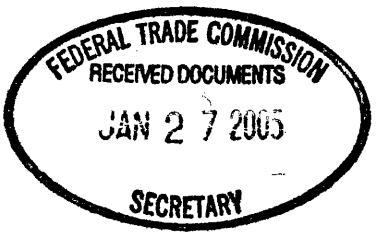
PUBLIC RECORD



UNITED STATES OF AMERICA
BEFORE FEDERAL TRADE COMMISSION
OFFICE OF ADMINISTRATIVE LAW JUDGES

In the Matter of	)	
BASIC RESEARCH, L.L.C, A.G. WATERHOUSE, L.L.C., KLEIN-BECKER USA, L.L.C., NUTRASPORT, L.L.C., SOVAGE DERMALOGIC LABORATORIES, L.L.C., d/b/a BASIC RESEARCH, L.L.C., OLD BASIC RESEARCH, L.L.C., BASIC RESEARCH, A.G. WATERHOUSE, BAN, L.L.C., d/b/a KLEIN-BECKER USA, NUTRA SPORT, and SOVAGE DERMALOGIC LABORATORIES, DENNIS GAY, DANIEL B. MOWREY, d/b/a AMERICAN PHYTOTHERAPY RESEARCH LABORATORY, and MITCHELL K. FRIEDLANDER	)	**DOCKET NO. 9318**
Respondents.	)	

RESPONDENTS' EMERGENCY MOTION TO STRIKE DR. ROBERT ECKEL AND DR. STEVEN HEYMSFIELD AS PETITIONER'S EXPERT WITNESSES AND FOR SANCTIONS AND OTHER RELIEF—EXPEDITED BRIEFING AND DECISION REQUESTED

During its pre-Complaint investigation and continuing into post-Complaint discovery in this case, the Federal Trade Commission ("FTC" or "the Commission") and Complaint Counsel propounded upon Respondents exhaustive discovery requests seeking, among other things, some of Respondents most private, confidential, valuable, and proprietary information and work product. Given the extremely sensitive and privileged nature of many of these documents and materials, including product formulations, product research and substantiation, and other proprietary work-product,

Respondents sought from this Court a pre-production protective order to ensure their compliance with Complaint Counsels' discovery requests would not result in the dissemination of their most protected and valuable information—particularly to competitors. To that end, the Court, early in this case, entered a Protective Order Governing Discovery Material ("Protective Order") specifically designed to protect against improper use or disclosure of Respondents' information.

Both before and after entry of the Order, Respondents, acting in good faith, produced to the FTC thousands of highly sensitive documents. Many of these documents were designated as attorneys' eyes only.

Respondents rightfully believed that the Court's August 2004 Protective Order would insulate Respondents' proprietary and confidential information from improper disclosure and, potentially improper use by the Federal Trade Commission and or its experts. Unfortunately, Respondents' faith that the Government would respect this Court's mandate was misplaced.

This Motion documents overwhelming evidence that Complaint Counsel ignored material provisions of the Protective Order prior to disseminating Respondents' attorneys' eyes only documents to its experts. During the same time those experts improperly received and reviewed Respondents' information, at least one of the experts was conducting and assisting in studies of potential weight-loss products for some of Respondents' direct and indirect competitors. Moreover, one of those experts was, at the same time, in negotiations with a competitor for in-house employment to head a division devoted to the development and production of a new line of products specifically designed for weight-loss. Incredibly, that expert, while in actual possession of

Respondents' confidential, proprietary information, including formulas, and product research and substantiation, negotiated a compensation package with a competitor that provides financial incentives based upon his success in getting to market new products that directly compete with Respondents' products.[1]

In clear violation of this Court's Order, designed to preclude the very dissemination that here occurred, Complaint Counsel provided Respondents' information to those two experts without providing Respondents advance notice. As a result, Respondents had no knowledge of these serious breaches and no opportunity to timely raise their concerns in advance of the incurable disclosures. These breaches by Complaint Counsel were unknown to Respondents until they received reports from Complaint Counsels' experts, and even then were unconfirmed until Respondents had a chance to question the experts about these issues during their recent depositions. Complaint Counsel has to the date of this filing never notified Respondents of these disclosures of Respondents' information or any disclosures to other individuals currently unknown to Respondents.

Complaint Counsels' failure to comply with this Court's Protective Order has caused Respondents incalculable harm and prejudice, as their most private, confidential and valuable commercial information now resides with at least one direct competitor and was held by Complaint Counsels' experts while conducting paid work for several of Respondents' direct and indirect competitors. The harm is extraordinary and incurable.

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[1] While Respondents no longer sell ephedra/ephedrine based dietary supplements, the formulas for these products still have value as there is no bar on filing a New Drug Application (NDA) for any ephedra/ephedrine based weight control compound.

For these reasons, as discussed more fully below, Respondents Basic Research, LLC, A.G. Waterhouse, LLC, Klein-Becker USA, LLC, Nutrasport, LLC, Sövage Dermalogic Laboratories, LLC, Ban, LLC, Dennis Gay, Daniel B. Mowrey, and Mitchell K. Friedlander (collectively "Respondents"), through undersigned counsel, jointly seek an Order pursuant to 16 C.F.R. § 3.38(b) and 16 C.F.R. § 3.42(h), that: (1) sanctions Complaint Counsel for their deliberate or grossly indifferent breach of this Court's Protective Order by excluding from these proceedings the two expert witnesses, Dr. Robert H Eckel ("Dr. Eckel") and Dr. Steven B. Heymsfield ("Dr. Heymsfield"), to whom Complaint Counsel improperly provided Respondents' confidential information; (2) commands the return to Respondents from Drs. Heymsfield and Eckel of all documents in their possession marked or designated confidential, together with sworn affidavits that reasonable efforts were made to confirm that no additional copies, either electronic or in hard form, exist, and that no other persons had access to the materials while in their possession; and (3) requires Complaint Counsel to file a declaration or sworn affidavit detailing in specific terms; (a) their handling of Respondents' protected confidential information, (b) the measures undertaken by Complaint Counsel to safeguard that information and comply with the Court's Protective Order, (c) the identities of all individuals who were provided access to or copies of the protected information, and (d) detailing the full scope of their violations of the Protective Order.

