Dietary fatty acid composition alters 11β-hydroxysteroid dehydrogenase type 1 gene expression in rat retroperitoneal white adipose tissue

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Abstract

The enzyme 11β-hydroxysteroid dehydrogenase type 1 (11β-HSD1) amplifies intracellular glucocorticoid action by converting inactive glucocorticoids to their active forms in vivo. Adipose-specific overexpression of 11β-HSD1 induces metabolic syndrome in mice, whereas 11β-HSD1 null mice are resistant to it. Dietary trans and saturated fatty acids (TFAs and SFAs) are involved in the development of metabolic syndrome, whereas polyunsaturated fatty acids (PUFAs) offer protection against this. Here, we report the effects of chronic feeding of different diets containing vanaspati (TFA rich), palm oil (SFA rich) and sunflower oil (PUFA rich) at 10% level on 11β-HSD1 gene expression in rat retroperitoneal adipose tissue. 11β-HSD1 gene expression was significantly higher in TFA diet-fed rats compared to SFA rich diet-fed rats, which in turn was significantly higher than PUFA rich diet-fed rats. Similar trend was observed in the expression of CCAAT-enhancer binding protein-α (C/EBP-α), the main transcription factor required for the expression of 11β-HSD1. We propose that TFAs and SFAs increase local amplification of glucocorticoid action in adipose tissue by upregulating 11β-HSD1 by altering C/EBP-α gene expression. The increased levels of glucocorticoids in adipose tissue may lead to development of obesity and insulin resistance, thereby increasing the risk of developing metabolic syndrome.

Introduction

11β-hydroxysteroid dehydrogenase type 1 (11β-HSD1) plays an important role in the development of obesity and insulin resistance. 11β-HSD1 reactivates inactive glucocorticoids (11-dehydrocorticosterone in rodents, cortisone in humans) to their active forms (corticosterone in rodents, cortisol in humans) [1]. It is expressed in various tissues including liver and adipose tissue. Mice over-expressing 11β-HSD1 in adipose tissue develop visceral obesity and insulin resistance [2]. 11β-HSD1 knockout mice are resistant to diet-induced obesity and have exhibited improved insulin sensitivity [3]. CCAAT/enhancer-binding protein α (C/EBPα) directly regulates the expression of 11β-HSD1 [4], where as liver x receptor-α (LXR-α) down regulates it by indirect mechanisms [5].

Dietary fatty acids play an important role in cellular physiology by altering membrane fluidity, signal transduction and gene expression. They are also implicated in the development of metabolic syndrome (visceral obesity, insulin resistance, type 2 diabetes mellitus, dyslipidemia, hypertension, and increased cardiovascular risk profile). Trans fatty acids (TFAs) and saturated fatty acids (SFAs) are reported to play an important role in the development of obesity and insulin resistance [6-8]. The mechanisms through which TFAs and SFAs increase obesity and insulin resistance are poorly understood. TFAs and SFAs are shown to decrease the insulin sensitivity in rat adipose tissue by up regulating genes like resistin [9], on the contrary, polyunsaturated fatty acids like linoleic (18:2 n-6) acid are shown to improve insulin sensitivity and down regulate the...
lipogenic genes in adipose tissue [10]. Although previous studies reported the effect of high fat diet on 11β-HSD1 gene expression in adipose tissue of rodents and humans [11,12], presently no studies have reported the impact of dietary fat with varied fatty acid composition on the expression of adipose tissue 11β-HSD1.

As 11β-HSD1 plays a key role in the adipose tissue metabolism and development of metabolic syndrome, a study was designed to assess the impact of dietary fats with high TFAs, SFAs and PUFA s contents on the expression of 11β-HSD1 in rat adipose tissue.

Materials and methods
Animals, diets and experimental design
Twenty four weanling female fischer rats were obtained from the National Centre for Laboratory Animal Sciences (NCLAS) and divided into three groups of 8 each and housed individually in a temperature (22 ± 2°C) and light controlled (12 h cycle) conditions. The study was approved by Institutional Animal Ethical Committee (IAEC). The animals were fed on isocaloric (1.78 kJ/100 g diet) semi-synthetic diet containing identical amounts of all dietary constituents, except the quality of dietary fats (fatty acid composition) for a period of 12 months. The salt and vitamin mixtures were prepared according to American Institute of Nutrition AIN-93 [13]. The fatty acid compositions of various dietary oils (g/100 g) were estimated by Gas chromatography [14] and shown in Table 1. Vanaspati (Indian partially-hydrogenated vegetable oil) was used for the preparation of trans fatty acid rich diet (TFA rich diet), while palmolein and sunflower oils were used for the preparation of saturated fatty acid (SFA rich) and polyunsaturated fatty acid (PUFA rich diet) rich diets respectively. The animals had free access to water and food. At the end of the experiment, animals were sacrificed by CO2 inhalation and the retroperitoneal adipose tissue was dissected and frozen immediately in liquid nitrogen and stored at -80°C for RNA isolation.

