Available online at www.sciencedirect.com
ScienceDirect
Biochemical and Biophysical Research Communications 361 (2007) 356–361
BBRC
www.elsevier.com/locate/ybbrc

Depot-specific effects of fatty acids on lipid accumulation in children's adipocytes

Matthew A. Sabin [a], Elizabeth C. Crowne [a], Claire E. Stewart [b], Linda P. Hunt [a], Stephen J. Turner [a], Gavin I. Welsh [a], Malcolm J. Grohmann [a], Jeff M. Holly [a,1], Julian P. Shield [a,*,1]

[a] *University of Bristol, Bristol, UK*
[b] *Manchester Metropolitan University, Manchester, UK*

Received 27 June 2007
Available online 16 July 2007

Abstract

Circulating concentrations of fatty acids are elevated in obesity, although their effect on regional fat deposition is relatively unexplored. With the increasing prevalence of childhood obesity, we aimed to investigate whether saturated and unsaturated fatty acids lead to differential lipid accumulation (LA) in children's subcutaneous and visceral adipocytes. To examine this, subcutaneous and peri-nephric pre-adipocytes, isolated from fat biopsies from 6 pre-pubertal children, were differentiated *in vitro* before being exposed to palmitate and/or oleate for 24 h. Lipid accumulation was then quantified by nile red staining. Palmitate significantly increased LA in visceral adipocytes at all doses $\geqslant$188 μM (e.g. Palmitate 750 μM: +30.0%[8.2]; $p < 0.01$), whilst only a dose of 375 μM led to a significant, but smaller, increase in LA in subcutaneous adipocytes (Palmitate 375 μM: +13.0%[4.3]; $p = 0.02$). In contrast, oleate significantly increased LA in subcutaneous (Oleate 1000 μM: +36.3%[14.0]; $p = 0.01$), but not visceral (Oleate 1000 μM: +16.2%[9.6]; $p = 0.25$) adipocytes. These data suggest that saturated and unsaturated fatty acids may exert depot-specific effects on lipid accumulation.

Keywords: Palmitate; Oleate; Subcutaneous; Visceral; Adipocyte; Children

Visceral adiposity is associated with the development of numerous metabolic complications including Type II diabetes and cardiovascular disease [1]. This relationship may be mediated by many factors but the elevated circulating levels of free fatty acids (FFAs) seen in adult [2] and childhood [3] obesity undoubtedly represent a significant causal link [4,5]. The mechanisms for this association remain unclear although differential effects of FFAs (including palmitate and oleate—the main circulating saturated and unsaturated FFAs, respectively) are evident in muscle [6,7], liver [8], pancreatic β-cells [9] and coronary artery endothelial cells [10]. While some studies have also examined the effects of FFAs on insulin signalling in adipocytes (yielding less significant findings than those seen in other cell types [11]), very few studies have examined their effect on intra-adipocyte lipid accumulation [12]. This would seem important given that variations in the type of dietary fat in early childhood may substantially alter the deposition and modelling of triacylglycerol in adipose tissue during growth—thus potentially increasing a child's susceptibility to later obesity [13]. It may also prove important in that the selective release of FFAs from adipose triacylglycerols, which may impact upon processes governing the development of Type II diabetes and cardiovascular

Abbreviations: DMEM, Dulbecco's modified eagle's medium; DHAP, dihydroxyacetone phosphate; FFA, free fatty acid; G3PDH, glycerol-3-phosphate dehydrogenase; HBBS, Hank's balanced salt solution; HiFBS, heat-inactivated fetal bovine serum; IBMX, 3-isobutyl-I-methyl-xanthine; IPA, isopropanol; NADH, nicotine adenine dinucleotide (reduced form); NR, nile red; PBS, phosphate buffered saline; 1% PSLG, 1%, penicillin/streptomycin with 1% L-glutamine; SDS, standard deviation score.

* Corresponding author. Fax: +44 117 342 0178.
E-mail address: j.p.h.shield@bris.ac.uk (J.P. Shield).

[1] These authors contributed equally to this work.

0006-291X/$ - see front matter © 2007 Elsevier Inc. All rights reserved.
doi:10.1016/j.bbrc.2007.06.184

disease, appears dependent upon chain length and the degree of saturation [14].

The increasing prevalence of childhood obesity now requires the generation of specific models with which to investigate the mechanisms responsible for the development of adiposity and associated co-morbidities in this population. The functional distinctions of visceral adipocytes cannot be examined in murine cell lines such as 3T3-L1 cells, and regional, depot-specific, differences in adipocyte function (that are so important for human morbidity) are very different and much less marked in rodents [15]. While the development of human adipocyte cultures has circumvented some of these issues, there is now evidence that child-specific studies are required to fully understand the processes occurring during childhood. For example, pre-pubertal children respond differently to an intra-lipid challenge when compared with pubertal children and adults [16]—an effect that is probably mediated by the absence of significant effects of sex steroids on triglyceride synthesis [17,18]. For these reasons, we have developed a unique *in vitro* cell culture model of human subcutaneous and visceral adipocytes derived from young children [19]. As early nutrition is known to be important for the development of obesity and associated metabolic disease [20], we hypothesised that saturated and unsaturated FFAs might display depot-specific effects on lipid accumulation in subcutaneous and visceral adipocytes derived from young children.