Alternatively, should the Court be disinclined to strike Dr. Heymsfield and/or Dr. Eckel from these proceedings, Respondents request the declaration from Complaint Counsel described above in addition to an Order from the Court commanding: (1) Dr. Heymsfield to make available immediately all documents in his possession responsive to

the subpoena specifications this Court made clear in an earlier ruling were applicable to this witness; (2) Complaint Counsel to make Dr. Heymsfield available for two days, following adequate opportunity for Respondents to review and digest the documents Respondents have yet to receive from Dr. Heymsfield or Complaint counsel, to ensure Respondents have a full and fair opportunity to resume and conclude their deposition; (3) Complaint Counsel to reimburse Respondents' costs and fees for having to conduct a second out-of-state deposition; and (4) setting a new date, ten days after completion of Dr. Heymsfield's deposition, for Respondents to respond to Complaint Counsels' motions for summary decision, in order to ensure a fair opportunity to respond to the arguments, issues and evidence presented therein, at least some of which will, no doubt, be based upon Dr. Heymsfield's opinions.

The instant Motion is filed as an emergency motion due to the immediate and pressing nature of these issues, the fact that the discovery cut-off has now passed, and the impending summary decision filing deadlines.[2] Timely clarification from the Court concerning the relief available is needed in order to properly plan for the resumption of Dr. Heymsfield's deposition, if the Court is reluctant to grant the relief requested and strike the FTC's experts. To that end, Respondents request an expedited briefing schedule and ruling from the Court.

In support of this Motion, Respondents state as follows:

I. FACTUAL BACKGROUND

A. The Protective Order Governing Discovery Material.

[2] As a showing of good faith, the filing of this Motion was shortly delayed due to settlement discussions between the parties.

On August 11, 2004, the Administrative Law Judge ("ALJ") entered a Protective Order Governing Discovery Material ("Protective Order") in this case to shield the parties and third parties against improper disclosure or use of confidential information submitted or produced in connection with this matter. Protective Order, p. 1. The Protective Order governs all confidential documents designated in the manner set forth in the Order. *See* Order at 4 ¶ 2. Those documents bearing the special designation "Restricted Confidential, Attorneys Eyes Only – FTC Docket No. 9318," contain competitively proprietary information and are subject to enhanced protection under the Order. *Id.* at 5 ¶ 2(b).

Under the express terms of the Protective Order, disclosure and dissemination of material marked "Restricted Confidential – Attorneys Eyes Only – FTC Docket No. 9318" ("Confidential Material") is limited to the parties and the Court. *Id.* at 7, ¶¶ 4(a), (b), (c) and (e). Dissemination of Confidential Material to expert witnesses is subject to the terms of Paragraphs 5 and 7 of the Protective Order. *Id.* at 9, ¶¶ 5 and 7. Even the process for copying the confidential information is spelled-out in the Order. Save for that which is "reasonably necessary to facilitate the conduct of this matter," Paragraph 7 prohibits copying or reproduction any of the Confidential Material, and any copies or reproductions that are reasonably necessary are afforded the same protections as originals under the Order. *Id.* at 9, ¶ 7.

The express language of the Protective Order requires Complaint Counsel to provide advance notice to Respondents of its desire to disclose to a third-party any Confidential Material. *Id.* at 5, ¶ 2(c). Complaint Counsel's notice must include, at a minimum, the name of the individual to whom disclosure is contemplated, together with

his or her professional address and affiliation. *Id.* Respondents then have five business days in which to object to the proposed disclosure. *Id.* The reason for this is obvious—to provide Respondents an opportunity to challenge proposed disclosure to someone connected directly or indirectly to a competitor. Complaint Counsel is also required by the Court's Order to obtain a written declaration from the proposed recipient swearing that he or she will comply with the terms of the Protective Order concerning the handling of the Confidential Material prior to receipt of the same. *Id.* at 7 ¶ 5.

The language of the Protective Order itself notes that disclosure of Confidential Materials, including without limitation, customer names, consumer complaints, strategic plans, trade secrets, customer specific evaluations or data, proprietary or engineering information, market research and analysis, and the like, "would cause substantial commercial harm or personal embarrassment to the disclosing party." *Id.* at 3, ¶ 20. It is Complaint Counsels' failure to comply with the mandatory provisions governing the disclosure of Respondents' confidential information to third-parties that has caused the very irreparable harm contemplated by the Court's Order, and which precipitates the instant Motion.

B. Respondents' Production of Confidential Material and Complaint Counsels' Subsequent Dissemination to Others in Contravention of the Protective Order.

In January 2001, several Respondents first learned they were under investigation by the FTC. During the course of that investigation, and continuing through the discovery phase of this action, initiated in June 2004, Respondents produced to the FTC and Complaint Counsel, thousands of requested documents, including some of Respondents' most valuable, confidential, and proprietary information and work

product.[3] The Protective Order clearly governs Respondents' productions following entry of the Order, but also expressly applies to documents produced in advance of the Order:

> All documents heretofore obtained by the Commission through compulsory process or voluntarily from any Party or Third Party, regardless of whether designated confidential by the Party or Third Party, and transcripts of any investigational hearings, interviews and depositions, that were obtained during the pre-Complaint stage of this Matter shall be treated as "Confidential," in accordance with paragraph 2(a) of this Order.

Protective Order at 6, ¶ 3.

Of course, Complaint Counsel also undertook responsibilities and duties as custodians of the confidential documents produced by Respondents irrespective of the Protective Order. Chief among the continuing responsibilities owed by Complaint Counsel to Respondents is the duty to vigorously protect the confidentiality of the private, commercial, and proprietary information provided in response to numerous requests and demands by the FTC, and to prevent the disclosure of that material in any manner that may injure or harm Respondents or their businesses.

