

# Serial Metabolic Measurements and Conversion to Type 2 Diabetes in the West of Scotland Coronary Prevention Study

## Specific Elevations in Alanine Aminotransferase and Triglycerides Suggest Hepatic Fat Accumulation as a Potential Contributing Factor

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To examine metabolic changes (lipids, liver enzymes, blood pressure, and weight) potentially associated with conversion to diabetes, we analyzed serial glucose and other metabolic measures obtained every 6 months within the West of Scotland Coronary Prevention Study trial. Changes in parameters for 86 men who converted to new-onset diabetes ("converters": two consecutive glucose levels  $\geq 7$  mmol/l) were compared with 860 "nonconverters" matched for age and treatment allocation. Eighteen months before the diagnosis, converters to diabetes had elevated ( $P < 0.01$ ) fasting glucose, weight, triglyceride, alanine aminotransferase (ALT), blood pressure, and white cell count and lower HDL cholesterol compared with nonconverters. The mean (SD) increase in fasting glucose over 18 months in converters was 1.80 (1.52) mmol/l, compared with 0.10 (0.57) in nonconverters. Of parameters measured, only ALT ( $P = 0.0005$ ) and triglyceride ( $P = 0.030$ ) increased significantly more over the 18 months in converters compared with nonconverters, but neither parameter increased significantly in nonconverters with high baseline glucose concentrations ( $>6.1$  mmol/l). Finally, only sustained increases in ALT predicted a higher risk for diabetes. We conclude that a relatively rapid rise in fasting glucose levels is frequent in converters to diabetes and that associated increases over time in ALT and potentially triglyceride suggest hepatic fat accumulation as a contributing factor for conversion to diabetes in men at risk. *Diabetes* 56:984–991, 2007

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ADA, American Diabetes Association; ALT, alanine aminotransferase; AST, aspartate aminotransferase; OGTT, oral glucose tolerance testing; SBP, systolic blood pressure; WOSCOPS, West of Scotland Coronary Prevention Study.

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The pattern of change in glucose concentrations before development of type 2 diabetes and associated precipitating factors have been the subject of recent studies. For example, elegant analyses by Ferrannini et al. (1) from the Mexico City diabetes study suggest that within a 3.25-year time frame, the onset of diabetes may very often be relatively rapid rather than gradual and can be partly explained by a fall in glucose-stimulated insulin response. In other words, in subjects at elevated risk of developing diabetes, i.e., obese insulin-resistant individuals, conversion to diabetes may take the form of rapid increment in glucose levels as a result of relatively small loss of  $\beta$ -cell function. Interestingly, although BMI was higher in the latter study, it did not change more rapidly in converters to diabetes compared with nonconverters (1). These data extended earlier evidence from a small study in Pima Indians where an abrupt rise in postprandial glucose occurred over 2 years from normality to diabetes (2). More recently, Lyssenko et al. (3) also reported deterioration in  $\beta$ -cell function before the onset of type 2 diabetes in a study of 2,115 subjects followed a median of 6 years apart.

Clearly, further prospective work is needed to confirm and extend such findings. In particular, because of the relatively wide time gap between visits in prior studies, the rapidity of change in glucose could be only broadly estimated. Identifying triggering events in transition to diabetes could be useful in the prevention or reversal of hyperglycemia as indicated by Ferrannini et al. (1). In the West of Scotland Coronary Prevention Study (WOSCOPS), a primary prevention statin study in middle-aged men with hypercholesterolemia, fasting glucose was measured every 6 months for a median follow-up of 4.9 years (4). As a result, we were able to diagnose new-onset diabetes based on the American Diabetes Association (ADA) criteria of two consecutive fasting plasma glucose concentrations  $\geq 7.0$  mmol/l. Such information, in conjunction with extensive baseline measures, has allowed us to link baseline metabolic syndrome status (5), C-reactive protein (6), and

alanine aminotransferase (ALT) levels (7) with subsequent risk for incident diabetes. Others have also shown baseline ALT to associate with incident diabetes (8–11) and metabolic syndrome (12). Clearly, however, the uncommon availability of 6-monthly fasting glucose concentrations for a median of 4.9 years in such a large population of mostly white men, together with concurrently measured fasting lipids, blood pressure, liver enzyme levels, and body weight, presents an excellent opportunity to determine the pattern of development of diabetes and examine potential metabolic triggers. Our hypothesis is that conversion to diabetes may occur more rapidly than previously appreciated and that acute changes in weight, lipids, or liver enzymes might accompany conversion to diabetes.

Finally, we recognize that our study relates to conversion to diabetes based on fasting blood measurements alone and that fasting and after-challenge hyperglycemia may represent phenotypes with distinct natural histories in the evolution of type 2 diabetes (13). Regardless, because fasting glucose concentrations are used to diagnose diabetes in many patients worldwide, our results could offer important scientific and clinical relevance.

