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Exercise enhancement of hepatic insulin-sensitising substance-mediated glucose uptake in diet-induced prediabetic rats

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Abstract

The sensitisation of insulin action in response to a meal (i.e. meal-induced insulin sensitisation, MIS) represents one of the major means of increased glucose disposal in peripheral tissues during the postprandial state. MIS occurs when the release of hepatic insulin-sensitising substance (HISS) stimulates skeletal muscle glucose uptake. Our previous study had demonstrated that the HISS pathway is impaired in age-associated insulin resistance, and in the rats which were part of that study, voluntary exercise improved the response to insulin by restoring HISS action. The present study tests the hypothesis that voluntary exercise would reverse insulin resistance in diet-induced models of insulin resistance, and that the benefits are attributed through the improvement in HISS action. In this study, two experimental diets, a high-fat diet (for 4 weeks) and 35 % sucrose solution (for 9 and 16 weeks), were used to induce insulin resistance in rats. These rats were assigned to the exercise/no-exercise intervention. The effect of 7 d voluntary running-wheel exercise was determined by measuring insulin- and HISS action in the exercised rats and comparing them with the non-exercised controls. Voluntary exercise reversed insulin resistance, caused by dietary manipulation, through restoration of the HISS action. The direct insulin action was not changed by either diet or exercise. The metabolic improvements and reduced adiposity correlated with the extent of reversal of HISS action induced by exercise. Exercise improves insulin sensitivity in diet-induced insulin resistance primarily by restoration of HISS-mediated glucose uptake.

Key words: Insulin resistance: Exercise: Diets: Hepatic insulin-sensitising substance

Exercise increases insulin sensitivity, and the adaptive changes induced by exercise confer long-lasting metabolic benefits[1]. The metabolic dysregulation associated with physical inactivity can initiate and accelerate the pathogenesis of insulin resistance; conversely, regular physical activity can retard the pathological progression and may even reverse the process[2]. The improvement in glycaemic control by exercise, in type 2 diabetes[3,4] and in ageing[5], is achieved through sustained metabolic adaptations. We demonstrated that voluntary running-wheel exercise for 7 d causes an enhancement in the whole-body glucose uptake response to insulin in healthy rats, primarily as a result of augmentation of the hepatic insulin-sensitising substance (HISS) action[5].

Postprandial glucoregulation involves the activation of a neurohormonal mechanism in the liver that allows insulin to release the putative hormone, HISS[6]. HISS is released only during the postprandial state, and results in augmented glucose uptake response to insulin[6–8]. This phenomenon of dramatic enhancement of insulin response by a meal, through the release of HISS, is termed meal-induced insulin sensitisation. HISS action accounts for approximately 50 % of post-meal glucose disposal in rats[9] and two-thirds of the same in humans[10]. In this phenomenon, two feeding signals are activated by the presence of food in the upper gastrointestinal tract, causing insulin to release HISS from the liver. Of these, one signal is delivered to the liver via the hepatic parasympathetic nerves acting on muscarinic receptors and subsequent activation of NO synthase[6,9]. The second is a chemical signal that is mediated through elevation of the hepatic glutathione (GSH) level by approximately 40 %[11,12]. Blocking either or both signals by pharmacological[11,13], pathological[14–17] or experimental means[9,13] leads to the blockade of HISS release.

If HISS release is blocked, meal-induced insulin sensitisation does not occur, and the postprandial glucose disposal and storage is solely determined by the direct action of insulin. This primary metabolic dysfunction leads to compensatory hyperinsulinaemia to manage elevated postprandial blood glucose. Insulin is lipogenic in nature, and therefore the increased serum insulin causes a shift in nutrient storage from muscle

Abbreviations: AMIS, absence of meal-induced insulin sensitisation; GSH, glutathione; Hf-4, high-fat diet for 4 weeks; HISS, hepatic insulin-sensitising substance; RIST, rapid insulin sensitivity test; Sc-9, 35 % sucrose solution for 9 weeks; Sc-16, 35 % sucrose solution for 16 weeks.

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glycogen to fat[18]. In ageing[15,17] and sucrose[16] models of obesity and diabetes, the absence of meal-induced insulin sensitisation (AMIS) causes progression to a predictable series of metabolic dysfunctions including adiposity and lipid abnormalities[15,16,18], cardiac[19] and vascular dysfunctions[20]. This cluster of dysfunctions secondary to the chronic impairment of HISS action is referred to as the AMIS syndrome.

Voluntary exercise improves HISS action in AMIS associated with ageing[5]. In the present study, we hypothesise that the 7 d voluntary exercise would reverse AMIS in diet-induced prediabetic rats through restoration of HISS-dependent glucose uptake. The dietary interventions incorporated either a high-fat diet or 35 % sucrose solution. The high-fat diet was given for 4 weeks and the 35 % sucrose supplement was provided for 9 and 16 weeks. The purpose of using three different metabolic interventions with two diet types was to demonstrate that these dietary stresses decrease the postprandial response to insulin by a common mechanism that could be intervened by exercise. The present study demonstrates that HISS-dependent glucose uptake is primarily affected in diet-induced AMIS, which can be reversed by 7 d of voluntary running.

Study design and methods

Animals and groups

Male Sprague–Dawley rats (4 weeks old) (Charles River) were pair-housed in a climate-controlled animal care facility. They were randomly assigned to the high-fat diet (Hf-4) or 35 % sucrose supplement (Sc-9 and Sc-16) groups. Experiments were pre-scheduled and conducted for each individual rat on a separate day, once the assigned groupage was met (Fig. 1). Healthy controls received a normal diet and no exercise. Animals were treated according to the Guidelines of the Canadian Council on Animal Care, and protocols were approved by the Protocol Management and Review Committees at the University of Manitoba.