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[3] Any challenge or dispute Complaint Counsel may now seek to raise concerning the propriety of any specific Confidential designation of documents here at issue should be summarily dismissed because Complaint Counsel has failed to raise any objection to the designation of any of the documents, as required under Paragraph 6 of the Protective Order. Moreover, Complaint Counsel is also barred from arguing the impropriety of any such designations based upon any such documents being otherwise available individually in the public domain, as the documents were produced to Complaint Counsel and the FTC as a collection or compilation, uniquely combined as they relate to the specific challenged products at issue here, thus justifying their status as trade secrets and giving Respondents a competitive advantage. See *Water Services, Inc.* v. *Tesco Chemicals, Inc.*, 410 F.2d 163, 173 (5th Cir. 1969); see also Restatement of the Law 3d, Unfair Competition (1995), § 39(f), p. 432 (stating "the fact that some or all of the components of [a] trade secret are well-known does not preclude protection for a secret combination, compilation, or integration of the individual elements."); *Salsbury Laboratories, Inc. v. Merieux Laboratories, Inc.*, 735 F.Supp. 1555, 1569 (M.D. Ga. 1989), aff'd 908 F.2d 706 (11th Cir. 1990); *Rivendell Forest Products v. Georgia-Pacific Corp.*, 28 F.3d 1042, 1042 (10th Cir. 1994); *Essex Group, Inc. v. Southwire Co.*, 501 S.E.2d 501, 503 (Ga. 1998).

1. Dr. Heymsfield.

Pursuant to notice previously provided and subpoena to the witness, Respondents began the deposition of Dr. Heymsfield on January 11, 2005, the date agreed upon by the parties. Prior to Complaint Counsel unilaterally suspending the deposition and walking out, over objection of all Respondents, it became clear from Dr. Heymsfield's testimony that Complaint counsel had materially violated the terms of this Court's Protective Order, that Complaint counsel had conducted itself in bad faith with respect to allowing Respondents sufficient access to Dr. Heymsfield, that Dr. Heymsfield had failed to meaningfully comply with Respondents' subpoena, and that Complaint Counsel had failed to comply with the terms of the Court's scheduling describing the documents to be exchanged by parties relating to experts.[4] Each of these issues has caused Respondents actual prejudice and harm. Cumulatively, the injury to Respondents is incalculable and incurable, thus entitling Respondents to the relief sought.

a. Complaint Counsel's Disclosure of Confidential Materials to Dr. Heymsfield in Violation of the Protective Order.

As discussed more fully below, Dr. Heymsfield's deposition testimony clearly establishes that Complaint Counsel provided Dr. Heymsfield with copies of Respondents'

[4] Because Complaint Counsel unilaterally suspended Dr. Heymsfield's deposition in the middle of examination by counsel for the entity Respondents on the very issues discussed herein, it is impossible for Respondents to know whether there are additional material breaches or issues that may bear on this Motion. Complaint Counsel intentionally interfered with Respondents' very ability to frame these issues for the Court's consideration by unilaterally removing Dr. Heymsfield once it became clear there were serious problems with their failure to comply with the Court's Order and Dr. Heymsfield's failure to comply with the subpoena. Respondents believe the prejudice flowing from these violations requires briefing as soon as possible, even though Respondents were deprived of the opportunity to fully develop this issue for the Court, and they submit this memorandum based upon the best information now available, however incomplete it may be.

confidential document and materials, and that Dr. Heymsfield reviewed those materials. It is not disputed that Complaint Counsel failed to comply with the express requirements of this Court's Protective Order, that notice in advance of disclosure be provided to Respondents for an opportunity to object. This failure led to extraordinary prejudice and harm to Respondents when their confidential documents and materials were provided to Dr. Heymsfield by Complaint Counsel.

During that portion of Dr. Heymsfield's deposition that was later unilaterally suspended by Complaint Counsel, counsel for Respondents started questioning Dr. Heymsfield about certain documents he received from Complaint Counsel during the course of his involvement in this case. During that questioning, Respondents' counsel showed Dr. Heymsfield a letter addressed to him from Complaint Counsel, acknowledging the enclosure of documents pertaining to Respondents' substantiation for Anorex, bate stamp numbered R0332 through R1811. Tr. Depo. Steven B. Heymsfield, p. 376 (January 11, 2005), attached to this Motion hereto as Exhibit 1 (and is cited in this Motion as "Heymsfield depo. at __"). Respondents' counsel then removed a representative sample of documents from that bate stamp range, numbered R1151 through R1252, and asked Dr. Heymsfield if the bottom of each of these documents read "Restricted Confidential Attorneys Eyes Only." *Id.* at 377. Dr. Heymsfield responded with a simple, "yes." *Id.* Respondents' counsel made the same inquiry with respect to two other document samples (bate stamped 826 through 1000, and 1094 through 1193) provided to Dr. Heymsfield with the September 27, 2004, letter from Complaint counsel. *Id.* at 378. Dr. Heymsfield again acknowledged that each page of these documents was marked "Restricted Confidential Attorneys Eyes Only." *Id.* at 378-9.

Respondents' counsel next asked if Dr. Heymsfield would agree that he had received "many, many of [Respondents'] 'Restricted Confidential Attorneys Eyes Only' documents," to which Dr. Heymsfield concurred.[5] *Id.* at 379.

Dr. Heymsfield's testimony clearly establishes Complaint Counsel provided him with Confidential Material covered by the Court's Protective Order. Respondents were never notified of this by Complaint Counsel, and given no opportunity to object. Had Respondents been given such an opportunity there is no question but that they would have objected for the reasons that follow, among others.

b. Dr. Heymsfield's Obvious and Serious Conflict of Interest.

Dr. Heymsfield's own deposition testimony proves that Complaint Counsel provided him with Respondents' confidential information and materials, including product formulations and other highly valuable and secret commercial data and research, at the same time Dr. Heymsfield was being paid by direct and indirect competitors of Respondents to conduct and assist with studies of other competing weight loss products. Even more astonishing is that Dr. Heymsfield was, while in possession of this same highly confidential, proprietary information and work product, negotiating with Merck & Company for employment as head of a research division at Merck charged with the task of researching, developing and getting to market weight loss products—products that

[5] To the extent Complaint Counsel may try to argue that no "new" documents were provided to Dr. Heymsfield after the Court issued the Protective Order, Respondents note that any such representation lacks merit. Among the documents provided Dr. Heymsfield in September 2004, after entry of the Order, were proprietary product formulations not produced by Respondents prior to discovery in this action. That is, Complaint Counsel did not have the formulation documents at the time they initially provided documents to Dr. Heymsfield before entry of the Order. There can be no serious dispute that Dr. Heymsfield received new, confidential documents and materials from Complaint Counsel following entry of the Protective Order.

would obviously compete directly with Respondents' products, the formulations for many of which were in Dr. Heymsfield's possession. Dr. Heymsfield's negotiations with Merck eventually led to his accepting a position as Executive Director of Clinical Research in Metabolism, with a healthy compensation package that includes financial bonuses directly tied to his success in creating and getting to market products that will complete with those of Respondents. *Id.* at 205-06.

