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Plasma Lactate and Diabetes Risk in 8,045 Participants of the Atherosclerosis Risk in Communities (ARIC) Study

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Abstract

Purpose—Determinants of oxidative capacity, such as fitness and level of adiposity, are strongly associated with type 2 diabetes. Whether decreased oxidative capacity itself is a cause or consequence of insulin resistance and diabetes is unknown.

Methods—We examined the association of plasma lactate, a marker of oxidative capacity, with incident diabetes in 8,045 participants from the Atherosclerosis Risk in Communities (ARIC) Study with no history of subclinical or diagnosed diabetes at baseline (1996–1998). Incident diabetes was self-reported during annual telephone calls.

Results—During a median follow-up of 12 years, there were 1,513 new cases of diabetes. In Cox proportional hazards models, baseline plasma lactate (per 10 mg/dL) was significantly associated with diabetes (HR 1.20, 95% CI: 1.01, 1.43), even after adjustment for diabetes risk factors, fasting glucose, and insulin. The upper quartile of baseline lactate (≥ 8.1 mg/dL) was also significantly associated with diabetes risk (HR 1.20, 95% CI: 1.02, 1.41) compared with the lowest quartile (< 5.1 mg/dL). Significant associations persisted among persons without insulin resistance (HOMA-IR < 2.6 units) (*P*-trend < 0.01).

Conclusions—These findings suggest that low oxidative capacity may precede diabetes. Future studies should evaluate the physiologic origins of elevated lactate to better understand its possible role in the pathogenesis of diabetes.

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Conflict of interest

The authors declare that there is no conflict of interest associated with this manuscript.

Keywords

Lactate; Diabetes risk; Cohort; ARIC

Introduction

Mitochondrial dysfunction, or decreased oxidative capacity, is strongly associated with type 2 diabetes [1]. This is supported by evidence of associations between insulin resistance and type 2 diabetes with increased glycolysis in muscle [2–4], decreased mitochondrial size and density [5–9], decreased oxidative gene expression [9–13], decreased oxidative phosphorylation [13–17], and decreased aerobic capacity [12,18,19]. Whether decreased oxidative capacity is a cause or consequence of insulin resistance, however, is unknown [20–22]. Longitudinal, population-based studies are a necessary approach to address this question but require a marker of decreased oxidative capacity.

Increased intracellular glycolysis is one consequence of mitochondrial dysfunction. Plasma lactate concentrations correlate with the rate of glycolysis and are thought to reflect mitochondrial oxidative capacity [23] as well as intracellular hypoxia [24]. Several cross-sectional studies have reported an association between elevated lactate concentrations and insulin resistance [25,26] as well as type 2 diabetes [27]. Furthermore, two prospective studies have found that lactate concentrations are associated with risk of diabetes during 9–13 years of follow-up [28,29] and even after adjustment for measures of adiposity [29]. Despite these observed associations, previous studies have not definitively determined whether lactate concentrations are associated with diabetes risk independently of insulin and glucose nor have they established the temporal relationship between lactate and insulin resistance. Thus, the objectives of this study were: (1) to examine the association of plasma lactate and diabetes risk with adjustment for fasting glucose and insulin, and (2) to determine whether elevations in lactate precede elevations in fasting glucose and insulin by examining the risk association among persons with and without insulin resistance at baseline.

Methods

Study Population

The Atherosclerosis Risk in Communities (ARIC) Study is a community-based prospective cohort of 15,792 predominantly black and white adults, ages 45–64 years at baseline. Participants were enrolled from 1987 to 1989 from 4 U.S. communities (Forsyth County, North Carolina; Jackson, Mississippi; suburban Minneapolis, Minnesota; and Washington County, Maryland) and followed for more than two decades [30–32]. In 1996–1998, 11,656 participants attended the fourth scheduled study visit and underwent physical examinations, medical interviews, and laboratory tests. This study visit served as the baseline for our prospective analysis because lactate was measured in visit 4 stored plasma specimens.

We excluded from our study population participants who were: (1) diagnosed or had subclinical diabetes at visit 4, i.e. prevalent cases (N=1,943); (2) not fasting eight or more

hours (N=485); (3) missing valid measurements of plasma lactate (N=170); (4) not contributing follow-up time (N=234); (5) underrepresented racial groups (N=31); or (6) missing information on relevant covariates (N=1,695). After accounting for redundant exclusions, the total excluded was 3,611, resulting in an analytic sample of 8,045. Prevalent diagnosed or subclinical diabetes was determined by self-reported physician diagnosis of diabetes, self-reported use of glucose-lowering medications, a measured fasting glucose ≥ 126 mg/dL, or a measured nonfasting glucose ≥ 200 mg/dL.

Written informed consent was obtained from all participants and the study protocol was approved by institutional review boards at each clinical site.