## RESEARCH DESIGN AND METHODS

**Sampling.** The WOSCOPS included 6,595 men (4). Sixty-nine men were known to be diabetic at baseline, and 21 were of unknown status; these 90 men were excluded from subsequent analyses. Two men were excluded because of missing data on age at baseline.

Incident diabetes was defined as two consecutive measurements of fasting glucose  $\geq 7$  mmol/l (ADA criteria), with the date of incidence taken as the date of the first of these measurements; 179 individuals satisfied this criterion. Of these, 81 case subjects were excluded because their date of incidence was within 12 months of randomization, with 45 having high fasting glucoses at baseline, and a further 12 were excluded because of missing data on at least one of the metabolic factors of interest (other than white cell count, for which samples were analyzed infrequently). This left 86 case subjects of incident diabetes by these criteria, for which complete metabolic data were available for the previous 18 months (the incident visit plus three previous visits). By this approach, we attempted to identify individuals that had genuinely converted to diabetes from a preceding nondiabetic state, thus minimizing the potential for residual confounding. Moreover, the 18-month time period allowed us to characterize the patterns of change in metabolic parameters more fully. For each case subject defined as above, 10 control subjects were sampled by computer at random from those available and matched to be in the same randomized group (statin or placebo) and to have had an age at baseline within 1 year of the case subject. Available control subjects must have had all metabolic factors measured at the visit of incidence of the case subject and at the three previous visits and must have had a glucose measurement taken at a visit after the visit of incidence for the associated case subject. Control subjects were therefore selected in such a way that they had sufficient data to have been classified as case subjects if their glucose measurements had satisfied the criteria for being a case subject.

**Laboratory methods.** Glucose was measured according to an automated enzymatic method, and plasma lipids and lipoproteins were measured according to the protocols of the lipid research clinics as described in detail previously (4). Blood counts, including white blood cell count, were performed with a Coulter STKR or S+1 automated cell counter. Liver enzymes were measured on fresh samples as described previously (7). Briefly, ALT and aspartate aminotransferase (AST) were determined on fresh samples using standard reagents by reaction rate assay based on the conversion of NADH to NAD. All AST and ALT analyses were conducted in the same laboratory with adherence to external quality control. The between-batch coefficient of variation for their determination was  $< 5\%$ .

**Statistics.** Statistical analyses were carried out using S-Plus for Windows v7.0. Baseline (i.e., at randomization) characteristics of case and control subjects are reported. The distribution of glucose levels relative to the 90th percentile of the glucose distribution among control subjects is provided. Mean (SD) values of the absolute changes in metabolic factors over the 18-month period of study were calculated for case and control subjects and are reported for illustrative purposes.

Two main analyses were applied. The first, using linear regression models, looked at patterns of glucose and other metabolic factors in case and control

subjects during the 18 months preceding the date of diagnosis of the case subjects. The second, using logistic regression, looked for associations between changes in metabolic factors during those preceding 18 months and the probability of becoming a case subject.

For the first analysis, linear mixed-effects models were applied to each metabolic factor over all time points, including random effects for each case-control cluster and each individual within clusters. Because of their skewed distributions, glucose, ALT, AST, total and HDL cholesterol, triglycerides, and white cell count were analyzed on a logarithmic scale, with estimated values transformed back to the original scale. All other metabolic factors were analyzed on their original scales. We report the results from two such models, including the following fixed effects. Model 1: a linear time effect, a case effect, and a time-by-case interaction; and model 2: a nonlinear time effect with separate case effects at each time point (i.e., separate mean values for case and control subjects at each time point).

The predicted values, with 95% CIs, from model 2 are tabulated and are shown graphically for selected variables. *P* values are reported for the case-by-time interaction effects from the two models. Within model 1, this highlights factors that change differentially, although in a smooth fashion, in the lead up to diagnosis among converters to diabetes compared with control subjects. Under model 2, this tests whether the patterns of change are arbitrarily different for case and control subjects and may detect more sudden changes in risk factors during the 18 months before diagnosis.

The second analysis applied conditional logistic regression models, with being a case subject as the response variable and conditioning on each case-control cluster. For each metabolic factor measured at the time of diagnosis and the three preceding visits, the differences between consecutive measurements were calculated (factors with skewed distributions were first transformed by taking logarithms, so that changes correspond to log ratios). Another series of models was fitted, including the following predictor variables. Model 3a: the change in metabolic factor between 18 and 12 months before diagnosis; model 3b: the change in metabolic factor between 12 and 6 months before diagnosis; model 3c: the change in metabolic factor between 6 months before diagnosis and the time of diagnosis; and model 4: all three of the above changes in the metabolic factor.

These models were used to estimate the odds ratio (OR) for being a case subject between an individual with a specified increase in the metabolic factor, relative to an individual with no change over the same period. Models 3a–3c estimate the ORs associated with increases at each time point univariately, without reference to changes over other time periods; model 4 estimates the same ORs, but estimates for each time period are controlled for changes in the metabolic factor during other periods. For those metabolic factors where evidence of an association with the incidence of diabetes was found, we report the estimated ORs graphically, with 95% CIs.

