Plasma free fatty acids do not provide the link between obesity and insulin resistance or β-cell dysfunction: results of the Reading, Imperial, Surrey, Cambridge, Kings (RISCK) study

Increasing the proportion of plasma monounsaturated fatty acids, as a result of dietary intervention, is associated with a modest improvement in insulin sensitivity

Running title:Plasma free fatty acids and insulin resistance

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Aim: To investigate whether changes in plasma free fatty acid (FFA) composition, as a result of dietary intervention, correspond to changes in insulin sensitivity.

Methods: The RISCK study was a 6-month randomised controlled dietary intervention study, which assessed the effect of modifying type of dietary fat and the glycaemic index (GI) of carbohydrates on insulin sensitivity. Fasting plasma FFA profiles and an insulin sensitivity index (Si), derived from intravenous glucose tolerance minimal-model analysis, were available from 533 participants, all at elevated risk of Type 2 diabetes. Bivariate correlations between changes in plasma saturated fatty acids (SFA), monounsaturated fatty acids (MUFA) (as a percentage of total plasma FFA) and changes in Si were assessed according to treatment group. Changes in dietary glycaemic index were adjusted for.

Results: Increasing total plasma FFA was associated with worsening Si (r=-0.152, P=0.001*)*. In the high MUFA/low GI diet study group, change in MUFA (% of total plasma FFA) was positively associated with change in Si (r=0.297, P=0.002). This association remained significant after adjusting for change in GI. Among MUFAs, change in oleic acid (18:1) was most strongly correlated with change in Si (r=0.266, P=0.005), as was change in minor plasma FFAs 22:1 (r=0.244, P=0.011) and 17:1 (r=0.196, P=0.042). In the high SFA/high GI diet study group, change in plasma SFAs was not significantly associated with change in Si.

Conclusion: Increasing the proportion of monounsaturated plasma FFAs as a result of dietary intervention may confer modest benefit for insulin sensitivity.

Introduction

Type 2 Diabetes Mellitus (T2DM) is a significant cause of morbidity and mortality and prevalence of the disease is anticipated to increase dramatically as a result of the obesity epidemic (1). There is substantial evidence that energy dense, high fat diets are diabetogenic and dietary modification targeted at reducing total energy and fat intake is an effective strategy in the prevention of T2DM, with superior outcomes to pharmacological interventions (2,3). However there is uncertainty regarding the effect of modifying dietary fat composition on insulin sensitivity.

Results from randomised trials have suggested that substituting saturated fat for unsaturated fat improves insulin sensitivity (4,5). However a recent randomised intervention study failed to demonstrate any benefit of reducing dietary saturated fat in a in weight-stable obese European population with the metabolic syndrome (6). Thus the optimum dietary approach with regard to fat and T2DM prevention remains unclear. There is also evidence that individual FFAs influence insulin sensitivity both positively and negatively, and to different (ref) (7). However it is currently unclear to what extent dietary fatty acid composition may affect one's propensity to developing impaired glucose tolerance through changes in insulin sensitivity.

To provide evidence based nutritional guidelines on dietary fat and T2DM prevention, controlled intervention studies are required. RISCK was a large intervention study, which manipulated dietary fat and carbohydrate composition in free-living individuals (8,9). The composition of plasma free fatty acids (FFAs) reflects dietary fat intake (10)**.** It follows that any beneficial or harmful effects of individual plasma FFAs on insulin sensitivity should be modifiable by diet. Initial results from the RISCK study indicated that iso-energetic replacement of dietary saturated fat with monounsaturated fat had no effect on insulin sensitivity (11). However it has not been considered whether changes in the proportion of individual plasma saturated fatty acids (SFA) and monounsaturated fatty acids (MUFA) were associated with changes in Si. We aimed to investigate whether changes in the plasma FFAcomposition, as a result of the dietary interventions implemented in the RISCK study, correspond to changes in insulin sensitivity.

