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Original Article

The effects of fasting and refeeding with a 'metabolic preconditioning' drink on substrate reserves and mononuclear cell mitochondrial function☆

Sherif Awad [a], Mary C. Stephenson [b], Elisa Placidi [b], Luca Marciani [c], Dumitru Constantin-Teodosiu [d], Penny A. Gowland [b], Robin C. Spiller [c], Kenneth C.H. Fearon [e], Peter G. Morris [b], Ian A. Macdonald [d], Dileep N. Lobo [a,*]

[a] Division of Gastrointestinal Surgery, Nottingham Digestive Diseases Centre NIHR Biomedical Research Unit, Nottingham University Hospitals, Queen's Medical Centre, Nottingham NG7 2UH, UK
[b] Sir Peter Mansfield Magnetic Resonance Centre, School of Physics and Astronomy, University of Nottingham, Nottingham, UK
[c] Division of Gastroenterology, Nottingham Digestive Diseases Centre NIHR Biomedical Research Unit, Nottingham University Hospitals, Queen's Medical Centre, Nottingham, UK
[d] Metabolic Physiology Group, School of Biomedical Sciences, University of Nottingham, Nottingham, UK
[e] Clinical and Surgical Sciences, University of Edinburgh, Edinburgh, UK

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SUMMARY

Background and aims: Preoperative fasting induces metabolic stress and leads to reduced postoperative insulin sensitivity, changes attenuated by preoperative carbohydrate loading. However, the mechanisms underlying these effects remain unknown. We investigated the dynamic changes in substrate metabolism and mononuclear cell mitochondrial function after fasting followed by refeeding with a drink [ONS (Fresenius Kabi, Germany)] designed to improve metabolic function preoperatively.

Methods: Twelve healthy volunteers took part in this study. They were fed a standardized meal and studied 4 h later (baseline 'fed' state), after 12 and 24 h of fasting, and 2, 4 and 6 h after ingestion of ONS (contained 100 g carbohydrate, 30 g glutamine, and antioxidants). Changes in liver and muscle glycogen and lipids were studied using ^{13}C and ^{1}H magnetic resonance spectroscopy. The activities of mitochondrial electron transport chain complexes I, II and IV in blood mononuclear cells were measured spectrophotometrically.

Results: Compared to the baseline fed state, 12 and 24 h fasts led to 29% and 57% decreases ($P < 0.001$) in liver glycogen content, respectively. Fasting for 24 h decreased mitochondrial membrane complexes I (-72%, $P < 0.05$), II (-49%, $P < 0.01$) and IV (-41%, $P < 0.05$) activities compared to those following a 12 h fast. A 23% increase ($P < 0.05$) in calf intramyocellular lipid (IMCL) content occurred after a 24 h fast. Liver glycogen reserves increased by 47% ($P < 0.05$) by 2 h following ingestion of ONS.

Conclusions: Short-term fasting (up to 24 h) affected mononuclear cell mitochondrial function adversely and increased IMCL content. Refeeding with ONS partially reversed the changes in liver glycogen.

1. Introduction

Preoperative fasting induces metabolic stress and leads to reduced postoperative insulin sensitivity.[1] This is of clinical importance given that the degree of postoperative insulin resistance correlates with the length of hospital stay,[2] and may lead to increased infectious complications due to associated hyperglycaemia.[3] Preoperative carbohydrate loading attenuates the decline in insulin sensitivity and leads to clinical benefits.[4] However, the mechanisms underlying the aforementioned effects are yet to be elucidated. Understanding these mechanisms may allow the optimization of interventions designed to minimize the development of postoperative insulin resistance.[5]

The postoperative reduction in insulin sensitivity is similar to that seen in other conditions associated with insulin resistance such as type 2 diabetes and ageing.[1,6] Studies on patients with these conditions suggested a link between the development of insulin resistance and impaired mitochondrial function in skeletal muscle.[1,7,8]

Abbreviations: CRP, C-reactive protein; EMCL, extramyocellular lipid; IMCL, intramyocellular lipid; MMC, mitochondrial membrane complex; MNC, mononuclear cell; MRS, magnetic resonance spectroscopy; NEFA, non-esterified fatty acids; OHB, β-hydroxybutyrate.

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* Corresponding author. Tel.: +44 115 8231149; fax: +44 115 8231160.
E-mail address: dileep.lobo@nottingham.ac.uk (D.N. Lobo).

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Compared to younger controls, elderly insulin-resistant subjects had a 40% reduction in the basal rates of muscle mitochondrial oxidative and phosphorylation activity.[8] Similar findings were noted in young lean insulin-resistant offspring of parents with type 2 diabetes, where severe defects in insulin stimulated muscle glucose metabolism were associated with an 80% increase in intramyocellular lipid content and a 30% reduction in rates of mitochondrial ATP production.[9] Further studies on semi-starved animals and malnourished patients have suggested that reduced energy intake may affect mitochondrial function adversely.[10–12] However, the effects of short-term fasting (up to 24 h) on mitochondrial function have never been studied in healthy humans. Finally, magnetic resonance spectroscopy (MRS) allows the *in vivo* assessment of glycogen and lipid levels using naturally abundant ^{13}C and ^{1}H nuclei, thereby enabling studies of energy metabolism.[13,14]

The aims of this study were two-fold: to study the dynamic changes in substrate metabolism and reserves following short-term fasting and refeeding with a drink designed to be given to patients preoperatively, and to determine whether mononuclear cell (MNC) mitochondrial activity is altered by this period of fasting/refeeding. Subjects were refed with a carbohydrate-based drink designed to improve perioperative metabolic function. The drink (Oral Nutritional Supplement [ONS, Fresenius Kabi, Bad Homburg, Germany]) also contained glutamine and antioxidants, the rationale behind this being that these ingredients may provide benefits in addition to those provided by carbohydrate loading alone.[15–20] We hypothesized that fasting would deplete liver glycogen reserves and impair MNC mitochondrial function, changes which would be reversed by ingestion of ONS.