## RESULTS

Thirty-nine case subjects and 390 control subjects (45.3%) were randomized to the pravastatin group with the remainder being allocated to receive placebo. Both groups had a mean (SD) age of 55.4 (5.7) years. Forty-three case subjects (50.0%) and 499 control subjects (58.0%) were smokers at baseline ( $\chi^2$  test;  $P = 0.19$ ).

**Differences before diagnosis.** Table 1 shows the predicted values for each metabolic factor at each time point with 95% CIs, and *P* values testing for case-control differences in general and case-by-time interactions, allowing for either linear or nonlinear time effects. It is notable that compared with nonconverters, men destined to develop diabetes (converters) had significantly higher weight, fasting glucose, triglycerides, systolic blood pressure (SBP), diastolic blood pressure, white cell count, and ALT, whereas HDL cholesterol was significantly lower during the 18 months before their diabetes diagnosis date (all  $P < 0.0001$ ). Total cholesterol was somewhat less elevated in converters ( $P = 0.031$ ), whereas albumin and AST levels were not different.

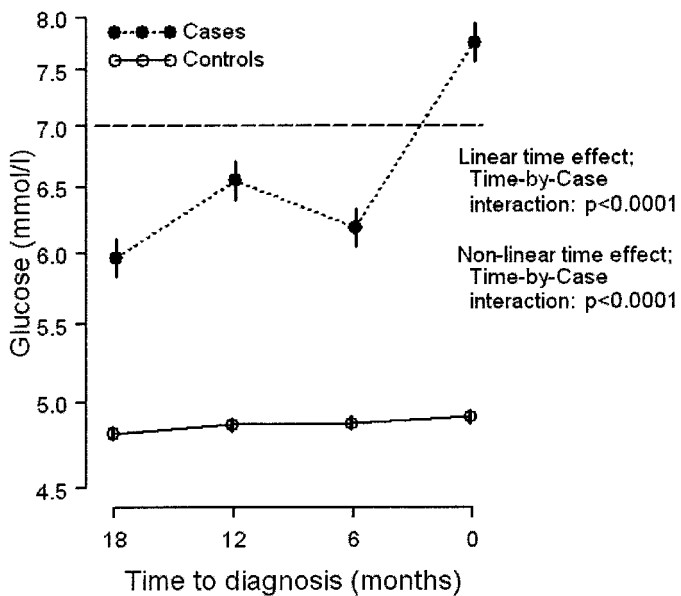
**Pattern of change in fasting glucose concentration.** Fasting glucose rose rapidly over the 18 months in converters by a mean of  $\sim 1.80$  mmol/l in converters compared with only 0.10 mmol/l in nonconverters. In fact, 86% of converters demonstrated a rise in fasting glucose above the 90th percentile from control subjects. Although the