Diet protocol

The three dietary-intervention models were previously shown to develop insulin resistance[14,21] that does not reverse spontaneously upon withdrawal of the dietary insults. The rats in the Hf-4 group were maintained on a high-fat diet (D12492, Research Diet, Inc.) for 4 weeks to develop insulin resistance. The energy content of the high-fat diet was 21·94 kJ/g (5·24 kcal/g) and consisted of protein (20 %), carbohydrate (20 %) and fat (60 %). During the final week before experimentation, they were returned back to the normal diet (Prolab RMH3000, PMI feeds) while receiving exercise/no-exercise intervention. The energy content of the normal diet was 17·17 kJ/g (4·10 kcal/g) and consisted of protein (25·97 %), carbohydrate (60·01 %) and fat (14·02 %). The rats in the Sc-9 and Sc-16 groups were maintained on the normal diet and normal drinking-water while having free access to a 35 % sucrose solution for a period of 9 and 16 weeks, respectively (Fig. 1). The sucrose supplementation was removed for the week before the experiments and the fatty chow was replaced by normal chow. The purpose of withdrawing the dietary interventions during the final week was to determine the effects of exercise at a stable disease state and to avoid the complex interference of the dietary stress with exercise.

Exercise intervention

To begin with, 7 d before the experiment, a single rat from the paired-cage was randomly selected and tail-marked for voluntary running. The other rat did not receive any exercise intervention and was used as a control. Both of the rats were maintained on the standard diet and normal water during this 1-week period. The exercised rat was kept in the voluntary

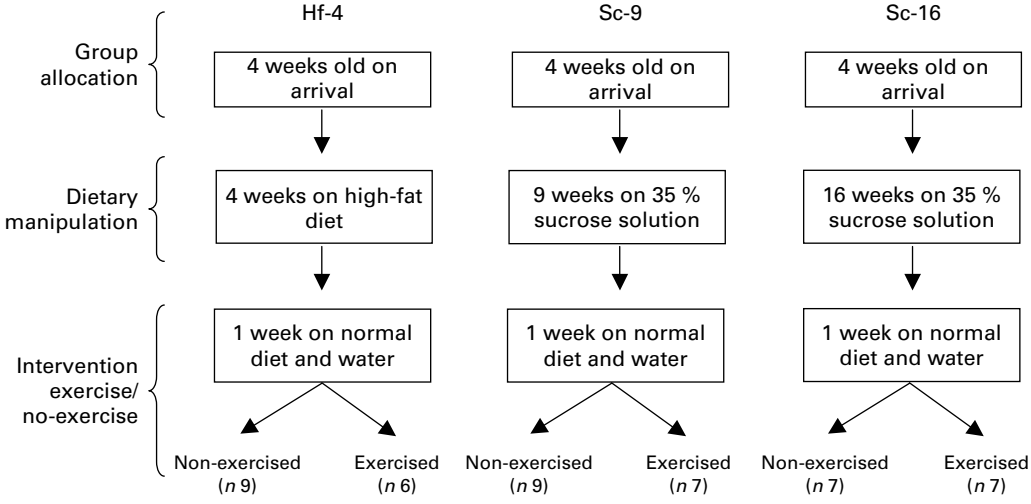

Fig. 1. Flow-chart of the study protocol illustrating the insulin-resistant rat models and intervention types. High-fat diet (Hf-4) or 35 % sucrose supplement (Sc-9/Sc-16) was used in specific age groups to induce insulin resistance. Following the period of dietary insult, the rats were maintained on a normal diet and drinking-water for 1 week while they received exercise/no-exercise intervention. Hf-4, high-fat diet for 4 weeks; Sc-9, 35 % sucrose solution for 9 weeks; Sc-16, 35 % sucrose solution for 16 weeks.

running-wheel cage (Lafayette Instrument) for approximately 18 h (15.00–09.00 hours) with free access to the running wheel; for the remaining 6 h (09.00–15.00 hours) the rat was returned to the cage-mate. Returning to the cage-mate during the non-active hours was adopted to satisfy the Central Animal Care Centre's preference for paired housing. The revolutions run by the exercised rat were recorded each day. The 7 d average exercise-performance was calculated as the running distance (km/d) considering the circumference of the wheel of 143·7 cm. We did not set any standard range for the exercise, or inclusion/exclusion criteria based on the exercise performance in rats. This approach allowed us to perform correlation studies of different metabolic factors against variable degrees of exercise. The acute experiments with the paired exercised/non-exercised rats were done on consecutive days.

Fast-feed protocol

All control and exercised rats underwent a fasting period of 12 h (19.00–07.00 hours) and a re-feeding period of 2 h (07.00–09.00 hours) before the acute experimentation. This fast-feed protocol maximises food intake, necessary to elicit an optimal feeding signal for HISS-release. In the case of exercised rats, experiments were done at least 24 h after the last exercise session in order to avoid the acute effects of exercise.

Adiposity measurement and surgical preparation

After feeding, the rat was weighed and anaesthetised with an intraperitoneal injection of sodium pentobarbital (54·7 mg/kg, CEVA Sante Animal S.A.). Whole-body adiposity was determined using bioelectrical impedance analysis as previously described[16,22]. The rat was then placed on its back and body temperature was monitored with a rectal probe thermometer. Temperature was maintained at 37·5 ± 0·5°C using a heated surgical table and a heat lamp above the table. An arterio-venous shunt, which allows uninterrupted blood flow from the artery to the vein, was established by cannulating the right femoral artery and vein (PE60 polyethylene tubing, Becton Dickinson) and connecting them with silicon tubing[23]. The arterio-venous-shunt was connected to a transducer for monitoring heart rate and arterial blood pressure after briefly occluding the venous end of the shunt. Blood sampling for glucose measurements was done by puncturing the arterial side of the shunt. Infusion of pharmacological agents was done through the venous side. Supplemental anaesthetic (5 ml/kg per h or 2·17 mg/kg per h of sodium pentobarbital in heparinised saline solution) was infused throughout the experiment.