Gene expression by semi quantitative reverse transcription-polymerase chain reaction
Total cellular RNA was extracted using a modification of the method of Chomczynski and Sacchi [15]. The integrity of the RNA was checked using 1% agarose gels stained with ethidium bromide. The RNA was quantified by spectrophotometric absorption at 260 nm. 1.0 µg of RNA was used to synthesize first strand cDNA. The reverse transcription (RT) reaction was carried out by incubating RNA with 0.5 µg oligo dT primer (Sigma) and 100 units of Molony murine leukemia virus reverse transcriptase (Finnzymes, Espoo, Finland) at 37°C for 60 min. Total reaction volume used in RT was 20 µL. An aliquot of cDNA was amplified in a 20 µL reaction mixture. PCR conditions were as follows, denaturation at 94°C for 1 minute, annealing for 45 seconds and polymerization for 70°C for 1 min with DyNAzyme II DNA polymerase (Finnzymes, Espoo, Finland). A final extension was carried out at 70°C for 7 min. The amount of RNA and the annealing temperature for different genes were standardized for linearity. Sequences of primers used for amplification are 11β-HSD1: forward primer-5'-GAGGAAGGGCTCCAG-3' and reverse primer-5' -GAGCCGAGATAAAGCC-3' (NM_012524), LXRα: forward primer-5'-GCCCCATGGACACCTA-3' and reverse primer-5' -TGAGGGTCCGGTGAA-3' (NM_031627), C/EBPα: forward primer-5' -GAGCCGAGATAAAGCCAA-3' and reverse primer 5'-CTTTCAGGCCGAC ACCA-3' (NM_012524), β-actin: forward primer-5' -ACCAACTGGGACGACATGGA-3' and reverse primer-5'-TCTCA AACATGATCTGGGTCA-3' (NM_031144). β-actin gene was used as an internal control. After amplification, 8 µL of reaction mixture were electrophoresed on agarose gel (2%) in Tris-borate EDTA buffer (pH 8.2). The ethidium bromide stained bands were visualized by a UV-transilluminator and analyzed densitometrically using Quantity One software program (Bio-Rad, version 4.4.0). Each experiment was repeated three times for similar results.

Statistics
Data were analyzed by one way ANOVA. Data are presented as mean ± SEM. Statistical significance was taken at level of P < 0.05 (two tailed).

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Table 1 Fatty acid composition of dietary oils (g/100 g of oil)

| Fatty acid | Vanaspati | Palmolein | Sunflower |
|------------|-----------|-----------|-----------|
| 14:0       | 0.9       | 0.79      | nd        |
| 16:0       | 38.4      | 35.6      | 6.55      |
| 16:1       | nd        | 0.16      | 0.13      |
| 18:0       | 5.16      | 4.50      | 4.67      |
| 18:1t      | 20.1      | nd        | nd        |
| 18:1c      | 29.4      | 45.6      | 44.0      |
| 18:2       | 43.1      | 12.4      | 43.6      |
| 20:0       | 0.48      | 0.55      | 0.45      |
| 18:3       | nd        | 0.25      | 0.44      |
| Σ SFA      | 45.0      | 41.5      | 11.7      |
| Σ MUFA     | 29.4      | 45.6      | 44.0      |
| LA         | 43.0      | 12.4      | 43.6      |
| ALNA       | nd        | 0.25      | 0.44      |

nd—not detected, ΣSFA—total saturated fatty acids, ΣMUFA—total monounsaturated fatty acids, LA—linoleic acid, ALNA—alpha-linolenic acid.

