European Journal of Clinical Nutrition (2005) 59, 291–298
 0954-3007/05 $30.00
www.nature.com/ejcn

ORIGINAL COMMUNICATION

Veganism and its relationship with insulin resistance and intramyocellular lipid

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Objective: To test the hypothesis that dietary factors in the vegan diet lead to improved insulin sensitivity and lower intramyocellular lipid (IMCL) storage.
Design: Case–control study.
Setting: Imperial College School of Medicine, Hammersmith Hospital Campus, London, UK.
Subjects: A total of 24 vegans and 25 omnivores participated in this study; three vegan subjects could not be matched therefore the matched results are shown for 21 vegans and 25 omnivores. The subjects were matched for gender, age and body mass index (BMI).
Interventions: Full anthropometry, 7-day dietary assessment and physical activity levels were obtained. Insulin sensitivity (%S) and beta-cell function (%B) were determined using the homeostatic model assessment (HOMA). IMCL levels were determined using *in vivo* proton magnetic resonance spectroscopy; total body fat content was assessed by bioelectrical impedance.
Results: There was no difference between the groups in sex, age, BMI, waist measurement, percentage body fat, activity levels and energy intake. Vegans had a significantly lower systolic blood pressure (−11.0 mmHg, CI −20.6 to −1.3, $P=0.027$) and higher dietary intake of carbohydrate (10.7%, CI 6.8–14.5, $P<0.001$), nonstarch polysaccharides (20.7 g, CI 15.8–25.6, $P<0.001$) and polyunsaturated fat (2.8%, CI 1.0–4.6, $P=0.003$), with a significantly lower glycaemic index (−3.7, CI −6.7 to −0.7, $P=0.01$). Also, vegans had lower fasting plasma triacylglycerol (−0.7 mmol/l, CI −0.9 to −0.4, $P<0.001$) and glucose (−0.4 mmol/l, CI −0.7 to −0.09, $P=0.05$) concentrations. There was no significant difference in HOMA %S but there was with HOMA %B (32.1%, CI 10.3–53.9, $P=0.005$), while IMCL levels were significantly lower in the soleus muscle (−9.7, CI −16.2 to −3.3, $P=0.01$).
Conclusion: Vegans have a food intake and a biochemical profile that will be expected to be cardioprotective, with lower IMCL accumulation and beta-cell protective.
Sponsorship: MRC PhD studentship.
European Journal of Clinical Nutrition (2005) **59,** 291–298. doi:10.1038/sj.ejcn.1602076
Published online 3 November 2004

Keywords: intramyocellular lipids; insulin resistance; insulin sensitivity; magnetic resonance spectroscopy; glycaemic index; vegan

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***Conflicts of interest*: LMG, JDB and PWS: MRC funding; AD: lecturer for Knoll, NovoNordisk, Novartis, Aventis and GlaxoSmithKline; GSF: receives research funding from Kellogg, Sugar Bureau, Roche and Nutricia.**
Received 10 May 2004; revised 19 July 2004; accepted 26 August 2004; published online 3 November 2004

Introduction

A central feature of the metabolic syndrome is the development of insulin resistance, which in turn appears to be the main underlying risk factor for coronary heart disease and type II diabetes (Zimmet *et al*, 2001). There is an extensive body of evidence to show that intramyocellular lipid (IMCL) is elevated in subjects who have reduced insulin sensitivity compared to those who are relatively insulin sensitive (Falholt *et al*, 1988; Phillips *et al*, 1996; Pan *et al*, 1997; Forouhi *et al*, 1999; Krssak *et al*, 1999). This has been recently shown to be true even in children and adolescents with prediabetes (Sinha *et al*, 2002; Weiss *et al*, 2003). Storing lipid in myocytes may be one of the primary causes of insulin resistance. The work of Randle *et al* (1963) has suggested this

to be the case. They proposed that increased IMCL may be the main cause of insulin resistance through the prolonged release of nonesterified fatty acids (NEFA) from myocytes and the subsequent competition for oxidation between these and circulating glucose (Randle *et al*, 1963). In recent years, this hypothesis has been extended by McGarry, who suggested that the metabolites of triacylglycerol interfere with phosphorylation processes within the cells (McGarry & Dobbins, 1999). McGarry suggested that the storage of triacylglycerols and triacylglycerol metabolites affects not only the insulin sensitivity of muscles and the liver but also pancreatic beta-cell function. This may be important as the development of frank diabetes requires an insulin-resistant state and the demise of beta-cell function.