Rapid insulin sensitivity test

The whole-body glucose uptake response to insulin was measured using the rapid insulin sensitivity test (RIST), as previously described[23]. Briefly, following surgery the rat was stabilised for 30 min. The baseline blood glucose was determined through sampling of the arterial blood (25 μl) at 5 min intervals, until three successive stable values were obtained. An intravenous-bolus insulin infusion (0·5 u/kg in 0·5 ml saline administered at 0·1 ml/min) was started and continued for 5 min. Approximately 1 min after the start of insulin infusion, the first glucose sample was taken and glucose infusion was commenced through the venous line. Arterial glucose levels were determined every 2 min, while glucose was infused at a variable rate to maintain euglycaemia. Arterial blood sampling and glucose infusion continued until the blood glucose level returned to control level and no further glucose infusion was required. The RIST index is the amount of glucose (mg/kg) infused over the test period to maintain euglycaemia, following the bolus infusion of insulin. A data acquisition system (National Instruments Lab-View, Austin, TX, USA) combined with application software (available on request from the authors) was used to record and analyse the mean arterial blood pressure, to calculate the RIST index and to provide real-time monitoring of adherence to the euglycaemic baseline. The software program calculated the accuracy and precision for the maintenance of the euglycaemic target baseline. If either deviated by more than 5 %, the entire RIST was considered to be invalid, and was discarded. The experimental protocol consists of two repeated RIST, separated by a stabilisation period of 30 min. The first RIST determined the dynamic glucose uptake response to both insulin and HISS. The second RIST was preceded by an intravenous infusion of atropine (1 mg/kg), which causes the inhibition of HISS-release, and therefore the post-atropine RIST index represents the HISS-independent component, or the direct action of insulin[9,13]. The difference between the two RIST represents the HISS-dependent glucose uptake.

Biochemical samplings and analysis

A sample (80 μl) for plasma insulin was collected at the start of the experiment; and prepared and preserved at −80°C for further assay of insulin. The plasma insulin measurement was done using an ultrasensitive ELISA kit (ALPCO Diagnostics). At the completion of both RIST, liver samples were collected and stored at −80°C for determination of the hepatic

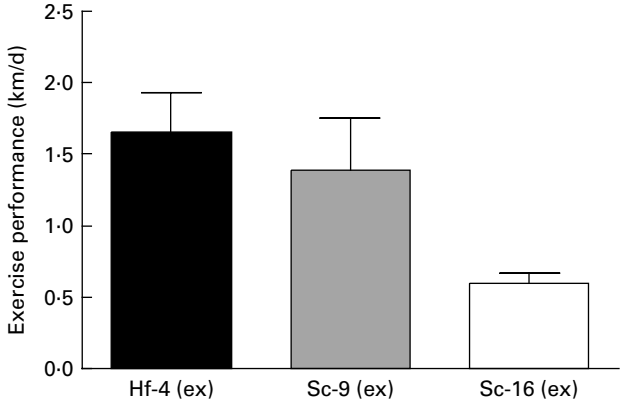

Fig. 2. The average distance run in the three intervention models of absence of meal-induced insulin sensitisation (7 d average exercise). Ex, exercised rats; Hf-4, high-fat diet for 4 weeks; Sc-9, 35 % sucrose solution for 9 weeks; Sc-16, 35 % sucrose solution for 16 weeks.

GSH level (Bioxytech GSH-420, OxisResearch). After liver sampling, the rat was euthanised and three regional fat pads (perinephric, epididymal and perienteric) were collected, weighed and compared with the bio-impedance estimate of total body fat content.

Data analysis

Values are presented as means with their standard errors. The comparisons between the control and intervention groups were done by using one-way ANOVA and unpaired *t* test as appropriate. Statistical significance was considered at $P<0{\cdot}05$. Linear regression analysis was performed to explore the correlations of any two variables by using statistical software (GraphPad Prism 5.0; Graph Pad Software Inc.).

Results

Exercise performance

The day-to-day exercise, for each individual rat over the 7 d training session, was recorded and demonstrated a gradual increase in distance run per d in all groups (data not shown). The average distance run in the Hf-4 (n 6), Sc-9 (n 7) and Sc-16 (n 7) groups is shown in Fig. 2.

Insulin/hepatic insulin-sensitising substance dynamic action

Insulin sensitivity was determined by the RIST, which quantifies the whole-body glucose uptake after a bolus infusion of insulin. The total glucose uptake response to insulin during the postprandial state has two major components: HISS-dependent and HISS-independent glucose uptake. HISS-independent glucose uptake represents the direct action of insulin and is the component remaining after atropine inhibition of HISS release.

The total glucose uptake in non-exercised rats of the Hf-4, Sc-9 and Sc-16 groups was decreased significantly compared to the healthy controls. Voluntary exercise caused at least a partial reversal of the response to insulin (combined HISS action and HISS-independent insulin action) in the diet-induced prediabetic groups (Fig. 3).