Vegetable oils were purchased from a local market, and used as a source of dietary fat (10%). Vanaspati, palmolein and sunflower oil were used for the preparation of trans fatty acid diet (TFA diet), saturated fatty acid diet (SFA diet) and polyunsaturated fatty acid diet (PUFA diet) respectively.
Results
Effect of dietary fatty acid composition on bodyweight gain and insulin resistance

Body weight gain increased significantly by TFA and SFA rich diet-fed rats compared to PUFA rich diet-fed rats (Table 2). There is no significance difference in bodyweight gain between TFA and SFA rich diet-fed rats (Table 2). There were no significant differences in fasting plasma insulin and glucose levels among the three groups (data not shown). TFA and SFA rich diet-fed rats had significantly lower insulin stimulated glucose uptake in diaphragm compared to PUFA-rich diet-fed rats and also there is no significance difference in insulin stimulated glucose uptake between TFA and SFA rich diet-fed rats (data not shown).

Effect of dietary fatty acid composition on 11β-HSD1 gene expression

Our main aim was to determine whether 11β-HSD1 gene expression in rat adipose tissue was altered in response to chronic dietary feeding of TFA, SFA and PUFA rich diets. 11-βHSD1 mRNA levels increased significantly by 78.5% and 214% in RPWAT of SFA and TFA rich diet-fed rats respectively compared to those observed in PUFA-rich diet-fed rats (Fig. 1A&1D). Compared to SFA-rich diet, TFA-rich diet increased the 11-β HSD1 mRNA levels significantly by 76%, in RPWAT of rats (Fig. 1A&1D).

Effect of dietary fatty acid composition on CCAAT/enhancer-binding protein α (C/EBP α) and Liver-X-Receptor-α (LXR-α) gene expression

C/EBP α mRNA levels increased significantly by 138% and 376% in RPWAT of SFA and TFA-rich diet-fed rats respectively, compared to those of PUFA rich diet-fed rats (Fig.1B&1D). TFA-diet increased the C/EBP α mRNA levels significantly by 172% in RPWAT as against those of SFA-diet fed rats (Fig.1B&1D). No differences in LXR-α mRNA levels among the groups were observed (Fig.1D).

Discussion

The major finding of the present study is that, the dietary fatty acid composition regulates the 11β-HSD1 expression in rat adipose tissue. 11β-HSD1 was upregulated to a great extent in rat adipose tissue by TFA rich diet, compared to SFA rich diet, which in turn was higher than that observed in PUFA-rich diet (TFA > SFA > PUFA).

The role of 11β-HSD1 in the development of metabolic syndrome is confirmed by transgenic studies. Mice over expressing 11β-HSD1 in adipose tissue are modestly obese and develop all other features of metabolic syndrome including insulin resistance [2]. 11β-HSD1 knockout mice are resistant to diet induced obesity, showed improved insulin sensitivity [3]. In the present study, consumption of both SFA and TFA rich diets increased 11β-HSD1 gene expression in rat retroperitoneal adipose tissue, compared to PUFA-fed rats. In line with the increased 11β-HSD1 gene expression, both TFA- and SFA-diet fed rats showed significantly increased body weight gain and increased peripheral insulin resistance compared to PUFA rich diet-fed rats. Although there is significance difference in 11β-HSD1 gene expression levels of SFA and TFA rich diet-fed rats, this did not result in change in body weight gain or insulin resistance parameters of these two groups. This observation support the previous studies reporting the association between dietary trans and saturated fatty acids with development of obesity and insulin resistance [6-10] and also provides the new insight into the mechanism through which TFA’s and SFA’s increase the risk of obesity and insulin resistance.

The mechanism by which dietary fatty acids regulate the expression of 11β-HSD1 may be through Peroxisome proliferator-activated receptor-γ (PPARγ). PPARγ is known to down regulate the expression of 11β-HSD1 [16]. The regulation by these receptors is indirect and involves other yet unidentified transcription factors. PPARγ has high affinity for unsaturated fatty acids compared to saturated fatty acids. Arachidonic (20:4 n-6) acid and its metabolites like 15-deoxy-Δ12, 14-prostaglandin J2 are high affinity ligands for PPARγ [17]. In 3T3 L1 adipocytes, arachidonic acid has been shown to decrease the lipogenic gene expression through its conversion to prostaglandins [18]. As dietary oleic (18:1 n-9) acid, is the precursor of the synthesis of arachidonic (20:4 n-6) acid, the diet rich in this fatty acid (PUFA-rich diet) might have provided higher amounts of lipids for PPARγ. This may be one of the possible mechanisms underlying the down regulation of 11β-HSD1 in adipose tissue of PUFA-fed rats. Both dietary SFAs and TFAs are shown to decrease the levels of n-6 PUFA (linoleic acid and arachidonic acid) in adipocyte plasma membrane phospholipids [18]. Decreased contents of linoleic and arachidonic acids might have decreased the availability of ligands for PPARγ, which could have resulted in the upregulation of 11β-HSD1 in adipose tissue of TFA and SFA-diet fed rats.