Materials and methods

Participants

Ethical approval was granted by the United Bristol Healthcare Trust Local Research Ethics Committee. The parents/guardians of 6 normal-weight children admitted to the Bristol Royal Hospital for Children for routine surgery (for non-septic, non-malignant conditions) were approached for this study. Following full explanation and written consent, small surgical biopsies (approximately 0.2–0.5 g) of subcutaneous and intra-abdominal peri-nephric fat were obtained by an experienced paediatric surgeon at the onset of the operation. Perinephric fat, although intra-abdominal, displays some differences in behaviour when compared with mesenteric and omental fat [1]. It should be noted, however, that the omentum is poorly formed in young children rendering sampling difficult. Furthermore, since most of the children were undergoing elective kidney operations, perinephric fat collection ensured no alteration to surgical procedures and provided a consistent source of intra-abdominal fat. All children were normotensive and fasting blood analysis demonstrated normal insulin sensitivity. Clinical details are shown in Table 1.

Materials

Cell culture plastics were obtained from Greiner Bio-One (Gloucestershire, England). HiFBS, DMEM/Ham'sF-12, and HBSS were from Gibco Invitrogen (Paisley, UK). Streptomycin and penicillin were from the hospital pharmacy. Phosphate buffered saline (PBS) was from Oxoid (Hampshire, England) and insulin (Actrapid) from NovoNordisk (Bagsværd, Denmark). Rosiglitazone was kindly donated by GlaxoSmithKline (Uxbridge, Middlesex). The FLUOstar fluorescent plate reader was from BMG Lab Technologies (Offenburg, Germany). All other reagents were from Sigma (Poole, England).

Table 1
Clinical details of the 6 children that provided paired subcutaneous and visceral fat samples for this study

Biopsy number	Sex	Decimal age (years)	Type of operation	BMI (kg/m^2)	BMI SDS
1	Female	8.8	Pyeloplasty	17.6	+0.60
2	Male	1.9	Pyeloplasty	16.3	−0.36
3	Male	1.1	Reimplantation of megaureter	18.1	+0.41
4	Female	4.8	Pyeloplasty	14.7	−0.63
5	Male	1.0	Nephrectomy	15.0	−2.22
6	Male	1.1	Nephrectomy	22.4	+2.89

BMI, body mass index; BMI SDS, BMI standard deviation score.

Methods

Cell culture and differentiation procedures. The techniques of human pre-adipocyte isolation, culture and differentiation were developed and fully characterized within our laboratory, and have been described in detail elsewhere [19]. Briefly, the adipose tissue was washed 3× in 10 ml HBSS, cut into 1 mm^3 pieces, and digested with 10 ml of 1 mg/ml Type II collagenase in HBSS for 60 min at 37 °C. Adipocytes were separated from the stromal-vascular cells by centrifugation at 100 g for 3 min and the pellet of sedimented pre-adipocytes was resuspended in pre-adipocyte growth media (DMEM/Ham's F12 with 15 mM Hepes and 15 mM $NaHCO_3$ supplemented with 20% HiFBS, 1% PSLG), and seeded onto a 75 cm^2 0.2% gelatin-coated tissue culture flask. This was maintained at 37 °C in a humidified atmosphere of 5% CO_2 and the media was changed every 72 h until confluency was attained. For differentiation, pre-adipocytes were seeded at a density of 18,000 cells/cm^2 in 6 well plates and after 16-h were induced to differentiate for 14 days using a basic serum-free pre-adipocyte differentiation media (DMEM/Ham's F12 with 15 mM Hepes and 15 mM $NaHCO_3$, containing 33 μM biotin, 10 μg/ml apo-transferrin, 1 μM dexamethasone, 0.2 nM 3,3,5-triiodothyronine, 17 μM pantothenate and 1% PSLG). The media was filter-sterilised prior to use and the following media changes were performed; *Day 0–3,* basic media supplemented with 100 nM insulin, 25 μM IBMX and 10 μM Rosiglitazone; *Day 3–7*, basic media supplemented with 100 nM insulin; *Day 7–10,* basic media supplemented with 100 nM insulin; *Day 10–14,* basic media alone. Experiments were performed on cells at passages 3–6 to ensure phenotypic conservation.

Determination of degree of differentiation. G3PDH measurement was used as a sensitive and precise marker of *in vitro* terminal adipocyte differentiation [21], using a modified method of Kozak and Jensen [22]. Cells were washed twice in ice-cold PBS and harvested by scraping in 100 μl of lysis buffer containing 0.05 M Tris–Cl, 1 mM EDTA, and 1 mM β-mercaptoethanol (pH 7.4). G3PDH activity was measured spectrophotometrically at 340 nm in a reaction mix containing 100 mmol/l triethanolamine–HCl (pH 7.5), 12.5 mmol/l EDTA, 0.1 mmol/l β-mercaptoethanol, and 0.12 mmol/l NADH. The reactions were initiated with the addition of 0.4 mmol/l DHAP and readings were taken every 30 s for 3 min to ensure linearity. Protein concentrations were quantified using a bicinchoninic acid protein assay (Pierce BCA™ Protein Assay).

Fatty acid dosing. FFA dosages and methodology were based upon a previous report that examined FFA effects in muscle [23]. Briefly, palmitate and oleate were dissolved in ethanol to make 75 and 400 mM solutions, respectively, and these were added to a 20% albumin solution (20 g FFA-depleted albumin dissolved in serum free media). Serial dilutions were performed in 20% albumin to ensure equal amounts of albumin in the final solutions. DMEM/Ham's F12 with 15 mM Hepes and 15 mM $NaHCO_3$ supplemented with 1% HiFBS and 1% PSLG media was then added to generate a final FFA-containing media containing 5% albumin and the required concentrations of palmitate and/or oleate. Final solutions were filter-sterilised through a 0.22 μM syringe filter. The mature adipocytes were washed twice in PBS and changed to the FFA-containing media for 24 h prior to staining. All experiments were performed alongside control (vehicle) wells containing 5% albumin in the absence of added FFAs.