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Metabolic regulation of leptin production in adipocytes: a role of fatty acid synthesis intermediates

Yoko Shirai, Shyunji Yaku, Masashige Suzuki*

Laboratory of Exercise and Nutrition, School of Sport Sciences, Waseda University, Tokorozawa, Japan 359-1192

Abstract

In addition to a signal arising from the physical "stretching" of the adipocytes, metabolic and endocrine regulation of leptin production seems to operate in adipocytes. There is however a paucity of literature examining direct role of fatty acid synthesis in regulating adipocytes leptin production. To clarify the relation between fatty acid synthesis and leptin release in adipocytes, we examined leptin release from primary cultured rat epididymal adipocytes with several substances relevance to de novo fatty acid syntyhesis. Bezafibrate (0.5 or 1.0 mM), known to inhibit acetyl-CoA carboxylase, decreased leptin release to 60.3 ± 7.2 or 47.3 ± 11.9%, while cerulenin (15, 30, or 75 mM), an inhibitor of fatty acid synthase, increased it by 20.5 ± 7.7, 58.5 ± 12.1 or 105.0 ± 35.0% of the control. Exogenous pyruvate (2.5, 5.0, or 10.0 mM) and malonyl-CoA (10, 20, or 40 mM), substrates and intermediate of fatty acid synthesis, increased leptin release by 11.0 ± 3.3, 21.5 ± 5.4, or 61.0 ± 10.7%, and 11.1 ± 3.0, 41.1 ± 9.7 or 56.7 ± 7.9% of the control, respectively. Considering difference in the site of action of bezafibrate and cerulenin along fatty acid synthesis pathway, one plausible explanation is that malonyl-CoA levels act as a signal of fuel availability to trigger leptin synthesis and/or secretion in adipocytes.

Keywords: Leptin secretion; Fatty acid synthesis; Malonyl-CoA; Rat adipocytes

1. Introduction

An adipocytes hormone, leptin is implicated in the regulation of food intake, energy expenditure, and body fat storage [1,2]. Circulating leptin concentrations are well correlated with body fat storage in humans [3–5] and animals [5–7], and an amount of leptin gene expression per cell correlates with the corresponding size of individual adipocytes [8]. A signal arising from the physical "stretching" of the adipocytes has been proposed as a component of signal-transduction system, by which fat cell senses its lipid storage [9]. In addition, metabolic and endocrine regulation of leptin production seem to operate in adipocytes, since changes in nutritional status acutely, within hours in some case, modify plasma leptin concentration. For example, circulating leptin diurnally changes in human and mice [10–13]. Furthermore, when meals are shifted 6.5 h without changing the light or sleep cycles, the plasma leptin rhythm is shifted by 5 to 7 h, suggesting that the diurnal variation in plasma leptin level is entrained to meal timing [10]. Plasma leptin concentration decreases after fasting [14–16] and increases after re-feeding [15,17]. Fasting produces a sharp decrease in leptin mRNA levels in adipose tissue and refeeding rapidly (3 to 6h) re-induces the expression of leptin gene [18,19].

Changes in nutritional status result in metabolic changes including blood levels of glucose, FFA and ketones [16,20,21]. These alterations are caused or followed by several hormonal changes including insulin, glucagon, catecholamine, cortisol, growth hormone and others [16,20,21]. Subcutaneous injection of insulin rapidly re-induces leptin gene expression in fasted mice within 4 h [18] and slightly after 1 hr in diabetic rat [19].

Glucose, insulin and glucocorticoids have been examined for their potential ability to regulate adipocytes leptin production **in vitro** [22,23]. Mueller et al. demonstrated that glucose uptake and metabolism are determinants of leptin production by adipocytes [24]. Furthermore it has been suggested that glucose oxidation or production of lipogenic precursors is implicated in adipocytes leptin production [25], but mechanistic basis for this causal role of glucose metabolism on leptin secretion remains to be clarified.

