Arachidonic acid and glucose uptake by freshly isolated human adipocytes

Ana C. A. Malipa¹, Roy A. Meintjes¹* and Marianne Haag²

¹Department of Anatomy and Physiology, Faculty of Veterinary Science, University of Pretoria, South Africa
²Department of Human Physiology, Faculty of Health, University of Pretoria, South Africa

Fatty acid (FA) and glucose transport into insulin-dependent cells are impaired in insulin resistance (IR; type 2 diabetes mellitus). Studies done on the effects of FAs on glucose uptake, and the influence of insulin on FA uptake by adipocytes, have yielded contradictory results. In this study, isolated human adipocytes were exposed to arachidonic acid (AA) and to insulin, and FA uptake as well as glucose uptake was measured. AA uptake into adipocyte membranes and nuclei was also investigated. Glucose uptake was inhibited by 57±8% after 30 min of exposure to arachidonate. AA was significantly taken up into adipocyte membranes (49.6±29% and 123±74%) at 20 and 30 min of exposure, respectively, and into nuclei (147.6±19.2%) after 30 min. Insulin stimulated AA uptake (24.1±14.1%) at 30 min by adipocytes from a non-obese subject, while inhibiting it (16.6±12%) in adipocytes from an obese subject. These results suggest that: (1) AA inhibits glucose uptake by adipocytes exposed over a short period, probably by a membrane-associated mechanism, (2) insulin-dependent AA uptake is dependent on the body mass index (BMI) of the donor and the insulin sensitivity of their adipocytes.

INTRODUCTION

Adipose tissue contributes meaningfully to the homeostasis of blood glucose, especially in the obese person.¹ Furthermore, high rates of lipolysis in the central fat depots of obese subjects⁵ could be an important source of fatty acids (FAs), not only for the normal roles of FAs in the body, but also for the eventual development of insulin resistance (IR).³⁻⁶ Both glucose and FA uptake into muscle and fat cells are impaired in IR in type 2 diabetes.⁹,¹⁰ Part of the regulation of FA transport is similar to the regulation of glucose transport: both FA transporters and glucose transporters are kept in different cytoplasmic vesicles from where they are recruited to the plasma membrane, following a stimulus (such as insulin or FAs)¹⁰,¹¹. Evidence has shown that FA uptake is facilitated by transport¹¹,¹² as well as by simple diffusion.¹³ Several plasma membrane¹⁴,¹⁵ and cytosolic proteins¹⁶,¹⁷ with affinity for FAs have been identified in adipocytes.

FAs have been shown to affect glucose uptake into adipocytes but the effect is highly controversial. While some authors have observed a stimulatory effect of FAs on glucose uptake,¹⁶,¹⁸⁻²⁰ others have shown an inhibitory effect.²¹⁻²³ Unsaturated FAs have also been shown to inhibit the stimulatory effect of insulin on glucose uptake,²³ but a high content of omega-3 FAs in the membrane was shown to improve the action of insulin,²⁰,²⁴ as well as the activity of membrane proteins such as the Na⁺/K⁻ ATPase pump, that is also involved in insulin action.²⁵ To our knowledge, the studies already done on the effects of FAs on glucose uptake by adipocytes were based on their exposure for more than 1 h to the FA.¹⁸,²⁰,²¹ Therefore, the present study investigates: (i) the effects of 30 min exposure of arachidonic acid (AA) (representing n⁻6 FAs) on glucose uptake by fresh human adipocytes, (ii) AA uptake into subcellular fractions (membranes...
and nucleus) as a first stage in identifying the mechanism whereby FA affects glucose uptake into adipocytes and (iii) to verify the effect of insulin on AA uptake in this model.

MATERIALS AND METHODS

Materials

Collagenase CLS type I (Worthington Biochemical Corporation, Lakewood, US). Polyamide nylon filter with 400 μm pore size (Neolab, Heidelberg, Germany). [1-14C] AA and 2-deoxy-D-[2,6-3H] glucose (Amersham Bioscience, Bucks, UK). AA, glucose, fatty acid free bovine serum albumin (FAFA), insulin, sucrose and all other chemicals (Sigma, St. Louis, USA).

Samples of omental fat were obtained from non-diabetic consenting women undergoing abdominal hysterectomy at the Pretoria Academic Hospital, Eugene Marais Hospital and Femina Clinic in Pretoria. The procedures were approved by the Ethical Committee of the Faculty of Health Sciences, University of Pretoria.