Dr. Heymsfield testified that negotiations between himself and Merck began sometime during the summer of 2004. *Id.* at 351. He further testified that he formally accepted employment with Merck in early October and began working there on November 1, 2004. *Id.* When asked if he had disclosed to Complaint counsel that he was in negotiations with Merck, Dr. Heymsfield answered, "yes... as soon as I signed my contract with Merck, I disclosed it to them." *Id.* Respondents' counsel then asked Dr. Heymsfield, "were you involved in any studies for any pharmaceutical or dietary supplement companies, any time during your tenure as an expert in this case, that is from the point where you were asked to act either in consulting or testifying capacity in this case?" *Id.* at 355. Dr. Heymsfield answered, "during that time period, I was actively engaged in research projects related to at least two drugs. One happened to be a Merck drug, the other is a drug from a company called Regeneron. For sure. There are probably many others, but those were two." *Id.* at 355-56.

As a follow-up, Respondents' counsel asked whether these two drugs were weight loss studies, to which Dr. Heymsfield responded, "yes." *Id.* at 356. Dr. Heymsfield went on to list several other companies that he worked with or was approached by while retained as an expert in this case, including Kellogg's, the National Dairy Counsel,

Healthy Tech, Tanita, Armeron, American Home Products, and Wyeth. *Id.* at 357-60. Dr. Heymsfield also acknowledged previous relationships with numerous other direct and indirect competitors of Respondents in the weight loss field. When asked for what other pharmaceutical companies Dr. Heymsfield had been a consultant, he answered that he had consulted for Hoffman LaRoche, Abbott Pharmaceuticals, Takida, Johnson & Johnson, Sinofy Pharmaceuticals, and Servier. *Id.* at 9-10. When asked specifically if he had ever been a consultant to any dietary supplement companies, Dr. Heymsfield's answered "I have been a consultant to companies such as SlimFast, Neutra System. I think those are the main ones. And companies like Weight Watchers... I did work at one point as a consultant for several dietary supplement companies. One is called Pacific Health." *Id.* at 10.

Had Respondents known that Dr. Heymsfield was affiliated in any way with these companies (especially the contemporaneous engagements with competitors while reviewing Respondents' confidential information), and had Respondents been provided the opportunity to object to disclosure of its confidential documents and materials to Dr. Heymsfield, as provided in the Court's Protective Order, they would unquestionably have done so.[6] Complaint Counsels' failure to comply with the Protective Order and notify Respondents of its desire to disclose Confidential Materials to Dr. Heymsfield deprived Respondents of their opportunity to prevent their most valuable, proprietary, and confidential material from landing squarely in the hands of a direct competitor—and to

[6] To the extent Complaint Counsel may suggest otherwise, Respondents note that Dr. Heymsfield's historical and contemporaneous relationships with competitors was not necessarily a basis for seeking to exclude him as an expert in this case. It was Complaint Counsels' act of providing him with confidential proprietary documents and materials while so engaged that caused the extraordinary harm here, the significance of which was unknown to Respondents until his deposition.

be sure, as the Executive Director of Clinical Research in Metabolism for Merck, a major pharmaceutical company, Dr. Heymsfield is Merck for all intents and purposes—actively engaged in developing weight loss products to bring to market to compete with Respondents' products. The prejudice is extraordinary and obvious. It is also incurable. The Court should grant Respondents Motion and provide the requested relief.

c. Post-Disclosure Breaches of the Protective Order by Dr. Heymsfield and Complaint Counsel.

Complaint Counsels' breaches of the Court's Protective Order did not end with disclosure of Respondents' Confidential Materials to an expert affiliated with direct and indirect competitors of Respondents. Complaint Counsel failed even to ensure Dr. Heymsfield, once he received from Complaint Counsel the documents he should not have received, was adequately educated on how to properly protect the confidentiality of the materials. During his deposition, counsel for Respondents asked whether Complaint Counsel had ever instructed Dr. Heymsfield on how to handle the Confidential Materials, to which Dr. Heymsfield responded, "they never gave me a lecture on how to handle these documents." *Id.* at 379-80. Respondents counsel next posed the question, "they never asked you to sign a declaration, obtaining your agreement to abide by the protective order that the court entered in this case; is that true?" *Id.* at 380. To this question, Dr. Heymsfield responded, "that is true." *Id.*

Additional questioning of Dr. Heymsfield also revealed he was completely unaware of the existence of a Protective Order in this matter. During the course of the deposition, Respondents' counsel asked and Dr. Heymsfield answered the following:

> Q. Did you know at the time that you were given this document that there had been a protective order entered by the court in this case

governing the handling and use of this document; do you know of that order yes or no?
A. No, I didn't know of that order.
Q. No one from the FTC told you about the order;... isn't that true?
A. I don't think that anyone ever told me specifically about that order.
Q. I'm showing you Exhibit 10, the protective order. You have no – do you have any recollection of ever read [sic] this is [sic] document?
A. No.

Id. at 364-5.[7]

As a result, Complaint Counsels' own expert witness, Dr. Heymsfield, was provided access to Respondents' Confidential Materials and, without Dr. Heymsfield's appreciation for the confidentiality of these documents, he admittedly scanned these documents and saved them to a computer owned not by himself, but by St. Luke's Hospital, in clear violation of the Protective Order. *Id.* at 372. Dr. Heymsfield also testified that these documents and materials were left for months in his Hospital office. Dr. Heymsfield's deposition demonstrates that Complaint Counsel provided Dr. Heymsfield with numerous sensitive confidential documents belonging to Respondents and marked Restricted Confidential Attorneys Eyes Only without Dr. Heymsfield's appreciation of the importance of such a designation, whether or not he was aware of the Protective Order, and that the failure to adequately counsel their witness led to additional

