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Journal

Obesity, 8(7)

ISSN

1930-7381

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Publication Date

2000-10-01

DOI

10.1038/oby.2000.66

Peer reviewed

Effects of Metformin and Vanadium on Leptin Secretion from Cultured Rat Adipocytes

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Abstract

MUELLER, WENDY M., KIMBER L. STANHOPE, FRANCINE GREGOIRE, JOSEPH L. EVANS, AND PETER J. HAVEL. Effects of metformin and vanadium on leptin secretion from cultured rat adipocytes. *Obes Res.* 2000;8:530–539.

Objective: We have reported that glucose utilization regulates leptin expression and secretion from isolated rat adipocytes. In this study, we employed two antidiabetic agents that act to increase glucose uptake by peripheral tissues, metformin and vanadium, as pharmacological tools to examine the effects of altering glucose utilization on leptin secretion in primary cultures of rat adipocytes.

Research Methods and Procedures: Isolated adipocytes (100 μL of packed cells per well) were anchored in a defined matrix of basement membrane components (Matrigel) with media containing 5.5 mM glucose and incubated for 96 hours with metformin or vanadium. Leptin secretion, glucose utilization, and lactate production were assessed.

Results: Metformin (0.5 and 1.0 mM) increased glucose uptake in the presence of 0.16 nM insulin by 37 $\pm$ 10% ($p < 0.005$) and 62 $\pm$ 8% ($p < 0.0001$) over insulin alone, respectively. Metformin from 0.5 to 5.0 mM increased lactate production by 105 $\pm$ 43% ($p < 0.025$) to 202 $\pm$ 52% ($p < 0.0025$) and at 1.0 and 5.0 mM increased the proportional rate of glucose conversion to lactate by 78 $\pm$ 18% ($p < 0.005$) and 166 $\pm$ 41% ($p < 0.0025$), respectively. At concentrations less than 0.5 mM, metformin did not affect leptin secretion, but at 0.5 mM, the only concentration that significantly increased glucose utilization without increasing glucose conversion to lactate, leptin secretion was modestly stimulated (by 20 $\pm$ 9%; $p < 0.05$). Concentrations from 1.0 to 25 mM inhibited leptin secretion by 25 $\pm$ 8% ($p < 0.005$) to 89 $\pm$ 4% ($p < 0.0001$). Across metformin doses, leptin secretion was inversely related to the percentage of glucose taken up and released as lactate ($r = -0.74$; $p < 0.0001$). Vanadium (5 to 20 μM) increased glucose uptake from 20 $\pm$ 7% ($p < 0.01$) to 34 $\pm$ 13% ($p < 0.02$) and increased lactate production at 5 μM by 17 $\pm$ 8% ($p < 0.025$) and 10 μM by 61 $\pm$ 20% ($p < 0.02$) but did not alter the conversion of glucose to lactate. Vanadium (5 to 50 μM) inhibited leptin secretion by 33 $\pm$ 6% ($p < 0.0025$) to 61 $\pm$ 8% ($p < 0.0001$).

Discussion: Both metformin and vanadium increase glucose uptake and inhibit leptin secretion from cultured adipocytes. The inhibition of leptin secretion by metformin is related to an increase in the metabolism of glucose to lactate. The inhibition by vanadium most likely involves direct effects on cellular phosphatases. We hypothesize that the effect of glucose utilization to stimulate leptin production involves the metabolism of glucose to a fate other than anaerobic lactate production, possibly oxidation or lipogenesis.

Key words: glucose uptake, lactate production, anaerobic metabolism

Introduction

The adipocyte hormone, leptin, has a central role in the regulation of food intake, energy expenditure, and body fat stores (1,2). Circulating leptin concentrations are well correlated with adipose stores in humans (3–5) and animals (5–7). However, leptin production is also acutely regulated by nutritional status. For example, circulating leptin decreases after fasting (6,8–10) or energy restriction (11,12) and increases after refeeding or overfeeding (9,13). These changes of circulating leptin are disproportionate to the relatively small changes of body fat. Although the regulation of leptin expression and secretion is incompletely understood, changes of insulin secretion during fasting and refeeding precede changes of circulating leptin concentrations. There is a growing body of evidence that suggests a role for insulin and glucose in mediating changes of circulating leptin levels in vivo. For example, infusion of small amounts of glucose to prevent the reductions of insulin and

Submitted for publication October 18, 1999.
Accepted for publication in final form February 22, 2000.
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glucose during fasting in humans also prevents the decrease in plasma leptin (8). Although insulin administration does not acutely increase plasma leptin concentrations in human subjects (14,15) increases have been reported after 4 to 6 hours during insulin infusions producing supraphysiological (16,17) or physiological (18) increments of plasma insulin levels. Similarly, prolonged hyperglycemia and hyperinsulinemia in response to extended glucose infusions increases plasma leptin after several hours in nonhuman primates (7) and human subjects (19). Lastly, leptin concentrations increase 4 to 6 hours after high carbohydrate meals, which induce large plasma insulin and glucose responses in humans (20).

