Increased FAT (fatty acid translocase)/CD36-mediated long-chain fatty acid uptake in cardiac myocytes from obese Zucker rats

S.L.M. Coort*, J.J.F.P. Luiken†, G.J. van der Vusse†, A. Bonen‡ and J.F.C. Glatz*

*Department of Molecular Genetics, Cardiovascular Research Institute Maastricht (CARIM), Maastricht University, P.O. Box 616, NL-6200 MD Maastricht, The Netherlands, †Department of Physiology, Cardiovascular Research Institute Maastricht (CARIM), Maastricht University, Maastricht, The Netherlands, ‡Department of Human Biology and Nutritional Sciences, Guelph University, Guelph, Ontario, Canada

Abstract
Disturbed cardiac lipid homoeostasis in obesity is regarded as a key player in the development of cardiovascular diseases. In this study, we show that FAT (fatty acid translocase)/CD36-mediated LCFA (long-chain fatty acid) uptake in cardiac myocytes from young adult obese Zucker rats is markedly increased, but insensitive to insulin. Basal and insulin-induced glucose uptake rates in these myocytes are not changed, suggesting that during the development from obesity to hyperglycaemic Type II diabetes, alterations in cardiac LCFA uptake precede alterations in cardiac glucose uptake.

In obesity, in which insulin resistance is the key metabolic defect, cardiovascular diseases are the most serious complication [1]. In the ‘obese heart’, TAG (triacylglycerol) accumulation occurs rapidly and is positively correlated with the development of insulin resistance [2–4]. It has been hypothesized that this cardiac TAG accumulation develops as a result of increased cardiac LCFA (long-chain fatty acid) uptake [5]. Under non-pathological conditions, a major part of the cardiac LCFA uptake is mediated by the sarcolemmal protein, FAT (fatty acid translocase)/CD36 [6]. Recently, it was found that upon physiological stimuli, such as insulin, FAT/CD36 translocates from intracellular stores towards the sarcolemma to increase LCFA uptake [7]. In the present study, we hypothesized that cardiac FAT/CD36-mediated LCFA uptake and its regulation by insulin are altered in obese Zucker rats.

We studied 11-week-old female lean and obese Zucker rats, a commonly used rodent model, to investigate obesity and insulin resistance [8]. Both body weight and heart weight were significantly higher in obese Zucker rats compared with their age-matched lean rats. Moreover, the obese Zucker rats, when compared with their lean controls, were markedly hyperinsulinaemic, had significantly elevated TAG plasma levels, and were euglycaemic (results not shown). Taken together, these characteristics demonstrate that the obese Zucker rats investigated were systemically insulin-resistant but still in a pre-diabetic state.

To investigate FAT/CD36-mediated LCFA uptake by the obese heart we used two models, i.e. giant sarcolemmal vesicles and isolated cardiac myocytes. In giant sarcolemmal vesicles LCFA transport across the sarcolemma can be examined separately from LCFA metabolism [9], whereas in cardiac myocytes LCFA uptake is closely linked to rapid LCFA metabolism [6]. LCFA uptake rates in giant sarcolemmal vesicles and cardiac myocytes, as derived from obese Zucker rats, were significantly elevated by 80 and 40%, respectively, compared with their lean controls (Figure 1). The total amount of FAT/CD36 in homogenates of obese rat hearts was unaltered (Figure 1). However, in obese Zucker rats both the membranes from giant sarcolemmal vesicles as well as the sarcolemma of cardiac myocytes displayed an elevated (+60 and +74%, respectively) amount of FAT/CD36, which in cardiac myocytes was found to coincide with a decreased (−50%) intracellular storage of this protein (Figure 1). Taken together, these data suggest that a permanent relocation of FAT/CD36 from an intracellular compartment to the sarcolemma is responsible for the elevated LCFA uptake by the obese rat heart.

In order to investigate whether this permanent relocation of FAT/CD36 to the sarcolemma is due to alterations in the regulation of FAT/CD36 translocation by insulin, we investigated the influence of insulin on LCFA uptake. For comparison we also measured DOG (deoxyglucose) uptake, which is predominantly mediated by the plasmalemmal glucose transporter 4 (GLUT4), in isolated cardiac myocytes from young adult Zucker rats. Basal DOG uptake rates were not different between lean and obese cardiac myocytes, and insulin, at a concentration of 10 nM, was able to induce DOG uptake in both lean and obese cardiac myocytes by 95 and 72% (P < 0.05), respectively (Figure 2A). These data are...
Cardiac LCFA uptake and FAT/CD36 subcellular distribution in lean and obese Zucker rats, as studied in sarcolemmal vesicles (A) and isolated cardiac myocytes (B).

(A) In giant sarcolemmal vesicles, derived from lean and obese Zucker rats, $[^{3}H]$palmitate (LCFA) transport was measured for 15 s at room temperature. Total FAT/CD36 protein in heart homogenates (10 µg), and sarcolemmal FAT/CD36 in giant sarcolemmal vesicles (5 µg) were determined by Western blotting. (B) In isolated cardiac myocytes, derived from lean and obese Zucker rats, $[^{14}C]$palmitate (LCFA) uptake was measured for 3 min at 37°C. Cardiac myocytes were fractionated and the FAT/CD36 protein content measured in both the sarcolemmal fraction and the intracellular membrane fraction by Western blotting. A monoclonal antibody (MO25) directed against human CD36, and kindly provided by Dr N.N. Tandon (Otsuka Maryland Research Institute Rockville, MD, U.S.A.), was used to detect FAT/CD36. Data are means ± S.E.M.; * significantly different from lean Zucker rats, $P < 0.05$. Giant sarcolemmal vesicle data were obtained from [5].

consistent with earlier findings of Kolter and co-workers [10], who demonstrated that in obese cardiac myocytes GLUT4 expression is not reduced and that its translocation machinery is still operational. As mentioned above, basal LCFA uptake rates by obese cardiac myocytes were significantly elevated by 40%, compared with lean cardiac myocytes (Figures 1B and 2B). Interestingly, insulin lost its ability to induce LCFA uptake in cardiac myocytes from obese Zucker rats, whereas it induced LCFA uptake by up to 30% in lean cardiac myocytes (Figure 2B).

In conclusion, the combined observations demonstrate a pivotal role of sarcolemmal FAT/CD36 in the altered cardiac LCFA uptake rate seen in obese Zucker rats. The increased plasma insulin concentration in the obese rats is most likely responsible for an increased sarcolemmal abundance of FAT/CD36, and hence an increased LCFA uptake rate. These findings also suggest that during the gradual development from obesity to hyperglycaemic Type II diabetes, abnormalities in cardiac LCFA uptake precede abnormalities in cardiac glucose uptake.
Figure 2 | Effects of insulin on substrate uptake by cardiac myocytes from lean and obese Zucker rats
Cardiac myocytes were preincubated for 15 min at 37°C in the absence or presence of 10 nM insulin, after which (A) [14C]palmitate (LCFA) and (B) [3H]DOG uptake rates in 3 min were simultaneously determined. Data are presented as means ± S.E.M.; *significantly different from basal uptake rate in lean cardiac myocytes, $P < 0.05$; #significantly different from basal uptake rate in obese cardiac myocytes, $P < 0.05$.

This study was supported by the Netherlands Heart Foundation (2000.156) and ZonMw (VIDI 016.036.305).

References
1 Taegtmeyer, H., McNulty, P. and Young, M.E. (2002) Circulation 105, 1727–1733

Received 7 September 2003