TABLE 1  
Change in metabolic parameters over time

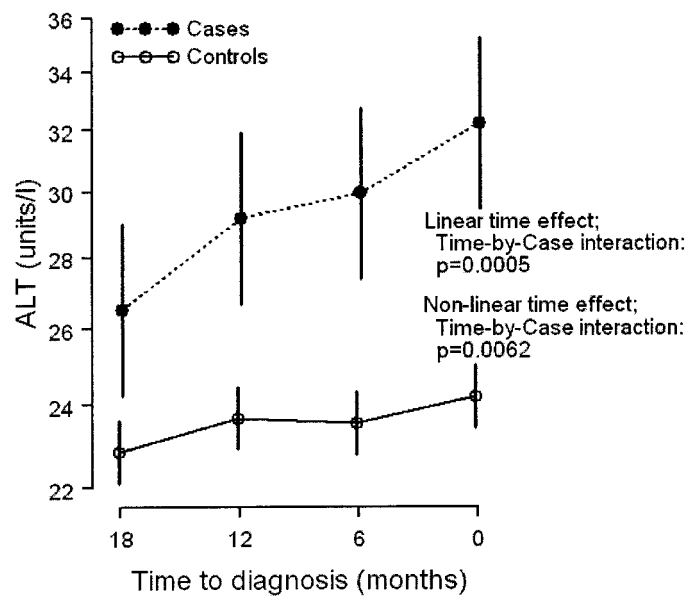
	Predicted value (95% CI) at each time point			Diabetes incidence	Case-control difference ( <i>P</i> )	Case-by-time interaction ( <i>P</i> )	
	-18 months	-12 months	-6 months			Linear time effect	Nonlinear time effect
Glucose (mmol/l)							
Control	4.81 (4.78-4.85)	4.86 (4.83-4.90)	4.88 (4.84-4.91)	4.91 (4.87-4.95)	<0.0001	<0.0001	<0.0001
Case	5.96 (5.83-6.10)	6.55 (6.40-6.70)	6.18 (6.04-6.33)	7.76 (7.58-7.94)			
ALT (units/l)							
Control	22.9 (22.1-23.6)	23.7 (22.9-24.5)	23.5 (22.8-24.3)	24.2 (23.4-25.0)	<0.0001	0.0005	0.0062
Case	26.5 (24.2-29.0)	29.2 (26.7-31.9)	29.9 (27.4-32.7)	32.2 (29.5-35.2)			
AST (units/l)							
Control	22.53 (22.09-22.98)	22.77 (22.32-23.22)	22.84 (22.40-23.30)	23.16 (22.71-23.62)	0.30	0.14	0.41
Case	22.44 (21.08-23.89)	23.51 (22.09-25.02)	23.82 (22.38-25.35)	24.17 (22.71-25.73)			
Cholesterol (mmol/l)							
Control	6.42 (6.27-6.59)	6.22 (6.07-6.38)	6.21 (6.06-6.37)	6.22 (6.06-6.38)	0.031	0.31	0.63
Case	6.52 (6.29-6.76)	6.41 (6.18-6.64)	6.40 (6.17-6.63)	6.40 (6.18-6.64)			
Triglycerides (mmol/l)							
Control	1.64 (1.58-1.69)	1.58 (1.52-1.63)	1.61 (1.55-1.66)	1.58 (1.53-1.64)	<0.0001	0.030	0.011
Case	2.21 (2.01-2.44)	2.26 (2.05-2.49)	2.16 (1.96-2.39)	2.39 (2.17-2.64)			
HDL cholesterol (nmol/l)							
Control	1.18 (1.16-1.20)	1.18 (1.16-1.20)	1.17 (1.15-1.18)	1.17 (1.15-1.19)	0.0001	0.65	0.95
Case	1.07 (1.02-1.13)	1.07 (1.02-1.13)	1.06 (1.01-1.11)	1.06 (1.01-1.11)			
Weight (kg)							
Control	76.6 (75.8-77.5)	77.0 (76.2-77.9)	77.3 (76.5-78.1)	77.5 (76.7-78.4)	<0.0001	0.31	0.39
Case	84.7 (82.3-87.1)	85.5 (83.2-87.9)	85.4 (83.1-87.8)	86.1 (83.7-88.4)			
SBP (mmHg)							
Control	132.2 (131.0-133.5)	131.8 (130.6-133.1)	133.1 (131.8-134.3)	132.7 (131.5-134.0)	0.0001	0.68	0.54
Case	137.0 (133.7-140.4)	139.1 (135.7-142.5)	139.0 (135.6-142.4)	138.8 (135.4-142.1)			
DBP (mmHg)							
Control	82.8 (82.2-83.4)	82.7 (82.1-83.3)	83.0 (82.4-83.6)	82.9 (82.3-83.5)	<0.0001	0.41	0.22
Case	85.4 (83.5-87.3)	86.9 (85.0-88.8)	85.6 (83.7-87.5)	86.9 (85.0-88.8)			
Albumin (g/l)							
Control	45.5 (45.3-45.7)	45.4 (45.2-45.6)	45.7 (45.5-45.9)	45.9 (45.7-46.1)	0.57	0.54	0.80
Case	45.4 (44.8-46.0)	45.6 (45.0-46.2)	45.9 (45.3-46.5)	46.0 (45.4-46.6)			
WCC (10 <sup>9</sup> /l)							
Control	6.27 (6.15-6.39); <i>n</i> = 637	6.22 (6.10-6.34); <i>n</i> = 616	6.26 (6.14-6.39); <i>n</i> = 506	6.27 (6.14-6.40); <i>n</i> = 421	<0.0001	0.82	0.86
Case	7.01 (6.60-7.45); <i>n</i> = 61	6.96 (6.55-7.39); <i>n</i> = 59	6.92 (6.50-7.38); <i>n</i> = 51	7.14 (6.69-7.63); <i>n</i> = 44			

Data are predicted values (95% CI) for each outcome variable in case subjects and control participants at each time point from mixed effects models allowing for random cluster and individual effects, with *P* values testing for case-by-time interactions using both linear and nonlinear time effects. Overall case-control difference in each parameter taking account of all time points is also presented. Note: numbers incomplete only for WCC; for all other factors, *n* = 86 for case subjects and 860 for control subjects. DBP, diastolic blood pressure; WCC, white cell count.