[7] Despite Dr. Heymsfield's plain testimony on this point, Complaint Counsel produced to Respondents, subsequent to the interrupted deposition, a copy of a declaration signed by Dr. Heymsfield acknowledging he was aware of and would comply with the terms of the Court's Protective Order. Respondents note the document was not in the material provided them by the witness or Complaint Counsel prior to the deposition. Whatever possible explanation may exist for why Dr. Heymsfield testified unequivocally that he had not previously seen the declaration or the Court's Protective Order, or even discussed either with Complaint Counsel, the testimony clearly establishes that Complaint Counsel failed to adequately educate their expert about the handling of the highly Confidential Materials. As discussed, this led to actual and harmful breaches of the Order by Dr. Heymsfield. Of course, whether or not Complaint Counsel obtained the signed declaration from Dr. Heymsfield, Complaint Counsel still breached the Protective Order by failing to provide Respondents advance notice of the disclosure, as noted herein.

breaches of the Court's Protective Order. Quite aside from Complaint Counsel's and Dr. Heymsfield's mis-handling of Respondents' Confidential Materials, an obvious additional concern to Respondents is that they cannot possibly know whether the confidentiality of the material it produced to Complaint Counsel in good-faith, and in reliance on the protections in the Protective Order, has been compromised by yet another party, causing still further harm and prejudice.

d. Complaint Counsels' Bad-Faith with Respect to Providing Respondents a Fair Opportunity to Depose Dr. Heymsfield.

Complaint Counsel has also acted in bad-faith with respect to allowing Respondents a fair and full opportunity to depose Dr. Heymsfield, a key witness in these proceedings. On January 6, 2005, the week before Dr. Heymsfield's deposition was to take place, Respondent Mitch Friedlander and counsel for corporate Respondents, Jeffrey Feldman, had a conversation with Complaint Counsel wherein Complaint Counsel was asked to make Dr. Heymsfield available as long as was necessary to complete his deposition on the day designated. Tr. Depo. Mitch Friedlander, p. 247 (January 6, 2005), attached to this Motion hereto as Exhibit 2. The conversation took place in the middle of what was only one of several very lengthy depositions taken by Complaint Counsel that week, some of which went into the evening hours. Complaint Counsel responded that they would speak to Dr. Heymsfield about his availability. Friedlander depo. at 248. Apprehensive about Complaint Counsel's simple assertion only that they would speak with Dr. Heymsfield about being available for an extended deposition, corporate Respondents' counsel made the following statement on the record:

> let's go back and remind the court and the record that yesterday I had a conversation which was not the first time about Dr.

> Heymsfield [sic] availability, and I would ask not – at least over the last two days – for assurance that Dr. Heymsfield would make himself available and be available for as long as we need him next week because there are one, two, three, four people that need to question him.
>
> I asked Ms. Kapin to call Dr. Heymsfield – I asked this yesterday – to determine whether or not he would be available and would be able to stay late if necessary. I have not gotten anything back.

Id. at 248. Complaint Counsel Laureen Kapin's response, also on the record, was "what I said, Jeff, is that I would talk with him. I have not had the opportunity to do that. I will do that." *Id.* Complaint Counsel apparently forgot that this conversation occurred on the record during Mr. Friedlander's deposition, as Complaint Counsel provided a completely different account of the conversation the next week during Dr. Heymsfield's deposition, in which Ms. Kapin stated that "for the record, we never talked about making Dr. Heymsfield tomorrow. He is not available tomorrow. And when you say you had discussed that with me previously, that is incorrect." Heymsfield depo at 68-9.

Notwithstanding Complaint Counsel's earlier assertion that Dr. Heymsfield would be consulted about making himself available until all parties had completed their questioning, Dr. Heymsfield was directly asked during his deposition: "before yesterday, at any time during the past week did you have any discussions with Complaint Counsel concerning the length of this deposition and your availability for the deposition?" *Id.* at 80. Dr. Heymsfield responded, "not in the past week that I recall." *Id.* Complaint Counsels' failure to consult Dr. Heymsfield about the need for him to remain until all parties had completed their questioning, together, it seems, with the testimony Dr. Heymsfield had given to that point concerning his failures to comply with subpoenas and the Protective Order, led to Complaint Counsel unilaterally suspending Dr. Heymsfield's

deposition in the middle of questioning by only the second Respondent with an opportunity to pose any questions.

After returning from a recess during Dr. Heymsfield's deposition, Complaint Counsel re-entered the deposition room and, at 7:11 p.m., over Respondents' counsel's objection, stated that they were going to recess Dr. Heymsfield's deposition. *Id.* at 392-93.

Complaint Counsel conducted many depositions during discovery in this case of much longer duration than Dr. Heymsfield's and not once did Respondents attempt to foreclose Complaint Counsel from completing their deposition. To the contrary, Respondents made their own offices available for an after-hours deposition after being forced to leave one location when it closed in the evening. Respondents have not once interfered with Complaint Counsels' ability to conduct and complete a full and fair deposition of a witness.

As a result of Complaint Counsels' deliberate failure even to consult their expert witness to ensure his schedule provided for a full and fair opportunity for Respondents to take his deposition on the day mutually agreed upon, and Complaint Counsels' misrepresentations on the record to Respondents about the same, Respondents are unable at this time even to adequately meet the allegations made by Dr. Heymsfield in his report, as only one of four Respondents was provided leave to complete their questioning of Dr. Heymsfield before Complaint Counsel simply walked-out with the witness. Of course, even the questioning that took place was necessarily incomplete, as discussed below, because Dr. Heymsfield elected to produce only about twenty percent of the documents in his file that he reviewed in this case.

Complaint Counsels' bad faith with respect to allowing Respondents a fair chance to fully question Dr. Heymsfield is ongoing. Even as of the date of this filing, Complaint Counsel is refusing to make Dr. Heymsfield available for deposition for any period longer than four hours, despite the fact that Dr. Heymsfield has well over four binders of documents he failed to previously produce, and despite that fact that only Respondent Mowery has completed his direct examination of this witness. Moreover, though Respondents believe that Dr. Heymsfield should be stricken, an adverse ruling on this Motion will force Respondents to return to New York City to finish the deposition Complaint Counsel unilaterally suspended, at a cost of many thousands of dollars in additional time and travel expenses.