Considering the physiological role of leptin, it is not surprising to learn that lipid metabolism is also closely linked to production and circulating level of this hormone. There is however a paucity of literature examining role of

* Corresponding author. Tel.: +81-33472-0121; fax: +81-33472-0121.

E-mail address: szk.dumbbell@tokyo-nl.com (M. Suzuki).

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fatty acid synthesis pathway in regulating asipocyte leptin production. In the present study, to clarify the relation between fatty acid synthesis and leptin release in adipocytes, we examined leptin release from primary cultured rat epididymal adipocytes incubated with several substances relevance to **de novo** FFA syntyhesis.

2. Materials and methods

2.1. Materials

Krebs-Ringer bicarbonate buffer, BSA, type II collagenase, bezafibrate, cerulenin, pyruvate, lactate and malonyl-CoA were purchased from Sigma Chemical Co. (St. Louis, MO, USA). Sodium fluoride (NaF) was purchased from Wako Pure Chemical Industries Ltd. (Tokyo, Japan). Insulin (Humalin R-40) was purchased from Eli Lilly Japan K.K. (Kobe, Japan).

2.2. Animals

The experimental protocols for this study were approved by the Institutional Animal Care and Use Committee at the University of Tsukuba. Male Sprague-Dawley rats (300 to 400g) were obtained from Nippon CLEA (Tokyo, Japan). Animals were housed in individual wire mesh cages in a temperature controlled (22 to 24°C) room with a 12h light-dark cycle and fed standard laboratory chow (CLEA rodent Diet CE-2; Nippon CLEA, Tokyo, Japan) and given water *ad libitum*.

2.3. Adipocytes preparation

Epididymal fat pads were resected, minced, and digested with type II collagenase according to Rodbell procedure [26] with minor modifications as described below. The cell preparation was performed at 37°C in Krebs-Ringer bicarbonate buffer (pH 7.4; containing 2% BSA, 10 mM D-glucose, 0.49 mM $MgCl_2$, 4.56 mM KCl, 119.78 mM NaCl, 0.7 mM Na_2HPO_4, 1.15 mM NaH_2PO_4, and 15 mM $NaHCO_3$). The cell suspension was separated from undigested tissue by filtration through a 450 mm nylon mesh and washed three times. For washing, cells were centrifuged at 500 rpm for 5 min, infranatant was discarded, and the adipocytes were respended in Krebs-Ringer bicarbonate buffer each time. An aliquot of isolated adipocytes was withdrawn to determine cell number with a hemocytometer. 1×10^6 adipocytes were distributed equally into 24-well culture dishes, and were incubated in a final volume of 2 mL Krebs-Ringer bicarbonate buffer containing 6 nM of insulin (approximately 20% cytocrit). An inhibitor (0.5 or 1.0 mM bezafibrate, or 15, 30, or 75 mM cerulenin, 5 mM NaF), pyruvate (2.5, 5.0, or 10.0 mM), or malonyl-CoA (10, 20, or 40 mM) were prepared 10 fold of final concentration in advance and added 200 mL to each medium. The cells were incubated at 37°C in air of 5% CO_2 for 3h. All preparations were performed under aseptic conditions.