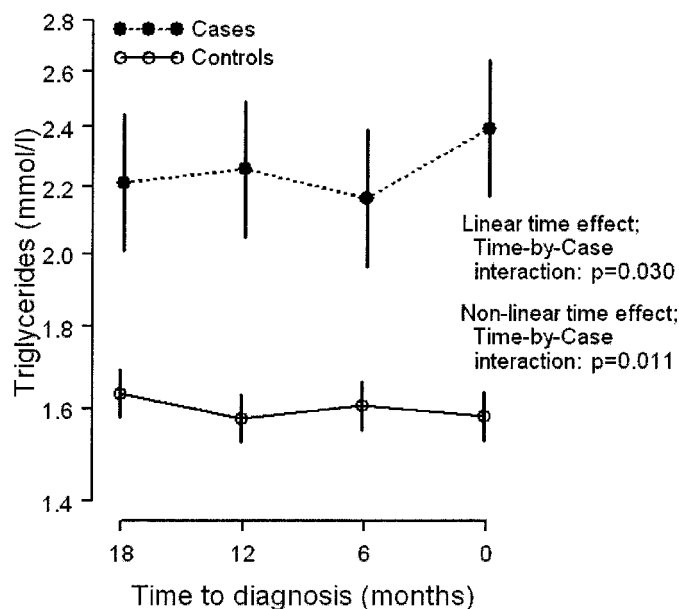
**A** Model predicted glucose with 95% CI in diabetic cases and controls



**B** Model predicted ALT with 95% CI in diabetic cases and controls



**C** Model predicted triglycerides with 95% CI in diabetic cases and controls



**FIG 1.** Predicted values with 95% CIs for glucose (A), ALT (B), and triglyceride (C) levels (shown on log-transferred axes) in case and control participants at each time point from mixed effects models allowing for random individual effect.

pattern of glucose change seems to include an apparent “rise” between 18 and 12 months before the development of diabetes, before a subsequent fall to 6 months, this is an artifact of the diagnostic criteria because the  $-6$  month value is constrained by having to be below 7 mmol/l, whereas the value at  $-12$  months is not. For interest, the mean glucose concentration at 24 months before diagnoses (6 months before the data presented in tables and figures) for those converters in whom this measure was available was 6.02 mmol/l. Thus, the pattern of data suggest that fasting glucose does rise abruptly over an 18-month period in those destined to be diagnosed as

having diabetes on the basis of the ADA criteria. The relatively minor slope of increment in mean glucose concentrations for nonconverters is striking in comparison with the change observed in converters (Fig. 1).

**Identifying potential triggering factors for rise in glucose concentration.** We were interested to examine whether changes in either weight or any other measured metabolic factor was significantly greater in converters to diabetes versus nonconverters. Data in Table 1 and Fig. 1 suggest that of all factors measured over time in WOSCOPS, changes in ALT ( $P = 0.0005$  linear model or 0.0062 nonlinear model) and to a lesser degree in trigly-

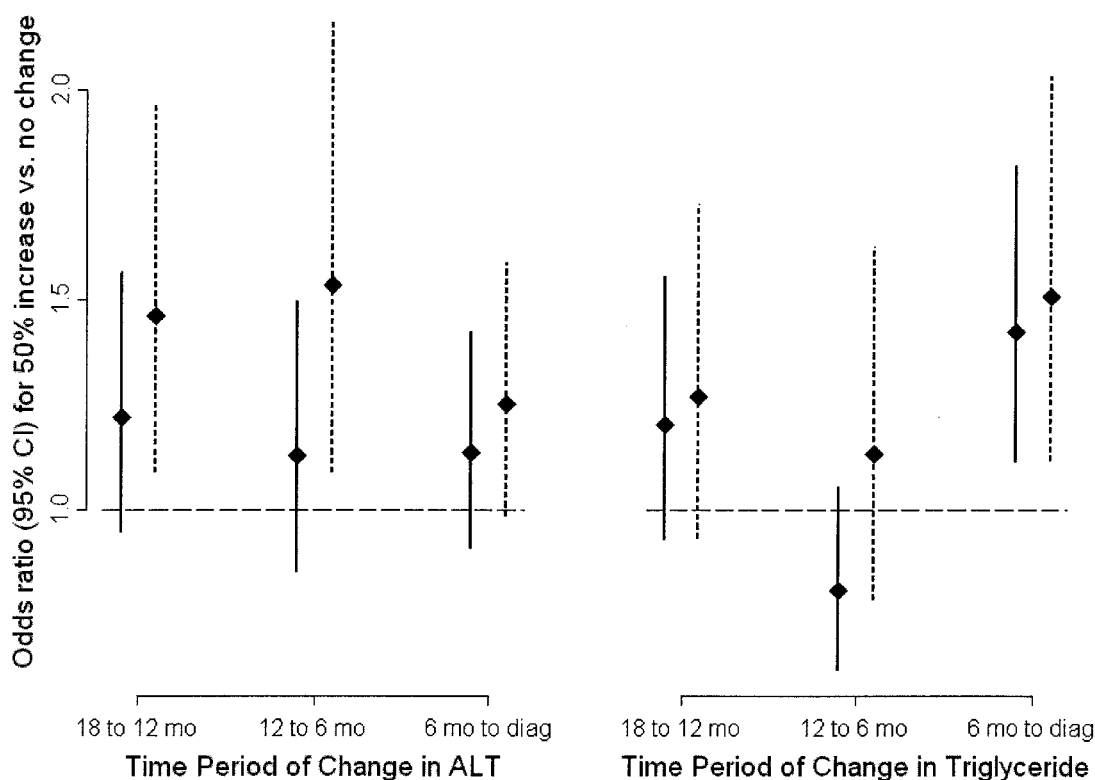


FIG 2. Estimated ORs with 95% CIs associated with 50% increases in ALT or triglyceride levels between 18 and 12 months prior to diagnosis, between 12 and 6 months prior to diagnosis, and between 6 months prior to and at diagnosis; estimates from univariate (solid lines) and multivariate (dashed lines) models.