Adipocyte isolation

30–50 g omental fat was dissected, minced finely with scissors and used to isolate adipocytes using collagenase.26 In a polypropylene tube, 5 g dissected, minced fat was mixed with 18 mg collagenase dissolved in 5 ml Krebs–Ringer buffer with glucose (KRB1, containing 25 mM Tris, 125 mM NaCl, 5 mM KCl, 1 mM KH2PO4, 2.5 mM MgSO4·7(H2O), 1 mM CaCl2·2(H2O), 4 mM glucose, pH 7.4). The volume was made up to 20 ml. The final concentration of collagenase in the mixture was 0.9 mg ml−1. The digestion of connective tissue was done at 37°C for 90 min, with gentle mixing by inverting the tube every 15 min. Isolated adipocytes were washed twice with Krebs–Ringer buffer without glucose (KRB2). Finally, adipocytes were resuspended with KRB2 at 30% lipocrit and kept at 37°C for 40 min to return to basal conditions.

Arachidonic acid solution

Six microliters of 328 mM AA stock solution in ethanol or dimethyl sulphoxide (DMSO) was diluted with 1986 μl 1% FAFA in KRB2 at 37°C to the following concentrations: 1 mM AA, 0.30% ethanol and 1% FAFA. In certain experiments [1–14C] AA was included (2–3 × 10^3 cpm nmol⁻¹ AA).

Effect of arachidonic acid on basal and insulin-stimulated glucose uptake

Three hundred and fifty microliters 1% FAFA in KRB2 was mixed with 300 μl isolated adipocytes (30% lipocrit) in a 4 ml polypropylene tube. The cells were pre-treated with 80 μl 1 mM 14C-AA in ethanol-FAFA or vehicle for 10 min. Subsequently 300 μl of insulin in KRB2 or vehicle was added at a concentration of 10 nM for 20 min. Then 100 μl 4.17 mM 3H-deoxyglucose (a non-metabolizable glucose analogue, 80–100 cpm nmol⁻¹) in KRB2 was added. 3H-deoxyglucose uptake was allowed for 6 min. The final concentrations in the reaction mix were: 96 μM AA, 0.52% FAFA, 0.03% ethanol, 502 μM 3H-deoxyglucose. To terminate the reaction, 35 μl 10.4 M phloretin (PHL) in DMSO-KRB2 were added (final concentration 200 μM PHL, 0.03% DMSO) and kept at 18°C for 5 min. The cells were then washed twice with KRB2 and lysed in 2% SDS at 80°C for 10 min. After cooling to room temperature, protein was determined using the Lowry method.27 Radioactivity in the samples was counted using a Beckman model L-17 scintillation counter. Deoxyglucose uptake was expressed in nanomole deoxyglucose/milligram protein/minute. Blanks were performed under the same conditions as the test, except that PHL was added at the beginning of the incubation. The value was subtracted from that of the test to correct for unspecific AA uptake.

Effect of insulin on arachidonic acid uptake

First, 0.9 ml isolated adipocytes (30% lipocrit) were placed in a 4 ml polypropylene tube. Metabolism of AA was inhibited by adding 50 μl 2 mM indomethacin (IDM, a cyclooxygenase inhibitor) and 50 μl 2 mM nordihydroguaiaretic acid (NDGA, a lipoxygenase inhibitor) to each tube and incubating for 2 min at 37°C. Then, 170 μl of KRB2 or insulin in KRB2, was added and adipocytes were sensitized with insulin (10–40 nM concentrations at this stage), for 5 min. Subsequently, 280 μl 0.52 mM 14C-AA in DMSO-FAFA (specific activity: 2–3 cpm nmol⁻¹ AA) were added and incubated for 30 min at 37°C. The final concentrations of the reaction mix were: adipocytes (19% lipocrit), 100 μM 14C-AA, 69 μM IDM, 69 μM NDGA, 0.19% FAFA, 0.05% DMSO and 10–40 nM insulin. The reaction was stopped by adding 59 μl 5 mM PHL (final concentration 196 μM PHL) and kept at 18°C for 5 min. Then, cells were washed twice with KRB2 and lysed with 1 ml 2% SDS at 80°C for 10 min. After cooling at room temperature,
aliquots were used to quantify protein by the Lowry method. Scintillation counting of $^{14}$C-AA in the samples was done in a model L17 Beckman counter. AA uptake was expressed in nanomole AA/milligram protein/minute. Non-specific AA uptake was corrected by subtracting blank from test values. The blank was performed under the same conditions except that PHL was added at the beginning of the incubation.