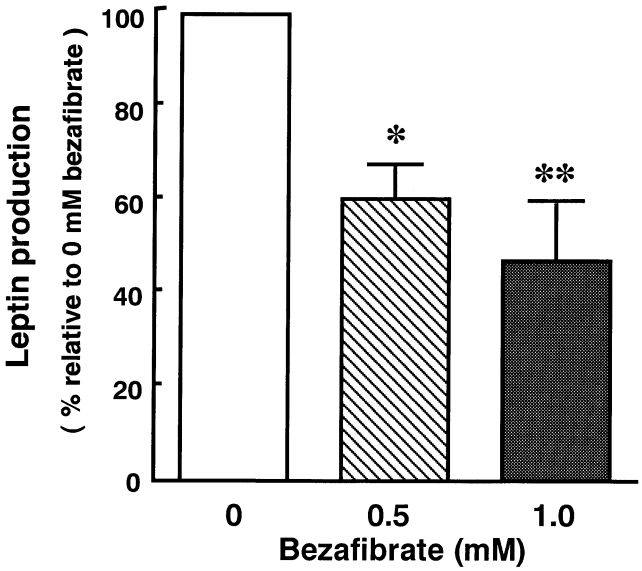

Fig. 1. Effects of 0 to 1.0 mM bezafibrate on leptin release from isolated rat adipocytes (1×10^6 cells). The medium contained 10 mM glucose and 6 nM insulin. Percentage changes in leptin release relative to 0 mM bezafibrate are expressed as means ± SE. The actual concentrations of leptin in the medium were 2.60 ± 0.53, 1.55 ± 0.33 and 1.10 ± 0.21 ng/mL, respectively ($n = 5$). *: $P < 0.05$, **: $P < 0.01$ compared with 0 mM bezafibrate.

2.4. Assays

Leptin concentrations in the medium were determined with enzyme-linked immunosorbent assay (ELISA) specific for rat (I.B.L. Co. Ltd. Takasaki, Japan). The limits of sensitivity and linearity for the leptin assay are 0.06 ng/mL and 3.60 ng/mL, respectively. The inter-assay variation for the assay at 1.42 ng/mL is 5.6%.

2.5. Data analysis

Values are expressed as the means ± SE. Differences among groups were determined by analysis of variance (ANOVA) followed by Dunett's test, and provability values of <0.05 were considered significant.

3. Results

3.1. Effects of bezafibrate on leptin production

Bezafibrate, an inhibitor of **de novo** free fatty acid synthesis, at 0.5 and 1.0 mM markedly reduced leptin production to 60.3 ± 7.2 and 47.3 ± 11.9% of the control (Fig. 1).

3.2. Effects of cerulenin on leptin production

Cerulenin, a potent inhibitor of fatty acid synthase, at 15, 30 and 75 mM increased leptin production by 20.5 ± 7.7,

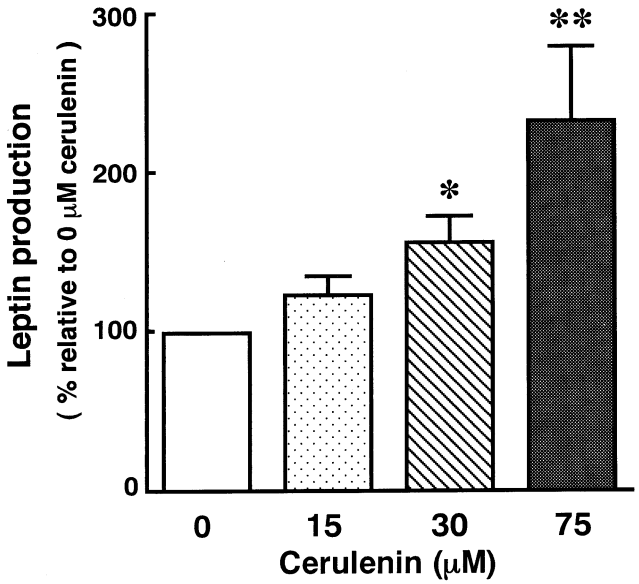

Fig. 2. Effects of 0 to 75 mM of cerulenin on leptin release from isolated rat adipocytes (1×10^6 cells). The medium contained 10 mM glucose and 6 nM insulin. Percentage changes in leptin release relative to 0 mM cerulenin are expressed as means ± SE. The actual concentrations of leptin in the medium were 1.41 ± 0.31, 1.63 ± 0.34, 2.19 ± 0.55 and 2.52 ± 0.46ng/mL, respectively ($n = 6$). *: $P < 0.05$, **: $P < 0.01$ compared with 0 mM cerulenin.

