British Journal of Nutrition (2001), **86**, 515–519

DOI: 10.1079/BJN2001421

Low iron status and enhanced insulin sensitivity in lacto-ovo vegetarians

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(Received 26 January 2001 – Revised 20 April 2001 – Accepted 14 May 2001)

The efficacy of insulin in stimulating whole-body glucose disposal (insulin sensitivity) was quantified using direct methodology in thirty lacto-ovo vegetarians and in thirty meat-eaters. All subjects were adult, lean (BMI $<23\,kg/m^2$), healthy and glucose tolerant. Lacto-ovo vegetarians were more insulin sensitive than meat-eaters, with a steady-state plasma glucose (mmol/l) of 4·1 (95 % CI 3·5, 5·0) *v.* 6·9 (95 % CI 5·2, 7·5; $P = 0{\cdot}0028$) respectively. In addition, lacto-ovo vegetarians had lower body Fe stores, as indicated by a serum ferritin concentration (μg/l) of 35 (95 % CI 21, 49) compared with 72 (95 % CI 45, 100) for meat-eaters ($P = 0{\cdot}0012$). To test whether or not Fe status might modulate insulin sensitivity, body Fe was lowered by phlebotomy in six male meat-eaters to levels similar to that seen in vegetarians, with a resultant approximately 40 % enhancement of insulin-mediated glucose disposal ($P = 0{\cdot}0008$). Our results demonstrate that lacto-ovo vegetarians are more insulin sensitive and have lower Fe stores than meat-eaters. In addition, it seems that reduced insulin sensitivity in meat-eaters is amenable to improvement by reducing body Fe. The latter finding is in agreement with results from animal studies where, no matter how induced, Fe depletion consistently enhanced glucose disposal.

Insulin: Glucose: Iron: Vegetarian diet

Meat-eaters have greater risk of developing both adult-onset diabetes and atherosclerosis (Snowdon *et al.* 1984; Snowdon & Phillips, 1985; Burr & Butland, 1988; Snowdon, 1988; Thorogood *et al.* 1994; Fraser & Shavlik, 1997). These age-related pathologies are also more common in insulin-insensitive (or resistant) individuals (Facchini *et al.* 2000). Although currently unknown, such inter-relationships among disease and metabolic defects on one hand, and disease and dietary habits on the other, suggest that meat-eating might also be associated with greater degrees of insulin resistance.

Meat-eaters, as compared with lacto-ovo (LO) vegetarians, have greater body Fe stores, and meat intake was indeed the only nutritional factor consistently shown to positively correlate with storage Fe (Bothwell *et al.* 1979; Worthington-Roberts *et al.* 1988; Lowik *et al.* 1990; Lyle *et al.* 1992; Shaw *et al.* 1995; Brussaard *et al.* 1997).

Insulin resistance is also positively correlated with the size of body Fe stores, when assessed by serum ferritin concentrations (Tuomainen *et al.* 1997; Fernandez-Real *et al.* 1998).

Since in animals Fe status is an important modulator of insulin-stimulated glucose disposal e.g. the lower the Fe status the lesser the degree of insulin resistance (Brooks *et al.* 1987; Borel *et al.* 1993; Farrel *et al.* 1988; Hostettler-Allen *et al.* 1993), we hypothesised that lacto-ovo vegetarians are less insulin resistant than meat-eaters.

The present study addressed two specific questions: (1) are meat-eaters more insulin resistant than lacto-ovo vegetarians; (2) does lowering of body Fe to a lacto-ovo vegetarian level improve insulin resistance.

Methods

Sixty lean normotensive glucose-tolerant individuals agreed to participate and gave written informed consent. Based on previous data, we performed sample size calculation using a two-sample comparison determining a required sample size of *n* 29 in each subject group (LO vegetarians and meat-eaters). BMI was $<23\,kg/m^2$. Although such stringent selection criteria were somewhat arbitrary, the goal was to exclude early clinical disease states such as hypertension, glucose intolerance and, particularly, obesity. All these conditions are closely related to insulin resistance and therefore confound the relationship between Fe, insulin and glucose metabolism.

Abbreviations: IST, insulin suppression test; LO, lacto-ovo; SSPG, steady-state plasma glucose.

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All participants were free of acute or chronic diseases, and not taking pharmaceuticals. Their health status was established by physical examination, blood cell count, routine chemical analyses, urine analysis and resting twelve-lead electrocardiography.

LO vegetarians were considered those who reported no meat consumption for the previous 5 years. Meat-eaters were those who ate meat (animal muscle) at least once daily for the previous 5 years.

