Insulin acutely upregulates protein expression of the fatty acid transporter CD36 in human skeletal muscle in vivo

JOURNAL OF PHYSIOLOGY AND PHARMACOLOGY 2008, 59, 1, 77–83
www.jpp.krakow.pl

E. CORPELEIJN[1], M. M.A.L. PELSERS[2], S. SOENEN[1], M. MENSINK[1], F. G. BOUWMAN[1], M. E. KOOI[3], W. H.M. SARIS[1], J. F.C. GLATZ[4], E. E. BLAAK[1]

INSULIN ACUTELY UPREGULATES PROTEIN EXPRESSION OF THE FATTY ACID TRANSPORTER CD36 IN HUMAN SKELETAL MUSCLE *IN VIVO*.

[1]From the Department of Human Biology, the Nutrition and Toxicology Research Institute Maastricht (NUTRIM), Maastricht University, Maastricht, The Netherlands; [2]Department of movement sciences, the Nutrition and Toxicology Research Institute Maastricht NUTRIM, Maastricht University, Maastricht, The Netherlands; [3]the Department of Radiology, University Hospital Maastricht, Maastricht, The Netherlands; [4]Department of Molecular Genetics, Cardiovascular Research Institute Maastricht (CARIM), Maastricht University, Maastricht, The Netherlands

Enhanced fatty acid uptake may lead to the accumulation of lipid intermediates. This is related to insulin resistance and type 2 diabetes mellitus. Rodent studies suggest that fatty acid transporters are acutely regulated by insulin. We investigated differences in fatty acid transporter content before and at the end of a hyperinsulinemic euglycemic clamp in skeletal muscle (m. vastus lateralis) of obese, glucose-intolerant men (IGT) and obese normal glucose tolerant controls (NGT). The fatty acid transporter FAT/CD36 protein content increased 1.5-fold ($P < 0.05$) after 3-hrs of insulin stimulation with no difference between IGT and control subjects. No change was seen in cytosolic fatty acid binding protein (FABPc) protein content. The increase in FAT/CD36 protein content was positively related to insulin resistance as measured during the clamp ($r = 0.56$, $P < 0.05$). An increase in FAT/CD36 protein content in skeletal muscle may result in a higher fractional extraction of fatty acids (larger relative uptake) after a meal, enhancing triglyceride accumulation in the muscle. We conclude that also in obese humans the FAT/CD36 protein content in skeletal muscle is dynamically regulated by insulin *in vivo* on the short term.

Keywords: *skeletal muscle, obesity, impaired glucose tolerance, lipid metabolism, FAT/CD36, insulin action*

INTRODUCTION

An imbalance between elevated plasma long-chain fatty acid (LCFA) availability, uptake and oxidation results in intramyocellular accumulation of LCFA metabolites, such as fatty acyl-CoA, ceramides, and diacylglycerol (1-4). Elevated levels of these LCFA metabolites are likely to induce defects in the insulin signalling cascade and are associated with the development of skeletal muscle insulin resistance and type 2 diabetes (1-5). Impaired utilization is not only reported in type 2 diabetic patients (6), but also in subjects with impaired glucose tolerance (7, 8), a 'prediabetic' state, suggesting that impaired fatty acid utilization may be an important early factor in the development of type 2 diabetes. It is not clear under which metabolic conditions the accumulation of triglycerides in skeletal muscle takes place, but increased storage can be due to increased circulating concentrations of LCFA and triglycerides (TG), as well as to an impaired suppression of plasma LCFA after a meal (9). Previously, it has been shown in type 2 diabetic patients that triglycerides can accumulate after high fat meals during the day (10). Not only an increased supply of lipids, but also an increased fractional extraction (relative uptake) of LCFA (plasma LCFA or TG-derived LCFA) can enhance the accumulation of fatty acids. Fatty acid transporters play a critical role in fractional fatty acid uptake, in particular when the fatty acid: albumin ratio is low, as is the case after a meal (11, 12). Indeed, the fatty acid transporter CD36 is sensitive to insulin, and a recent study in cardiomyocytes has shown that insulin can rapidly, within hours, increase CD36 mRNA expression as well as protein content, which contributed to an increased fatty acid uptake capacity (13).