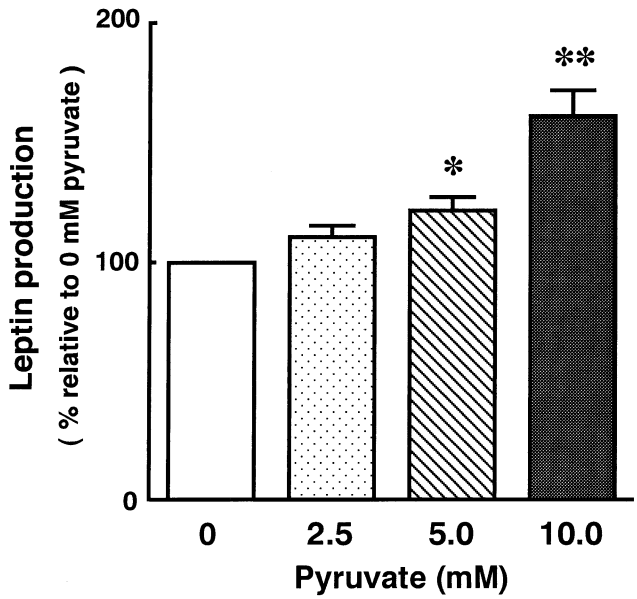

Fig. 3. Effects of 0 to 10 mM of pyruvate on leptin release from isolated rat adipocytes (1×10^6 cells). The medium contained 10 mM glucose and 6 nM insulin. Percentage changes in leptin release relative to 0 mM of pyruvate are expressed as means ± SE. The actual concentrations of leptin in the medium were 2.11 ± 0.53, 2.32 ± 0.57, 2.50 ± 0.57 and 3.46 ± 0.94ng/mL, respectively ($n = 8$). *: $P < 0.05$, **: $P < 0.01$ compared with 0 mM of pyruvate.

58.5 ± 12.1 and 105.0 ± 35.0% of the control, and the effects at 30 and 75 mM were statistically significant (Fig. 2).

3.3. Effects of pyruvate and lactate on leptin production

Pyruvate, at 2.5, 5.0 and 10.0 mM increased leptin production by 11.0 ± 3.3, 21.5 ± 5.4, and 61.0 ± 10.7% of the control, and the effects at 5.0 and 10.0 mM were statistically significant (Fig. 3). Lactate (5 mM), another metabolite in glycolytic pathway, showed no significant effects on leptin production (7.8 ± 5.3%, $P > 0.25$).

3.4. Effects of malonyl-CoA on leptin secretion

Five mM NaF markedly reduced leptin secretion (~50%). Malonyl-CoA at 10, 20 and 40 mM in the presence of NaF increased leptin secretion by 11.1 ± 3.0, 41.1 ± 9.7 and 56.7 ± 7.9% of the control and the effects at 20 and 40 mM were statistically significant (Fig. 4). Without NaF, malonyl-CoA showed no effects on leptin production (data not shown).

4. Discussion

To understand fully the influence of nutritional status on leptin physiology, it is necessary to clarify mechanisms regulating leptin production in the fat cell. Leptin level in blood changes within hours after a meal and initiation of fasting [14,18,21,27,28], suggesting a short-term regulation of leptin production by metabolic and/or endocrine signals. The present study evaluated acute effect of inhibition of **de**