eride concentration ( $P = 0.030$  linear model or  $0.011$  nonlinear model) were more marked in converters than nonconverters. Other parameters showed significant trends over time and/or differences between converters and nonconverters, although none of these factors (all  $P > 0.10$ ) demonstrated different trends over time between the two groups. In particular, weight, AST, SBP, and HDL cholesterol did not change more rapidly in men who converted compared with those who did not convert to diabetes (Table 1). Moreover, although numbers were smaller for white cell count, this parameter also did not seem to change more rapidly in converters versus nonconverters. **Predicting risk of incident diabetes from magnitude of change in parameters between each 6-month period before date of incident diabetes.** We estimated the ORs for incident diabetes associated with changes in metabolic factors measured at consecutive time points up to the time of diagnosis, both with and without adjustment for changes during other periods. Only changes in fasting glucose, ALT, and triglycerides showed any association with risk for incident diabetes (Fig. 2 shows data for ALT and triglyceride). Changes between any two time points in all of the other parameters (weight, blood pressure, white cell count, AST, cholesterol, or HDL cholesterol) were not significantly associated with development of diabetes.

Univariately, changes in ALT are not associated with diabetes risk, but in the multivariate model, greater increases in ALT are associated with increased risk of diabetes. This is a consequence of the negative correlation between consecutive changes in ALT, i.e., the correlation between the  $-18$ - to  $-12$ -month change in ALT and the  $-12$ - to  $-6$ -month change is  $-0.41$ ; the correlation between the changes from  $-12$  and  $-6$  months and from  $-6$

months to diagnosis is  $-0.52$ . A large increase in ALT between  $-18$  and  $-12$  months is often followed by a reduction, so an isolated increase in ALT over this time period is therefore not necessarily associated with the risk of diabetes 12 months later. The multivariate model, however, estimates the effect of changes in ALT between  $-18$  and  $-12$  months of diagnosis, while controlling for subsequent changes. According to this model, if the changes in ALT for two individuals are the same between  $-12$  months and the time of diagnosis, a 50% greater increase in ALT between  $-18$  and  $-12$  months corresponds to an OR for developing diabetes of  $1.5$  (95% CI  $1.1-2.0$ ),  $P = 0.011$ . Similarly, if all other relative changes in ALT are the same, a 50% greater increase between  $-12$  and  $-6$  months corresponds with an OR for becoming diabetic of  $1.5$  ( $1.1-2.1$ ),  $P = 0.014$ . In other words, if ALT increases and stays high, then there is an association with diabetes risk.

For changes in triglyceride levels, a different pattern of association with incident diabetes is observed. Changes prior to 6 months before diagnosis are not found to be associated with incidence, but greater increases during the final 6 months are associated with increased odds of being a case subject under both the univariate and multivariate models, e.g., adjusting for changes prior to 6 months before diagnosis, a 50% greater increase in triglycerides between  $-6$  months and diagnosis corresponds to an OR for being diabetic of  $1.5$  ( $1.1-2.0$ ),  $P = 0.007$ .

**Comparison of changes over time in ALT and triglyceride in converters versus nonconverters with high baseline glucose concentrations.** Twenty-two of the 860 control subjects (2.6%) had a glucose level  $>6.1$  mmol/l at 18 months before the date of diagnosis of their corre-

sponding case subject. The geometric mean ALT levels of the case subjects and the "high-glucose" control subjects at 18 months before diagnosis were 26.5 and 24.9 units/l, respectively, an estimated case-to-control subject ratio of 1.1 (95% CI 0.9–1.3,  $P = 0.51$ ). At the time of diagnosis, the geometric mean ALT levels were 32.2 and 22.7 units/l, an estimated case-to-control subject ratio of 1.4 (1.1–1.8,  $P = 0.0024$ ). Similarly, the geometric mean triglyceride levels in case subjects and high-glucose control subjects at 18 months before diagnosis were 2.21 and 1.94 mmol/l, an estimated case-to-control subject ratio of 1.1 (0.9–1.5,  $P = 0.28$ ). At diagnosis, the geometric mean triglyceride levels were 2.39 and 1.75 mmol/l, an estimated ratio of 1.4 (1.1–1.8,  $P = 0.015$ ). These data confirm a significant increase in ALT and triglyceride only in converters versus nonconverters despite similar baseline glucose concentrations in both groups.