Baseline measurements included weight, height, blood pressure, a 75 g oral glucose-tolerance test and fasting serum ferritin (Franco, 1987). Insulin resistance was quantified using a standard insulin suppression test (IST). After an overnight fast, subjects were given a fixed-dose simultaneous intravenous infusion of somatostatin (at 5 μg/min), glucose (13 mmol/m^2 per min) and insulin (180 pmol/m^2 per min). The infusion lasted 180 min and venous blood samples were drawn every 10 min during the last 30 min for measurement of plasma glucose (Schmidt, 1961) and insulin (Hales & Randle, 1963). Since somatostatin inhibits endogenous insulin secretion and insulin plasma clearance has little inter-individual variability, this constant rate of insulin infusion allows comparable steady-state levels, of about 350 pmol/l, in all individuals. These physiological, postprandial-like, levels fully suppress hepatic glucose production.

The mean value of the four measurements during the last 30 min was used to calculate the steady-state plasma glucose (SSPG) concentration. At SSPG concentrations, the rate of glucose infusion equals whole-body glucose disposal (metabolism). Under these circumstances, the higher the SSPG, the less effective insulin is in stimulating glucose disposal and the more insulin resistant the individual. This method, although not as sensitive as the glucose-clamp technique in quantifying insulin action, is operator-independent and, therefore, more suitable for paired studies (Greenfield *et al.* 1981).

For the phlebotomy trial in meat-easters, we estimated a sample size of *n* 6 for a one-sample comparison of means. Six meat-eaters agreed to undergo Fe lowering by phlebotomy according to the method described by Haskins *et al.* (1952). Monthly or bimonthly 0·5 l phlebotomy is very effective in reducing the size of body Fe stores in Fe overloading (Van Oost *et al.* 1983) and in normal individuals (Finch *et al.* 1977).

The IST was repeated once serum ferritin concentrations reached values comparable with those of LO vegetarians.

Assessment of physical activity relied on self-reporting the number of exercise sessions per week resulting in sweating. This type of questionnaire method is best correlated with aerobic power (Siconolfi *et al.* 1985).

Statistics

All results are expressed as means and 95 % confidence intervals. Non-normally-distributed variables were serum ferritin concentration and SSPG. Inter-group comparisons were performed using the unpaired Student's *t* test and Mann-Whitney *U* test. In the six meat-eaters, where Fe was lowered, SSPG values at follow-up were compared with baseline results by two-tailed Student's paired *t* test after log transformation. Linear and multiple regression models were used to evaluate the relationships among insulin resistance (SSPG), serum ferritin concentration and demographic variables. All calculations were performed using a commercial statistical software package (Statistica; Statsoft Inc., Tulsa, OK, USA) for the MacIntosh (mod.iMAC; Apple Computers, Cupertino, CA, USA).

Results

Table 1 illustrates demographic and metabolic characteristics of the two groups. The LO vegetarians were not different from the meat-eaters in terms of age, gender and BMI.

Serum ferritin concentration (μg/l; 35 (95 % CI 21, 49) *v.* 72 (95 % CI 45, 100); $P = 0{\cdot}0012$) and insulin resistance (SSPG (mmol/l); 4·1 (95 % CI 3·5, 5·0) *v.* 6·9 (95 % CI 5·2, 7·5); $P = 0{\cdot}0028$) were lower in LO vegetarians *v.* meat-eaters respectively. When data from all subjects were pooled, the two variables positively correlated (R 0·80; $P = 0{\cdot}0001$); i.e. the higher the serum ferritin concentration the greater the degree of insulin resistance. Such linear correlation (shown in Fig. 1) was independent of gender, BMI and age (Table 2).

In six male meat-eaters a mean of 2·9 (95 % CI 1·9, 4·0) phlebotomies were performed to reduce body Fe stores to a level similar to that of LO vegetarians. Following this intervention serum ferritin concentration in the six meat-eaters fell from a mean of 85·3 (95 % CI 65·1, 105·5) μg/l

Table 1. Group characteristics for meat-eaters and lacto-ovo (LO) vegetarians participating in the study

(Mean values and 95 % confidence intervals)

	Meat-eaters (*n* 30)		LO vegetarians (*n* 30)		Statistical significance of difference between groups
Variable	Mean	95 % CI	Mean	95 % CI	
Age (years)	40	36, 44	41	37, 45	NS
Gender (F:M)	20:10		20:10		NS
BMI (kg/m^2)	21·9	21·3, 22·5	21·6	21·0, 22·2	NS
SSPG (mmol/l)	6·9	5·2, 7·5	4·1	3·5, 5·0	$P = 0{\cdot}0028$
SSPI (pmol/l)	395	332, 488	366	330, 402	NS
Ferritin (μg/l)	72	45, 100	35	21, 49	$P = 0{\cdot}0012$

SSPG, steady-state plasma glucose concentration; SSPI, steady-state plasma insulin concentration.