Plasma Lactate

Plasma specimens were collected during visit 4 (1996–1998) and frozen at -70°C . Lactate was measured in 2011 on a Roche Hitachi 911 auto-analyzer, using an enzymatic reaction that converts lactate to pyruvate [33]. The inter-assay coefficient of variation was 4.4% (based on the average of 3 laboratory plasma control pools with means 8.9, 11.5, and 36.0 mg/dL). Normal blood lactate levels range between 4.5 mg/dL and 18.0 mg/dL (0.5 mmol/L – 2.0 mmol/L).

Incident Diabetes

Among persons with no diagnosed or subclinical diabetes at baseline (prevalent cases), we identified incident cases of diabetes between baseline (ARIC visit 4, 1996–1998) and April 18th, 2011 (the last date of follow-up available) during annual telephone calls to all participants. Annual telephone follow-up response rates averaged over 90% throughout this study. Participants were classified as incident cases if they reported a physician diagnosis of diabetes or diabetes medication use. Date of self-report was used as a proxy for the date of diagnosis. This definition of incident diabetes in the ARIC Study has been shown to be valid and highly specific for the classification of newly diagnosed cases [34,35]. Participants who did not develop diabetes were censored on the date of the last annual follow-up response.

Other Measurements

Trained personnel collected all data using standardized protocols with extensive quality control measures, as described previously [36,37]. Age, sex, race, education attainment, parental history of diabetes, coronary heart disease, and smoking status were assessed via self-report. Height, weight, and waist circumference at the umbilical level were measured during physical examinations. Body mass index (BMI) was calculated as measured weight in kilograms divided by height in meters squared. Low density lipoprotein cholesterol (LDLc), high density lipoprotein cholesterol (HDLc), triglycerides, fasting glucose, and fasting insulin were measured in serum. Triglycerides and insulin were log-base 10 transformed to normalize their distributions. Serum fasting glucose and glucose measured after a two-hour oral glucose tolerance test (OGTT) were quantified using a hexokinase method. We calculated the homeostatic model assessment insulin resistance index (HOMA-IR) as $((\text{fasting insulin in } \mu\text{u/mL}) \times (\text{fasting glucose in mg/dL})) / 405$. Physical activity, was assessed during the first visit of ARIC (1987–1989) using a modified Baecke Physical Activity questionnaire. Physical activity was ultimately only examined in sensitivity

analyses due to the length of time elapsing between assessment and lactate specimen collection.

Statistical Analysis

We used Cox proportional hazard models to examine the association between baseline plasma lactate (divided into quartiles or as a continuous variable) and risk of incident diabetes using three nested models. Model 1 was adjusted for age, sex, and race-center. Model 2 was adjusted for all variables in Model 1 plus LDLc, HDLc, $\log_{10}$ triglycerides, hypertension status, history of coronary heart disease (yes or no), smoking status (never, former, or current), parental history of diabetes (yes or no), high school education or higher (yes or no), BMI, waist circumference, and serum uric acid. Model 3 was adjusted for all variables in Model 2 plus fasting glucose and fasting insulin. We further evaluated the association of lactate with incident diabetes by strata of glucose (<100 mg/dL versus 100 to 125 mg/dL), insulin (<15 μ U/mL versus 15 μ U/mL) [38], and HOMA-IR (<2.6 versus 2.6) [39] with adjustment for the variables in Model 2. We plotted a restricted cubic spline model to show the shape of the continuous relationship between plasma lactate and diabetes risk after adjustment for covariates in Model 2. In this model, we used the 25th percentile as the reference level and placed knots at the 25th, 50th, and 75th percentiles. We also compared the distribution of lactate between persons who did not develop diabetes and those who developed diabetes using two-sample Kolmogorov-Smirnov equality-of-distributions tests. The medians of the distributions were compared via quantile regression. We performed sensitivity analyses substituting OGTT in place of serum glucose in Model 3 or restricting our analyses to ARIC participants with a plasma lactate < 18 mg/dL. A cutoff of 18 mg/dL was chosen as this corresponds to the clinical value of mild hyperlactatemia (~2 mmol/L) [40]. We also performed a sensitivity analysis adjusting our models for physical activity.

Results

The mean plasma lactate in the overall population (N = 8,045) was 6.93 mg/dL (SD, 2.78), ranging from 2.2 to 55.5 mg/dL (Table 1). Ninety-nine percent of the study population (N = 7,995) had a baseline lactate concentration within the normal range, i.e. < 18 mg/dL. Age, smoking status, and LDLc did not differ significantly across quartiles of baseline lactate. However, male gender, black race, BMI, waist circumference, education status, family history of diabetes, hypertension status, and coronary heart disease status were associated with lactate concentrations. Serum uric acid, triglycerides, fasting glucose, fasting insulin, and HOMA-IR all demonstrated a positive trend with higher lactate quartiles (all *P*-trends < 0.001). HDLc was inversely associated with lactate levels at baseline (*P*-trend < 0.001).