## DISCUSSION

On the basis of serial 6-monthly fasting glucose measurements in a large population, development of diabetes proceeded rapidly within an 18-month time period in middle-aged white men at elevated risk. Glucose concentrations underwent a change of 1.80 mmol/l from a mean of 5.96–7.76 mmol/l over 18 months immediately preceding the development of diabetes. By contrast, in men who do not convert, fasting glucose increased an average of 0.06 mmol/l per year, in keeping with Mexico City Study data. More importantly, our serial data suggest that a large change in glucose concentrations in converters to diabetes is associated with specific but minor increases in triglyceride in the 6 months before diagnosis and more strongly to a sustained increase in ALT concentrations over the prior 18 months. By contrast, although weight, systolic and diastolic blood pressure, and white cell count were higher 18 months before conversion to diabetes in converters, subsequent changes in such parameters were not significantly greater in converters relative to nonconverters. Finally, in further support of a link between conversion to diabetes and specific changes in ALT and triglyceride concentrations, ALT and triglyceride concentrations did not increase in the subgroup of nonconverters ( $n = 22$ ) who had elevated baseline glucose concentrations ( $>6.1$  mmol/l). Given that ALT and triglyceride levels even within the normal range correlate with increasing hepatic fat content (14,15), our observations are consistent with hepatic fat accumulation as a potential trigger for the conversion from pre-diabetes to diabetes. In other words, in "metabolically stressed" subjects at elevated risk for diabetes, recognized by higher baseline levels of several known risk factors, continued hepatic fat accumulation may lead to a point of metabolic instability "triggering" a large rise in hepatic glucose synthesis sufficient to be diagnosed with diabetes. The implications of our findings are twofold; first, our results add support for the importance of altered hepatic metabolism, via excess fat accumulation, in the pathogenesis of type 2 diabetes; and second, our data suggest that preventing hepatic fat accumulation may help prevent conversion to diabetes in some individuals at risk. Because previous studies have shown that increased liver fat (as reflected by elevated ALT) predicts the metabolic syndrome (12), it is clear that there is a role for liver fat accumulation earlier and later in the process of insulin resistance and development of diabetes.

It is clear that ALT levels even within the "normal" range

correlate with increasing liver fat (14). Excess fat in liver, via accumulation of total fatty acyl-CoA, impairs insulin-stimulated IRS-1 and IRS-2 tyrosine phosphorylation and ultimately decreases insulin activation of glycogen synthase and promotes increased gluconeogenesis (16). Elevated ALT within the high normal range independently predicts type 2 diabetes (7,11) and metabolic syndrome (12). It is also a principal diagnostic feature of nonalcoholic fatty liver disease (17). ALT levels correlate to measured liver fat in several studies as recently reviewed (9) and are judged to be an acceptable marker of hepatic steatosis for epidemiological studies. The use of ALT in clinical care for this purpose is attracting interest but requires further study.

With respect to lipids, hepatic fat accumulation, potentially arising in part from an excessive supply of fatty acids from visceral adipose tissue, may be the proximate cause of the characteristic atherogenic dyslipidemia seen in type 2 diabetes, specifically by triggering overproduction of large triglyceride-rich VLDL particles (15). In other words, hepatic fat accumulation might account for greater changes in both ALT and triglycerides in converters versus nonconverters to diabetes. In further support for our suggestion, only sustained (but not transient) increases in ALT levels in either of the 6-month periods between –18 and –6 months before diagnosis (multivariate bars in Fig. 2) were associated with incident diabetes.

Our 6-monthly serial measurements of fasting glucose allowed us to estimate the time period required for the emergence of diabetes from a period of metabolic stress, and as such, our work complements information from prior studies. In the Mexico City Study, glucose tolerance measurements were made at 3.25-year intervals or greater; shorter timescales for conversion to diabetes could not be examined (1). Similarly, although data from both the Mexico City Study (1) and the Botnia study (3) suggested a critical role for a loss of  $\beta$ -cell function in the conversion to type 2 diabetes in subjects at risk, it was not possible to rule out other, potentially earlier or co-existent triggering factors, such as continued hepatic fat accumulation, for the conversion to type 2 diabetes. Because of the absence of concurrent measures of insulin (not possible to rapidly separate fasting samples in out-patient center) in our study, we could not determine whether  $\beta$ -cell dysfunction precedes liver fat accumulation or whether the two processes occur simultaneously. Clearly, because suppression of hepatic glucose production is a major regulatory role of insulin, continued hepatic fat accumulation (and thus greater hepatic insulin resistance) allied to impairment of  $\beta$ -cell function (and thus further impediment of hepatic glycogen synthesis) could act synergistically to trigger a large rise in glucose concentrations in subjects at risk for diabetes. Such suggestions require direct examination in future prospective studies. Regardless of the exact relationship between these two related processes, because glucose concentrations in the postabsorptive state (i.e., after overnight fast) are largely dependent on the rate of hepatic glucose output, the role of the liver in conversion to diabetes in subjects at risk, at least on the basis of fasting measurements, is likely critical. Certainly, in animal models, hepatic rather than skeletal muscle insulin resistance has been shown to be primary in development of metabolic syndrome (18). Recent data from the Insulin Resistance Atherosclerosis Study demonstrating greater PAI-1 increments over a 5-year time frame in converters to diabetes compared with nonconverters are also consistent

with our findings (19) because PAI-1 is partially hepatic derived.