In vitro studies have shown that insulin increases leptin expression and secretion in isolated rodent (21–23) and human (15,24) adipocytes. It has not, however, been clear whether the effect of insulin to increase leptin production is a direct consequence of increased insulin signaling or might be indirectly mediated by insulin's actions on glucose metabolism. Several in vivo studies have provided support for the latter explanation. First, glucose administration induces increases of *ob* mRNA expression, which are more closely related to changes of plasma glucose than to plasma insulin concentrations (25,26).

In addition, the decrease in plasma leptin during marked caloric restriction in humans is better correlated with the decrease in plasma glucose than with changes in insulinemia (11,12). Furthermore, we have found that low plasma leptin levels in insulin-deficient streptozotocin diabetic rats are acutely increased by insulin administration in proportion to the degree of glucose lowering (27). Further support from in vitro experiments for a role for adipose glucose utilization in the regulation of leptin production is provided by our recent report that increased glucose metabolism is an important mediator of insulin-stimulated leptin expression and secretion. Blockade of glucose uptake or inhibition of glycolysis decreases *ob* gene expression and leptin secretion in isolated rat adipocytes (28). However, glucose uptake, by itself, only seems to be important in that glucose must first be taken up by the adipocytes before it can be metabolized. Rather than glucose uptake per se, the inverse relationship observed, between the proportional conversion of glucose to lactate and leptin secretion by isolated adipocytes (28), suggests that a regulatory step for glucose metabolism to mediate changes of leptin production involved the metabolism of glucose to a point beyond the anaerobic metabolism of glucose-derived pyruvate to lactate.

Metformin and vanadium are two antidiabetic agents, which are able to enhance glucose uptake and utilization by peripheral tissues (29,30). In the present study, we employed metformin and vanadium as pharmacological tools to examine the effects of altering adipocyte glucose utilization on leptin production in primary cultures of isolated adipocytes. Glucose utilization, lactate production, and leptin secretion were measured over 96 hours in isolated rat adipocytes cultured in a basement membrane matrix that maintains adipocyte differentiation.

Research Methods and Procedures

Materials

Media (Dulbecco's modified Eagle's medium [DMEM]) and fetal bovine serum (FBS) were purchased from Life Technologies (Grand Island, NY). The media was supplemented with 6 mL each of minimal essential medium amino acids, penicillin/streptomycin (5000 U/mL/5000 μg/mL), and nystatin (10,000 U/mL; all from Life Technologies) per 500 mL of DMEM. Bovine serum albumin fraction V, 4-(2-hydroxyethyl)-1-piperazineethansulfonic acid (HEPES), collagenase (*Clostridium histolyticum*, type II; specific activity, 456 U/mg), insulin, and metformin were purchased from Sigma Chemical Co. (St. Louis, MO). Matrigel matrix was purchased form Becton Dickinson (Franklin Lakes, NJ). Bis(maltolato) oxovanadium(IV) (BMOV), an organified form of vanadium (31), was a gift from Drs. John McNeill and Violet Yuen, Department of Pharmaceutical Sciences, University of British Columbia, Vancouver, BC, Canada. Six-well Falcon plates were purchased from Fisher Scientific (Pittsburgh, PA). Nylon filters were purchased from Tetko (Kansas City, MO).

Animals

Male Sprague-Dawley rats (3 to 6 months of age) were obtained from Charles River (Wilmington, MA). Animals were housed in hanging wire cages in temperature-controlled rooms (22 °C) with a 12-hour light-dark cycle and fed Purina chow diet (Ralston-Purina, St. Louise, MO) and given deionized water ad libitum. Animal use and care was in accordance with the National Institutes of Health Guide for the Use and Care of Laboratory Animals and conducted in facilities accredited by the American Association for Accreditation of Laboratory Animal Care. The study protocol was approved by the Administrative Animal Use and Care Committee at University of California, Davis.

Methods

Cell Isolation/Preparation. Adipocytes were prepared from epididymal fat pads from male Sprague-Dawley rats weighing 300 to 600 g. Epididymal fat depots were resected from halothane-anesthetized rats under aseptic conditions, and adipocytes were isolated by collagenase digestion by the Rodbell method (32) with minor modifications as previously described (28). The isolated adipocytes were then incubated for 30 minutes at 37 °C before being plated and cultured on Matrigel-coated plates.

Adipocyte Culture. Adipocytes were maintained in culture anchored to a basement membrane matrix (Matrigel, Becton Dickinson). Although all in vitro systems have

inherent advantages and disadvantages, advantages of this system compared with cultures containing free-floating adipocytes are that the matrix simulates their normal basement membrane attachment and that the cells are maintained in close proximity to each other, allowing direct cell-to-cell contact. Together the cell contact and basement membrane attachment help to maintain differentiation, because adipocytes have a strong tendency to dedifferentiate in long term (>24-hour) culture. In addition, the matrix and the small amount of serum in the media both contain growth factors, which are also likely to help maintain cell differentiation. Furthermore, the adipocytes in this system are not exposed to toxic levels of oxygen at the interface of the media and the incubator atmosphere, as opposed to free-floating adipocytes which aggregate at the surface of the media. An advantage of the system over those containing minced adipose tissue is that all of the cells in the culture are equally exposed to the nutrients and the oxygen dissolved in the media. Thus, although clearly different from the in vivo situation, we believe that this system provides a more physiological environment than most systems for maintaining adipocytes in long term culture. In the case of the present studies, the goal was to examine the direct actions of metformin and vanadium on leptin production and adipocyte metabolism. Therefore, the advantage of employing in vitro experimentation for this purpose over in vivo models was that it was possible to control confounding variables, such as effects of these agents on food intake, which would indirectly influence leptin production via changes of insulin secretion (18,20). Unlike an in vivo system, in this study the environment surrounding the adipocytes within the individual wells of each culture plate was identical with the exception of the presence or absence and the concentration of metformin or vanadium, allowing assessment of the direct effects of the treatments.