During a median of 11.9 years of follow-up (range: 4.8 months to 13.7 years), there were 1,513 new, self-reported cases of diagnosed diabetes. There was a significant, graded relationship across categories of baseline plasma lactate and risk of diabetes (Models 1 & 2, *P* trends <0.001) (Table 2). Variables responsible for attenuating the association between plasma lactate and risk of diabetes from Model 1 to Model 2 were triglycerides, serum uric acid, parental history of diabetes, hypertension status, and BMI. In Model 3, although glucose and insulin further attenuated much of the relationship between lactate and incident

diabetes, there was still a significant association between lactate and incident diabetes (Model 3, P trend = 0.004). Similarly, when modeled as a continuous variable, plasma lactate (per 10 mg/dL) was significantly associated with a 1.20 times greater risk of incident diabetes even after adjusting for fasting glucose and insulin (95% CI: 1.01, 1.43; P = 0.04). The trend of association across quartiles of lactate and as a continuous variable was attenuated in sensitivity analyses adjusting for glucose measured after a 2-hour OGTT in place of fasting glucose (Supplemental Table 1). Restricting our analysis to participants with a lactate < 18 mg/dL, strengthened our findings (Supplemental Table 2), while adjustment for physical activity had virtually no impact on our findings (Supplemental Table 3).

Figure 1 demonstrates the adjusted hazard ratios for incident diabetes according to plasma lactate concentration. In general, plasma lactate values above the 50th percentile (i.e. 6.3 mg/dL) demonstrated a linearly increasing relationship with diabetes risk. Similarly, plasma lactate concentrations below the 25th percentile were associated with a lower diabetes risk. There was no evidence of a threshold effect for the risk of diabetes. Kolmogorov-Smirnov comparison of the probability density of plasma lactate by diabetes case status supported the above findings, in that the baseline lactate concentrations were higher in incident cases of diabetes versus non-cases of diabetes (P -value < 0.001). Similarly, the median value of baseline lactate concentration among incident cases of diabetes was 7.1 mg/dL versus 6.2 mg/dL among non-cases of diabetes (P -value < 0.001 via quantile regression).

In general, there was no evidence of effect modification by strata of insulin, glucose, and HOMA-IR (Table 3). However, trends in the association of plasma lactate with risk of incident diabetes were stronger among participants with a fasting glucose between 100 and 126 mg/dL or a fasting insulin < 15 μ U/mL (both P -trends < 0.001) compared to participants with a fasting glucose < 100 mg/dL or fasting insulin $\geq$ 15 μ U/mL. Similarly, there were no appreciable differences in trends by strata of HOMA-IR (both P -trends < 0.01). Further adjustment for glucose and insulin measures attenuated the observed trends in lactate (Supplemental Table 4).

Discussion

This report represents one of the largest and longest prospective cohort studies of the association between plasma lactate and diabetes risk in a community-based population. We observed a robust, graded relationship between plasma lactate and subsequent risk of diagnosed diabetes during over a decade of follow-up. The graded association, which was observed across normal values of lactate (< 18 mg/dL), was independent of traditional diabetes risk factors. The association was attenuated, but still significant, after adjustment for fasting glucose and insulin. Adjustment for 2-hour OGTT further attenuated the association.

Our study supports growing evidence that lactate concentrations within the normal range are a marker of diabetes risk. Previous cross-sectional studies have reported that variation in fasting lactate is partially explained by insulin sensitivity [25] and that lactate is elevated among persons with diabetes [26,41]. Furthermore, one prospective study of 766 Swedish men, found that an elevated lactate was associated with 2.4 times the risk of diabetes [28]. In

a later case-cohort study of 1,077 men and women, we demonstrated that the highest quartile of lactate was associated with 2.1 times the risk of diabetes in the lowest quartile of lactate independently of measures of adiposity. However, when adjusted for concurrent fasting insulin or fasting glucose, the association was attenuated, which may be due to inadequate power [29]. In the current study, we were able to use the larger sample and extended follow-up of the annual ARIC follow-up questionnaire to re-examine the association between lactate and diabetes. In this study, not only was lactate associated with diabetes risk after adjusting for fasting glucose and insulin, lactate was also associated with diabetes risk among individuals with a normal or mildly elevated HOMA-IR.

Fasting lactate is primarily a marker of extrahepatic glucose utilization. While the majority of plasma lactate is generated by muscle, population variation in lactate is largely explained by extramuscular tissue [42,43], especially adipose [44]. There is mounting evidence that excess adiposity contributes to elevations in lactate [44]. This is believed to be due to cellular hypoxia related to adipose size [45,46] as well as inadequate capillarity and perfusion [47]. However, differences in adipose tissue do not fully explain lactate variation [25,29] and in our study we found lactate levels were associated with diabetes independently of measures of adiposity at baseline.