However, to date, the correlational data that show a relationship between IMCL and insulin sensitivity are not able to separate cause from effect. Currently, acute intervention studies have shown that insulin sensitising manipulations can modulate IMCL storage (Krssak *et al*, 2000; Brechtel *et al*, 2001) but there is very little data on the impact of long-term physiological interventions on IMCL and insulin sensitivity. Various dietary factors have been shown to reduce insulin sensitivity in humans. Large epidemiological studies suggest that high fat diets are detrimental to insulin sensitivity (Marshall *et al*, 1994; Feskens *et al*, 1995). Moreover, saturated fatty acids appear to have the most significant detrimental impact on insulin sensitivity (Vessby *et al*, 2001). The type of carbohydrate in the diet also appears to affect insulin sensitivity. Sucrose has been shown to be more detrimental than starch (Reiser *et al*, 1979, 1981; Coulston *et al*, 1985), while classifying carbohydrates by their glycaemic index (GI) shows that low GI carbohydrates have a beneficial effect on insulin sensitivity compared to high GI carbohydrates (Wolever *et al*, 1992; Frost *et al*, 1996; Salmeron *et al*, 1997a, b). Taking all this evidence into account, a group of particular interest would be subjects that follow a vegan dietary regimen. These diets are generally based on cereals, pulses, nuts, vegetables and fruits, avoiding the consumption of all foods from animal origin (Key *et al*, 1999a). In the 1980s, there was a surge of literature from studies investigating the health consequences of following a vegetarian/vegan dietary regimen (Burr & Sweetnam, 1982; Thorogood *et al*, 1987, 1989, 1990; Dwyer, 1988; Frentzel-Beyme *et al*, 1988; Snowdon, 1988). These studies found that vegetarian/vegan subjects had significantly lower body mass indexes (BMI), lower blood pressure and the incidence of, and mortality from, ischaemic heart disease (IHD), and type II diabetes was lower in these populations also (Key *et al*, 1999b). Further analysis showed that these subjects had cardioprotective lipid profiles, for example lower total cholesterol and lower low-density lipoprotein (LDL-) cholesterol (Appleby *et al*, 1999; Key *et al*, 1999a). These findings would indirectly predict vegan subjects to have a relatively high insulin sensitivity. However, there are no data available regarding the insulin sensitivity or IMCL of vegan/vegetarian subjects, mainly due to the fact that at the time of the above studies, it was not fully recognised that abnormalities of lipid metabolism and carbohydrate metabolism were linked through insulin resistance. It is reasonable to hypothesise that vegan subjects may be more insulin sensitive than omnivore subjects, given the low incidence of insulin resistance-based diseases among this population (Key *et al*, 1999b), but this hypothesis has yet to be investigated. There has been a small amount of interventional work performed using vegetarian/vegan dietary interventions in type II diabetic subjects, which has shown improvements in glycaemic control (Nicholson *et al*, 1999). Considering the basis of the vegan diet, it might be expected that this dietary regimen would promote insulin sensitivity through its low fat content, particularly saturated fat, and low GI. However, to date, there is no published literature to show that the vegan diets are of low GI.

In the current study, we have assessed the dietary intakes, insulin sensitivity and the IMCL concentrations of vegan subjects and compared these with matched omnivore controls. The study aimed to determine if vegan subjects are more insulin sensitive than omnivores, and to determine what dietary factors may influence their insulin sensitivity. Additionally, we aimed to determine if IMCL storage was lower in vegan subjects than omnivore controls and whether this relates to insulin sensitivity or some specific dietary factors.

Subjects and methods

Study sample

A total of 24 Caucasian vegan subjects (12 male, 12 female) (mean age 42.4 ± 2.8 y, mean BMI 24.6 ± 1.39 kg/m^2) were recruited through an advertisement in The Vegan Society (UK) newsletter and from a cohort who took part in a previous study at the centre. All subjects had been following a vegan diet (defined as a diet free from all meat and animal produce, including cheese, milk and eggs) for a minimum of 3 y prior to participating in the study.