Our data suggest that dietary fatty acids may regulate the 11β-HSD1 gene expression directly by altering C/EBP α gene expression. CCAAT/Enhancer-binding

Table 2 Body weight changes in experimental groups

|                     | PUFA-diet | SFA-diet | TFA-diet |
|---------------------|-----------|----------|----------|
| Initial Body weight | Mean      | SEM      | Mean     | SEM      | Mean     | SEM      |
| (g)                 | 55.5      | 2.5      | 55       | 3.0      | 54       | 2.5      |
| Final Body weight   | 185       | 2.7 b    | 220      | 3.5 b    | 195      | 4.0 b    |
| (g)                 | 129       | 3.0 b    | 165      | 4.5 b    | 145      | 5.0 b    |
| Weight gain (g)     | 129       | 3.0 b    | 165      | 4.5 b    | 145      | 5.0 b    |

Values are means ± SEM of 8 rats. Statistical significance was taken at P ≤ 0.05 level (by one-way ANOVA). Comparisons were made between the three experimental groups.
proteins are essential for the preadipocyte differentiation and maintenance of adipocyte phenotype [19]. C/EBP \( \alpha \) is a known potent transcriptional activator of 11\( \beta \)-HSD1 [5]. It has already been shown that in adipocytes, C/EBP \( \alpha \) regulates the expression of key genes involved glucose and lipid metabolism [20-22]. Chronic challenging with TFA and SFA rich diets increased the expression of C/EBP \( \alpha \)-m RNA levels in rat adipose tissue compared to...
PUFA-rich diet. Between TFAs and SFAs, TFA appeared to be more potent stimulator of C/EBP α. The mechanisms by which dietary fatty acids regulate the C/EBP α are unclear.

In conclusion, diets rich in trans fatty acids and saturated fatty acids increased 11β-HSD1 gene expression in RPWAT of rats as compared PUFA enriched diet. The differential regulation of 11β-HSD1 gene in RPWAT of rat by different dietary fatty acids may be through regulation of C/EBP-α gene. These results suggest that in adipose tissue, diets rich in TFAs and SFAs increase the local amplification of glucocorticoids than PUFA-rich diets. Thus, the increased local conversion of inactive to active glucocorticoids in adipose tissue may increase the risk of developing obesity and insulin resistance.

Competing interests
The authors declare that they have no competing interests.

Authors’ contributions and information
SSVP proposed the hypothesis, carried out PCR analysis and wrote the manuscript. JSM collected the tissues and isolated the RNA. URP and QSSYH designed and carried the experiment. VA drafted the manuscript and had overall supervision and gave final approval of the manuscript to be published. All authors have read and approved the final manuscript.