Despite all the foregoing, Complaint Counsel is still failing to act in good-faith, refuses to comply with the discovery rules and chooses not to extend to Respondents the same courtesies extended to them. The Court should grant Respondents the relief requested.

e. Dr. Heymsfield's Intentional Failure to Comply with Respondents' Subpoena and this Court's Expert Witness Disclosure Requirements.

1. Heymsfield Deliberately Ignored the Respondents' Subpoena for Documents.

As noted, it also became apparent during his deposition that Dr. Heymsfield simply chose not to comply with the subpoena issued by Respondents. On October 12, 2004, Respondents issued a subpoena to Dr. Heymsfield, lawfully ordering him to produce certain specifically identified documents in advance of his deposition.[8] Among

[8] The FTC filed a Motion for Protective Order as to a number of the document specifications in Dr. Heymsfield's subpoena; however, the FTC had no objection to the

the documents requested were all documents Heymsfield reviewed in preparation of his expert witness report, all correspondence between Dr. Heymsfield and the FTC concerning this matter and Dr. Heymsfield's entire file pertaining to this matter. Subp. Steven B. Heymsfield, Exhibit A(1), (2), (6), (October 12, 2004), attached to this Motion as Exhibit 3. However, when questioned during his deposition about his production pursuant to the subpoena, Dr. Heymsfield acknowledged that he elected not to produce to Respondents the requested materials.

Of particular concern was Heymsfield's decision to withhold documents that he considered in reaching his decisions in this case. Dr. Heymsfield's stated in his expert report that he considered several categories of documents. One category encompassed documents the FTC sent to him for his review. Another category were documents that Dr. Heymsfield pulled from his own files. Indeed, Dr. Heymsfield's expert report states that: "My report is based on material provided to me by the Federal Trade Commission (FTC) and on my own extensive files and literature search." Expert Report of Steven B. Heymsfield, p. 9, ¶ 40. Specification numbers 6 and 7 of Respondents' subpoena to Dr. Heymsfield called for the production of "All documents reviewed by you in connection with you work on this matter" and "All materials consulted by you or relied on by you in forming any opinion in connection with this matter." *See* Exhibit 3. Despite having an appreciation that the subpoena required production of all documents that he considered, even those in his personal files, Dr. Heymsfield decided to provide only those documents that he deemed "directly relevant" to his opinion.

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first seven specifications. All of the documents withheld by Dr. Heymsfield were responsive to one of more of these first seven requests.

Notably, Respondents' counsel asked Dr. Heymsfield if he "would agree that [he] did not list the files, [his] own files that [he] considered." Heymsfield depo at 297. Dr. Heymsfield responded simply, "that's correct," later stating "I produced to [Respondents] all the papers I relied *often*. I didn't produce my files." *Id.* at 297, 299 (emphasis added). Dr. Heymsfield also admitted that he "only provided the material that [he] thought was directly relevant to [his] report, but there is other material." *Id.* at 318. Moreover, when asked which documents Dr. Heymsfield produced apart from those provided to him by Complaint Counsel, Dr. Heymsfield responded "likely... none of them, because the FTC's was the most consolidated clear set of papers as opposed to mine which are pretty worn." *Id.* at 302. Thus, Dr. Heymsfield's production apparently consisted of no more than a portion of Complaint Counsels' own file to Dr. Heymsfield, though Dr. Heymsfield himself admittedly reviewed and relied upon other documents in his own files.

In attempting to determine the extent of Dr. Heymsfield's breach of the subpoena and disregard of Respondents' right to fully and fairly examine him, Respondents' counsel asked approximately how many notebooks of material Dr. Heymsfield received from Complaint Counsel and how much of that material he actually produced to Respondents. Specifically, Respondents' counsel asked, "of the total amount of information that you received from the FTC... what percentage of it have you produced to [Respondents]?" *Id.* at 324. Dr. Heymsfield's response was remarkable, stating that he "provided to [Respondents]... a little more than one photo book. There's probably another four or five notebooks full of material which I did not specifically use in my

report, and that I only reviewed in a very cursory way as a screening, which I did not send." *Id.* at 324-5.

Thus, Dr. Heymsfield also admits to failing to produce the vast majority of documents provided to him by Complaint Counsel. Failure to produce all of the documents Dr. Heymsfield received from Complaint Counsel *and* all of the documents Dr. Heymsfield consulted in formulating his opinion, severely prejudices Respondents, making it impossible for Respondents to have conducted a thorough examination of Dr. Heymsfield, and rendering Respondents unable to adequately formulate rebuttal argument against Dr. Heymsfield and his report. It effectively nullifies even the effect of the testimony obtained during his partial deposition because Respondents' counsel could not possibly be adequately prepared to depose Dr. Heymsfield without knowing what documents he reviewed and relied upon.

This Court's recent ruling concerning a single document the Respondents withheld (on the basis of privilege) from the production of Respondents' expert linguist, Professor Larry Solan, clearly establishes an expert's responsibility to provide, "all data, documents, or information considered by a testifying expert witness in forming the opinions to be proffered," including "documents considered but rejected by the testifying expert in reaching [the] opinions." Order on Complaint Counsel's Motion to Compel a Document from Respondents' Testifying Expert Solan, at 2 (citations omitted). This Court was clear that each expert expected to testify at trial must provide "all documents reviewed, consulted, or examined . . . in connection with forming his or her opinion on the subject on which he or she is expected to testify...." *Id.*

These documents were requested by Respondents in the subpoena served on Dr. Heymsfield. He was aware of the request and simply chose not to comply with Respondents' lawful request and the Subpoena issued by this Court. His failure to comply deprived Respondents (at least those who had any opportunity to examine Dr. Heymsfield at all) of an opportunity to fully explore the basis for his opinions and the materials considered. Respondents are now forced to prepare motions for summary decision and prepare for the hearing in this matter having been deprived of a meaningful opportunity to examine a key witness in this proceeding. The reason for that is simple—the Commissions' expert simply decided to not to comply with the terms of the valid and lawful subpoena from this Court. As a matter of equity, and in the context of the numerous other issues raised by the conduct of Complaint Counsel and Dr. Heymsfield, it is only fair that Dr. Heymsfield should be stricken entirely from this proceeding.