Method

RISCK was a large randomised controlled dietary intervention study conducted across five leading U.K. nutritional research centres (Reading, Imperial, Surrey, Cambridge and Kings). The study was performed in free-living participants at elevated risk of metabolic disease, and assessed the effect of modifying type of dietary fat and the glycaemic index of carbohydrate on metabolic risk factors. The study design has been described in full elsewhere (8,11).

Subjects

Men and women aged 30-70 who met selection criteria (supplementary table 2) were recruited from the general population. Participants were screened to identify individuals at increased metabolic risk but below a level warranting clinical intervention.

Study design

720 participants were enrolled and randomised to five dietary intervention groups by a computerised system designed to balance age, sex, waist measurement and HDL cholesterol between groups (supplementary table 1). 548 participants completed the study (23.9% dropout rate). The study provided isoenergetic dietary substitutions for fat and carbohydrate rich foods while allowing subjects to eat *ad libitum*. Key exchangeable sources of dietary fat (cooking oils, spreads and margarine) and carbohydrates (e.g. pasta, rice, bread, cereal) were substituted for study foods with measured FA profiles and glycaemic indices. In order that diets were isocaloric, carbohydrate levels were modified to ensure the changes to fat intake were balanced by carbohydrate intake.

All subjects received the high-SFA and high glycaemic index (HS/HGI) reference diet for a four-week run-in period, which represented the average UK diet (~18% saturated fat; GI of 63). After a run-in period baseline measurements were performed and one of the five trial diets prescribed for 24-weeks. The food

exchange model successfully achieved the targets for dietary fat and carbohydrate in each of the five diets(9).

At the beginning and end of the dietary intervention baseline characteristics were assessed. Fasting blood samples were collected for plasma FFA profiles and a short intravenous glucose tolerance test (IVGTT) performed from which insulin sensitivity index (Si) was derived via minimal modelling. FFA concentrations were assessed with an enzymatic colorimetry assay.

Data analysis

Statistical analysis was performed with IBM SPSS statistics software. Correlations are presented as Pearsons's *r* values along with corresponding P-values. Si results were assigned standardized values. Results with z-score > ±3.3 were defined as outliers (12) and removed from analysis.

Bivariate correlations of baseline (post run-in period) total plasma FFA concentration, total saturated, unsaturated, monounsaturated), polyunsaturated (PUFA), omega-3 (n-3) PUFA, and omega-6 (n-6) PUFA and Si were assessed for the whole cohort and within individual treatment groups.

Previous work has confirmed that compared to the other intervention groups, RISCK study subjects who consumed high MUFA (HM) diets had significantly elevated plasma MUFA levels following the intervention (9). The association between change in the percentage of the total FFA pool comprising of total MUFAs and change in Si was assessed both for all subjects receiving HM diets and for the two individual HM sub-groups (high monounsaturated fat, high GI [HM/HGI] and high monounsaturated fat, low GI [HM/LGI]). The association between change in percentage of individual MUFAs and change in Si was also assessed within this group. Partial correlations were performed to adjust for change in GI between run-in and post intervention to assess the unique effect of the change in MUFA concentration on Si. Additionally linear regression was performed to adjust for age, sex, ethnicity and for % change in body mass across the study period. β co-efficients, where reported, are standardised. When changes in individual fat subtypes

have been assessed against change in Si, changes in other subgroups were controlled for except for where the variables were excessively co-linear.

Results

Subjects

Of the 549 participants who completed the RISCK study, IVGTT results and FFA profiles were available for 533. Subjects' baseline characteristics are shown in table 1.

Total plasma FFA concentration at baseline was inversely correlated with Si (r= -0.108, P= 0.013). Total saturated FFA was also inversely associated with Si (r=-0.106, P=0.014) as were total unsaturated FFA (r=-0.088, P=0.042), total PUFA (r=-0.098, P=0.041) and n-6 PUFA (r=-0.093, P=0.032). There was no association between total MUFA or n-3 PUFA and Si.