**Uptake of AA into adipocyte membrane and nuclear fractions**

Three ml adipocytes (30% lipocrit) were pre-treated with 125 $\mu$L 2 mM IDM and 125 $\mu$L 2 mM NDGA, both in DMSO-KRB2 (concentration 100 $\mu$M of both IDM and NDGA at this stage), for 5 min at 37°C to minimize AA metabolism. Subsequently, 625 $\mu$L 0.52 mM AA in DMSO-FAFA containing 4–8 $\mu$L of $^{14}$C-AA was added (specific activity: 2–3 cpm mmol$^{-1}$ AA). AA uptake was performed for 0–30 min in a water bath at 37°C. The final concentrations in the reaction mixture were: adipocytes (23% lipocrit), 84 $\mu$M AA, 65 $\mu$M IDM, 65 $\mu$M NDGA, 0.16% FAFA and 0.05% DMSO. Thereafter, the uptake was stopped by adding 157 $\mu$L 5.1 mM PHL in DMSO-KRB2, to a final concentration of 198$\mu$M PHL and 0.05% DMSO, for 5 min at 18 °C. Adipocytes were washed once with KRB2, twice with ice-cold buffer (TES: 250 mM sucrose, 20 mMTris, 1 mM phenyl-methyl-sulphonyl fluoride (PMSF) and 1 mM EDTA, pH 7.4) and resuspended with 10 ml of the same buffer. Adipocytes were homogenized with 10 strokes using a Potter homogenizer. The homogenate was centrifuged at 800g for 10 min at 4 °C using a Rotixa 120 R Hettich centrifuge to pellet the nuclei. The nuclei were washed once again under the same conditions. The supernatant of the first centrifugation was ultracentrifuged at 10000g for 20 min at 4°C using a model L-17 Beckman ultracentrifuge to pellet the crude membranes. The pellet was centrifuged once again under the same conditions to wash the membranes. Subsequently, membranes and nuclei were dissolved in 2% SDS at 80°C for 10 min. After cooling at room temperature, protein determinations were done by the Lowry method. $^{14}$C-AA taken up in the membrane and nuclear fractions was measured using a model L-17 Beckman ultracentrifuge to pellet the crude membranes. The experiment was repeated eight times and three representative experiments ($n = 3$) were combined to present the results.

![Figure 1](image-url)  
Figure 1. Effect of arachidonic acid on glucose uptake into fresh human adipocytes: Adipocytes were pre-treated with 100 $\mu$M AA, 40 nM insulin (I 40 nM) and 40 nM insulin plus 100 $\mu$M AA for 30 min. Uptake of $^3$H-deoxyglucose (DOG) (5 mM) was performed for 6 min. $^3$H-deoxyglucose uptake was expressed in nanomole/milligram protein/minute. ANOVA with Bartlett’s post-hoc test was used to analyse the data, $p < 0.05$ was considered as significant. A significant increase of basal glucose uptake was observed when the cells were exposed to 40 nM insulin. The exposure to 100 $\mu$M AA significantly depressed basal glucose uptake. The experiment was repeated eight times and three representative experiments ($n = 3$) were combined to present the results.
Beckman scintillation counter. The $^{14}$C-AA uptake was expressed in nanomole/milligram protein.

RESULTS

Effects of arachidonic acid on glucose uptake

The influence of AA on glucose uptake was investigated, measuring $^3$H-deoxyglucose taken up into isolated human adipocytes after their sensitization with 100 μM AA for 30 min. The results (Figure 1) showed that basal glucose uptake (0.42 ± 0.03 nmol mg protein min$^{-1}$ in the control) was significantly inhibited by AA with an average of 57 ± 13%. Insulin significantly increased glucose uptake by an average of 40 ± 21% compared to the control. However, the addition of AA had no influence on the stimulation of DOG by insulin.

Time frame of arachidonic acid uptake into subcellular fractions

AA uptake into human adipocyte subcellular fractions (membranes and nucleus) was determined over 30 min, in order to identify the site of subcellular action behind the observed inhibitory effect of AA on glucose uptake into adipocytes. To this end, $^{14}$C-AA taken up by membranes and nuclei over 30 min was determined. The results (Figure 2A and B) showed that AA was significantly taken up into both adipocyte crude membrane (123 ± 73%) and nuclear (147 ± 19%) adipocyte fractions after 30 min in relation to the uptake of AA at zero minute (membranes 1.77 ± 0.06 nmol AA mg$^{-1}$ protein and nuclear 2.25 ± 0.10 nmol mg$^{-1}$ protein).