MATERIALS AND METHODS

Nine obese men with impaired glucose tolerance (IGT) and eight obese men with normal glucose tolerance (NGT), matched for age and BMI, participated in the study. Inclusion criteria were obesity (BMI > 30 kg/m^2), diastolic blood pressure < 100 mm Hg, no major health problems, and no use of medication that could influence the measurements. The NGT men had no family history of diabetes. Subjects were screened for glucose metabolism with a standard oral glucose tolerance test (75 g glucose) with capillary blood sampling at baseline and after 2 hrs. Subjects were included according to the WHO criteria of 1999 for capillary plasma (IGT: fasting < 7.0 mmol/l, 2hr postload > 8.9 and < 12.2 mmol/l). Three subjects with glucose values (fasting < 8.0 mmol/l and 2hr postload < 14.8 mmol/l) above the cutoff points were included as well. The experimental protocol was approved by the local Medical Ethical Committee of the Maastricht University. All subjects gave written informed consent.

The NGT and IGT men underwent measurements for body composition using hydrostatic weighing, aerobic capacity using an incremental exhaustive bicycle test and insulin sensitivity using a hyperinsulinemic euglycemic clamp (1 mU*kg BW^{-1}*min^{-1}). The glucose infusion rate (GIR, mmol glucose/min) per kg fat free mass (FFM) was determined during a steady state of 30 min. after at least 120 min of insulin infusion. Muscle biopsies were taken before and after insulin-

stimulation at the end of the steady state of the clamp, freed from any visible fat and blood and immediately frozen in liquid nitrogen or for immunofluorescence in isopentane at its melting point.

Muscle type FABPc was measured by means of ELISA (Hycult Biotechnology, Uden, the Netherlands) (14), while CD36 protein was analysed with a in-house developed sandwich-type ELISA (15). Biopsy lipid content was analysed using Oil Red O staining (16). Slides were incubated with a primary antibody against adult human slow myosin heavy chain (A4.951, Developmental Studies Hybridoma Bank, Iowa City, USA) to determine fibre type and a rabbit polyclonal antiserum against human laminine (pLam, Sigma) to visualize myocyte membranes. Images were captured using a Nikon E800 fluorescence microscope (Uvikon, Bunnik, the Netherlands) and a colour CCD camera (Basler A101 C) with 240 times magnification. Per biopsy, at least 50 different cells were analyzed using Lucia 5.49 software.

Plasma FFA and glucose were analyzed in EDTA plasma using standard enzymatic techniques automated on the COBAS Fara centrifugal analyzer (for FFA: FFA-C test kit, Wako chemicals, Neuss, Germany; for glucose: Roche Unikit III, Hoffman-la-Roche, Basel, Switzerland). Insulin was analyzed using a fluoroimmunometric assay (autoDELFIA Insulin, PerkinElmer, Turku, Finland) with no cross-reactivity with proinsulin or split forms of proinsulin.

Results are given as mean ± sem. A two-tailed Student's t-test for independent samples was used to compare groups. Correlations were tested using Pearson's correlation coefficient (r). $P < 0.05$ was considered statistically significant. Statistical analysis was performed using SPSS 10.0 for Macintosh.

RESULTS AND DISCUSSION

No differences in CD36 or FABPc content were found between the obese IGT men and obese controls (*Table 1*). Skeletal muscle CD36 protein increased 1.5 fold after 3 hours of insulin-stimulation ($p < 0.05$, figure 1A), the change in CD36 protein content was comparable between NGT and IGT subjects ($p = 0.62$, *Fig. 1A*). Two men (one IGT and one NGT) showed a decrease. In contrast, skeletal muscle FABPc protein content did not change ($p = 0.22$, *Fig. 1B*). The rapid increase in CD36 protein content indicates that the uptake of plasma LCFA into skeletal muscle may be actively regulated by fatty acid transporters at the level of skeletal muscle itself, and not only in a passive way by plasma lipid supply. Insulin directly activates glucose transporters, but also appears to activate fatty acid transport. This can be very relevant, considering that LCFA from chylomicrons and VLDL may become available for uptake in a later stage after meal intake (9). Indeed, Chabowski and coworkers found the same remarkable dynamic upregulation of CD36 protein content, already after 1 hour of insulin stimulation in rat cardiomyocytes (13). This was preceded by an increase in mRNA expression. Insulin also induced the translocation of CD36. In that study, a large part of the newly synthesized CD36 protein was translocated to the plasma membrane, suggesting that the new proteins may directly contribute to the fatty acid uptake capacity of the muscle cell. Also in humans, insulin induces the translocation of CD36 to the plasma membrane in response to insulin infusion (17). Apparently, insulin has two fast effects: within minutes, it induces the translocation of endosomal CD36 protein to the sarcolemma, and within hours

Table 1. General and metabolic characteristics of the impaired glucose tolerant subjects (IGT) and normal glucose tolerant controls (NGT).