Matched omnivore controls were recruited from the staff of the centre and from volunteers who had participated in previous studies at the centre. Each subject was matched for gender, ethnicity, age (± 5 y) and BMI (± 2 kg/m^2). The aim was to match each vegan volunteer to at least one omnivore control. A total of 25 control subjects who matched with the vegan volunteers (11 male, 14 female) were recruited (mean age 42.4 ± 3.32 y, mean BMI 23.4 ± 0.55 kg/m^2).

Exclusion criteria included diabetes, coronary heart disease and any metabolic disorder or drug treatment known to affect carbohydrate or lipid metabolism. Subjects were also excluded if they followed a special diet other than veganism or if they had contraindications for an MRI scan, such as claustrophobia or metal prostheses. All subjects gave informed written consent. The study was granted ethical approval by the Imperial College School of Medicine, Hammersmith Campus Research Ethics Committee (ref: 99/5690).

Methods

Anthropometry. Weight (kg), height (m), BMI (kg/m^2) and waist to hip ratio (W/HR) were measured for each subject. Body fat percentage was measured using the InBody 3.0 (Biospace Co. Ltd, Korea) system, which is a multifrequency segmental bioelectrical impedance method (Thomas *et al*, 2000). Blood pressure was measured using an automated sphygmomanometer, subjects were rested in a seated position, three repeat measurements were performed and the mean reading recorded.

Insulin sensitivity. The homeostatic model assessment (HOMA) method was used to assess insulin sensitivity (%S) and beta-cell function (%B) in the subjects. This test requires three fasting blood samples taken at 10 min intervals following a 12 h overnight fast. This was achieved using a butterfly blood collection system. A measure of 10 ml of blood was taken into lithium heparin tubes, containing 4000 kIU aprotinin (Bayer, Newbury, UK); between sampling, 10 ml flushes of 0.9% sodium chloride solution were used to keep the line patent for the whole of the test period. The samples were immediately centrifuged for 10 min at 3000 rpm at 4°C and plasma separated and stored in aliquots at −20°C.

Plasma glucose concentrations were determined using a glucose-oxidase-based autoanalyser (Technicon, Axon Bayer Diagnostic, Newbury, Berkshire, UK). Insulin concentrations were determined using radioimmunoassay (Albano *et al*, 1972). The interassay and intra-assay coefficients of variation for this assay were less than 10% and the detection limit of the assay was 2 pmol/l with 95% confidence. To minimise interassay variation, all samples were included in one assay and were analysed on the first freeze–thaw.

Fasting lipid profiles. Total cholesterol, high-density lipoprotein (HDL) cholesterol, LDL-cholesterol, triacylglycerols and nonesterified fatty acid (NEFA) concentrations were measured using the fasting blood samples taken for the HOMA.

Total cholesterol was measured using the Wako L-Type Cholesterol test kit, HDL-cholesterol using the Wako L-Type HDL-Cholesterol test kit, triacylglycerols using the L-Type Triacylglycerol test kit and NEFA using the Wako NEFA C test kit (all from Wako Chemicals, Alpha Laboratories Ltd, Eastleigh, Hampshire, UK). LDL-cholesterol was calculated using the Friedwald *et al* (1972) equation.

Dietary assessment. Seven-day prospective food diaries were collected from the subjects. Portion sizes were assessed and validated using a photographic aid (Nelson *et al*, 1997). The assessments were analysed for macro- and micronutrient composition using the DietPlan 5™ (Forestfield Software Ltd, Horsham, UK) computer package. The glycaemic index (GI) of the subjects' diets were calculated using the published tables of glycaemic indices for individual foods (Foster & Miller, 1995) and using the formula derived by Wolever and Jenkins (1986) for calculating the GI of mixed meals. The dietary assessments were validated using the subjects' estimated energy requirement (EER), calculated using the Schofield equation and Department of Health (1991) recommendations for activity requirements (Department of Health, 1991). Dietary assessments that varied from the EER by $\pm$2000 kJ were rejected as inaccurate assessments of dietary intake. In analysing the vegan dietary records, many of the food compositions were not available in the database used. Therefore, the analysis on the food labels was used where available and where not available, the nearest food alternatives were used.

All assessments were within $\pm$2000 kJ of the subjects' EERs and so were accepted as accurate representations of their dietary intakes.