In culturing each suspension, Matrigel was first thawed on ice to a liquid and uniformly applied to the surface of culture dishes (300 μL of Matrigel/35-mm well). After the incubation period, 150 μL of the adipocyte suspension (2:1 ratio of packed cells to media) were plated on the liquid Matrigel matrix. Adipocytes from each suspension were thoroughly mixed with a transfer pipette before plating to insure that a similar number of adipocytes with a similar size distribution were added to the control and experimental wells for each suspension. The warmth of the added cells and media caused the Matrigel to gel around the adipocytes, effectively anchoring them to the culture dish. After a 30-minute incubation at 37 °C, 2 mL of warm culture medium was added. The cells were maintained in an incubator at 37 °C for 96 hours with 6% CO_2. Aliquots of adipocytes from each animal were divided into wells, with the different concentrations of either metformin or vanadium (as detailed below). In each plate an appropriate control well contained adipocytes from the same animal. Adipocytes were incubated with media (DMEM) containing 5.5 mM (100 mg/dL) glucose plus 5% FBS at five concentrations of Metformin (0.1, 0.25, 0.5, 1.0, 5.0, and 25.0 mM). A low basal concentration of insulin (0.16 nM) was added to the incubations performed with metformin, because metformin is thought to act in part by increasing insulin signaling (33,34). Vanadium was added at four concentrations (5.0, 10.0, 20.0, and 50.0 μM) in DMEM with 5.0 to 5.5 mM glucose and 1% FBS. Adipocytes were cultured with vanadium without added insulin, because vanadium action is considered to be largely independent of insulin (35,36). To examine the responses to insulin in the adipocytes obtained from each adipocyte suspension in the vanadium experiments, a separate well containing 1.6 nM insulin was included for each suspension. In all experiments, aliquots of media (300 μL, 15% of the media volume) were collected from culture wells and replaced with fresh media containing the appropriate concentrations of metformin or vanadium at 24, 48, 72, and 96 hours.

Assays. Leptin concentrations in the medium were determined with a sensitive and specific radioimmunoassay for rat leptin (37) with reagents obtained from Linco Research, St. Charles, MO. Glucose and lactate were measured with a glucose analyzer (model 2300, YSI, Yellow Springs, OH).

Data Analysis. The uptake of glucose was assessed by measuring the concentration of glucose in the media in each well before and at 24, 48, 72, and 96 hours of incubation and calculating the decrease over 96 hours after correcting for the amount of glucose that was removed during each 24-hour media sampling and the amount added by the replacement of fresh media (15% of total volume). Lactate production was calculated as the increase of media lactate at 24, 48, 72, and 96 hours by correcting for the amount of lactate removed by sampling and added with media replacement. To examine the relationship between adipocyte carbon flux and leptin secretion in adipocytes cultured with metformin or vanadium, the amount of carbon released as lactate per amount of carbon taken up as glucose over 96 hours was calculated as lactate production/glucose utilization and expressed as a percentage (28). Cumulative leptin production was calculated as the change of media leptin concentrations at 24, 28, 72, and 96 hours with correction for the amount of leptin removed during sampling. The area under the curve for leptin production between 0 and 96 hours was calculated by the trapezoidal method. The experimental results from each adipocyte suspension prepared from a single animal were analyzed in relation to a control well from the same suspension. Given the

individual variation in leptin responses between animals and/or suspensions, it was not appropriate to compare means from control and treated adipocytes from different animals and/or suspensions. Therefore, the means for all the controls in the metformin ($n = 18$) and vanadium ($n = 14$) experiments contain a larger sample size (n) of animals and/or suspensions than all except the 1.0 mM metformin dose, which was studied in every experiment. Thus, when the number of wells with a particular concentration of metformin or vanadium differed from the total number of control wells (e.g., 0.5 mM metformin; $n = 9$), each result was compared only to that obtained in a corresponding control well from the same suspension in a pair-wise comparison. To examine the relationships between the medium concentrations of metformin or vanadium, glucose uptake, lactate production, and glucose conversion to lactate, and leptin secretion, simple and multiple linear regression analyses were performed with a statistics software package (StatView for Macintosh, Abacus Concepts, Inc., Berkeley, CA).

Because metformin exhibited toxic effects on adipocyte metabolism at concentrations greater than 5.0 mM, results from cultures incubated with metformin at concentrations greater than 5.0 mM were not included in these analyses. The relationship between lactate production from glucose and leptin secretion was also examined within the control groups alone. Data are expressed as means + SEM.