Lactate is also a marker of impaired oxidative respiration [48] due to mitochondrial dysfunction [23]. There is currently much debate regarding the role of oxidative capacity in the pathogenesis of diabetes [20,21]. Several studies have shown a relationship between mitochondrial dysfunction and insulin resistance [5,49] as well as diabetes [50,51]. Furthermore, changes in mitochondrial size, density, and activity have been observed among individuals with insulin resistance and diabetes [6,9,52,53]. While some posit that insulin resistance may induce mitochondrial change [54], our study suggests that these changes, manifesting as elevations in lactate, may be observed among individuals with a normal to slightly elevated fasting insulin concentrations as well as a low HOMA-IR. While much of the association between lactate and diabetes may be explained by traditional risk factors, including glucose and insulin, the residual association suggests a role for oxidative capacity in the pathogenesis of diabetes.

There are several limitations to this study. We had only a single plasma lactate measurement obtained from samples that had been stored 12–15 years. Furthermore, we did not have measurements of glycated hemoglobin or fasting glucose to identify incident cases of undiagnosed diabetes. While self-report of diagnosed diabetes has been shown to be a valid and specific means of identifying cases of diabetes in the ARIC population [35], this case definition may underestimate some risk factor associations [34]. Finally, as with any observational study, residual confounding is a concern. Along these lines, it is important to recognize the difficulty of establishing order of events even in prospective studies, particularly when variables are determined by co-occurring risk factors. Strengths of this study include rigorous assessment of diabetes risk factors, a large, diverse population, and over a decade of follow-up.

In conclusion, our results show baseline elevations in plasma lactate were associated with an increased risk of incident diabetes independent of fasting glucose and insulin, but not 2-hr

OGTT. Furthermore, lactate was associated with diabetes among participants without evidence of insulin resistance at baseline. This finding suggests that lactate elevations precede insulin resistance. Additional research is needed to better understand the determinants of resting lactate variation and the pathways linking lactate variation to diabetes risk.

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SPJ researched data, wrote manuscript, intellectual content. JHY wrote manuscript, intellectual content, critical review. ES reviewed/edited manuscript. ERM reviewed/edited manuscript. FB reviewed/edited manuscript. SPJ is the guarantor of this work, had full access to all the data, and takes full responsibility for the integrity of data and the accuracy of data analysis.

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References

1. Pagel-Langenickel I, Bao J, Pang L, Sack MN. The role of mitochondria in the pathophysiology of skeletal muscle insulin resistance. *Endocr Rev.* 2010; 31:25–51. [PubMed: 19861693]
2. Simoneau JA, Colberg SR, Thaete FL, Kelley DE. Skeletal muscle glycolytic and oxidative enzyme capacities are determinants of insulin sensitivity and muscle composition in obese women. *FASEB J.* 1995; 9:273–8. [PubMed: 7781930]
3. Kelley DE, Slasky BS, Janosky J. Skeletal muscle density: effects of obesity and non-insulin-dependent diabetes mellitus. *Am J Clin Nutr.* 1991; 54:509–15. [PubMed: 1877507]
4. Del Prato S, Bonadonna RC, Bonora E, Gulli G, Solini A, Shank M, et al. Characterization of cellular defects of insulin action in type 2 (non-insulin-dependent) diabetes mellitus. *J Clin Invest.* 1993; 91:484–94. [PubMed: 8432857]
5. Petersen KF, Befroy D, Dufour S, Dziura J, Ariyan C, Rothman DL, et al. Mitochondrial dysfunction in the elderly: possible role in insulin resistance. *Science.* 2003; 300:1140–2. [PubMed: 12750520]
6. Kelley DE, He J, Menshikova EV, Ritov VB. Dysfunction of mitochondria in human skeletal muscle in type 2 diabetes. *Diabetes.* 2002; 51:2944–50. [PubMed: 12351431]
7. Menshikova EV, Ritov VB, Toledo FGS, Ferrell RE, Goodpaster BH, Kelley DE. Effects of weight loss and physical activity on skeletal muscle mitochondrial function in obesity. *Am J Physiol Endocrinol Metab.* 2005; 288:E818–825. [PubMed: 15585590]
8. Toledo FGS, Menshikova EV, Azuma K, Radiková Z, Kelley CA, Ritov VB, et al. Mitochondrial capacity in skeletal muscle is not stimulated by weight loss despite increases in insulin action and decreases in intramyocellular lipid content. *Diabetes.* 2008; 57:987–94. [PubMed: 18252894]
9. Morino K, Petersen KF, Dufour S, Befroy D, Frattini J, Shatzkes N, et al. Reduced mitochondrial density and increased IRS-1 serine phosphorylation in muscle of insulin-resistant offspring of type 2 diabetic parents. *J Clin Invest.* 2005; 115:3587–93. [PubMed: 16284649]
10. Sparks LM, Xie H, Koza RA, Mynatt R, Hulver MW, Bray GA, et al. A high-fat diet coordinately downregulates genes required for mitochondrial oxidative phosphorylation in skeletal muscle. *Diabetes.* 2005; 54:1926–33. [PubMed: 15983191]