	NGT	IGT	P-value
	n = 9	n = 8	
Age (yrs)	57.1 ± 2.6	58.1 ± 2.7	0.79
Capillary glucose fasting (mmol/l)	5.7 ± 0.6	6.8 ± 1.0	**0.035**
Capillary glucose 2-hour OGTT (mmol/l)	6.7 ± 1.2	12.97 ± 1.6	**0.001**
Body mass index (kg/m^2)	34.2 ± 1.5	32.6 ± 0.6	0.28
Body fat (%)	34.7 ± 1.5	32.7 ± 1.1	0.51
Fat free mass (kg)	69.5 ± 10.8	62.7 ± 3.8	0.084
Waist-hip ratio	1.02 ± 0.02	1.03 ± 0.01	0.59
VO_2max (ml O_2* kg FFM^{-1}*min^{-1})	40.5 ± 2.0	38.9 ± 1.8	0.57
Triglycerides (mmol/l)	1.73 ± 0.29	1.54 ± 0.27	0.65
Glucose - fasting (mmol/l)	5.7 ± 01	6.2 ± 0.2	0.059
Glucose - SS (mmol/l)	4.5 ± 0.1	4.4 ± 0.1	0.40
Insulin - fasting (mmol/l)	16.8 ± 4.1	14.1 ± 1.8	0.092
Insulin - SS (mmol/l)	111 ± 6	108 ± 9	0.76
FFA - fasting (mmol/l)	539 ± 54	696 ± 81	0.64
FFA - SS (mmol/l)	140 ± 19	167 ± 13	0.27
GIR (µmol*kgFFM^{-1}*min^{-1})	32.2 ± 4.5	21.2 ± 3.7	0.085
muscle CD36 protein (ng/g wet weight)	20.0 ± 4.1	16.5 ± 4.3	0.57
muscle FABPc protein (µg/g wet weight)	93.6 ± 13.6	83.5 ± 13.8	0.61
IMTG (Oil Red O, lipid stained area fraction)	0.058 ± 0.034*(n=5)*	0.068 ± 0.041*(n=4)*	0.85
Fibre type area (% type 1 fibre)	46.5 ± 8.7 *(n=5)*	51.3 ± 11.6 *(n=4)*	0.75

Mean ± sem. Student's t-test for unpaired samples, two-tailed. FFM = fat free mass, GIR = glucose infusion rate, IGT = impaired glucose tolerance, IMTG = intramyocellular triglycerides, NGT = normal glucose tolerance, SS = steady state (last half hour) during insulin-stimulation (clamp). n = 9 for NGT and n = 8 for IGT unless indicated otherwise.

this is followed by an increase in total CD36 protein, which is also immediately available for translocation. Both adaptations lead to more sarcolemmal CD36 and an increased fatty acid uptake capacity after a meal. This may be an important adaptation for a rapid storage of meal-derived fatty acids.

It is remarkable that despite the increase in CD36 protein, we did not find an increase in muscle FABPc. If the muscle increases its fatty acid uptake capacity, would it then not be necessary to also increase the intracellular capacity to transport fatty acids? Studies with FABPc knock-out mice have indicated the involvement of FABPc in shuttling LCFA from the sarcolemma to intracellular sites of oxidation or esterification, but rather in a permissive than in a regulatory fashion (18, 19). Even a reduction of FABPc protein of 50% is sufficient to maintain LCFA trafficking. Thus, in comparison to CD36, the need to increase FABPc protein content is limited.

The increase in CD36 protein content upon insulin stimulation (*Fig. 1A*) was comparable between groups (p = 0.62). The change in CD36 protein in relation to insulin resistance was further investigated in the group as a whole.