Physical activity assessment. Physical activity levels were assessed using the Baecke physical activity questionnaire (Baecke *et al*, 1982). This assesses physical activity levels during occupational, sporting and leisure time activities.

Magnetic resonance spectroscopy (MRS). IMCL (*in vivo*): ^{1}H-MRS was used to assess IMCL storage, in brief (as we have published earlier detailed methodology elsewhere (Rico-Sanz *et al*, 1998; Forouhi *et al*, 1999)), spectra were acquired using a 1.5 T Marconi Medical Eclipse system from the soleus (predominantly oxidative muscle fibres), tibialis (predominantly glycolytic fibres) and gastrocnemius (mixed fibre type) muscles of the left lower leg. Subjects lay in a supine position with the left leg immobilised in a 30-cm diameter quadrature birdcage coil. A clamp device was used to immobilise and measure the positioning and orientation of the leg to ensure that the leg was in an identical position for follow-up scans. Transverse T1-weighted MR images (TR 600, TE 16ms) were acquired for placement of the ^{1}H-MRS voxel, with a slice thickness of 5 mm, a 20 cm field of view and 192×256 data matrix. Spectra were obtained without water suppression using a standard PRESS sequence with TE/TR = 135/1500 ms, 8 cm^3 voxel and 256 averages. Spectra were analysed using the MRUI software package (van den Boogaart *et al*, 1996) (available from http://carbon.uab.es/mrui). Peak areas for each signal were obtained and lipid resonances were quantified with reference to total creatine after correcting for T_1 and T_2 (Rico-Sanz *et al*, 1998). The reproducibility of this technique has earlier been calculated by the group; the interexamination coefficient of variation is 13.6 $\pm$ 3.5% (Rico-Sanz *et al*, 1998).

Lipoprotein profile (*in vitro*): The plasma lipoprotein profile was obtained using *in vitro* MRS. Briefly, plasma samples were thawed and then spun at 10 000 *g* for 1 min. Aliquots (0.6 ml) were placed into a MRS tube holding a coaxial tube containing 8.18 mM sodium trimethylsilyl-[2,2,3,2,^{2}H$_4$]propionate (TSP) in deuterium oxide. MRS was performed on a Varian Unity + spectrometer operating at

500 MHz proton resonance frequency. Single pulse experiments were performed at 45°C with 128 transients and a delay of 3.2 s. Chemical shifts were referenced to TSP (0 ppm) in the coaxial tube. Quantification was performed by measuring intensity of peaks of interest (lipids) relative to that of TSP.

Statistical analysis. Statistical analyses were performed using SPSS Release 11.0 for Windows.

The results in tabular and graphical form are shown as mean ± s.d. Data were tested for normality using the Shapiro–Wilk W test. The unpaired Student's *t*-test, Pearson's correlation coefficient and multiple regression analysis were used for statistical analysis of normally distributed data and the Mann–Whitney test was used for not normally distributed data. Statistical significance was taken as $P<0.05$.

Results

Of the 24 vegan subjects recruited, three of the subjects could not be matched and so results are shown for a total of 21 vegan subjects and 25 omnivore controls.

Anthropometrics

The anthropometrical characteristics of the groups are shown in Table 1. Each subject in the vegan group was individually matched for sex, age and BMI, and the results for the complete groups show that the matching was satisfactory, with no significant differences in age and BMI between the groups. There were also no significant differences in WHR, percentage body fat or physical activity scores. However, there was a significant difference in the subjects' systolic blood pressures, with the vegans having significantly lower values.

Dietary assessments

Seven-day dietary assessments were collected from all subjects. The vegan and omnivore macronutrient intakes are shown in Table 2.

There was no difference in total energy intake between the vegan and omnivore subjects; however, the energy distributions did differ significantly between the groups. There was a trend for total fat intake to be lower in the vegan subjects but this did not reach statistical significance. However, there were highly significant differences in the types of fat; it has to be remembered that the data here are reported from the