Results

Effects of Metformin

The effects of metformin on glucose uptake, lactate production, and leptin secretion were examined. Metformin at a concentration of 0.1 mM did not affect glucose uptake compared with the corresponding control suspensions containing insulin (0.16 nM) alone. At 0.25 mM, glucose uptake was increased ($\%\Delta = +9 \pm 8\%$), but the effect was not statistically significant perhaps due to the smaller number of trials ($n = 5$) performed at this concentration. Metformin stimulated glucose uptake at concentrations of 0.5 mM ($+37 \pm 10\%$, $p < 0.005$) and 1.0 mM ($+62 \pm 8\%$, $p < 0.0001$) compared with that in the corresponding control suspensions (Table 1, Figure 1). At 5.0 mM, glucose uptake was not significantly different from control. Higher concentrations of metformin (≥25.0 mM) markedly inhibited glucose uptake most likely reflecting a toxic effect of metformin at these very high concentrations. Metformin had no significant effect on lactate production at concentrations lower than 0.5 mM but increased lactate production at concentrations of 0.5 mM ($+105 \pm 43\%$, $p < 0.025$), 1.0 mM ($+186 \pm 31\%$, $p < 0.0001$), and 5.0 mM ($+202 \pm 52\%$, $p < 0.0025$) vs. insulin alone (Table 1, Figure 1). At concentrations of 25.0 mM, lactate production was markedly inhibited ($p < 0.0001$), because glucose utilization was almost completely suppressed. Concentrations of metformin of 0.5 mM and below did not affect the proportional conversion of glucose to lactate (Table 1). However, glucose conversion to lactate was increased at a concentration of 1.0 mM, and this effect was marked at 5.0 mM with more than twice the amount of glucose released as lactate (Table 1). Although 1.0 mM metformin did increase mean glucose uptake over control rates, a significantly larger proportion of the glucose that was taken up was released as lactate. The concentration of 0.5 mM was the only level of metformin that induced a significant increase of glucose utilization without increasing the proportion of glucose carbon released as lactate (Table 1).

At concentrations of metformin lower than 0.5 mM, leptin secretion was unaffected. With metformin at 0.5 mM, the area under the curve (AUC) for leptin secretion over 96 hours was significantly greater ($+20.5 \pm 9\%$, $p < 0.05$) than control (Figure 2). Metformin inhibited leptin secretion at concentrations of 1.0 mM ($-25 \pm 8\%$, $p < 0.005$), 5.0 mM ($-89 \pm 4\%$, $p < 0.0001$), and by 90% at toxic concentrations of 25.0 mM ($p < 0.0001$) (Figure 2).

Within the 18 control wells, leptin secretion was inversely related to the conversion of glucose to lactate ($r = -0.61$; $p < 0.01$). At metformin concentrations from 0 to 5.0 mM, leptin secretion was inversely proportional to the log of the metformin concentration ($r = -0.53$; $p < 0.0001$), to lactate production ($r = -0.53$; $p < 0.0001$), and to the proportional conversion of glucose to lactate across metformin doses ($r = -0.74$; $p < 0.0001$)(Figure 3) but was not related to glucose uptake ($r = 0.13$; $p = 0.27$) by simple regression. By multiple regression analysis, leptin secretion was inversely related to the conversion of glucose to lactate ($p < 0.0001$) but not to the log of the metformin concentration ($p = 0.91$), lactate production ($p = 0.39$), or glucose uptake ($p = 0.62$). Leptin secretion was only increased over control by metformin at 0.5 mM, which was also the only concentration that significantly increased glucose uptake without shunting a greater proportion of the glucose into lactate production (Table 1). A similar inverse relationship ($r = -0.73$; $p < 0.0025$) between leptin production and anaerobic glucose metabolism to lactate was observed in 32 control wells containing either no insulin or a low insulin concentration of 0.16 nM (~20 μU/mL) (Figure 4).

Effects of Vanadium

The effects of vanadium on glucose uptake, lactate production, and leptin secretion were examined in adipocytes cultured with concentrations of vanadium of 0 to 50 μM. Vanadium at 5.0 μM ($+20 \pm 7\%$, $p < 0.01$), 10.0 μM ($+38 \pm 12\%$, $p < 0.02$), and 20.0 μM ($+34 \pm 13\%$, $p < 0.02$) increased glucose uptake, compared with rates of glucose uptake in the corresponding control suspensions (Table 2, Figure 5). The effect of vanadium at these con-

Table 1. Effects of metformin in the presence of 0.16 nM insulin on glucose uptake, lactate production, and the percentage of glucose carbon taken up that was released as lactate by isolated rat adipocytes over 96 hours in culture (mean ± SEM)

[Metformin] (mM) + Insulin (0.16 nM)	Glucose uptake (μmol) over 96 hours	Lactate production (μmol) over 96 hours	Glucose to lactate (%)
Control ($n = 18$)	7.5 ± 0.7	5.7 ± 0.5	40.9 ± 3.6
0.1 ($n = 4$)	6.5 ± 0.9	5.9 ± 0.6	47.2 ± 6.3
0.25 ($n = 5$)	11.0 ± 1.4	8.6 ± 1.3	40.2 ± 5.9
0.5 ($n = 9$)	11.0 ± 1.2†	9.5 ± 1.2†	44.9 ± 5.1
1.0 ($n = 18$)	11.6 ± 0.7‡	14.4 ± 0.9‡	63.8 ± 3.4*
5.0 ($n = 15$)	8.3 ± 0.6	14.4 ± 1.0‡	85.6 ± 4.1‡