11. Patti ME, Butte AJ, Crunkhorn S, Cusi K, Berria R, Kashyap S, et al. Coordinated reduction of genes of oxidative metabolism in humans with insulin resistance and diabetes: Potential role of PGC1 and NRF1. *Proc Natl Acad Sci USA*. 2003; 100:8466–71. [PubMed: 12832613]
12. Mootha VK, Lindgren CM, Eriksson K-F, Subramanian A, Sihag S, Lehar J, et al. PGC-1alpha-responsive genes involved in oxidative phosphorylation are coordinately downregulated in human diabetes. *Nat Genet*. 2003; 34:267–73. [PubMed: 12808457]
13. Stump CS, Short KR, Bigelow ML, Schimke JM, Nair KS. Effect of insulin on human skeletal muscle mitochondrial ATP production, protein synthesis, and mRNA transcripts. *Proc Natl Acad Sci USA*. 2003; 100:7996–8001. [PubMed: 12808136]
14. Short KR, Nair KS, Stump CS. Impaired mitochondrial activity and insulin-resistant offspring of patients with type 2 diabetes. *N Engl J Med*. 2004; 350:2419–2421. author reply 2419–2421. [PubMed: 15175449]
15. Petersen KF, Dufour S, Befroy D, Garcia R, Shulman GI. Impaired mitochondrial activity in the insulin-resistant offspring of patients with type 2 diabetes. *N Engl J Med*. 2004; 350:664–71. [PubMed: 14960743]
16. Bruce CR, Anderson MJ, Carey AL, Newman DG, Bonen A, Kriketos AD, et al. Muscle oxidative capacity is a better predictor of insulin sensitivity than lipid status. *J Clin Endocrinol Metab*. 2003; 88:5444–51. [PubMed: 14602787]
17. Lowell BB, Shulman GI. Mitochondrial dysfunction and type 2 diabetes. *Science*. 2005; 307:384–7. [PubMed: 15662004]
18. Wisløff U, Najjar SM, Ellingsen O, Haram PM, Swoap S, Al-Share Q, et al. Cardiovascular risk factors emerge after artificial selection for low aerobic capacity. *Science*. 2005; 307:418–20. [PubMed: 15662013]
19. Eriksson KF, Lindgärde F. Impaired glucose tolerance in a middle-aged male urban population: a new approach for identifying high-risk cases. *Diabetologia*. 1990; 33:526–31. [PubMed: 2253828]
20. Holloszy JO. “Deficiency” of mitochondria in muscle does not cause insulin resistance. *Diabetes*. 2013; 62:1036–40. [PubMed: 23520283]
21. Goodpaster BH. Mitochondrial deficiency is associated with insulin resistance. *Diabetes*. 2013; 62:1032–5. [PubMed: 23520282]
22. Perry CGR, Kane DA, Lanza IR, Neufer PD. Methods for assessing mitochondrial function in diabetes. *Diabetes*. 2013; 62:1041–53. [PubMed: 23520284]
23. Avogaro A, Toffolo G, Miola M, Valerio A, Tiengo A, Cobelli C, et al. Intracellular lactate- and pyruvate-interconversion rates are increased in muscle tissue of non-insulin-dependent diabetic individuals. *J Clin Invest*. 1996; 98:108–15. [PubMed: 8690781]
24. Arieff AI, Graf H. Pathophysiology of type A hypoxic lactic acidosis in dogs. *Am J Physiol*. 1987; 253:E271–276. [PubMed: 3631257]
25. Lovejoy J, Newby FD, Gebhart SS, DiGirolamo M. Insulin resistance in obesity is associated with elevated basal lactate levels and diminished lactate appearance following intravenous glucose and insulin. *Metab Clin Exp*. 1992; 41:22–7. [PubMed: 1538640]
26. Reaven GM, Hollenbeck C, Jeng CY, Wu MS, Chen YD. Measurement of plasma glucose, free fatty acid, lactate, and insulin for 24 h in patients with NIDDM. *Diabetes*. 1988; 37:1020–4. [PubMed: 3292322]
27. Chen YD, Varasteh BB, Reaven GM. Plasma lactate concentration in obesity and type 2 diabetes. *Diabetes Metab*. 1993; 19:348–54. [PubMed: 8293860]
28. Ohlson LO, Larsson B, Björntorp P, Eriksson H, Svärdsudd K, Welin L, et al. Risk factors for type 2 (non-insulin-dependent) diabetes mellitus. Thirteen and one-half years of follow-up of the participants in a study of Swedish men born in 1913. *Diabetologia*. 1988; 31:798–805. [PubMed: 3234634]
29. Juraschek SP, Shanta GPS, Chu AY, Miller ER 3rd, Guallar E, Hoogeveen RC, et al. Lactate and Risk of Incident Diabetes in a Case-Cohort of the Atherosclerosis Risk in Communities (ARIC) Study. *PLoS ONE*. 2013 In press.
30. The Atherosclerosis Risk in Communities (ARIC) Study: design and objectives. The ARIC investigators. *Am J Epidemiol*. 1989; 129:687–702. [PubMed: 2646917]