**Existing diabetes preventative mechanisms and hepatic fat.** Whatever the exact mechanism for continued hepatic fat accumulation, modalities that can influence hepatic fat accumulation may prevent diabetes in subjects at elevated risk. For example, Petersen et al. (20) noted that moderate weight loss with very low-fat diet normalizes fasting hyperglycemia in patients with poorly controlled type 2 diabetes by mobilizing a relatively small pool of intrahepatic lipid. Similarly, we recently noted that greater physical activity correlated independently to lower liver enzymes (in particular  $\gamma$ -glutamyl transferase), another parameter that correlates with liver fat and predicts type 2 diabetes (21). Finally, increased liver fat content appears also to correlate to dietary fat intake (22) and low-fat and high-fiber diets appear to decrease diabetes risk (23).

With respect to drug treatment, glitazones lower hepatic fat directly and lower liver enzyme levels (24,25), in particular ALT, as observed in the rosiglitazone arm of the recently reported DREAM (Diabetes Reduction Assessment with Ramipril and Rosiglitazone Medication) study (26), whereas metformin has been variably linked to improvements in liver enzymes (27,28). Finally, although ramipril did not significantly reduce progression to diabetes in subjects at risk, it did significantly increase regression to normoglycemia and was associated with a significantly greater reduction in ALT concentrations in the 1st year compared with placebo (29).

**Strengths and limitations.** We acknowledge that the current study represents a post hoc analysis of men with elevated cholesterol and that men with levels of ALT >70 units/l were excluded from WOSCOPS. However, because cholesterol is not generally predictive of diabetes and because others have shown higher ALT levels to predict diabetes (8,10,11), we feel such limitations are not significant concerns. Clearly, our results are not necessarily applicable to women or other ethnic groups. We also acknowledge that the absence of an oral glucose tolerance test (OGTT) is a relative weakness and that some men with a fasting glucose <7 mmol/l may have had 2-h glucose concentrations in the diabetes range. Of interest, Meigs et al. (13) recently demonstrated that fasting hyperglycemia and hyperglycemia after challenge may represent distinct phenotypes in the progression to type 2 diabetes. They also suggested that progression to diabetes is somewhat dependent on diagnostic thresholds. That said, to conduct OGTTs in over 6,000 men every 6 months for 5 years would have been difficult. We also accept that direct measurements of liver fat accumulation were not conducted but rather inferred based on considerable prior data linking elevated ALT (even with the current normal range) and liver fat levels, as reviewed by Yki-Jarvinen (14). However, data herein on triglyceride (albeit of borderline significance, thus requiring confirmation in future studies) and previously for PAI-1 (19) and supportive evidence in animals (18) provide some confidence that hepatic fat accumulation is an important mechanism contributing to conversion to diabetes. We acknowledge that our results are observational data demonstrating only a temporal association between two processes, and therefore no definitive cause and effect relationship can be inferred. Finally, although these results were conducted in the context of a statin trial, and statins can raise transaminases transiently, it is important to note that case and

control subjects were matched on statin allocation and that there was no interaction between treatment allocation and change in ALT over time in case or control subjects (data not shown).

In regard to strengths, although there are many useful prospective studies on potential predictors of diabetes, nearly all have used only baseline measurements. Many such studies have also discussed novel assays often not routinely available in clinical practice. Our study is potentially the first to consider serial changes in a range of routinely available risk markers (lipids, blood pressure, liver enzymes, and weight) for diabetes, and as such, our data are of potential clinical and scientific relevance. The 6-monthly glucose concentrations allowed us to better define rapidity of conversion to diabetes. Although women were not included, the homogenous nature of the cohort (>99% white men) helped avoid potential confounding issues of sex and ethnicity. Moreover, many primary care physicians, at least in the U.K., increasingly use the ADA fasting glucose criteria to diagnose diabetes (two consecutive fasting glucose  $\geq 7.0$  mmol/l), and hence our diagnosis of diabetes is clinically relevant to many practicing physicians. Finally, our statistical analyses were carefully considered with several models presented, with findings consistent throughout.

**Conclusion.** We conclude that a rapid (within 18 months) increase in fasting glucose is frequent in converters to diabetes and that associated specific increases over time in ALT in particular, and possibly triglyceride, in converters versus nonconverters suggest hepatic fat accumulation as a potential triggering event for conversion to diabetes in men at risk.

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