* $p = 0.01$; † $p = 0.005$; ‡ $p = 0.0005$; vs. corresponding controls from the same adipocyte suspensions.

centrations was comparable to that of insulin at 1.6 nM, which increased glucose uptake by 38 ± 8% ($p < 0.0001$)(Table 2, Figure 5). Vanadium at 50.0 μM did not significantly affect glucose uptake (%Δ = −4 ± 14%). Vanadium at 5.0 μM increased lactate production by + 17 ± 8% ($p < 0.025$). Mean lactate production in the six wells that served as controls for the 10.0 μM concentration of vanadium was lower than average; however, lactate production was increased in five of six corresponding experimental wells. Thus, vanadium at 10 μM increased lactate production by 61 ± 20% ($p < 0.02$) despite absolute lactate production being similar to the mean of the total 14 control wells. At 20.0 and 50.0 μM, lactate production was not significantly different from that of the control (Table 2).

Insulin at 1.6 nM increased leptin secretion over 96 hours by 59 ± 15% ($p < 0.005$) and the 0- to 96-hour AUC by 38 ± 8% ($p < 0.0001$)(Figure 5). Leptin secretion was unaffected by vanadium at a concentration of 5 μM. Higher concentrations of 10, 20, and 50 μM consistently inhibited leptin secretion over 96 hours by −33 ± 6% ($p < 0.0025$), −53 ± 7% ($p < 0.0001$), and −61 ± 8% ($p < 0.001$), respectively (Figure 6). Across vanadium concentrations, leptin secretion at 96 hours was positively correlated with glucose uptake ($r = 0.35$; $p < 0.02$) and inversely related to the log of the vanadium concentration ($r = -0.44$; $p < 0.0001$), to lactate production ($r = -0.30$; $p < 0.025$), and to the conversion of glucose to lactate ($r = -0.58$; $p <$

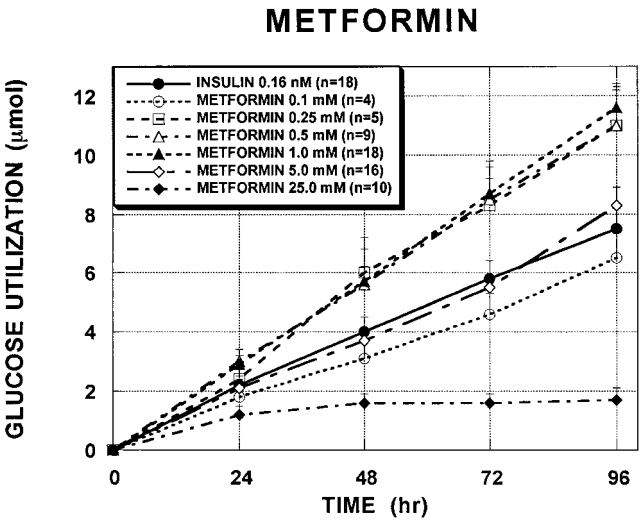

Figure 1. Glucose utilization (corrected for media sampling and replacement) over 96 hours by isolated rat adipocytes in primary culture with insulin at 0.16 nM and metformin at concentrations from 0 to 25.0 mM.

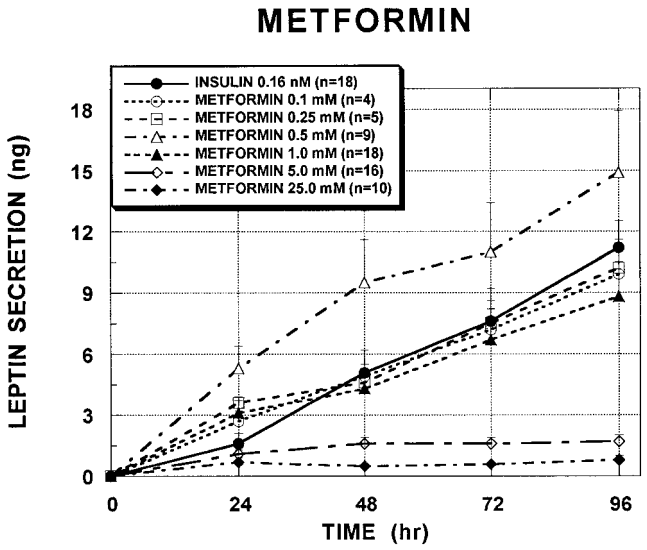

Figure 2. Leptin secretion (corrected for media sampling and replacement) over 96 hours by isolated rat adipocytes in primary culture with insulin at 0.16 nM and metformin at concentrations from 0 to 25.0 mM.