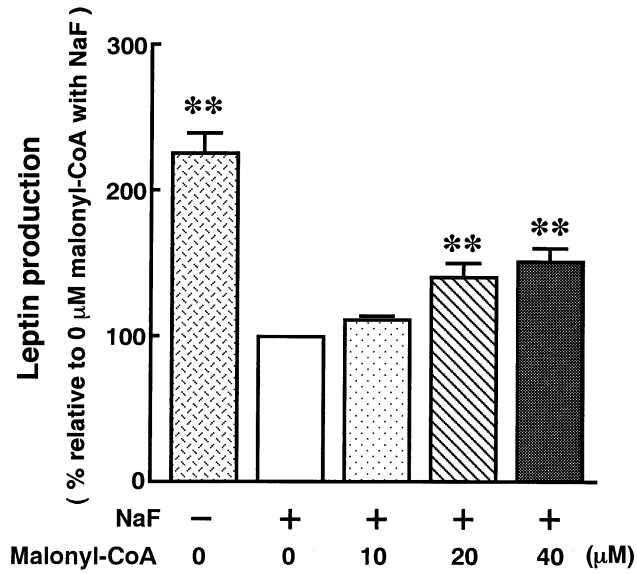

Fig. 4. Effects of 0 to 40 mM of malonyl-CoA on leptin release from isolated rat adipocytes (1×10^6 cells). The medium contained 10 mM glucose, 6 nM insulin and 5 mM NaF. Percentage changes in leptin release relative to 0 mM of malonyl-CoA with NaF are expressed as means ± SE. The actual concentrations of leptin in the medium were 1.55 ± 0.18, 0.66 ± 0.07, 0.74 ± 0.10, 0.85 ± 0.09 and 1.01 ± 0.10 ng/mL, respectively ($n = 6$). **: $P < 0.01$ compared with 0 mM of malonyl-CoA.

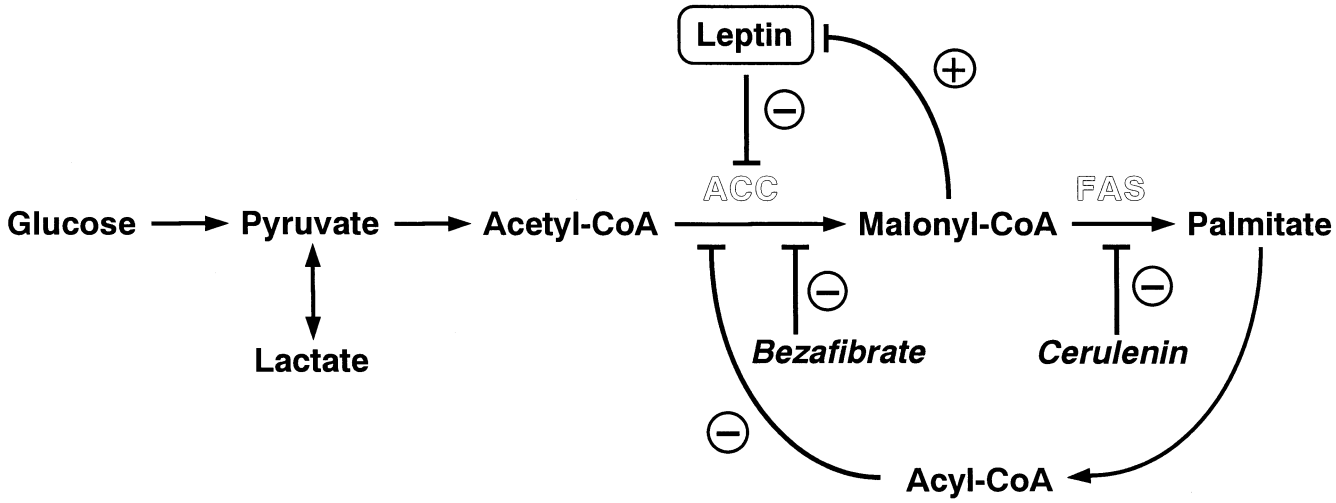

Fig. 5. Proposed mechanism for regulation of leptin secretion in adipocytes. Acetyl-CoA carbokylase is inhibited by long-chain fatty acyl-CoA [43] and leptin [35].