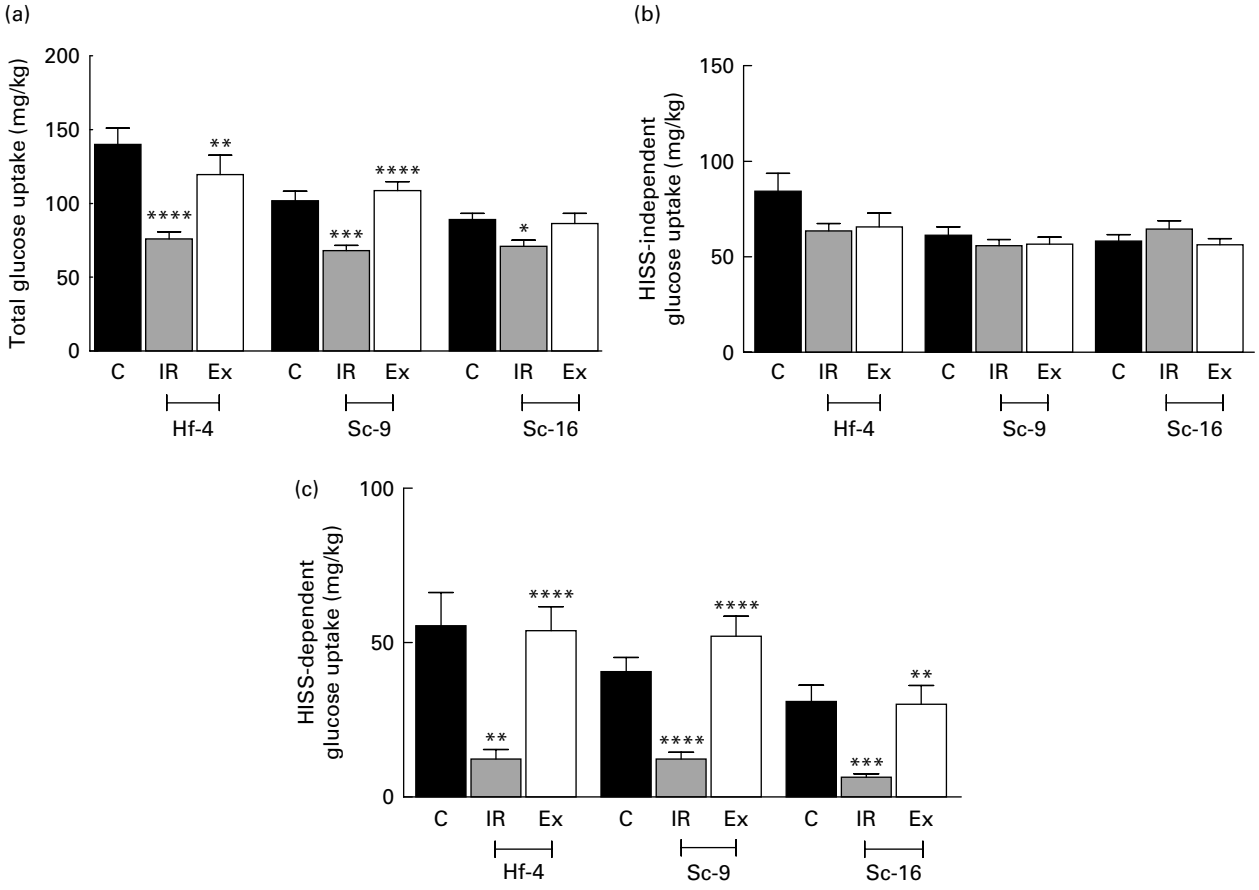

Fig. 3. (a) Total glucose uptake in insulin-resistant (IR) rats was compared with that in exercised (Ex) rats and age-matched healthy controls (C). The rapid insulin sensitivity test was used to determine (b) hepatic insulin-sensitising substance (HISS)-independent and (c) HISS-dependent glucose uptake. Postprandial insulin sensitivity was decreased significantly by dietary insult and reversed by exercise. The development of insulin resistance was caused primarily due to the blockade of HISS action; conversely, exercise reversed insulin resistance by restoring the HISS pathway. HISS-independent glucose uptake (or direct insulin action) was not affected by the diet or exercise. Hf-4, high-fat diet for 4 weeks; Sc-9, 35 % sucrose solution for 9 weeks; Sc-16, 35 % sucrose solution for 16 weeks. Values are means, with standard errors represented by vertical bars. Mean value was significantly different from that of the control rats: * $P<0{\cdot}05$, ** $P<0{\cdot}01$, *** $P<0{\cdot}001$, **** $P<0{\cdot}0001$.