2. Subjects and methods

2.1. Study design and ethics

This prospective observational healthy volunteer study was approved by the University of Nottingham Medical School Research Ethics Committee. Informed written consent was obtained prior to enrolment and the study was carried out according to the principles of the Declaration of Helsinki of the World Medical Association.

2.2. Subjects

Twelve male Caucasians (aged 18–35), suitable for magnetic resonance scanning, with a normal body mass index (20–25 kg/m^2) and no history of abdominal surgery or gastrointestinal disorders were studied. Subjects were excluded if they smoked or had a personal/family history of diabetes.

2.3. Interventions

After recruitment volunteers completed a 3-day food diary which was used to standardize the meal given at the commencement of the study. Volunteers abstained from alcohol and caffeine for 24 h, and from strenuous exercise for 3 days prior to the study. Throughout the study the volunteers were asked to rest, have water *ad libitum*, and were transported by taxi to eliminate the possible confounding effect of exercise.

2.4. Standardized meal and the study drink

The aim of the standardized mixed-meal was to load liver and muscle glycogen reserves thus representing the baseline 'fed' state. Microdiet® (Downlee Systems Ltd, Highpeak, UK) software was used to alter the quantities of the meal constituents to provide 40% of the volunteers' daily energy intake with a macronutrient energy contribution of 50% from carbohydrate, 35% from fat and 15% from protein. Volunteers consumed the meal within 15 min and time 0 was defined as the end of the meal. The study drink (ONS) was designed to improve insulin sensitivity, immune function and antioxidant defenses and contained 100 g carbohydrate, 30 g glutamine, 1.5 g vitamin C, 500 mg vitamin E, 2 g green tea extract, 10 mg β carotene, 20 mg zinc and 300 μg selenium. The drink was reconstituted from powdered form into a suspension (400 ml) using bottled water.

2.5. Study protocol

Volunteers reported for the study at 0745 hours after a 12 h fast. Height, weight and body mass index were recorded and the fast commenced after ingestion of the standard meal (Fig. 1). After 4 h blood was sampled by venepuncture and MRS scans were performed to study the subjects in the baseline 'fed' state. The 4 h time point was chosen given that the mean (95% CI) T_{100} gastric emptying time of ONS was 156 (138–174) min[21] and that previous studies have demonstrated maximal postprandial increases in liver glycogen within 4–6 h of ingestion of a mixed-meal.[22,23] Blood sampling and MRS scanning were repeated after 12 h of fasting following which the volunteers were allowed home with instructions to remain fasted. Blood sampling and MRS scanning were repeated after 24 h of fasting had elapsed. Volunteers then ingested ONS (within 5 min) following which blood samples were obtained and MRS scans performed at 2 hourly intervals until 8 h had elapsed. The aforementioned measurements were selected to allow a full a study of the *in vivo* metabolic changes following fasting and refeeding.

2.6. Magnetic resonance techniques

MRS was performed on a Philips 3T system. A transmit/receive body coil was used for ^{1}H imaging and MRS for measurements of liver volume and hepatic and skeletal muscle (soleus) lipid concentrations, respectively. A 14 cm ^{13}C surface probe with quadrature proton decouple coils was used for measurement of hepatic and calf (gastrocnemius) muscle glycogen. Liver volumes were measured using T1-weighted, breath-hold turbo field echo scan with resolution $2 \times 2 \times 7$ mm^3, 36 slices, matrix 180×182, TR = 3.11 ms, total scan time 14.4 s. The images were analyzed by drawing regions of interest in Analyze6 (Biomedical Imaging Resources, Rochester, MN) and values are reported as volume (litres) and % change from baseline. The coefficient of variation (CV) for repeated measurement of liver volume was 0.8%. Hepatic ^{1}H spectra were acquired from a $30 \times 30 \times 30$ mm voxel positioned in the right lobe using a respiratory triggered PRESS sequence and the

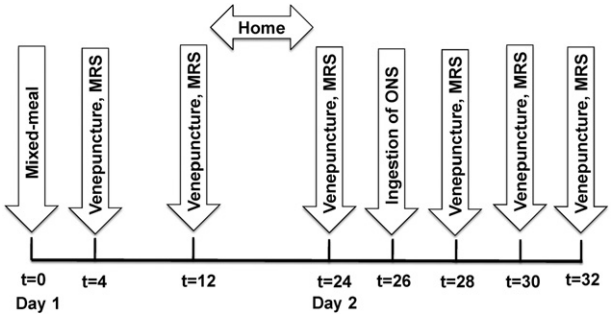

Fig. 1. Experimental protocol. The baseline 'fed' state was studied 4 h after ingestion of a standardized mixed-meal. At 4, 12, and 24 h venepuncture was performed and magnetic resonance spectroscopy (MRS) performed. Subjects ingested the study drink (Oral Nutritional Supplement [ONS, Fresenius Kabi, Bad Homburg Germany]) at 26 h following which venepuncture and MRS were repeated at 2 hourly intervals for 6 h.