2. Complaint Counsel and Heymsfield Violated this Court's Expert Witness Disclosure Requirements.

Complaint Counsel also failed to disclose all cases in which Dr. Heymsfield has testified as an expert and, with respect to one of those cases, Complaint Counsel and Dr. Heymsfield failed to disclose the full nature of Dr. Heymsfield's testimony in that case. For example, in Complaint Counsel's expert witness disclosure, Complaint Counsel and Dr. Heymsfield represented that Dr. Heymsfield had testified in five (5) cases, none of which involved the FTC as a party. However, during his deposition, Dr. Heymsfield disclosed, for the first time, that he had testified via deposition and at trial as an expert in a case for the FTC. *See, e.g.,* Heymsfield Dep. at 41:21-42:3.

Additionally, in their expert witness disclosure, Complaint Counsel and Dr. Heymsfield represented that Dr. Heymsfield had testified via deposition in the case of

Parks v. Cytodyne Technologies, Inc. However, Respondents independently learned that in addition to testifying via deposition in the *Parks* case, Dr. Heymsfield also testified at the trial of that case.

Complaint Counsel's and Dr. Heymsfield's failure to fully disclose all cases in which Dr. Heymsfield has testified as an expert is important because, aside from the fact such failure is a violation of the Court's order, Dr. Heymsfield has offered testimony in this case which is irreconcilably inconsistent with testimony he has previously offered.

For example, Dr. Heymsfield has testified in this case that several of the studies that Respondents' rely on to support their ephedra, aspirin and caffeine products are irrelevant because the active ingredients in the studies did not include aspirin but rather a willow bark derivative called salicin. Dr. Heymsfield has opined in this case that aspirin and salicin are not the same thing and therefore studies that involve salacin cannot substantiate aspirin based products. In this regard, Dr. Heymsfield's testified at his deposition as follows:

> Q. So is it your opinion that aspirin and salicin are the same thing?
>
> A. No. I think there's some questions about comparable efficacy of willow bark compared to acetylsalicylic acid, which is aspirin.

Yet in direct contrast to that testimony, Dr. Heymsfield testified during the *Parks* trial that salicin and aspirin are herbal equivalents, and that salicin and aspirin are the same thing. For example, during questioning at the *Parks* trial concerning the use of ephedrine, caffeine, and aspirin in a scientific study conducted by, *inter alia*, Dr. Patricia Daly, and whether Cytodyne's Xenadrine product contained aspirin, the following colloquy occurred:

Q. Now Xenadrine RFA-1, if we're to believe what's on the label, says it has salicin, correct?

A. That's correct.

Q. And that's a -- is that an herb?

A. Well, it's -- I believe there's an herbal source. It's called white willow bark and it's salicin, that's right.

Q. Is that an active ingredient?

A. Not by itself. It's transformed in the body to acetylsalicylic acid which is aspirin.

Transcript of Proceedings, *Parks v. Cytodyne Technologies, Inc.*, March 13, 2003, at 1590:15-23.

During Dr. Heymsfield's testimony at the *Parks* trial, there was also testimony concerning a scientific study which Dr. Heymsfield had done involving a product which has ephedra, caffeine, and salicin from white willow bark, but not aspirin. During that discussion, Dr. Heymsfield specifically testified that salicin (the source of which is white willow bark) is the same thing as aspirin:

Q. And that product [the product which was the subject of Dr. Heymsfield's study], I understand, did not have synephrine; is that correct?

A. That's correct.

Q. Did not have tyrosine?

A. That's correct.

Q. And did it have aspirin or -

A. Yes.

Q. Okay.

A. --It did.

Q. It did have aspirin but did not have synephrine?

A. Had the same thing. White willow bark, I believe. I could be wrong about that.

Id. at 1682:21-1683:4.[9]

Dr. Heymsfield's inconsistencies on the aspirin/salacin dichotomy issue are important because, *inter alia*, in his rebuttal expert report in this case Dr. Heymsfield stated that "An average weight loss of 2 pounds/months for ephedrine/ephedra as reported in the Rand Report is considered extremely weak efficacy." Heymsfield Rebuttal Report at 8. Yet in his original expert report, Dr. Heymsfield characterized the weight loss from ephedrine or ephedra products as "modest." *See, e.g.,* Heymsfield Expert Report at 10. Moreover, in his prepared testimony to Congress, Dr. Heymsfield characterized the weight loss resulting from ephedrine, caffeine and aspirin in a much different light, testifying that:

> The collective studies strongly support the premise that ephedrine, particularly in combination with caffeine and also aspirin, promote ***significant*** short-term (≥ 3-6 months) weight loss when ingested as part of an intervention program including dietary and lifestyle management. Long-term (>6 months) controlled trials with large and diverse subject

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[9] Similarly, in written testimony to Congress Dr. Heymsfield also expressly testified that salicin is an herbal equivalent of, and the same thing as, aspirin, testifying as follows:

> Ephedrine and ephedra alkaloids alone have modest weight loss effects and their efficacy appears to be enhanced by addition of caffeine and aspirin either as the pharmaceutical grade ingredients or as their natural counterparts such as Guarana and Willow-bark, respectively.
>
> Addition of caffeine (i.e., "Guarana") arid aspirin (i.e., Willow-bark) to MaHuang purportedly potentiates the actions of ephedrine.

Prepared Witness Testimony: Heymsfield, Steven, presented to The Committee on Energy and Commerce W "Billy" Tauzin, Chairman, July 23, 2003 ("Heymsfield Congressional Testimony") at 3.

> populations are lacking. The evidence for ephedra efficacy is summarized in the recent Rand Report.

Heymsfield Congressional Testimony at 3.

Given the clear and irreconcilable inconsistencies between Dr. Heymsfield's testimony in this case and his testimony in other proceedings, Complaint Counsel's and Dr. Heymsfield's failure to fully disclose all cases wherein Dr. Heymsfield has testified as an expert witness is prejudicial to Respondents, and warrants an appropriate remedial order from the Court.