Table 1 Anthropometrical results of the vegan and omnivore subjects

	Vegan (n = *21*)	*Omnivore* (n = *25*)	*95% CI for difference* (P)
Age (y)	35.0 (23–69)	36.0 (24–75)	−10.6 to 6.3 (0.9)
Weight (kg)	66.6 ± 8.94	68.4 ± 13.53	−8.80 to 5.12 (0.6)
BMI (kg/m^2)	22.7 ± 2.42	23.4 ± 2.77	−2.26 to 0.86 (0.4)
Waist to hip ratio	0.84 ± 0.05	0.86 ± 0.06	−0.056 to 0.156 (0.3)
Systolic blood pressure (mmHg)	112.7 ± 11.93	123.7 ± 18.70*	−20.6 to −1.3 (**0.03**)
Diastolic blood pressure (mmHg)	66.9 ± 8.88	70.1 ± 9.91	−9.0 to 2.5 (0.3)
Body fat (%)	21.8 ± 7.22	22.8 ± 7.94	−5.7 to 3.7 (0.7)
Physical activity	8.1 (5.7–12.2)	7.9 (6.7–11.1)	−1.04 to 0.76 (0.8)

Data are mean ± s.d. except when the data are not normally distributed, in these cases they are represented as medians and ranges. BMI denotes the subjects' degree of obesity. Unpaired *t*-tests were used to test for differences between the groups, and a Mann–Whitney test was used on the non-normal data.
*$P<0.05$ shown in bold text.

Table 2 Macronutrient intakes of vegan and omnivore subjects

	Vegan (n = *21*)	*Omnivore* (n = *25*)	*95% CI for difference* (P)
Energy (MJ)	9.8 ± 1.95	10.4 ± 2.78	2.11–0.79 (0.4)
Protein (%)	11.9 (6.3–14.3)	15.1 (8.6–20.1)*	−5.0 to −2.1 (<**0.001**)
CHO (%)	54.7 ± 6.98	44.0 ± 5.87*	6.9–14.5 (<**0.001**)
Fat (%)	32.2 ± 6.11	35.8 ± 7.35	−7.6 to 0.5 (0.08)
SAFA (%)	4.89 ± 1.23	10.9 ± 3.53*	−7.7 to −4.4 (<**0.001**)
MUFA (%)	9.04 ± 2.42	9.40 ± 2.60	−1.9 to 1.2 (0.6)
PUFA (%)	7.97 ± 3.38	5.18 ± 2.73*	1.0–4.6 (**0.003**)
NSP (g)	34.5 (17.0–56.0)	13.0 (7.7–30.5)*	15.8–25.6 (<**0.001**)
GI	77.8 ± 4.54	81.5 ± 5.45	−6.7 to −0.7 (**0.01**)

Data are mean ± s.d. for normally distributed data, for not normally distributed data the median and ranges are shown. Protein, carbohydrate (CHO) and fat intakes are shown as percentages of total food energy. Saturated fatty acids (SAFA), monounsaturated fatty acids (MUFA), polyunsaturated fatty acids (PUFA), glycaemic index (GI) and nonstarch polysaccharides (NSP) represent fibre intake. Unpaired *t*-tests were used to test for differences between the vegan and omnivore subjects (Mann–Whitney tests were used for not normally distributed data).
*$P<0.05$ shown in bold text.

food table where analysis is not complete so the total of the fatty acid profile does not match the total fat intake. The vegan subjects had significantly lower saturated fat intakes than the omnivores and this corresponded with a significantly increased intake of polyunsaturated fats but no difference in monounsaturated fats. Some of these dietary differences appear to be reflected in the plasma lipid profile. *In vitro* MRS results of plasma samples show that the total amount of triacylglycerol within all the lipoprotein particles was significantly lower and the relative index of unsaturation was significantly higher in vegan subjects compared to omnivores, Table 3. These differences appear to arise principally from VLDL particles.

Carbohydrate intakes were significantly higher in the vegans than the omnivores, and protein intakes were significantly lower in the vegans than the omnivores. Fibre (NSP) intakes were significantly higher in the vegans than the omnivores and the GI of the vegan diet was significantly lower than that of omnivore diet.

Biochemistry

Table 4 shows the biochemical results for the vegans and omnivores. Vegan subjects had lower total cholesterol concentrations than the controls but this did not reach statistical significance; however, the plasma triacylglycerol concentrations were significantly lower in the vegans. Fasting glucose was significantly lower in the vegans and beta-cell function (%B), assessed through the HOMA technique, was significantly higher. However, insulin sensitivity (%S) as assessed through the HOMA technique was not different between the two groups.