Effect of insulin on arachidonic acid uptake

To assess insulin-induced AA uptake, adipocytes from obese and non-obese subjects were exposed to $^{14}$C-AA for 30 min. The $^{14}$C-AA taken up by adipocytes pre-treated with different concentrations of insulin were compared with the control. As shown in Figure 3A, insulin stimulated AA uptake into adipocytes from a non-obese subject (BMI = 25). The AA uptake at zero nanomolar insulin (control)
was 0.06 ± 0.01 nmol mg protein/min. At 40 nM, insulin increased AA uptake by 31 ± 4%. In contrast, insulin inhibited AA uptake after 30 min by adipocytes from an obese subject (BMI = 30). This inhibition was significant at 40 nM insulin (0.06 ± 0.005 nmol AA mg protein min⁻¹), compared to the AA uptake at zero nanomolar insulin (0.08 ± 0.01 nmol mg protein min⁻¹).

DISCUSSION

High levels of plasma saturated FAs have been correlated with the development of IR and type 2 diabetes. Brief in vitro exposure (<30 min) of adipocytes to saturated FAs has been reported to stimulate insulin-stimulated glucose uptake (ISGU), while prolonged (>1 h) exposure has impaired such uptake.28,29 The dietary effect of unsaturated FAs (both mono-unsaturated and poly-unsaturated) on ISGU uptake is also controversial. Some research groups found that dietary omega-3 and omega-6 FAs stimulated ISGU in adipocytes.23,29 Of note is the work of Nugent and coworkers in 2001,20 who demonstrated that exposure of 3T3-L1 adipocytes to AA in culture for 4–48 h enhanced ISGU. In contrast, there is also evidence that olate and polyunsaturated fatty acids (PUFAs)30,31 impair ISGU in adipocytes. To our knowledge there is no literature available about short-term exposure of human adipocytes to AA. In contrast with all the above mentioned results, the present study showed no effect of AA on ISGU after 30 min exposure (Figure 1).

The present study has, however, shown that, in contrast to insulin, AA depressed basal glucose uptake (facilitated by Glut 1) into adipocyte cells after 30 min exposure (Figure 1). Little is known about the regulation of GLUT1, but it is possible that AA may act via a PKC-dependent or independent mechanism23,30,32 with subsequent inhibition of GLUT1 translocation to the plasma membrane.21 Since a cyclo-oxygenase inhibitor7 was not used in this assay, the inhibitory effect of AA on glucose uptake observed in this study could also be due to the action of eicosanoids.15 Because the minimal period required to express the glucose transporter genes has been reported to be 30 min,33 the possibility of AA or its metabolite inhibiting the expression of the GLUT1 gene21,34,35 may also be possible. The results of the present study are in contrast with the findings of Fong et al.36 in 1999 who have shown a stimulatory effect of AA exposure on basal (insulin-independent) glucose uptake.

Other mechanisms by which FAs affect glucose uptake have been proposed in the literature: (i) an effect on phospholipid membrane composition6,18,24; (ii) activation of the peroxisome proliferator-activator receptor (PPAR)-γ, which has the consequence of stimulating GLUT4 rather than GLUT1 gene expression37 and (iii) an effect on the stability of mRNA of the glucose transporter.21 The mechanism(s) involved in our results remain to be established.

Figure 3. Influence of insulin concentration on AA uptake measured for 30 min into fresh human adipocytes: Adipocytes were pre-incubated with 100 μM IDM and NDGA. Subsequently, cells were sensitized with insulin (0, 10, 20 and 40 nM) for 20 min. AA uptake was performed for 10 and 30 min. AA uptake was expressed in nanomole/milligram protein/minute. Comparisons between the control (zero nanomolar) and uptake at different insulin concentrations were done with ANOVA using Bartlett’s post-hoc test. Three experiments were conducted, n = 4. Data (mean ± SD) from one representative experiment is presented, p < 0.05 was considered to be significant. (A) Data from a non-obese subject (BMI = 24.5 kg m⁻²). (B) Data from an obese subject (BMI = 30.5 kg m⁻²); a significant difference was observed only at 40 nM insulin"
To aid in determining the cellular locus of AA action on glucose uptake, AA uptake into subcellular fractions (nuclei and crude membranes) was performed over a time span of 30 min. FA transport into and out of the cell is known to take place through simple diffusion \(^{13}\) and highly regulated protein-mediated transport. \(^{11}\) The results showed that AA was significantly taken up into both adipocyte crude membrane and nuclear adipocyte fractions after 30 min, in relation to AA content at zero minute. By extrapolation, this suggests that the effect of AA on glucose uptake by fresh isolated adipocytes may take place by a membrane-based mechanism but eventual participation of gene expression may also be possible after 30 min of exposure of this cell type to AA.