31. Jackson R, Chambless LE, Yang K, Byrne T, Watson R, Folsom A, et al. Differences between respondents and nonrespondents in a multicenter community-based study vary by gender ethnicity. The Atherosclerosis Risk in Communities (ARIC) Study Investigators. *J Clin Epidemiol.* 1996; 49:1441–6. [PubMed: 8970495]
32. White AD, Folsom AR, Chambless LE, Sharret AR, Yang K, Conwill D, et al. Community surveillance of coronary heart disease in the Atherosclerosis Risk in Communities (ARIC) Study: methods and initial two years' experience. *J Clin Epidemiol.* 1996; 49:223–33. [PubMed: 8606324]
33. Barham D, Trinder P. An improved colour reagent for the determination of blood glucose by the oxidase system. *Analyst.* 1972; 97:142–5. [PubMed: 5037807]
34. Bielinski SJ, Pankow JS, Rasmussen-Torvik LJ, Bailey K, Li M, Selvin E, et al. Strength of Association for Incident Diabetes Risk Factors According to Diabetes Case Definitions: The Atherosclerosis Risk in Communities Study. *American Journal of Epidemiology.* 2012;1093/aje/kwr326
35. Schneider ALC, Pankow JS, Heiss G, Selvin E. Validity and Reliability of Self-Reported Diabetes in the Atherosclerosis Risk in Communities (ARIC) Study. 2012 In Press.
36. Duncan BB, Schmidt MI, Pankow JS, Ballantyne CM, Couper D, Vigo A, et al. Low-grade systemic inflammation and the development of type 2 diabetes: the atherosclerosis risk in communities study. *Diabetes.* 2003; 52:1799–805. [PubMed: 12829649]
37. Schmidt MI, Duncan BB, Sharrett AR, Lindberg G, Savage PJ, Offenbacher S, et al. Markers of inflammation and prediction of diabetes mellitus in adults (Atherosclerosis Risk in Communities study): a cohort study. *Lancet.* 1999; 353:1649–52. [PubMed: 10335783]
38. McLaughlin T, Abbasi F, Cheal K, Chu J, Lamendola C, Reaven G. Use of metabolic markers to identify overweight individuals who are insulin resistant. *Ann Intern Med.* 2003; 139:802–9. [PubMed: 14623617]
39. Ascaso JF, Pardo S, Real JT, Lorente RI, Priego A, Carmena R. Diagnosing insulin resistance by simple quantitative methods in subjects with normal glucose metabolism. *Diabetes Care.* 2003; 26:3320–5. [PubMed: 14633821]
40. Mizock BA, Falk JL. Lactic acidosis in critical illness. *Crit Care Med.* 1992; 20:80–93. [PubMed: 1309494]
41. Crawford SO, Hoogeveen RC, Brancati FL, Astor BC, Ballantyne CM, Schmidt MI, et al. Association of blood lactate with type 2 diabetes: the Atherosclerosis Risk in Communities Carotid MRI Study. *Int J Epidemiol.* 2010; 39:1647–55. [PubMed: 20797988]
42. Perriello G, Jorde R, Nurjhan N, Stumvoll M, Dailey G, Jenssen T, et al. Estimation of glucose-alanine-lactate-glutamine cycles in postabsorptive humans: role of skeletal muscle. *Am J Physiol.* 1995; 269:E443–450. [PubMed: 7573421]
43. Yki-Järvinen H, Bogardus C, Foley JE. Regulation of plasma lactate concentration in resting human subjects. *Metab Clin Exp.* 1990; 39:859–64. [PubMed: 2198437]
44. Qvisth V, Hagström-Toft E, Moberg E, Sjöberg S, Bolinder J. Lactate release from adipose tissue and skeletal muscle in vivo: defective insulin regulation in insulin-resistant obese women. *Am J Physiol Endocrinol Metab.* 2007; 292:E709–714. [PubMed: 17077346]
45. Sandqvist MM, Eriksson JW, Jansson PA. Increased lactate release per fat cell in normoglycemic first-degree relatives of individuals with type 2 diabetes. *Diabetes.* 2001; 50:2344–8. [PubMed: 11574418]
46. Crandall DL, Fried SK, Francendese AA, Nickel M, DiGirolamo M. Lactate release from isolated rat adipocytes: influence of cell size, glucose concentration, insulin and epinephrine. *Horm Metab Res.* 1983; 15:326–9. [PubMed: 6350139]
47. Ye J. Adipose tissue vascularization: its role in chronic inflammation. *Curr Diab Rep.* 2011; 11:203–10. [PubMed: 21327583]
48. Py G, Lambert K, Perez-Martin A, Raynaud E, Préfaut C, Mercier J. Impaired sarcolemmal vesicle lactate uptake and skeletal muscle MCT1 and MCT4 expression in obese Zucker rats. *Am J Physiol Endocrinol Metab.* 2001; 281:E1308–1315. [PubMed: 11701447]

