1. Effect of metformin on oxygen consumption by brown adipocytes of normal (open bars) and STZ diabetic (hatched bars) mice. Brown adipocytes were preincubated for 30 min without or with metformin (10^{-4} mol/L) and the maximum rate of oxygen consumption was measured during the initial 10 min of subsequent test incubations without or with metformin (10^{-4} mol/L), and/or noradrenaline (10^{-6} mol/L). Values are means ± SE, N = 6. * $P < 0.05$ versus similarly treated brown adipocytes of normal mice. Noradrenaline significantly ($P < 0.05$) stimulated oxygen consumption above basal with brown adipocytes of normal and STZ diabetic mice.

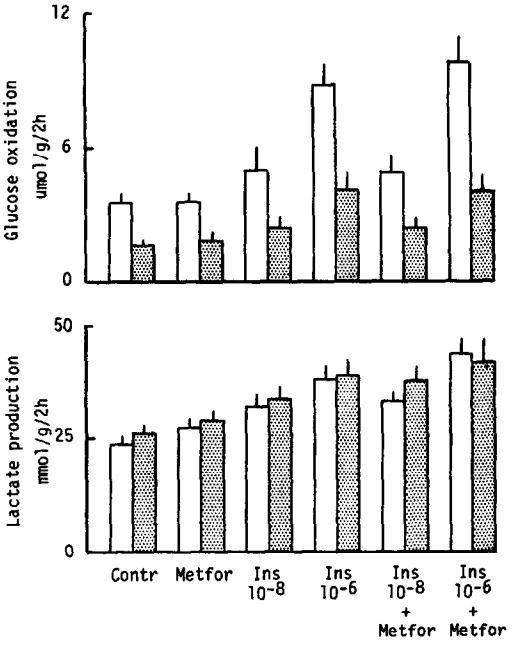

Fig. 2. Effect of metformin on glucose oxidation and lactate production by BAT of normal (open bars) and STZ diabetic (hatched bars) mice. Tissue was incubated for 2 hr with 10 mmol/L glucose, without or with metformin (10^{-4} mol/L) and/or insulin (10^{-8} and 10^{-6} mol/L). Values are means ± SE, N = 6–8. Glucose oxidation was consistently significantly ($P < 0.05$) lower in tissue from STZ diabetic mice than normal mice. 10^{-6} mol/L insulin significantly ($P < 0.05$) stimulated glucose oxidation.

Statistical analysis. Groups of data have been expressed as means ± SE, and compared using Student's *t*-test. Differences were considered to be significant if $P < 0.05$.

Results and Discussion

Cellular respiration. Isolated brown adipocytes of normal mice showed a typically prompt and marked (8-fold) increase in the rate of oxygen consumption when exposed to 10^{-6} mol/L noradrenaline (Fig. 1). Noradrenaline-stimulated oxygen consumption was lower with brown adipocytes of STZ diabetic mice. Preincubation for 30 min with 10^{-4} mol/L metformin did not significantly alter basal or noradrenaline-stimulated oxygen consumption by brown adipocytes of normal or STZ diabetic mice.

Glucose oxidation. Basal and insulin-stimulated glucose oxidation were lower during 2 hr incubations with isolated BAT of STZ diabetic mice than normal mice (Fig. 2). The presence of 10^{-4} mol/L metformin did not significantly alter basal or insulin-stimulated glucose oxidation by BAT of either normal or STZ diabetic mice. Lactate production was slightly raised by insulin, but was not significantly affected by metformin.

Cytochrome oxidase and uncoupling protein. After administration of metformin (250 mg/kg/day) to normal mice for 7 days there was no significant effect on body weight, food and fluid intake, or basal glycaemia as noted previously [5] (data not shown). The weight of interscapular brown fat was not significantly altered, although the mean value was higher in the metformin-treated group (121 ± 8 versus 108 ± 12 mg/mouse, mean ± SE, N = 6). Cytochrome oxidase activity, which provides an indication of mitochondrial mass, was similar in the metformin-treated and placebo groups (23.5 ± 1.2 and 24.4 ± 2.7 nmol/min/mg tissue, respectively; mean ± SE, N = 6). Semi-quantitative measurements of mitochondrial uncoupling protein were also similar in the two groups of mice.

Discussion. The hypothesis that metformin might increase energy expenditure by increasing the metabolism of BAT was not substantiated by the present study. Fatty acids are the major substrate for oxidation by BAT [14], and failure of metformin to increase either basal or noradrenaline-stimulated oxygen consumption by this tissue is consistent with clinical evidence that metformin does not affect plasma concentrations of glycerol or non-esterified fatty acids [1, 4]. The lack of effect of metformin on glucose oxidation by BAT may be contrasted with evidence that the same concentration of metformin can produce a small increase in glucose oxidation by white adipose tissue [7]. However, the control of glucose uptake and cellular respiration by BAT differs from white adipose tissue in that these processes are also stimulated by noradrenaline in BAT via a mechanism dissociated from insulin [15]. A tendency for metformin treatment to increase the amount of interscapular BAT corresponds with evidence that metformin does not enhance lipolysis in white adipose tissue [1, 4].

Using indirect calorimetry to assess energy expenditure, a study of three insulin-dependent diabetic patients revealed an increase in noradrenaline-stimulated oxygen consumption when insulin therapy was supplemented with metformin [16]. In adult humans, increased BAT metabolism has been variously estimated to account for as much as 14% [6] or as little as 0.2% [17] of the increase in oxygen consumption in response to adrenergic stimulation. Adrenergic stimulation is known to increase energy utilization through an increase and redistribution of cardiac output [18], and skeletal muscle is quantitatively the most important site of energy utilization during such stimulation [6]. Since metformin increases glucose utilization by skeletal muscle in diabetic states [13], muscle is a more likely site at which metformin increases metabolic energy expenditure. The intestine is another site of increased glucose utilization by metformin [7, 19], and increased glucose cycling offers a further potential mechanism through which the drug could be increasing energy expenditure [20].

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