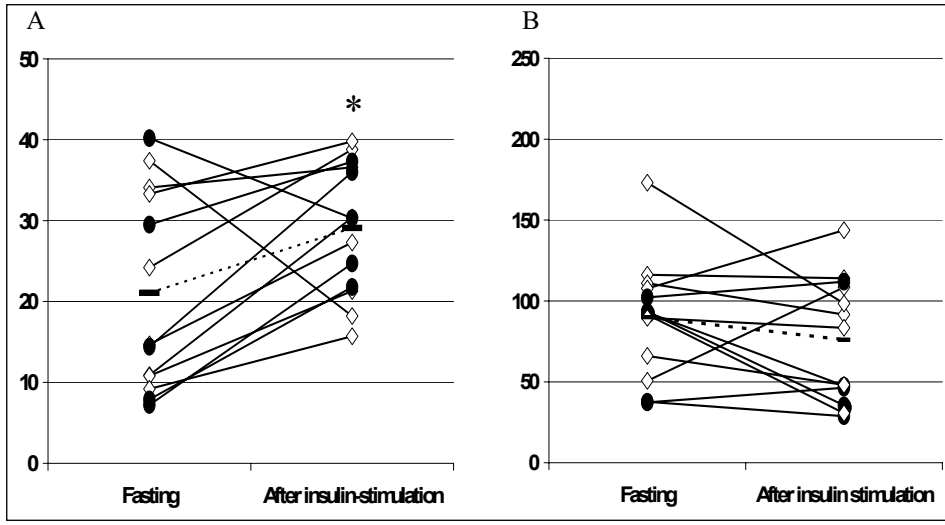

Fig. 1. Skeletal muscle protein content (µg/g wet weight) of CD36 (1A) and FABPc (1B) during fasting and after insulin stimulation in obese men. Open diamonds are normal glucose tolerant controls; filled circles are impaired glucose tolerant subjects; flat object with dotted line is the mean. * $P < 0.05$, paired Student's t-test, two-tailed.

Interestingly, a positive association was found between the increase in CD36 protein and GIR (Pearson $r = 0.564$, $p = 0.045$). Correction for a possible confounding by baseline values, by dividing the change in CD36 protein content by fasting CD36 protein content, did not reduce the association ($r = 0.640$, $p = 0.020$). Although this finding seems contra-intuitive, it has been shown that in insulin resistance, CD36 protein translocation is impaired (20), showing an increased amount of CD36 at the sarcolemma and a reduced translocation after insulin stimulation *in vitro*. A larger increase in CD36 protein may be a mechanism to compensate for a reduced translocation effect. On the other hand, an increased fatty acid transporter capacity during the time that meal-derived LCFA are highly available from chylomicrons and VLDL is likely to increase intramyocellular lipid storage, and thus these subjects may have become more insulin resistant.

In conclusion, CD36 protein is regulated in a remarkably dynamic manner by insulin *in vivo* in human skeletal muscle of obese subjects. This is a promising finding because an increase in CD36 protein content in the late postprandial phase may play an important role in an increased fractional extraction of fatty acids from plasma, enhancing intramyocellular triglyceride storage. It also emphasises that the uptake of free fatty acids may be regulated at the level of skeletal muscle by fatty acid transporters and not only by plasma lipid supply. In addition, our data suggest that this regulation may depend on the degree of insulin resistance.

Acknowledgements: Antibody MO25 was kindly provided by Dr. N. N. Tandon, Otsuka America Pharmaceutical, Inc., Rockville, MD, USA. We thank our volunteers without whom this study would not have been possible. We thank Jos Stegen, Gert Schaart, Joan Senden, Eveline Peeters-Tielen, Judith Huskens and Dorien Mintjes for their excellent assistance. Supported by grants from the Dutch Diabetes Research Foundation (DFN 98.901 and DFN 2000.00.020). JFCG is Netherlands Heart Foundation Professor of Cardiac Metabolism.