Change in total plasma FFA concentration across the study period within the whole cohort was inversely associated with Si (r=-0.152, P=0.001). In the high saturated fat (SF) group, changes in SFA ($\%FFA_{total}$) was not associated with a change in Si. However in high MUFA groups, increases in % plasma MUFA was associated with positive changes in Si (r=0.218, P=0.001). This remained significant when adjusting for change in dietary GI (r=0.212, P=0.003). When a linear regression model adjusting for age, sex, ethnicity and change in body weight was applied this remained significant (β=0.233, P=0.001). When high MUFA groups were separated into HM/HGI and HM/LGI groups the association with increasing Si was only significant in the low GI group (r=0.297, P=0.002). After controlling for change in GI, this association remained significant (r=0.303, P=0.002). The relationship also remained after adjustment for age, sex, ethnicity and change in body weight (β=0.321, P=0.001)

In the HM groups, change in oleic (18:1) was most strongly positively correlated with change in Si (r=0.204, P=0.003), as was change in minor plasma MUFAs 22:1 (r=0.180, P=0.008) 18:1 (r=0.150, P=0.035) and 17:1 (r=0.145, P=0.035). Similarly in the HM/LGI group, change in oleic acid (18:1) was

most strongly correlated with change in Si (r=0.266, P=0.005), as was 22:1 (r=0.244, P=0.011) and 17:1 (r=0.196, P=0.042). These associations remained significant after adjustment for change in GI.

No association was observed between change palmitoleic acid and change in Si when HM groups were considered together or individually. It may also be noted that within HM groups, change in total SFA and PUFA (%total FFA) were positively associated with change in Si (r=0.184, P=0.007; r=0.208, P=0.002 respectively). However, the positive relationship between percentage change in MUFA (%total FFA) and change in Si remained when the effects of change in SFA (%total FFA) was controlled for (table 2). The effect of changes in PUFA concentrations of Si could not be accurately controlled for due to excessive co-linearity with MUFA concentration.

Discussion

Original analysis of data from the RISCK study indicated that replacement of dietary saturated fat with monounsaturated fat as a result of dietary intervention did not result in a significant improvement in insulin sensitivity (11). However it has not previously been considered how changes in biochemical markers of adherence to the dietary interventions, such as changes in the proportion of FFA subclasses, may correspond to changes in insulin sensitivity. Our re-assessment of the RISCK dataset suggest that increasing the proportion of the plasma fatty acid pool comprising monounsaturated fatty acids (most notably oleic acid) following adherence to a high MUFA dietary intervention may be associated with a modest improvement in insulin sensitivity.

There is conflicting evidence from randomised controlled trials on the effect of dietary fat composition on Si, and the potential benefits of high MUFA diets. The KANWU study, a 3-month dietary intervention study similar in design to RISCK, noted improvements in Si following a reduction in the proportion of dietary saturated FFA and a corresponding increases in MUFA (5). A finding replicated by a smaller intervention study that compared the effects of a high MUFA diet and high SF diet on Si (4) but not by the original results of the RISCK study **or** another large pan-European dietary intervention study, LIPGENE, that reported no effect on Si following isoenergetic replacement of high SF diets with high MUFA or low fat/high carbohydrate diets (6). . This highlights the current uncertainty regarding the optimal dietary strategy for T2DM prevention.

Previous studies have suggested a link between high saturated fat diets (and serum SFA content) and increased progression to impaired fasting glycaemia and T2DM (13 14) . However there is also evidence that individual SFAs differentially influence insulin sensitivity (ref7). A recent large scale cohort study found that individual SFAs were associated both positively and negatively with incidence of T2DM (Forouhi et al). This further suggests it may be an oversimplication to consider an entire FA subclass as protective or deleterious with respect to T2DM risk and demonstrates the importance of considering the individual effects of FFAs. At baseline there was a weak inverse association between total saturated fat

concentration and Si, consistent with the literature. While there was an inverse correlation between baseline total omega-6 PUFA and Si in the RISCK dataset this finding is less consistent with the literature that suggests a diet lacking essential n-6 PUFAs such as linoleic acid may promote insulin resistance (15) . Dietary intervention studies with sustained high PUFA consumption are required to establish the metabolic effects of PUFA on Si.