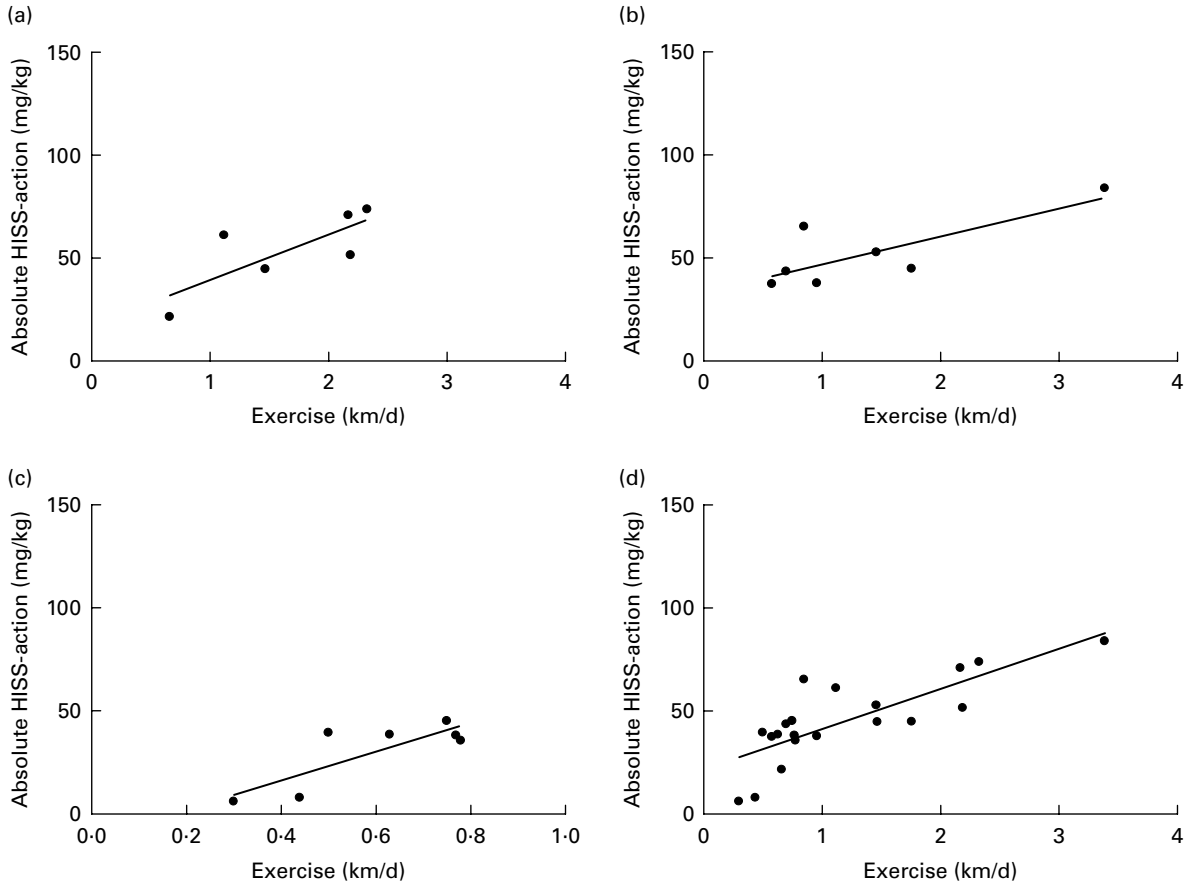

Fig. 4. Positive correlation between exercise and hepatic insulin-sensitising substance (HISS) action signifies a performance-dependent enhancement in insulin response in exercised rats of groups: (a) Hf-4-fed rats (r^2 0·60; slope 22·0 (SE 9·0); NS), (b) Sc-9-fed rats (r^2 0·61; slope 13·5 (SE 4·9); $P<0·05$), (c) Sc-16-fed rats (r^2 0·66; slope 70·00 (SE 22·4); $P<0·05$) and (d) all exercised insulin-resistant rats (r^2 0·62; slope 19·5 (SE 3·6); $P<0·0001$). Note that the exercise axis is on a different scale for the oldest rats, which exercised the least. Hf-4, high-fat diet for 4 weeks; Sc-9, 35 % sucrose solution for 9 weeks; Sc-16, 35 % sucrose solution for 16 weeks.

HISS-dependent glucose uptake was significantly decreased by the intervention diets and reversed by voluntary exercise. HISS-independent glucose uptake was mostly unaltered by diet and exercise. The improvement in HISS action accounted for the major contribution of exercise-induced enhancement in the whole-body response to insulin. The whole-body glucose uptake improves in proportion to the amount of performed exercise, and a linear relationship exists between the HISS-dependent glucose uptake and the running distance (Fig. 4).

Body weight, adiposity, fat pad mass and muscle mass

There was a corresponding increase in fat pad content with the increase in whole-body adiposity; a linear relationship (r^2 0·75) exists between total fat pad and the percentage fat mass. The extent of decrease in adiposity or fat content by exercise depends on the amount of voluntary running, and an inverse relationship (r^2 0·35) exists between the fat pad mass and the running distance (data not shown). There was also an inverse relationship between HISS action and fat pad mass and/or serum insulin concentration (Fig. 5). These findings are consistent with our previous observations[5,16] and the hypothesis that the metabolic abnormalities are secondary to the impaired HISS action.

Voluntary exercise caused a tendency to decrease body weight in rats of all groups. The body weight of non-exercise *v.* exercise in the Hf-4, Sc-9 and Sc-16 groups was 425·6 (SEM 11·2) *v.* 397·5 (SEM 10·4) g, 570·9 (SEM 18·8) *v.* 516·0 (SEM 29·4) g and 702·3 (SEM 44·0) *v.* 657·1 (SEM 18·6) g, respectively. The whole-body adiposity (percentage fat mass) was decreased significantly by exercise in most of the study groups. The combined mass of perinephric, epididymal and perienteric fat pads tended to decrease with exercise in all groups, but the changes were not statistically significant. The lean body mass was determined by subtracting total body fat (calculated from percentage fat mass) from body weight. Similar to our previous findings in healthy ageing rats[5], the muscle mass was not altered by exercise in the insulin-resistant groups (Fig. 6).

Postprandial blood glucose, serum insulin and hepatic glutathione

The metabolic status of the rats was determined by assessing the postprandial parameters including postprandial glycaemia