novo fatty acid synthesis by bezafibrate and cerulenin on leptin production in cultured adipocytes. Bezafibrate, an inhibitor of acetyl-CoA carboxylase [29,30], decreased leptin production while cerulenin, a natural fatty acid synthase inhibitor [31,32], increased it. Although bezafibrate is known to act as multifunctional reagent through the activation of peroxisome proliferator-activated receptor (PPAR)-a, it is unlikely the case in adipocytes, since dominant form of PPARs in the adipocytes is g-subtype and a-subtype in the adipocytes is only weakly expressed [33,34]. Considering the difference in sites of action of these inhibitors along fatty acid synthesis pathway, one possible explanation is that malonyl-CoA is a signal for adipocytes leptin production (Fig. 5). Palmitate, which inhibits acetyl-CoA carboxylase, may also downregulate leptin production by blocking **de novo** fatty acid synthesis prior to malonyl-CoA [35]. Plasma leptin concentration correlated negatively with dietary intake of polyunsaturated fatty acids [36]. And plasma FFA and leptin levels reciprocally change with nutritional intervention in humans and experimental animals [16,21]. Furthermore, infusion of isoproterenol or epinephrine, conditions known to raise plasma free fatty acids, decreases plasma leptin level [37,38]. Consisting with the intervention studies with whole body, **in vitro** study demonstrated that fatty acids suppressed leptin production [36,39].

Inhibition of glucose transport or phosphorylation and glycolysis causes a suppression of leptin release in cultured rat adipocytes [24]. The rate of glucose metabolism is a determinant of leptin production, and the sitimulation occurs downstream of phosphofructokinase. Consistently, exogenous pyruvate stimulated leptin secretion (Fig. 3) but a stimulation by lactate failed to reach statistical significance in the present study. It is reported that a significant portion (42.4 ± 4.3%) of glucose taken up by adipocytes was released as lactate but the production of lactate was inversely proportional to the leptin response [25]. From these observations, Mueller et al. [25] postulated that the effect of glucose metabolism to stimulate leptin production involves glucose oxidation and/or the production of lipogenic precursors. Since glycolytic intermediates and products contribute as precursors of **de novo** fatty acid synthesis, it is plausible that the effect of glucose metabolism to regulate leptin secretion in adipocytes is mediated by changes in intracellular malonyl-CoA level (Fig. 5).

None of the previous studies and our present study concerning regulation of leptin secretion in cultured adipocytes measured intracellular malonyl-CoA concentration, since a technique to measure that in the cell after incubation was not available. In the present study, malonyl-CoA concentration in culture medium was raised to higher above the assumed intracellular level [40,41], assuming its limited permeability through the plasma membrane. Incubation of the cell with exogenous malonyl-CoA stimulated leptin production consistent with the notion that malonyl-CoA acts as a signal of leptin production (Fig. 4).

Systemic treatment with a fatty acid synthase inhibitor (synthetic compound C75) led to an inhibition of feeding and a dramatic weight loss in mice [42]. Contrary to our observations concerning the effect of fatty acid synthase inhibitor on leptin secretion in isolated adipocytes, serum leptin levels were reduced rather than elevated in C75-treated mice. Amount of C75 injection was limited (30 mg/kg) because of its toxicity **in vivo** study, and it might not be enough to enhance serum leptin levels. Since C75 possesses central nervous system mechanism to suppress feeding, reduction in leptin levels might be secondary results of anorexia and emaciation. Indeed, intracerebroventricular micro-injection with cerulenin and C75 led to an inhibition of feeding and a dramatic weight loss in mice. From these observations, malonyl-CoA level is assumed to regulate feeding in central nervous system.

In summary, blockade of **de novo** fatty acid synthesis by bezafibrate inhibited but that by cerulenin and exogenous malonyl-CoA and pyruvate stimulated leptin production in rats isolated adipocytes. Our data support the possibility that malonyl-CoA levels act as a signal of the availability of fuels in adipocytes and act as a trigger of leptin production. The exact signaling pathway downstream of malonyl-CoA remains to be identified.

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