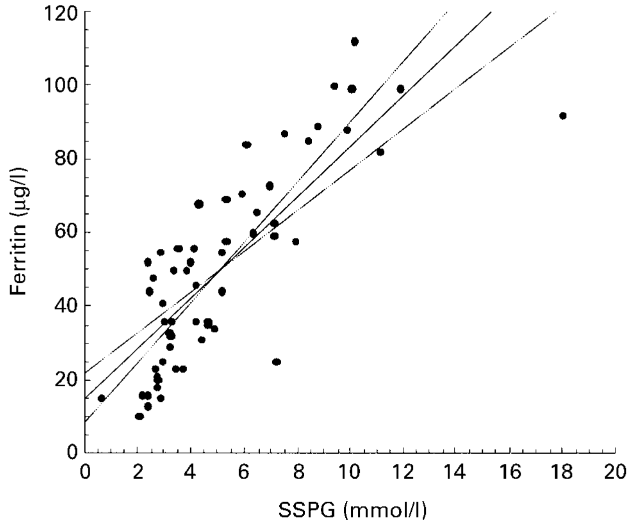

Fig. 1. Linear regression analysis, showing 95 % CI bands (– · · · –), of steady-state plasma glucose concentration (SSPG) *v.* serum ferritin concentration for all meat-eaters and lacto-ovo vegetarians (*n* 60). For details of subjects and procedures, see pp. 515–516 and Table 1. $y = 14{\cdot}894 + 6{\cdot}856x$; R^2 0·652.

before phlebotomy to a mean of 27 (95 % CI 17·3, 34·8) µg/l after phlebotomy ($P = 0{\cdot}0004$).

The effect of Fe lowering on insulin resistance is shown in Fig. 2. Individual values are shown for the six subjects before and after phlebotomy. Despite a similar steady-state plasma insulin concentration (pmol/l; 401 (95 % CI 311, 491) *v.* 364 (95 % CI 298, 430), physical activity level (no. of sessions of sweat-inducing exercise per week; 3·0 95 % CI 2·4, 3·6 *v.* 3·0 (95 % CI 2·2, 3·8) and body weight (kg; 89 (95 % CI 85, 93 *v.* 90 (95 % CI 84, 96) before and after phlebotomy respectively, SSPG was 41 % lower after phlebotomy (from 8·8 (95 % CI 6·8, 10·8) mmol/l before phlebotomy to 5·2 (95 % CI 3·8, 6·6) mmol/l after phlebotomy; ($P = 0{\cdot}0008$), indicating enhancement of insulin-stimulated glucose disposal following Fe depletion.

Discussion

The main finding of our investigation was that LO vegetarians are less insulin resistant than habitual meat-eaters.

Table 2. Multiple regression analysis for the pooled data from meat-eaters and lacto-ovo vegetarians (*n* 60). Dependent (outcome) variable SSPG. Independent (predictor) variables age, gender, BMI, PA ferritin. Model R^2 0·66, SE 0·13, $P = 0{\cdot}00001$

Variable	Beta coefficient	SE	Statistical significance: *P*
Age	0·11	0·08	0·19
Gender	0·07	0·09	0·40
BMI	0·08	0·08	0·29
PA	−0·03	0·08	0·09
Ferritin	0·76	0·08	0·00001

SSPG, steady-state plasma glucose concentration; PA, physical activity level (no. of sessions of sweat-inducing exercise per week).

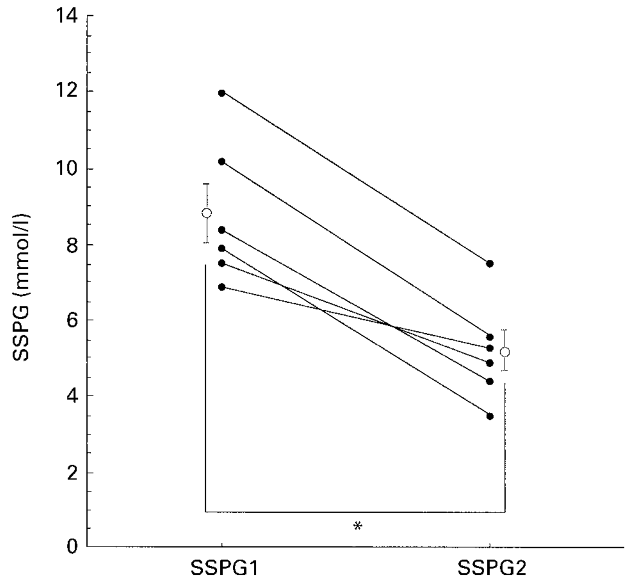

Fig. 2. Individual steady-state plasma glucose concentrations (SSPG) before (SSPG1) and after iron lowering (SSPG2) in six male meat-eaters (●). For details of subjects and procedures, see pp. 515–516. (○), Mean values with their standard errors represented by vertical bars for six subjects. Mean values were significantly different: *$P = 0{\cdot}0008$.