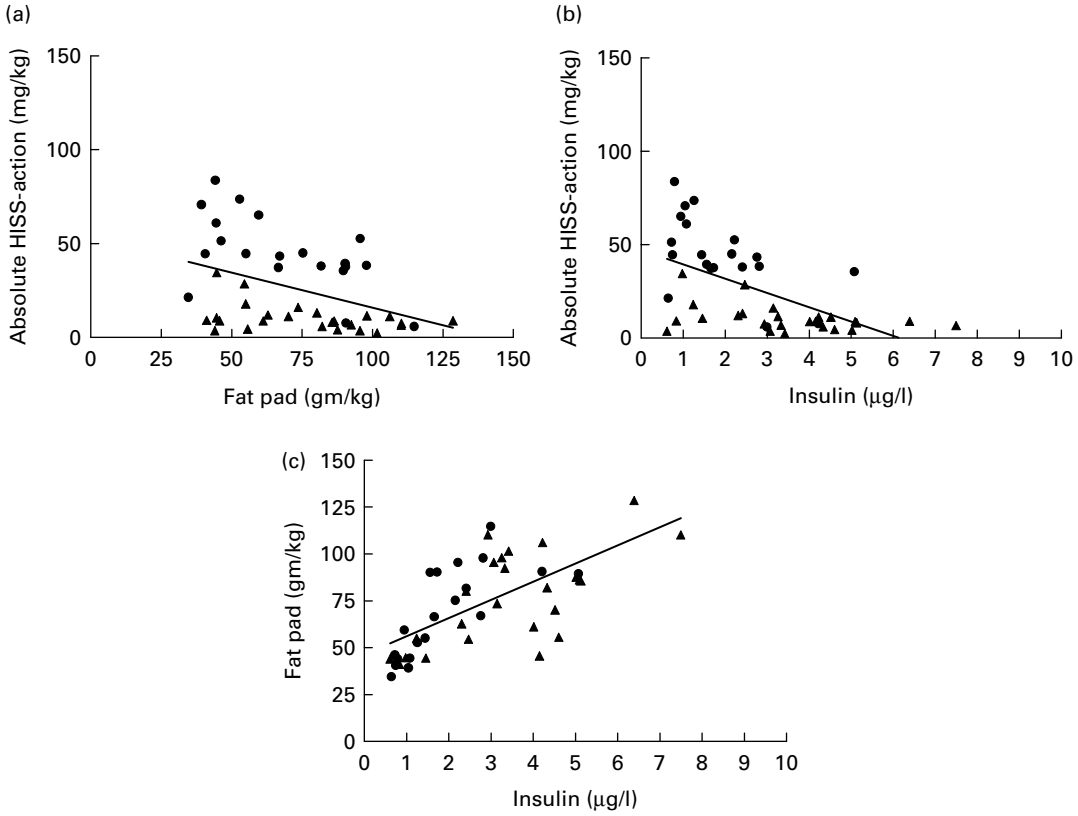

Fig. 5. Impairment in the hepatic insulin-sensitising substance (HISS) pathway causes progressive development of multiple metabolic abnormalities including hyperglycaemia, resultant hyperinsulinaemia, increased oxidative stress and accumulation of body fat. The inverse relationship between (a) HISS action and fat pad mass (r^2 0·17; slope −0·37 (SE 0·13); $P<0·01$) or (b) HISS action and postprandial serum insulin concentration (r^2 0·34; slope −7·71 (SE 1·64); $P<0·0001$), and the positive correlation between (c) serum insulin level and fat pad mass (r^2 0·44; slope 9·70 (SE 1·66); $P<0·0001$) are consistent with the pathologies being secondary to the impaired HISS action. In the exercised groups (●), the HISS action was recovered and a higher HISS action was correlated with lower adiposity and serum insulin level. In the non-exercised groups (▲), the HISS pathway was almost blocked, leading to progressive adiposity and hyperinsulinaemia over the range of a very low HISS action.

and plasma insulin concentration. The blood glucose level tended to decrease with exercise in all groups, and was statistically significant in the Sc-9 group. The plasma insulin concentration demonstrated statistically significant decreases in the exercised rats, signifying that the postprandial insulinaemia was improved by voluntary running. The hepatic GSH level was decreased significantly by exercise in the Hf-4 group, but remained unchanged in others (Table 1).

Discussion

Voluntary exercise for 7 d is able to prevent the progression to the AMIS syndrome in healthy ageing rats[5]. However, chronic dietary manipulations with high-fat diet or sucrose supplementation impair the postprandial response to insulin in the prediabetic models of AMIS[14,21]. These observations compelled us to test the hypothesis that exercise would reverse the diet-induced AMIS through restoration of HISS-dependent glucose uptake. The present study showed that the 7 d voluntary running improves the postprandial glucose uptake response to insulin, in the diet-induced AMIS models, through recovery of the HISS action.

Technical considerations

The protocol was designed to utilise three established models of AMIS that employ two different diet types, a high-fat diet and a 35 % sucrose supplement. These animal models were not age-matched on normal diets. The data for different ages are previously reported[5]. What we wanted to test is whether exercise would be capable of reversing AMIS in the three-diet/age models that were previously shown to have a reduced postprandial insulin response, secondary to the absence of HISS action. The sucrose diet was given for 9 weeks when full blockade of HISS release would have existed for at least 7 weeks[21]. By increasing the duration of the sucrose insult to 16 weeks, it was expected that signs and symptoms of the AMIS syndrome would show a predictable progression of pathologies.

During the final week before experimentation, the rats were returned to the normal diet and were allocated to the exercise/no-exercise subgroups. Removal of the dietary insult was done in order to avoid acute effects of the diet and to carry out the testing in a stable diseased state. Both the high-fat diet and sucrose supplementation cause an absence of HISS action[14,21]. HISS action does not recover spontaneously for at least a week following withdrawal of the dietary insult, and

returning the rats to the normal diet and water. HISS-dependent glucose uptake remained low in non-exercised rats after 1 week of withdrawal of the intervention diets (Fig. 3).

The exercised rats received 7 d voluntary running-wheel exercise, and the insulin and HISS action were measured at least 24 h after the last bout of exercise. This interval was adopted to minimise the acute effects of exercise and to avoid the complications of testing immediately after the last session. The studies relate to the chronic effects of dietary imbalance and the sustained effects of exercise. The beneficial metabolic effects of exercise persisted after 24 h, though the onset and duration of exercise effect on AMIS were not examined. A more prolonged exercise is expected to provide more metabolic benefits, since each rat showed a gradual increase in day-to-day running distance over the 7 d session (data not shown) and degree of improvement correlated with the distance run (Fig. 4).