IMCL storage

The IMCL results can be seen in Table 4. IMCL storage in the soleus muscle was significantly lower in the vegans than in the omnivores. In the tibialis and gastrocnemius muscles, the differences were nonsignificant.

Discussion

In this study, we investigated the effect of veganism on IMCL and insulin sensitivity against a matched omnivore control group. We hypothesised that veganism, because of the chronic low-fat, highly complex carbohydrate diet, would have positive effects on insulin sensitivity and possibly IMCL. As reported in other studies, the dietary intake of the

Table 3 MRI assessment of muscle triacylglycerol storage and lipoprotein triacylglycerols in vegans and omnivores

	Vegan (n = 21)	*Omnivore (n = 25)*	*95% CI for difference (P)*
Soleus (IMCL/Cr_{tot})	11.7 (6.1–24.6)	16.9 (2.7–44.7)*	−13.20 to −3.29 (**0.01**)
Tibialis (IMCL/Cr_{tot})	6.5 (2.5–10.1)	5.9 (2.7–35.7)	−5.82 to 0.59 (0.4)
Gastrocnemius (IMCL/Cr_{tot})	10.7 (4.5–201.8)	10.3 (4.7–44.7)	−9.68 to 26.4 (0.4)
Lipoprotein TG content[a]	1.74 (1.62–1.86)	2.81 (2.28–2.79)	0.47–1.66 (**0.0001**)
Unsaturation-index[b]	0.26 (0.23–0.28)	0.17 (0.14–0.18)	−0.15 to −0.015 (**0.018**)

Data are medians and ranges because of their non-normal distribution. Muscle triacylglycerol values are expressed as intramyocellular lipid (IMCL) to (total) creatine ratio.
[a]Plasma TG content was determined from–CH_3 lipid signal intensity.
[b]unsaturation-index was determined from the ratio of C = C/CH_3 and Mann–Whitney tests were used to determine differences between the groups.
*$P < 0.05$ shown in bold text.

Table 4 Biochemistry results for vegans and omnivores

	Vegan (n = 21)	*Omnivore (n = 25)*	*95% CI for difference (P)*
Cholesterol (mmol/l)	3.73 ± 0.72	4.18 ± 0.94	−0.96 to 0.06 (0.08)
HDL-cholesterol (mmol/l)	1.22 ± 0.25	1.32 ± 0.41	−0.32 to 0.09 (0.3)
LDL-cholesterol (mmol/l)	2.25 ± 0.60	2.30 ± 0.96	−0.53 to 0.44 (0.9)
Cholesterol/HDL-cholesterol ratio	3.15 ± 0.69	3.50 ± 1.59	−1.10 to 0.41 (0.4)
Triacylglycerols (mmol/l)	0.56 (0.07–2.12)	1.18 (0.53–1.91)*	−0.93 to −0.37 (<**0.001**)
NEFA (mmol/l)	606 ± 231	551 ± 164	−62.5 to 173.0 (0.3)
Fasting glucose (mmol/l)	3.99 (3.03–4.75)	4.03 (3.67–5.10)*	−0.73 to −0.09 (**0.05**)
Fasting insulin (pmol/l)	45.0 ± 12.59	41.7 ± 17.64	−5.98 to 12.56 (0.5)
HOMA (%S)	116 (83.1–270.0)	138 (64.3–416.0)	−58.63 to 16.92 (0.3)
HOMA (%B)	141 ± 38.1	109 ± 35.2*	10.3–53.9 (**0.005**)

Data are mean ± s.d. for normally distributed data and median and ranges for not normally distributed data. HDL- and LDL-cholesterol denote high- and low-density lipoprotein-cholesterol, respectively. Nonesterified fatty acids (NEFA), homeostatic model assessment (HOMA). Unpaired *t*-tests were used to test for differences between the groups, for not normally distributed data, Mann–Whitney tests were used.
*$P < 0.05$ shown in bold text.