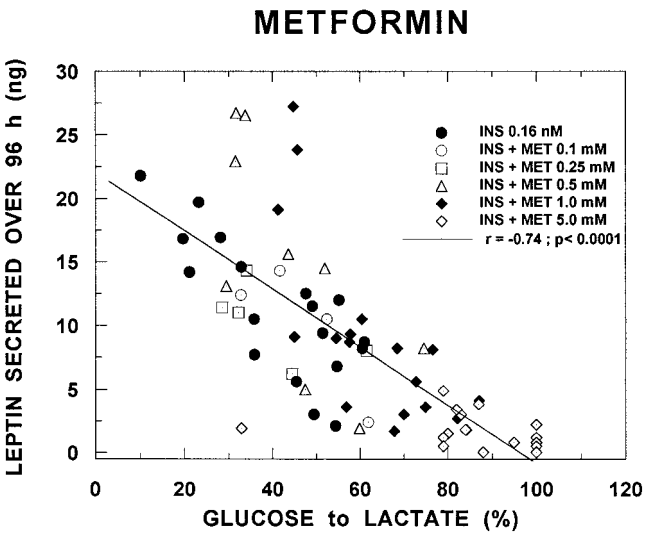

Figure 3. Relationship between the percentage of glucose taken up and released as lactate and leptin secretion over 96 hours by adipocytes in primary culture with insulin (INS) at 0.16 nM and metformin (MET) at concentrations from 0 to 5.0 mM. Leptin secretion, glucose utilization, and lactate production are corrected for media sampling and replacement.

0.0001) (data not shown). By multiple regression analysis, leptin secretion at 96 hours was more closely related to glucose conversion to lactate ($p < 0.0001$) than to absolute lactate production ($p < 0.02$) or the log of the vanadium concentration ($p < 0.005$) and was not related to absolute glucose uptake ($p = 0.22$). Despite the relationship between leptin secretion and the conversion of glucose to lactate across vanadium concentrations, unlike with metformin, the percentage of glucose released as lactate was not altered by any concentration of vanadium (Table 2). In contrast, insulin at 1.6 nM, which significantly decreased the proportional conversion of glucose to lactate (i.e., anaerobic glucose metabolism) (Table 2), stimulated leptin secretion (Figure 6).

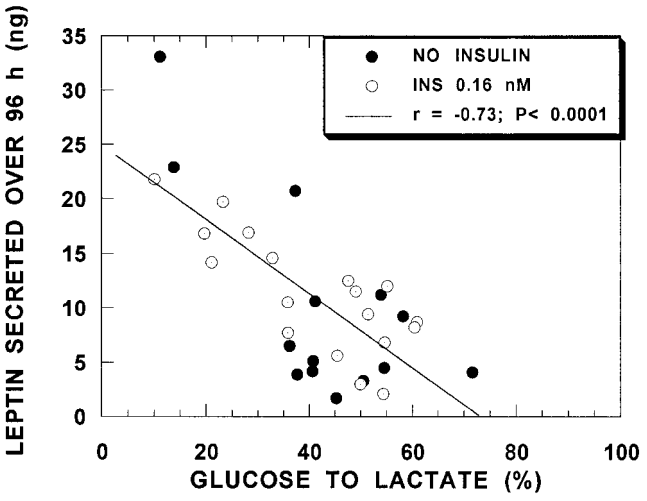

Figure 4. Relationship between the percentage of glucose taken up and released as lactate and leptin secretion over 96 hours by adipocytes in primary culture in 32 control wells containing no added insulin or insulin at a low concentration of 0.16 nM. Leptin secretion, glucose utilization, and lactate production are corrected for media sampling and replacement.

Discussion

We have recently reported that insulin-mediated glucose metabolism is an important factor regulating leptin expression and secretion in isolated rat adipocytes (28). Some previous studies have shown that drugs in the thiazolidinedione class, which are used in the treatment of type 2 diabetes, can inhibit leptin production in vitro and in vivo (38,39). In the present study, we examined the effects of metformin and vanadium, two other antidiabetic drugs known to increase cellular glucose utilization, on leptin secretion, glucose uptake, and lactate production in isolated cultured rat adipocytes. Our goal was to use these compounds as tools to examine their effects for altering adipocyte glucose utilization on leptin secretion. Therefore, we used concentrations within a range that were found to produce significant increases of adipocyte glucose uptake. Particularly in the case of metformin, these concentrations (0.25 to 5.0 mM) were far above the range of plasma concentrations (0.005 to 0.02 mM) observed in patients treated with therapeutic doses of metformin (40). In fact, at therapeutic concentrations, metformin seems to act primarily to inhibit hepatic glucose production with limited, if any, effects on peripheral glucose uptake (41–43). At therapeutic concentrations, metformin generally has little direct effect on glucose utilization in vitro (44,45). At concentrations higher than those achieved in serum with therapeutic metformin administration, metformin stimulates glucose transport by rat (46,47) and human adipocytes (48), and in rat and human skeletal muscle (see reviews in 29,34,48,49). At the cellular level, high concentrations of metformin increase insulin receptor binding, along with tyrosine kinase activity, glucose transport, and glycogen synthesis (33,34).

In the present study, metformin concentrations ranging from 0.5 to 5.0 mM increased both glucose uptake and lactate production. In addition to increasing absolute lactate production, metformin at 1.0 and 5.0 mM increased the percentage of glucose carbon that was metabolized to lactate and released into the culture media by 80% to 170%. At high concentrations of metformin (= 25.0 mM), both glucose uptake and lactate production were markedly inhibited, most likely due to a toxic effect of very high levels of metformin on cellular metabolism. Metformin at 0.5 mM modestly increased leptin secretion by ~20%.