The RIST, which is a transient euglycaemic clamp in response to a bolus of insulin, was used to measure the whole-body glucose uptake in response to insulin. In the fed state, the response to insulin has two components, HISS-dependent and HISS-independent glucose uptake. HISS-dependent glucose uptake results through the insulin-induced release of HISS from the liver and its action in peripheral tissues. HISS-independent glucose uptake represents the direct action of insulin in the periphery. If HISS release is blocked, the remaining response is attributed entirely to the direct action of insulin. The RIST was done before and after atropine infusion that blocks the hepatic parasympathetic feeding signal, and therefore blocks HISS-release. Atropine produces an effect similar to the blockade of hepatic NO synthase or hepatic denervation. Atropine is a useful tool in this regard, as it is capable of eliminating the HISS response in fed animals and has no effect on direct insulin actions[13].

Voluntary exercise provides sustained metabolic benefits in diet-induced insulin resistance

The absence of HISS release is compensated in type 2 diabetes by elevated insulin secretion[16], which leads to numerous metabolic abnormalities. It raises the possibility that an intervention which potentiates the HISS pathway will slow/

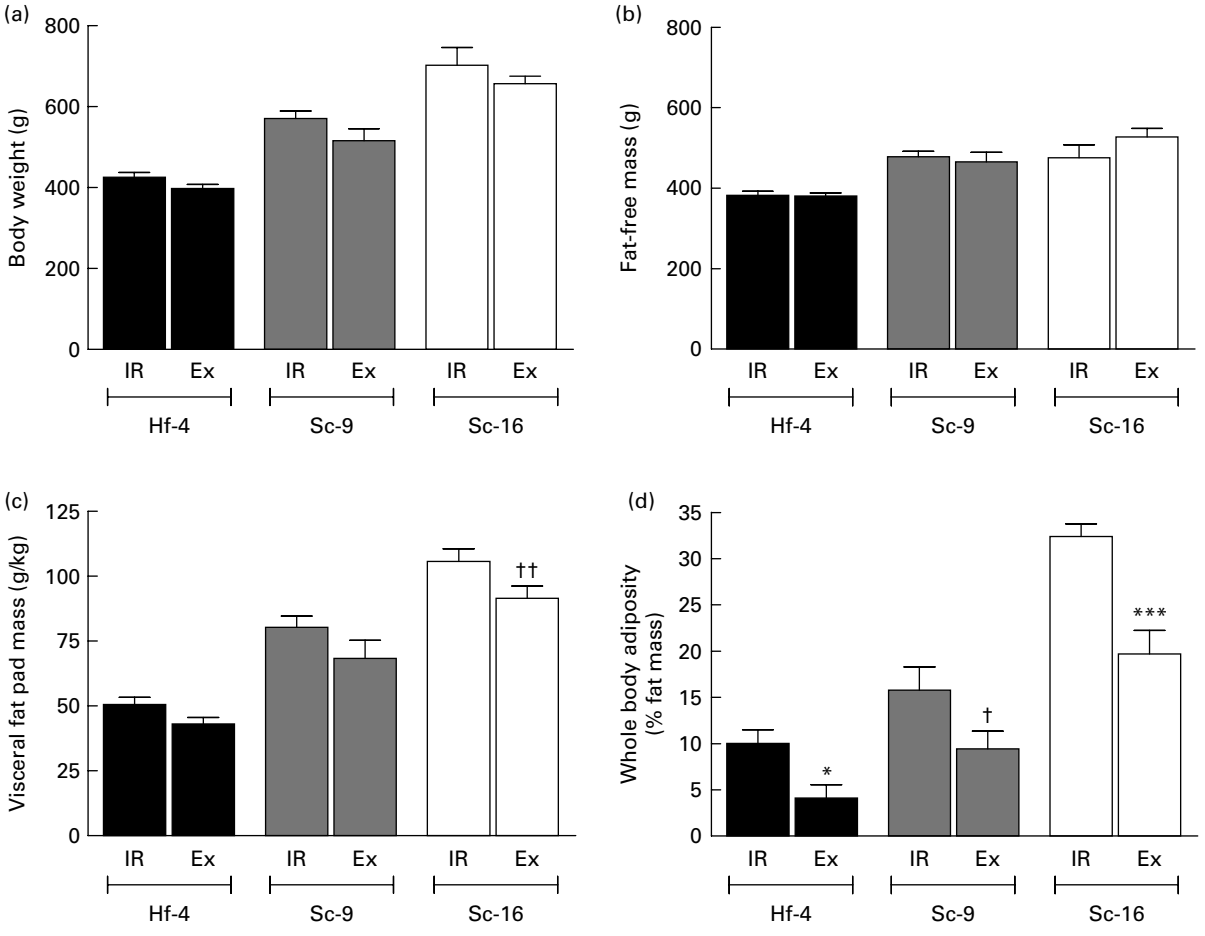

Fig. 6. (a) Body weight, (b) fat-free mass, (c) visceral fat pad mass and (d) whole body adiposity. The 7 d voluntary exercise (Ex) reduced (a) body weight, (c) visceral fat mass and (d) adiposity (percentage fat mass) in all groups. (b) Lean body weight or fat-free mass was not changed significantly by exercise, signifying that the increase in muscle mass is not the primary mechanism by which the 7 d voluntary training causes insulin sensitisation in diet-induced insulin-resistant (IR) rats. Hf-4, high-fat diet for 4 weeks; Sc-9, 35 % sucrose solution for 9 weeks; Sc-16, 35 % sucrose solution for 16 weeks. Values are means, with standard errors represented by vertical bars. Mean value was significantly different from that of the IR rats: * $P<0{\cdot}05$, *** $P<0{\cdot}001$. Mean value was marginally significantly different from that of the IR rats: † $P=0{\cdot}08$, †† $P=0{\cdot}059$.