The fact that total, saturated and unsaturated FFA concentration at baseline were inversely correlated suggests that, regardless of fat type, if total consumption (and therefore plasma fat concentration) increases beyond a point, insulin sensitivity deteriorates, as is the consensus view. Interestingly, the KANWU study only reported an improvement in insulin sensitivity following substitution of SF for MUFA when fat consumption contributed <37% of total energy intake (5). This would imply that provided fat consumption is kept within recommended limits, manipulation of dietary fat type to ensure higher proportions of fat subgroups, such as MUFAs, may benefit insulin sensitivity. Modification of dietary fat quality as opposed to quantity may be a more achievable strategy at a public health level given that current efforts aimed at reducing total fat consumption have been widely unsuccessful.

The optimal dietary strategy for prevention of T2DM with regard to fat consumption remains uncertain. Our data suggests a modest benefit of increasing the proportion of dietary monounsaturated fat (most significantly the major MUFA, oleic acid) on insulin sensitivity. Longer-term interventions would be required to assess the absolute risk reductions for T2DM in populations adhering to this characteristically 'Mediterranean' dietary model, replete in MUFA sources such as olive oil. On the basis of current evidence however, dietary recommendations for T2DM prevention are likely to mirror those for cardiovascular disease prevention; reducing total saturated fat intake to <10% and substituting saturated fat sources for unsaturated fats where possible (16) .

The strength of this data is that changes in Si have been assessed against changing FFA levels following a long (6 month) intervention period. A limitation of this analysis is that causality cannot be assumed on the basis of correlation. Furthermore data on physical activity was unavailable; hence the effects of exercise on

FFA levels could not be adjusted for. Since the study was conducted solely in individuals at elevated metabolic risk generalizability is limited. However the effects of FFAs and dietary modification in T2DM prevention are most pertinent in this high-risk group.

Progression from euglycemia to T2DM is usually a gradual process over time of β-cell dysfunction and of declining insulin sensitivity. Small dietary changes over many years may confer substantial benefit in slowing this progression. At a population level, substituting 'high risk' sources of dietary fat for those that may have a small protective effect, such as monounsaturated fatty acids, may help to combat the rapid rise in T2DM prevalence.

Table 1

Characteristic	**M** ***(n=227)***	**F** ***(n=306)***
Age (y)	53.1 (10.3)	51.9 (9.5)
Ethnicity [n (%)]		
White	187 (87.2)	236 (77.1)
South/SE Asian	21 (9.3)	31 (10.1)
Black	12 (5.3)	28 (9.2)
Far East	1 (0.4)	2 (0.7)
Other	4 (1.8)	7 (2.3)
BMI (kg/m^2)	28.5 (3.8)	28.8 (5.3)
Waist (cm)	102.2 (10.4)	94.2 (12.1)
Average Insulin (pmol/L)	68.4 (32.0)	63.1 (29.5)
Average Glucose (mmol/L)	5.7 (0.5)	5.5 (0.6)
Insulin sensitivity ($x10^{-4}$ $mL \cdot \mu U^{-1} \cdot min^{-1}$)[4]	2.9 (1.9)	3.3 (2.1)
AIRg ($mL \cdot \mu U^{-1} \cdot min^{-1}$)[7]	489.4 (364.5)	466.5 (339.5)
Systolic Blood Pressure (mmHg)	134.3 (15.1)	125.5 (15.3)
Diastolic Blood Pressure (mmHg)	82.5 (9.0)	77.4 (9.0)
Total Cholesterol (mmol/L)	5.5 (0 .9)	5.6 (1.0)
HDL Cholesterol (mmol/L)	1.3 (0.3)	1.5 (0.4)
Triglyceride (mmol/L)	1.7 (0.8)	1.4 (0.6)
Smokers [n (%)]	17 (7.5)	16 (5.2)
Anti-hypertensive medication [n (%)]	44 (19.4)	51 (16.7)
HRT [n (%)]	-	34 (11.1)
Oral contraceptive [n (%)]	-	10 (3.3)
Thyroxine [n (%)]	2 (0.9)	22 (7.2)