Table 2. Effects of insulin (1.6 nM) or vanadium on glucose uptake, lactate production, and the percentage of glucose carbon taken up that was released as lactate by isolated rat adipocytes over 96 hours in culture (mean ± SEM)

[Vanadium] (μM); no insulin added	Glucose uptake (μmol) over 96 hours	Lactate production (μmol) over 96 hours	Glucose to lactate (%)
Control (n = 14)	6.8 ± 0.5	5.6 ± 0.6	42.4 ± 4.3
1.6 nM Ins (n = 14)	9.4 ± 0.9§	5.7 ± 0.7	33.0 ± 3.5‡
5.0 (n = 12)	7.8 ± 1.1§	5.7 ± 0.6*	40.5 ± 4.7
10.0 (n = 6)	8.2 ± 1.3‡	5.2 ± 0.8†	36.0 ± 6.5
20.0 (n = 12)	8.7 ± 1.2‡	6.3 ± 0.6	41.6 ± 5.0
50.0 (n = 13)	6.9 ± 1.02	4.9 ± 0.5	53.3 ± 9.4

* p = 0.05; † p = 0.02; ‡ p = 0.0025; § p = 0.0005; vs. corresponding control wells from the same adipocyte suspensions.

Importantly, this was the only concentration of metformin tested that increased glucose uptake without shunting a greater proportion of glucose into lactate production. In contrast, at concentrations of 1.0 mM and higher, leptin secretion was modestly to markedly suppressed.

A significant proportion of glucose taken up by adipose tissue is metabolized to lactate and released (50). At metformin concentrations ≤ 5.0 mM, leptin secretion was inversely related to the amount of glucose taken up by the adipocytes, converted to lactate, and released into the media. We have previously observed that the stimulation of leptin secretion by insulin is associated not only with increased glucose utilization, but with a decrease in the proportional conversion of glucose to lactate (28), a finding that was also observed within the control groups in the present study (Figure 4). Thus, when lactate production is increased, less carbon derived from glucose is available to enter the tricarboxylic acid cycle either for oxidation or use in de novo lipogenesis. Together, these data suggest that it is not glucose uptake, per se, but its metabolism beyond pyruvate and lactate in the adipocyte that is involved in the action of glucose to stimulate leptin secretion. Thus, the anaerobic metabolism of glucose does not stimulate leptin production. The entry of glucose into the hexosamine biosynthetic pathway and the production of UDP-glucosamine have been suggested as a mechanism by which glucose utilization can

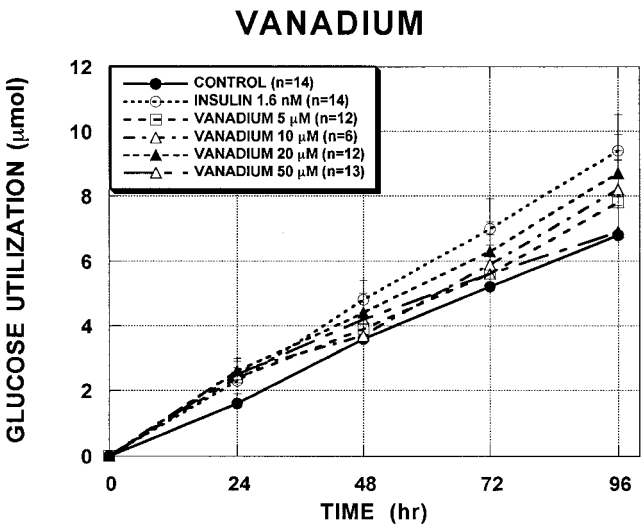

Figure 5. Glucose utilization (corrected for media sampling and replacement) over 96 hours in by isolated rat adipocytes in primary culture with vanadium at concentrations from 0 to 50.0 μM or with insulin at 1.6 nM.

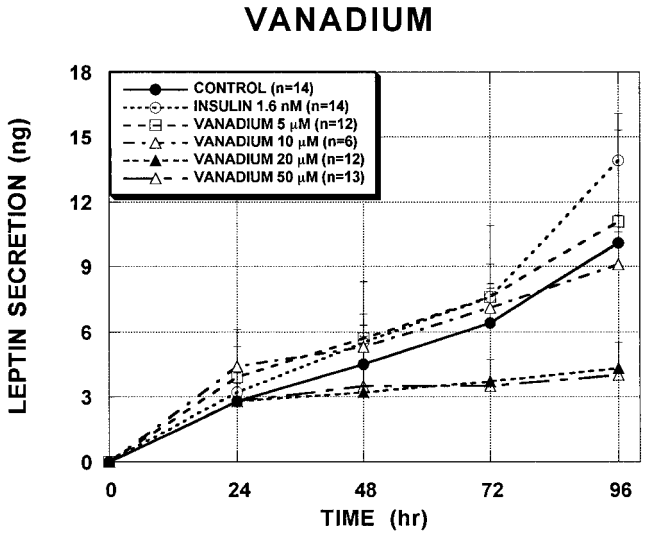

Figure 6. Leptin secretion (corrected for media sampling and replacement) over 96 hours by isolated rat adipocytes in primary culture with vanadium at concentrations from 0 to 50.0 μM or with insulin at 1.6 nM.

stimulate leptin production in adipose tissue (51). However, our results indicate that glucose can be metabolized to lactate, a point well beyond where glucose enters the hexosamine pathway, without stimulating leptin production. Therefore, if the hexosamine pathway were to be the sole mechanism by which glucose regulates leptin production, one would need to postulate that either anaerobic glucose metabolism and/or metformin itself, have an inhibitory effect on glucose entry into this pathway. The results of the present study suggest that it is more likely that the effect of glucose metabolism to stimulate leptin production involves glucose oxidation and/or the production of lipogenic precursors.