vegan group was significantly different from that of the omnivore group (Appleby *et al*, 1999). The vegan group had significantly higher intakes of carbohydrate, PUFA and NSP and significantly lower intakes of protein, SFA and a lower overall GI. Similar intake profiles have been reported by others (Appleby *et al*, 1999). The nutrient profile of the vegan diet would be expected to be less atherogenic than the omnivore intake (Dwyer, 1988; Appleby *et al*, 1999), and the biochemical profile of the vegan subjects substantiates this hypothesis. The vegan subjects were found to have significantly lower triacylglycerol levels and fasting glucose concentrations when compared to omnivore controls and a trend towards a lower total cholesterol level. The MRS of the plasma lipoproteins demonstrates that there was a significantly lower amount of total triacylglycerol carried in the lipoprotein particles of the vegan group, and the triacylglycerol in the lipoprotein particles, when controlled for the total amount of fat in the vegans' diets, had a higher amount of unsaturated fat; again supporting a less atherogenic picture in the vegans. Also of note is the lower systolic blood pressure, an observation which has been reported elsewhere (Dwyer, 1988; Key *et al*, 1999a). These findings are supported in other larger cohorts where vegan/vegetarian cohorts were shown to be at a lower atherogenic risk (Appleby *et al*, 1999). It has to be remembered that these observations are made in two well-matched healthy cohorts, and may further emphasise the positive observations made in the vegan group.

In this study, we used an indirect method of assessing insulin sensitivity. The HOMA has been shown by others to have a close correlation with insulin sensitivity measured through the hyperinsulinaemic glucose clamp (Hermans *et al*, 1999). In our study, we were not able to demonstrate a significant difference in HOMA %S between vegans and omnivore controls. However, we did detect a significantly improved beta-cell function (HOMA %B) in the vegan subjects, which is suggested to be protective against type II diabetes (van Haeften, 2002). If we consider, in addition, the lower plasma triacylglycerols and fasting glucose concentrations that were found in the vegan cohort, it would suggest that veganism may be protective for the beta-cell and decrease the risk of diabetes. Such an hypothesis is supported by the data showing a lower incidence of type II diabetes in vegan subjects compared to meat-eaters (Dwyer, 1988; Key *et al*, 1999b).

It is of interest that the soleus muscle had a significantly lower IMCL in the vegan cohort compared to omnivores. The soleus muscle fibres, in the main, are oxidative muscle fibres and therefore a major site of glucose metabolism. If lipid storage in myocytes has effects on glucose disposal and insulin sensitivity, then a decrease in IMCL in the soleus muscle should have a major effect on insulin sensitivity. However, similar findings to ours have been previously reported in which subjects who are predisposed to insulin resistance have increased IMCL compared to healthy controls; however, no difference is measured in their insulin sensitivity (Szczepaniak *et al*, 1999). Their increased deposition of IMCL appears to occur prior to a measurable demise in their insulin sensitivity. McGarry discusses this finding in his 2001 Banting Lecture and proposes that the lipotoxicity that is occurring in the muscles, liver and pancreas of these 'at risk' individuals is what predisposes and causes the beta-cell failure that characterises type II diabetes (McGarry, 2002).

Similarly, in cohorts of teenage children whose parents have conditions related to insulin resistance compared to teenagers whose parents do not, higher levels of IMCL have been observed before detectable changes in insulin sensitivity (Jacob *et al*, 1999). It is possible that the lower circulating TG and the high ratio of polyunsaturated fat in the vegan volunteers played a role in the lower IMCL values. It is possible that high background IMCL levels are the initial metabolic abnormality that initiates insulin resistance, when the person is exposed to an adverse environment such as an excessive energy intake. The observations that beta-cell function appears increased in the vegan volunteers is of interest and it could be argued that it fits in with McGarry's theory of lower circulating TAG and higher polyunsaturated fatty acid ratio having positive effects on beta-cell function (McGarry, 2002).

Our results support the hypothesis that chronic changes in nutritional intake, specifically fat and carbohydrate, lead to changes in IMCL and beta-cell function. The higher intake of low GI carbohydrates, low intake of saturated fat and higher intake of polyunsaturated fat lead to an improved beta-cell function and a low level of IMCL, which would be expected to reduce the risk of the insulin resistance syndrome. The nonsignificant differences in traditional lipid risk factors are possibly due to the well-matched control group; it is also possible that at high levels of BMI, the observations between the groups may be greater.

In conclusion, we have demonstrated that the vegans have a food intake and a biochemical profile that will be expected to be cardioprotective and that veganism is beta-cell protective.

Acknowledgements

The authors would like to thank the MRC and Philips (Cleveland, OH, USA) for their assistance during this study. We would also like to thank Ms Cherie Tong for her help with the vegan dietary analysis.

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