49. Iossa S, Mollica MP, Lionetti L, Crescenzo R, Tasso R, Liverini G. A possible link between skeletal muscle mitochondrial efficiency and age-induced insulin resistance. *Diabetes*. 2004; 53:2861–6. [PubMed: 15504966]
50. Reardon W, Ross RJ, Sweeney MG, Luxon LM, Pembrey ME, Harding AE, et al. Diabetes mellitus associated with a pathogenic point mutation in mitochondrial DNA. *Lancet*. 1992; 340:1376–9. [PubMed: 1360090]
51. Van den Ouweland JM, Lemkes HH, Ruitenbeek W, Sandkuijl LA, de Vijlder MF, Struyvenberg PA, et al. Mutation in mitochondrial tRNA(Leu)(UUR) gene in a large pedigree with maternally transmitted type II diabetes mellitus and deafness. *Nat Genet*. 1992; 1:368–71. [PubMed: 1284550]
52. Björntorp P, Scherstén T, Fagerberg SE. Respiration and phosphorylation of mitochondria isolated from the skeletal muscle of diabetic and normal subjects. *Diabetologia*. 1967; 3:346–52. [PubMed: 4244686]
53. Kim JY, Hickner RC, Cortright RL, Dohm GL, Houmard JA. Lipid oxidation is reduced in obese human skeletal muscle. *Am J Physiol Endocrinol Metab*. 2000; 279:E1039–1044. [PubMed: 11052958]
54. Hoeks J, van Herpen NA, Mensink M, Moonen-Kornips E, van Beurden D, Hesselink MKC, et al. Prolonged fasting identifies skeletal muscle mitochondrial dysfunction as consequence rather than cause of human insulin resistance. *Diabetes*. 2010; 59:2117–25. [PubMed: 20573749]

Figure 1.

Adjusted hazard ratios (solid line) for incident self-reported diabetes between ARIC visit 4 and April, 2011 according to baseline concentrations of plasma lactate values from a restricted cubic spline model. Dashed lines are the 95% confidence intervals. The models were expressed relative to the 25th percentile of lactate with knots specified at the 25th, 50th, and 75th percentiles and were adjusted for age, gender, race-ARIC center, education, diagnosis of hypertension, prevalent coronary disease, smoking status, parental history of diabetes, body mass index, waist circumference, serum uric acid, log₁₀-transformed triglycerides, low density lipoprotein cholesterol, and high density lipoprotein cholesterol. The plot was truncated at the 1st and 99th percentiles of lactate. The hazard ratios are shown on a natural log scale. In addition, overlaid are kernel density plots depicting the distribution plasma lactate by participants who did not develop diabetes (dash) and participants who did develop diabetes (solid).

Table 1

Baseline characteristics of the study population without a history of diagnosed diabetes overall and by quartiles of lactate at baseline, the Atherosclerosis Risk in Communities Study, 1996–1998

	Overall (N = 8,045)	Mean (SD) or %				P for Trend [‡]
		Q1 (N = 2,055)	Q2 (N = 1,984)	Q3 (N = 2,044)	Q4 (N = 1,962)	
Plasma lactate, mg/dL *	6.9 (2.8)	4.4 (0.5)	5.7 (0.3)	7.1 (0.5)	10.7 (3.0)	NA
Age, yr	62.7 (5.7)	62.2 (5.7)	62.9 (5.6)	62.7 (5.6)	62.7 (5.7)	0.06
Male, %	42.5	35.3	45.9	47.6	41.4	<0.001
Black, %	16.7	11.5	14.5	17.2	23.7	0.005
High School Education or Higher, %	50.4	53.3	50.0	50.8	47.4	0.001
Family history of diabetes, %	23.5	20.5	24.7	23.5	25.2	0.003
Hypertension, %	42.6	34.1	39.1	44.5	53.3	<0.001
History of Coronary Heart Disease, %	7.2	5.9	6.6	7.4	9.0	<0.001
Leisure index, units**	2.4 (0.6)	2.5 (0.6)	2.4 (0.5)	2.4 (0.5)	2.4 (0.6)	<0.001
Smoking Status						
Never, %	42.7	43.6	42.3	41.6	43.2	0.84
Former, %	43.2	41.9	42.7	44.4	43.7	0.21
Current, %	14.1	14.4	14.9	13.9	13.0	0.13
Body mass index, kg/m ²	28.2 (5.3)	26.9 (5.0)	27.8 (5.1)	28.5 (5.1)	29.5 (5.6)	<0.001
Waist circumference, cm	100.2 (13.9)	96.7 (13.7)	99.2 (13.6)	101.4 (13.3)	103.5 (14.2)	<0.001
Serum uric acid, mg/dL	5.6 (1.5)	5.1 (1.4)	5.5 (1.3)	5.7 (1.5)	6.0 (1.5)	<0.001
Triglycerides, mg/dL [†]	118 (87 to 165)	99 (75 to 131)	110 (83 to 149)	128 (95 to 175)	148 (104 to 207)	<0.001
LDL cholesterol, mg/dL	123.1 (33.1)	122.2 (31.1)	124.7 (33.3)	123.6 (34.3)	121.8 (33.5)	0.25
HDL cholesterol, mg/dL	51.5 (16.7)	55.0 (17.3)	51.5 (16.9)	49.7 (15.8)	49.8 (16.2)	<0.001
Fasting glucose, mg/dL	98.9 (9.4)	95.8 (8.5)	98.6 (8.8)	99.8 (9.1)	101.6 (10.1)	<0.001
Fasting insulin, μ U/mL	11.4 (7.8)	9.3 (6.2)	10.5 (6.5)	12.0 (9.2)	13.8 (8.2)	<0.001
HOMA-IR, units	2.8 (2.1)	2.2 (1.6)	2.6 (1.7)	3.0 (2.5)	3.5 (2.3)	<0.001