Table 1. Metabolic profiles in the three intervention rat models with or without exercise‖
(Mean values with their standard errors)

	Hf-4 groups				Sc-9 groups				Sc-16 groups			
	Non-exercised (*n* 9)		Exercised (*n* 6)		Non-exercised (*n* 9)		Exercised (*n* 7)		Non-exercised (*n* 7)		Exercised (*n* 7)	
	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM
Postprandial blood glucose (mg/dl)	114·8	2·8	106·8*	2·6	111·3	2·4	103·6†	2·6	109·0	5·4	103·8§	5·1
Serum insulin (μg/l)	2·25	0·53	0·92†	0·10	3·74	0·35	1·65‡	0·26	4·65	0·66	3·03†	0·46
Hepatic glutathione (μmol/g)	5·30	0·26	4·12†	0·41	6·39	0·24	5·65§	0·27	6·06	0·17	6·17§	0·27

Hf-4, high fat diet for 4 weeks; Sc-9, 35 % sucrose solution for 9 weeks; Sc-16, 35 % sucrose solution for 16 weeks.
* Mean value tended to decrease from that of non-exercised rats of the Hf-4 group ($P=0·07$).
† Mean values were significantly different from those of non-exercised rats of Hf-4 Sc-9 and Sc-16 groups ($P<0·05$).
‡ Mean value was significantly different from that of non-exercised rats of Sc-9 group ($P<0·001$).
§ Mean values were not significantly different from those of non-exercised rats of Sc-9 and Sc-16 groups.
‖ The metabolic parameters were adversely affected by high-fat diet or 35 % sucrose supplement, and led to the development of hepatic insulin-sensitising substance (HISS)-dependent insulin resistance. Voluntary exercise reversed the diet-induced HISS-dependent insulin resistance and improved the associated conditions.

reverse the progression to AMIS, prediabetes and diabetes. Voluntary exercise was used as an intervention to modulate the HISS pathway in the diet-induced models of AMIS. In our study, exercise restored HISS-dependent glucose uptake in diet-induced prediabetic rats, and the benefits were seen 24 h after the last bout of exercise. These benefits might be obtained through the adaptive effects of exercise that resulted in the augmentation of HISS action. The sustained metabolic improvements correlate with the beneficial changes in other risk factors like hyperinsulinaemia, adiposity and body composition.

Hepatic insulin-sensitising substance, the primary pathway attenuated by dietary stress and improved by exercise

Both of the diets significantly decreased the response to insulin due to attenuation of the HISS pathway. These diets impaired only HISS-dependent glucose uptake, while the direct insulin action remained mostly unaffected (Fig. 3). The 7 d voluntary exercise reversed the diet-induced AMIS, and the improved insulin response was achieved through restoration of HISS action. There was no significant impact of exercise on the direct insulin action. The exercise-induced metabolic benefits correlated directly with the enhancement in HISS-dependent glucose uptake. Increased HISS release might be attained through augmentation of the feeding signals (hepatic parasympathetic nerve activity and GSH concentration) required for HISS release. Hepatic GSH level was decreased with exercise in the high-fat diet group and remained unaltered in the Sc-9 and Sc-16 groups (Table 1). Therefore, it is possible that the increased HISS action was mediated by the nerve feeding signal, or by some yet unknown mechanism.

Exercise-induced metabolic benefits are secondary to the improvement in hepatic insulin-sensitising substance action

Inability of post-meal signals to release HISS causes inadequate glucose utilisation and storage in peripheral tissues. The chronic shift in nutrient storage from muscle glycogen to fat, as a consequence of impaired HISS action, results in a progressive and predictable series of metabolic, cardiac and vascular dysfunctions[16,18–20]. The 7 d voluntary exercise increased postprandial insulin response and tended to decrease postprandial blood glucose in all prediabetic groups. The plasma insulin concentration was significantly decreased by exercise (Table 1). The whole-body adiposity and fat pad mass tended to decrease with exercise. The inverse relationship between HISS action and serum insulin or fat content (and the positive correlation between insulin concentration and fat mass) suggests that the impact of exercise on various metabolic parameters was attained secondary to the augmentation of HISS-dependent glucose uptake (Fig. 5).

Since skeletal muscle accounts for >85 % of glucose utilisation[24,25], it could be possible that an increase in skeletal muscle mass might cause the insulin sensitisation response to exercise. However, the metabolic characteristic of the skeletal muscle is a more important determinant than the muscle mass for glucose metabolism[25]. Our previous study with ageing rats[5] demonstrated that the muscle mass increased with age, but was unaffected by 1 week of voluntary exercise. The increased muscle mass with age does not compensate for decreased HISS action, and exercise benefits in ageing are obtained primarily through enhancement/restoration of the HISS pathway. The present study supports our previous findings and indicates that improved metabolic dynamics of the muscle through HISS, but not an increased muscle mass, is the primary mechanism by which 7 d voluntary exercise provides insulin sensitisation in diet-induced insulin resistance.

Conclusion

The present study was designed to test the ability of exercise to restore the HISS pathway in the diet-induced models of AMIS. A high-fat diet and 35 % sucrose supplement were used to induce HISS-dependent insulin resistance in rats, and the 7 d voluntary running was utilised as an exercise intervention. The intervention diets reduced the postprandial response to insulin mostly by impairment of the HISS action, which was reversed by voluntary exercise. The impairment

of HISS action correlated with a cluster of dysfunctions, consistent with AMIS being the initiator of an AMIS syndrome. The mechanism by which exercise improves the HISS action and any possible cross-interaction between exercise and other therapeutic factors (e.g. antioxidants) on the HISS pathway need yet to be examined.

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