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References
1. Stewart PM, Krozowski ZS: 11β-Hydroxysteroid dehydrogenase. Vitam Horm 1999, 57:249-324.
2. Masuzaki H, Paterson J, Shinyama H, Morton NM, Mullins JJ, Seckl JR, Flier JS: A transgenic model of visceral obesity and the metabolic syndrome. Science 2001, 294:2166-2170.
3. Morton N, Paterson J, Masuzaki H, Holmes MC, staels B, Fievez C, Walker B, Flier J, Mullins J, Seckl JR: Reduced 11β-Hydroxysteroid dehydrogenase type 1-mediated intra-adipose glucocorticoid regenration: a novel protective adaptation to and treatment for the metabolic syndrome. Programmed Abstracts of the 85th Annual Meeting of the Endocrine Society, 2003, June 19-22, abstract OR39-S-114.
4. Williams LJ, Lyons V, Maldeol J, Rajan V, Darlington GJ, Poli V, Seckl JR, Chapman KE: C/EBP regulates hepatic transcription of 11β-Hydroxysteroid dehydrogenase type 1. A novel mechanism for cross-talk between the C/EBP and glucocorticoids signaling pathways. J Biol Chem 2000, 275:30232-30239.
5. Stirling TM, Oppermann U, Steffensen KR, Steffensen KB, Schuster GU, Gustafsson JA: Liver x receptors down regulate 11β-Hydroxysteroid dehydrogenase type 1 expression and activity. Diabetes 2002, 51:2426-2433.
6. Lovejoy JC, Champagne CM, Smith SR, Smith R Steven, DeLany P;ames, Bray AG;eorge, Lefevre Michael, Denkens M'vonne, Roed C'rner: Relationship of dietary fat and serum cholesterol ester and phospholipid fatty acids to markers of insulin resistance in men and women with a range of glucose tolerance. Metabolism 2001, 50:86-92.
7. Lefevre M, Lovejoy J, Smith S: Acute effects of dietary trans fatty acids on postprandial insulin, glucose and triglyceride levels. FASEB J 1999, 13: A54.
8. Dorfman Elszuazene, Laurente Didier, Gounaindes Sjhon: Metabolic Implications of Dietary Trans-Fatty Acids. Obesity 2009, 17:1200-7.
9. Sarvanan Natarajan, Hasseb Addul, Ehtesam Znaseeen, Ghafoorunissa : Differential effects of dietary saturated and trans-fatty acids on expression of genes associated with insulin sensitivity in rat adipose tissue. Eur J Endocrinol 2005, 153:159-165.
10. Jump DB, Clark SD: Regulation of gene expression by dietary fat. Annu Rev Nutr 1999, 19:63-90.
11. Drake AJ, Livingstone DE, Andrew R, Andrew R, Seckl JR, Morton NM, Walker BR: Reduced adipose glucocorticoid reactivation and increased hepatic glucocorticoid clearance as an early adaptation to high fat feeding in wistar rats. Endocrinology 2005, 146:913-9.
12. Strimson RH, Johnstone AM, Homer NZ, Homer NZ, Wake DJ, Morton NM, Lobley GE, Walker BR: Dietary macronutrient content alters cortisol metabolism independently of body weight changes in obese men. J Clin Endor Metabol 2007, 92:4480-4.
13. Reeves PG, Nielsen FH, Fahey GC Jr: AIN-93 purified diets for laboratory rodents: final report of the American Institute of Nutrition ad hoc writing committee on the reformulation of the AIN-76 rodent diet. J Nutr 1993, 123:1939-1951.
14. Vajreswari A, Rupalatha M, Rao PS: Effect of altered dietary n-6 to n-3 fatty acid ratio on erythrocyte lipid composition and membrane-bound enzymes. J Nutr Sci Vitamino 2002, 48:365-370.
15. Chomczyski P, Sacchi N: Single step method of RNA isolation by acid guanidinium thiocyanate phenol chloroform extraction. Anal Bioch 1987, 162:156-9.
16. Berger J, Tanen M, Elbrecht A, Elbrecht A, Hverenanowski-Vatsaka A, Moller DE, Wright SD, Thieringer R, Peroxisome proliferators-activated receptor-γ ligands inhibit adipocyte 11β-Hydroxysteroid dehydrogenase type 1 expression and activity. J Biol Chem 2001, 276:12629-12635.
17. Forman MB, Tontonog Peter, chem Jasmine, Brun Plegiena, spigelman MBrucc, Evan M: 15-deoxy-D12, 14-prostaglandin J2 is a ligand for the adipocyte determination factor PPAR γ. Cell 1995, 83:803-812.
18. Mater KM, kelle pan David, Beren WG: Arachidonic acid inhibits lipogenic gene expression in 3T3-L1 adipocytes through a prostanoid pathway. J Lipid Res 1998, 39:1327-1334.
19. Darlington GJ, Se Ross, MacDougall DA: The role of C/EBP genes in adipocyte differentiation. J Biol Chem 1998, 273:30057-30062.
20. Wang ND, Finegold MJ, Bradley A, Bradley A, Ou CN, Abdelsayed SV, Wilde MD, Taylor LR, Wilson DR: Impaired energy homeostasis in C/EBPα knockout mice. Science 1995, 269:1108-1112.
21. Wedel A, Ziegler-Heitbrock HWL: The C/EBP family of transcription factors. Immunobiology 1995, 193:171-185.
22. Rosen ED, Walkey CJ, Piuigserter P, Spiegelman BM: Transcriptional regulation of adipogenesis. Gene Dev 2000, 14:129-1307.

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