FA uptake into intact cells is notoriously difficult to measure because the FAs are rapidly channelled into metabolic processes. \(^{9,34}\) The data of this experiment are, however, considered to be reliable, since the metabolism of AA was minimized by pre-treatment of the adipocytes with IDM \(^{30}\) and NDGA \(^{20,38}\) But, because carnitine acyl-transferase was not inhibited, it is likely that some amount of FA could have moved into mitochondria. However, because the AA uptake in the present work was done in crude membranes, which include plasma membranes and mitochondria and other cytosolic organelles, except the nucleus, the AA eventually taken up by mitochondria was measured together with plasma membranes.

Insulin influences both glucose and FA transport into and out of cells. \(^{10,11,20}\) Although insulin is reportedly a negative regulator of FA transport protein 1 (FATP1) mRNA, \(^{39}\) there is also evidence suggesting that insulin stimulates FA uptake. \(^{10,20}\) Insulin was also shown to profoundly suppress FA transport from adipocytes. \(^{40}\) This finding was supported by Luiken et al. \(^{41}\) in 2002, who showed that adipocytes from streptozotocin-induced diabetic (Diabetes Mellitus type 1) rats had increased their FA transport across the plasma membrane, releasing FAs, with a simultaneous increase of FA binding protein, plasma membrane (FABPpm) expression and increased amounts of this FA transporter in the plasma membrane.

To assay insulin-induced AA uptake, adipocytes from obese and non-obese subjects were exposed to \(^{14}\)C-FA for 30 min after pre-sensitization with insulin for 20 min. In a non-obese subject, insulin stimulated AA uptake into adipocytes. The maximal insulin concentration (40 nM) was not enough to have a saturating effect. Although the methods used were different, this study agrees with the finding of Hamilton and Kamp \(^{11}\) in 1999 in their studies using 3T3-L1 adipocytes: Insulin was shown to stimulate FATP1 translocase to the plasma membrane of adipocytes. \(^{10,27}\) Insulin was also reported to stimulate the translocation of FAT/CD36 from intracellular vesicles to the plasma membrane of myocytes, resulting in enhanced palmitate uptake. \(^{33}\) Adipocytes also express FAT/CD36. \(^{33,42}\) Therefore, beside the more probable mechanism that involves FATP1 translocation, the translocation of FAT/CD36 protein could also be involved in increased AA uptake observed in the present study. Furthermore, it was also observed that in an obese subject, insulin decreased AA uptake (30 min) by adipocytes. This could result from the fact that the cells from this obese subject are already IR, thus depressing AA uptake. Also, the obese subject was of African origin and the non-obese subject of Caucasian origin so ethnic differences cannot be ruled out.

Taking the results of the present study into consideration, it is concluded that (i) 30 min exposure of fresh human isolated adipocytes to AA depressed basal glucose uptake, (ii) the mechanism by which AA depresses glucose uptake primarily involves AA action on the membrane and only after 30 min of exposure the effect of AA perhaps can also be attributed to modulation of gene expression, (iii) the effect of insulin on AA uptake is also influenced by the origin of adipocytes and their sensitivity to insulin.

ACKNOWLEDGEMENTS

This work was supported by research grants to M. Haag from the National Research Foundation of South Africa and the Medical Research Council of South Africa. A contribution from the World Bank to A. Malipa (a grantee from Mozambique) for her M Sc studies in South Africa is also gratefully acknowledged. Our sincere thanks to Dr A. Mouton and Prof G. Dreyer from the Dept. of Gynaecology, University of Pretoria, and Dr M. Groot from Eugene Marais Hospital in Pretoria for providing us with omental fat from hysterectomy operations. We also acknowledge the guidance of Prof R. Apatu, Department of Physiology, University of Pretoria, in ultracentrifuge techniques. We are grateful to Ms Alida Koorts for statistical evaluation of our data.

REFERENCES