Serum ferritin concentrations were also lower in LO vegetarians, and this finding is in agreement with those of previous studies comparing healthy vegetarians with omnivores (Helman & Darnton-Hill, 1987; Worthington-Roberts *et al.* 1988; Lowik *et al.* 1990; Lyle *et al.* 1992; Donovan & Gibson, 1995; Shaw *et al.* 1995; Brussaard *et al.* 1997). In healthy individuals serum ferritin is highly correlated with Fe stores measured by either quantitative phlebotomy (Walters *et al.* 1973) or bone-marrow Fe staining (Lipschitz *et al.* 1974; Hallberg *et al.* 1993, 1997). Thus, in our study subjects lower serum ferritin concentrations indicated decreased body Fe stores.

There are several reasons why LO vegetarians have a lower Fe status than meat-eaters. First, meat is a powerful enhancer of intestinal non-haem-Fe absorption (Hallberg & Rossander, 1982; Lynch, 1997). Second, haem-Fe absorption is less inhibited than non-haem-Fe by polyphenols, phytates and the size of body Fe stores (Hallberg *et al.* 1979; Lynch, 1997). These mechanisms explain why meat intake is the only nutritional factor found to predict Fe status in normal individuals (Bothwell *et al.* 1979; Worthington-Roberts *et al.* 1988; Lowik *et al.* 1990; Lyle *et al.* 1992; Shaw *et al.* 1995; Brussaard *et al.* 1997; Lynch, 1997). Third, LO vegetarians often consume considerable amounts of Ca-enriched products (in particular, cheese). A variety of studies have shown that Ca salts also significantly inhibit intestinal Fe absorption as well (Anderson *et al.* 1940; Cook *et al.* 1991; Hallberg *et al.* 1991). Thus, low *v.* high dietary Fe bioavailability is likely to account for differences in Fe status among vegetarians and meat-eaters.

The Fe-lowering trial using phlebotomy demonstrated that Fe is a key factor in the difference in insulin resistance among LO vegetarians and meat-eaters. Studies in animals (Brooks *et al.* 1987; Borel *et al.* 1993; Farrel *et al.* 1988;

Hostettler-Allen *et al.* 1993), in which Fe-poor diets had phlebotomy-like effects, and *in vitro*, in which Fe chelation enhanced glucose transporter 1-mediated glucose uptake (Potashnik *et al.* 1995), support this conclusion. Thus, increasing body Fe leads to greater serum ferritin and insulin resistance (Tuomainen *et al.* 1997; Fernandez-Real *et al.* 1998), thereby worsening the risk of diabetes (Salonen *et al.* 1998). In addition, well-known associations, such as that among glucose intolerance, haemochromatosis (Dandona *et al.* 1983) and secondary Fe overload (Merkel *et al.* 1988) are also seemingly related to Fe-induced insulin resistance.

Fe status influenced insulin sensitivity similarly when methods other than the IST were used. For example, glucose tolerance improved after phlebotomy and at comparable serum ferritin values (Facchini, 1998).

It could also be argued that the observed reduction in insulin resistance was caused (rather than by Fe lowering *per se*) by low reproducibility of the IST, operator-dependent bias, or by the fact that insulin resistance *per se* is a time-sensitive variable.

However, the IST is, in contrast to the euglycaemic clamp, an operator-independent technique, and therefore the most appropriate in the setting of a paired study design (Greenfield *et al.* 1981). Moreover, it was demonstrated previously that not only does the IST have good short-term reproducibility (Sheu *et al.* 1992), but also that insulin resistance is a fairly stable metabolic variable over long-term follow-up (Facchini *et al.* 1999).

Thus, it seems unlikely that methodological issues accounted for the enhancement of insulin sensitivity. Non-methodological factors, such as weight loss and/or increased physical activity could also have explained our findings (Olefsky *et al.* 1974; Rosenthal *et al.* 1983; Hughes *et al.* 1984; James *et al.* 1984). However, in our six meat-eating subjects these variables were unchanged from baseline. Thus, the most obvious interpretation of our results is that Fe status markedly affected glucose metabolism.

In conclusion, LO vegetarians are more insulin sensitive than meat-eaters. This metabolic feature appears to be secondary to decreased body Fe in LO vegetarians compared with meat-eaters.

Whether or not Fe status, directly or via its modulatory effect on insulin-stimulated glucose disposal, is relevant in the reduction in diabetes and atherosclerotic cardiovascular disease in LO vegetarians (Snowdon *et al.* 1984; Snowdon & Phillips, 1985; Snowdon, 1988; Burr & Butland, 1988; Thorgood *et al.* 1994; Fraser & Shaulik, 1997) remains unknown. Our data, if reproduced, should serve as a stimulus for larger intervention studies in which the relative importance and interaction of Fe lowering, insulin resistance and vegetarianism on outcome development can be assessed over long-term follow-up.

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