Abbreviations. Q, quartile; NA, not applicable; SD, standard deviation; LDL, low density lipoprotein; HDL, high density lipoprotein; HOMA-IR, homeostatic model assessment insulin resistance index

* Lactate mg/dL may be converted to mmol/L by multiplying by 0.111

[†] Presented as the median (IQR). To evaluate trend, this variable was log₁₀ transformed to normalize its distribution.

[†] P-trend evaluated with linear or logistic regression using the median lactate value for each quartile as an ordinal variable.

^{**} Leisure index was assessed at ARIC visit 1

Table 2

Adjusted hazard ratios (95% confidence intervals) for baseline lactate (in quartiles) and risk of diagnosed diabetes

	Model 1	Model 2	Model 3
Quartiles of Lactate			
2.2 – 5.1 mg/dL	1.00 (reference)	1.00 (reference)	1.00 (reference)
5.2 – 6.3 mg/dL	1.27 (1.08, 1.50)	1.10 (0.93, 1.29)	1.00 (0.85, 1.17)
6.4 – 8.0 mg/dL	1.42 (1.22, 1.67)	1.11 (0.94, 1.30)	0.95 (0.81, 1.12)
8.1 – 55.5 mg/dL	2.29 (1.97, 2.65)	1.51 (1.29, 1.77)	1.20 (1.02, 1.41)
<i>P</i> for Trend*	< 0.001	< 0.001	0.004
Lactate per 10 mg/dL	1.99 (1.77, 2.23)	1.50 (1.28, 1.75)	1.20 (1.01, 1.43)
<i>P</i> -value	<0.001	<0.001	0.036

Model 1: Age, gender, race-ARIC center

Model 2: Model 1 + education, diagnosis of hypertension, prevalent coronary disease, smoking status, parental history of diabetes, body mass index, waist circumference, serum uric acid, log₁₀triglycerides, low density lipoprotein cholesterol, high density lipoprotein cholesterol

Model 3: Model 2 + fasting glucose + log₁₀fasting insulin

*
P-value for trend evaluated using an ordinal variable based on the median lactate in each quartile.

Table 3

Adjusted hazard ratios (95% confidence intervals) for baseline lactate (in quartiles) and risk of diagnosed diabetes stratified by categories of fasting glucose, fasting insulin, or HOMA-IR at baseline

	Plasma Lactate Quartile (mg/dL)					P-trend [†]
	2.2 – 5.1	5.2 – 6.3	6.4 – 8.0	8.1 – 55.5		
Fasting Glucose						
<100 mg/dL	1.00 (reference)	0.93 (0.71, 1.22)	0.95 (0.72, 1.25)	1.24 (0.94, 1.64)		0.070
100–<126 mg/dL	1.00 (reference)	1.06 (0.86, 1.31)	1.03 (0.84, 1.26)	1.38 (1.14, 1.68)		<0.001
Insulin						
<15 µU/mL	1.00 (reference)	1.03 (0.85, 1.24)	1.08 (0.89, 1.30)	1.50 (1.24, 1.81)		<0.001
15 µU/mL	1.00 (reference)	1.20 (0.86, 1.69)	1.03 (0.74, 1.42)	1.32 (0.96, 1.80)		0.055
HOMA-IR						
<2.6 units	1.00 (reference)	1.03 (0.82, 1.30)	0.95 (0.75, 1.22)	1.40 (1.09, 1.79)		0.009
2.6 units	1.00 (reference)	1.12 (0.88, 1.42)	1.12 (0.90, 1.41)	1.42 (1.14, 1.77)		<0.001

* Cox proportional hazards models are adjusted for age, gender, race-ARIC center, education, diagnosis of hypertension, prevalent coronary heart disease, smoking status, parental history of diabetes, body mass index, waist circumference, serum uric acid, log10triglycerides, low density lipoprotein cholesterol, and high density lipoprotein cholesterol

[†] P-value for trend evaluated using an ordinal variable based on the median lactate in each quartile.