Table 2 – linear regression models

Group	Dependent variable	Independent variable	Control variables	β coefficient	Standard error	Standardized *β coefficient*	R^2	R^2change	P-value
HM	Si	Change in MUFA % total FFA	Age, sex, ethnicity, change in bodyweight	0.040	0.012	0.233	0.041	0.054	0.001
HM/LGI	Si	Change in MUFA % total FFA	Age, sex, ethnicity, change in bodyweight	0.059	0.017	0.321	0.094	0.099	0.001
HM	Si	Change in MUFA (% total FFA)	Change in SFA (% total FFA)	0.085	0.036	0.475	0.034	0.025	0.020

Supplementary table 1 – Dietary regimes and targets for fat and carbohydrate consumption as a percentage of total energy intake.

Diet	Fat (% of total energy; M/F)	SFA (% of total energy; M/F)	MUFA (% of total energy; M/F)	PUFA (% of total energy; M/F)	Carbohydrate (% of total energy; M/F)
HS/HGI	38.6/39.3	17.8/18.5	12.3/12.4	5.4/5.7	40.7/43.5
HM/HGI	38.8/38.4	11.0/11.0	20.0/19.5	5.2/5.6	40.6/44.2
HM/LGI	38.8/38.4	11.0/11.0	20.0/19.5	5.2/5.6	40.6/44.2
LF/HGI	26.2/27.2	9.3/9.4	9.8/10.3	4.4/5.1	49.7/52.4
LF/LGI	26.2/27.2	9.3/9.4	9.8/10.3	4.4/5.1	49.7/52.4

The 5 dietary regimens were as follows: high saturated FA and high glycaemic index (HS/HGI; the reference diet), high monounsaturated fatty acid (MUFA) and high GI (HM/HGI), high MUFA and low GI (HM/LGI), low fat and high GI (LF/HGI), and low fat and low GI (LF/LGI). Levels of fat and carbohydrate as a percentage of total energy intake achieved by the four intervention diets are shown. The target for fat as a percentage of total energy intake was 38% in both high SF and high MUFA diets and 28% in low fat diets. Target carbohydrate intake was 55% in low fat diets compared to 45% in the high SF and high MUFA diets. The four intervention diets aimed to reduce saturated fat intake to 10% of total energy intake. Desired MUFA intake in HM diets was 20% and 12% in the LF and HS/HGI (reference) diets.

Supplementary table 2

Point system for selection of subjects at elevated risk of metabolic disease:

Fasting glucose concentration >5.5 mmol/L or insulin concentration >40 pmol/L = 3 points; body mass index (BMI; in kg/m^2) >30 or waist >102 cm for men and >88 cm for women = 2 points; BMI of 25–30 or waist >94 cm for men and >80 cm (women) = 1 point; treated hypertension = 2 points; systolic BP >140 mm Hg = 1 point; diastolic BP >90 mm Hg = 1 point; HDL cholesterol concentration <1.0 mmol/L for men and <1.3 mmol/L for women = 2 points; and serum triacylglycerol concentration >1.3 mmol/L = 1 point (11). A score of ≥ 4 qualified entry into the study.

Exclusion criteria

The exclusion criteria for recruitment of participants to RISCK were as follows: history of ischemic heart disease; 10-year CVD risk >30%; diabetes mellitus, cancer, pancreatitis, cholestatic liver disease, renal disease; use of lipid lowering medication; systemic corticosteroids, androgens, phenytoin, erythromycin, drugs for regulation of haemostasis (excluding aspirin); exposure to an investigational agent ≤30 days prior to the study; gastrointestinal disorders or use of drugs affecting gastrointestinal motility/absorption; history of alcoholism or substance misuse; current or planned pregnancy or birth in past 12 months; allergy to or intolerance of intervention foods; unwillingness to adhere to study protocol or to provide informed consent; weight change of >3kg in the two months before the study; intake of >1g eicosapentanoic acid and docosahexaenoic acid/acids; smoking >20 cigarettes per day (11).

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