REFERENCES

1. Itani SI, Ruderman NB, Schmieder F, Boden G. Lipid-induced insulin resistance in human muscle is associated with changes in diacylglycerol, protein kinase c, and ikappab-alpha. *Diabetes* 2002; 51: 2005-2011.
2. Bachmann OP, Dahl DB, Brechtel K, *et al*. Effects of intravenous and dietary lipid challenge on intramyocellular lipid content and the relation with insulin sensitivity in humans. *Diabetes* 2001; 50: 2579-2584.
3. Jensen MD. Fatty acid oxidation in human skeletal muscle. *J Clin Invest* 2002; 110: 1607-1609.
4. van Loon LJ, Goodpaster BH. Increased intramuscular lipid storage in the insulin-resistant and endurance-trained state. *Pflugers Arch* 2006; 451: 606-616.
5. Kelley DE, Mandarino LJ. Fuel selection in human skeletal muscle in insulin resistance: A reexamination. *Diabetes* 2000; 49: 677-683.
6. Blaak EE, van Aggel-Leijssen DP, Wagenmakers AJ, Saris WH, van Baak MA. Impaired oxidation of plasma-derived fatty acids in type 2 diabetic subjects during moderate-intensity exercise. *Diabetes* 2000; 49: 2102-2107.
7. Mensink M, Blaak EE, van Baak MA, Wagenmakers AJ, Saris WH. Plasma free fatty acid uptake and oxidation are already diminished in subjects at high risk for developing type 2 diabetes. *Diabetes* 2001; 50: 2548-2554.
8. Turpeinen AK, Takala TO, Nuutila P, *et al*. Impaired free fatty acid uptake in skeletal muscle but not in myocardium in patients with impaired glucose tolerance: Studies with pet and 14(r,s)-[18f]fluoro-6-thia-heptadecanoic acid. *Diabetes* 1999; 48: 1245-1250.
9. Frayn KN. Adipose tissue as a buffer for daily lipid flux. *Diabetologia* 2002; 45: 1201-1210.
10. Ravikumar B, Carey PE, Snaar JE, *et al*. Real-time assessment of postprandial fat storage in liver and skeletal muscle in health and type 2 diabetes. *Am J Physiol Endocrinol Metab* 2005; 288: E789-797.
11. Febbraio M, Abumrad NA, Hajjar DP, *et al*. A null mutation in murine cd36 reveals an important role in fatty acid and lipoprotein metabolism. *J Biol Chem* 1999; 274: 19055-19062.
12. Glatz JF, Bonen A, Luiken JJ. Exercise and insulin increase muscle fatty acid uptake by recruiting putative fatty acid transporters to the sarcolemma. *Curr Opin Clin Nutr Metab Care* 2002; 5: 365-370.
13. Chabowski A, Coort SL, Calles-Escandon J, *et al*. Insulin stimulates fatty acid transport by regulating expression of fat/cd36 but not fabppm. *Am J Physiol Endocrinol Metab* 2004; 287: E781-789.
14. Wodzig KW, Pelsers MM, van der Vusse GJ, Roos W, Glatz JF. One-step enzyme-linked immunosorbent assay (elisa) for plasma fatty acid-binding protein. *Ann Clin Biochem* 1997; 34 (Pt 3): 263-268.
15. Pelsers MM, Lutgerink JT, Nieuwenhoven FA, *et al*. A sensitive immunoassay for rat fatty acid translocase (cd36) using phage antibodies selected on cell transfectants: Abundant presence of

fatty acid translocase/cd36 in cardiac and red skeletal muscle and up-regulation in diabetes. *Biochem J* 1999; 337 (Pt 3): 407-414.
16. Koopman R, Schaart G, Hesselink MK. Optimisation of oil red o staining permits combination with immunofluorescence and automated quantification of lipids. *Histochem Cell Biol* 2001; 116: 63-68.
17. Bandyopadhyay GK, Yu JG, Ofrecio J, Olefsky JM. Increased malonyl-coa levels in muscle from obese and type 2 diabetic subjects lead to decreased fatty acid oxidation and increased lipogenesis; thiazolidinedione treatment reverses these defects. *Diabetes* 2006; 55: 2277-2285.
18. Binas B, Danneberg H, McWhir J, Mullins L, Clark AJ. Requirement for the heart-type fatty acid binding protein in cardiac fatty acid utilization. *FASEB J* 1999; 13: 805-812.
19. Luiken JJ, Koonen DP, Coumans WA, *et al*. Long-chain fatty acid uptake by skeletal muscle is impaired in homozygous, but not heterozygous, heart-type-fabp null mice. *Lipids* 2003; 38: 491-496.
20. Bonen A, Parolin ML, Steinberg GR, *et al*. Triacylglycerol accumulation in human obesity and type 2 diabetes is associated with increased rates of skeletal muscle fatty acid transport and increased sarcolemmal fat/cd36. *FASEB J* 2004; 18: 1144-1146.

Received: August 23, 2007
Accepted: January 7, 2008

Author's address: E. Corpeleijn, PhD, Department of Human Biology, Faculty of Health, Medicine and Life Sciences, Maastricht University, P.O. Box 616, 6200 MD Maastricht, The Netherlands. Phone +31 433881638. Fax +31 433670976; e-mail: E.Corpeleijn@hb.unimaas.nl