Metformin at 0.1 and 0.25 mM did not effect glucose metabolism or leptin secretion. Thus, metformin at therapeutic levels is unlikely to affect leptin production in vivo. Of the concentrations of metformin tested in this study, only 0.5 mM increased glucose uptake without shunting a larger proportion of the glucose into lactate. As previously discussed, this was the only concentration of metformin that had effects on glucose metabolism that did not inhibit leptin secretion, and, in fact, leptin secretion was modestly increased at 0.5 mM. Thus, only when glucose uptake *and* its metabolism beyond lactate were simultaneously increased, did we observe an increase, and not an inhibition, of leptin secretion. Therefore, it seems that only within a very narrow concentration range is metformin able to have a net effect to increase glucose uptake as well as its metabolism beyond lactate in isolated adipocytes. Accordingly, the effects of metformin to inhibit leptin secretion at most concentrations examined is likely to be a result of its effects to direct pyruvate metabolism into lactate and away from other potential pathways for pyruvate metabolism such as oxidation or lipogenesis.

The use of vanadium-containing compounds in the treatment of diabetes has been widely investigated in animals (52,53), and a few clinical trials have been conducted in human patients (54,55). A compound structurally similar to the form of organified vanadium used in this study (BMOV) has recently entered Phase 1 clinical trials. To our knowledge, the present study is the first report examining the effects of a vanadium compound on leptin production in vivo or in vitro.

Vanadium stimulated glucose uptake at concentrations up to 20 μM, whereas glucose uptake was not affected by a concentration of 50 μM. Lactate production was modestly increased at the lower concentrations of vanadium. We found that vanadium at a low concentration of 5.0 μM did not affect leptin production, however, concentrations of 10.0 μM and higher inhibited leptin secretion from isolated adipocytes by 30% to 60%. Although the amount of leptin secreted was inversely proportional to the percentage conversion of glucose to lactate across the concentrations of vanadium tested, this relationship was significantly weaker than that observed across metformin concentrations.

Furthermore, the proportion of glucose taken up and released as lactate was unaffected by vanadium at any concentration. Thus, in contrast to what was observed with metformin, the ability of vanadium to inhibit leptin secretion seems to be independent of any effects on glucose metabolism or lactate production, most likely because it does not increase the proportion of glucose fluxing into anaerobic metabolism.

The observed effects of vanadium result from one or more of the multiple known biological actions of vanadium in cells. These include the inhibition of protein tyrosine phosphatases and the activation of cytosolic protein-tyrosine kinases, resulting in the alteration of cellular tyrosine phosphorylation content (30,56). Vanadium has also been shown to exert direct inhibitory effects on a number of other cellular enzymes, including acid, alkaline, and dual-function phosphatases, ATPases, glucose-6-phosphatase, and fructose-2,6-bisphosphatase (30,55). At high concentrations, vanadium might exert some toxic effects on the cells, an effect which could underlie the lack of effect of the highest concentration of vanadium to stimulate glucose uptake, as well as the inhibition of leptin production at the two highest concentrations examined. In particular, the effects of vanadium to inhibit the activity of one or more enzymes involved in cellular energy metabolism could both inhibit leptin production and, at high concentrations, impair the ability of the cell to utilize energy derived from glucose metabolism.

In vanadium-treated animals, plasma vanadium concentrations have been estimated to be in the 10 to 20 μM range and in human clinical trials in the 1 to 5 μM range (53). Although it is unlikely based on the present results that the concentration of vanadium achieved in humans would be sufficient to affect leptin production, previous human studies employed low doses of vanadyl sulfate or sodium metavanadate, which are molecular forms that exhibit poor bioavailability. The potential effects on leptin secretion of the more readily absorbed forms of vanadium, such as the organified vanadium compound (BMOV) used in the present study (31), should therefore be considered.

In summary, both metformin and vanadium inhibit leptin secretion from primary cultures of rat adipocyte at concentrations that significantly increase glucose utilization. The inhibition of leptin production by metformin, but not by vanadium, is related to an increased conversion of glucose to lactate (i.e., anaerobic metabolism). This effect of metformin, coupled with our previous findings (28), suggests that the effect of glucose utilization to stimulate leptin production is not mediated by glucose uptake per se but involves the metabolism of glucose beyond pyruvate to a fate other than lactate, possibly oxidation or lipogenesis. Thus, metformin is a useful tool for examining the effects of

increasing anaerobic glucose metabolism. Further research, including examination of the potential roles of glucose oxidation and lipogenesis, needs to be conducted to determine the precise biochemical and molecular mechanisms by which glucose metabolism regulates leptin production.

Acknowledgments

This work was supported in part by NIH Grants DK-50129 and DK-35747, the Juvenile Diabetes Foundation, the American Diabetes Association, and the United States Department of Agriculture. We thank Drs. John McNeill and Violet Yuen, in the Department of Pharmaceutical Sciences, University of British Columbia, Vancouver, BC, Canada, for generously providing the BMOV used in the study.

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