2. Dr. Eckel.

On or about October 21, 2004, Respondents received the report of Dr. Eckel, an expert retained by Complaint Counsel to testify concerning the scientific validity of certain representations allegedly made by Respondents in various of their advertisements. Report of Robert H. Eckel, M.D., ¶ 13, attached to this Motion hereto as Exhibit 4 (and is cited in this Motion as "Eckel Report ¶ __"). Dr. Eckel's report included a disclosure describing the documents that Complaint Counsel provided to him in his capacity as their expert:

> The staff of the Federal Trade Commission (FTC) have provided the following materials for my review: Complaint and Exhibits A-L (product advertisements); Respondents' CLAIM SUBSTANTIATION FOR "TUMMY FLATTENING GEL"; Respondents' CLAIM SUBSTANTIATION FOR DERMALIN Apg"; Respondents' CLAIM SUBSTANTIATION FOR "CUTTING GEL"; DermTech report dated 6/11/03 titled "Evaluation of the Percutaneous Absorption of Aminophylline, *in vitro*, Using the Human Cadaver Skin Model"; DermTech report dated 9/01/02 titled "Determination of the Percutaneous Absorption of Aminophylline, *in vitro*, Using the Human Cadaver Skin Model"; and DermTech report dated 12/06/01 titled "Evaluation of the Percutaneous Absorption of Aminophylline, *in vitro*, Using the Human Cadaver Skin Model". A hard copy and CD-Rom were both supplied.

Complaint Counsel ostensibly provided these documents to Dr. Eckel on or about July 1, 2004. A letter reflecting Complaint Counsels' transmittal of these documents is sequentially numbered FTC 5426 and is attached to this Motion as Exhibit 5.

Among other things, Dr. Eckel's report states that the concentration of aminophylline in Dermalin-Apg, Cutting Gel, and Tummy Flattening Gel is [redacted] and then, using the identical language used in the Protective Order, cites this formulation information as "('Restricted, Confidential-Attorneys Eyes Only')." Eckel report, ¶ 26. This proprietary and trade secret formulation information was very clearly designated "RESTRICTED, CONFIDENTIAL – ATTORNEYS EYES ONLY, FTC DOCKET NO. 9318." It can be traced back to Exhibit "A" to respondents' Answers to Complaint counsel's First Set of Interrogatories on Behalf of Basic Research, LLC, A.G. Waterhouse, LLC, Klein-Becker usa, LLC, Nutrasport, LLC, and Sovage Dermalogic Laboratories, LLC, dated September 16, 2004, *over a month after execution of the Protective Order.* A copy of Respondents' Confidential formulation documents, as produced by Respondents, is filed under seal as Exhibit 6.

Dr. Eckel's reference to the proper concentration of aminophylline, his reference to the same as confidential, combined with the date the formulation information was produced to Complaint Counsel, make clear that Complaint Counsel provided Dr. Eckel with Confidential Material produced by Respondents and governed by the terms of the Protective Order. However, just as they had failed to do with the highly confidential, private, proprietary documents, and information provided to Dr. Heymsfield, Complaint Counsel breached the express terms of the Protective Order by failing to provide

Respondents with any advance notice whatsoever that Confidential Material would be disclosed to Dr. Eckel.

Had Complaint Counsel complied with the terms of the Protective Order and provided Respondents with notice of an intent to disclose Respondents' confidential documents and material to Dr. Eckel, Respondents would have timely objected and, if necessary, would have pursued the issue with the Court, as Dr. Eckel is or has been affiliated with several of Respondents' competitors in the weight loss industry, including Merck and SlimFast. See, Dr. Eckel's *curriculum vitae* attached as Report 1 and incorporated at Exhibit 4.

Respondents move, based upon these numerous instances of intentional, deliberate and careless irregularities surrounding Complaint Counsels' experts, Drs. Heymsfield and Eckel, including several violations of the plain language of this Court's Protective Order, and the severe prejudice flowing therefrom, that the Court grant Respondents the relief requested, including the striking of these witnesses. As explained below, this remedy is available and is the only fair way to deal with the incurable harm to Respondents.

II. ARGUMENT

A. SANCTIONS AGAINST COMPLAINT COUNSEL SHOULD BE IMPOSED FOR FAILING TO COMPLY WITH THE PROTECTIVE ORDER

Complaint Counsel should be sanctioned for violating the Protective Order. They should be held fully accountable for any acts, omissions or practices that violated the Protective Order. The Court has this authority pursuant to 16 C.F.R. § 3.38(b):

> If a party or an officer or agent of a party fails to comply...with an order including but not limited to, an order...of the Administrative Law

> Judge,... the Administrative Law Judge or the Commission, or both... may take such action in regard thereto as is just, including but not limited to the following:
>
> (1) Infer that the admission, testimony, documents or other evidence would have been adverse to the party;
>
> (2) Rule that for the purposes of the proceeding the matter or matters concerning which the order or subpoena was issued be taken as established adversely to the party;
>
> (3) Rule that the party may not introduce into evidence or otherwise rely, in support of any claim or defense, upon testimony by such party, officer, or agent, or the documents or other evidence;
>
> (4) Rule that the party may not be heard to object to introduction and use of secondary evidence to show what the withheld admission, testimony, documents, or other evidence would have shown; and
>
> (5) Rule that a pleading, or part of a pleading, or a motion or other submission by the party, concerning which the order or subpoena was issued, be stricken, or that a decision of the proceeding be rendered against the party, or both.

The ALJ plainly possess statutory authority to impose sanctions against Complaint Counsel for violations of its Orders and the discovery rules. The Protective Order here violated by Complaint Counsel is a discovery order, and 16 C.F.R. § 3.38(b) is, therefore, applicable. Failure to comply with an ALJ's order may subject a violating party to contempt. 16 C.F.R. § 3.42(h). There is wide latitude to determine whether there has been contemptuous behavior and wide latitude to determine proper sanctions. *Id.*; *Gifford v. Heckler*, 741 F.2d 263, 266 (9th Cir. 1984).

The importance of protective orders and the role they serve in facilitating discovery in litigation are widely acknowledged. *Beam Sys., Inc. v. Checkpoint Sys.*, 1997 WL 364081, *2 (C.D. Cal. 1997). Without such orders, litigants would be forced to choose between fully presenting their claims and/or defenses or forgoing such claims

and/or defenses in order to keep competitively sensitive, proprietary information confidential. *Id.* Protective orders also preclude discovery materials from being used as a sword through threats against a producing party that disclose confidential information. *Joy v. North*, 692 F.2d 880, 893 (2d Cir. 1982). Accordingly, "it is essential that protective orders be respected." *Beam Sys.*, 1997 WL 364081, *2. Whether a violation of a protective order is merely careless or willful is irrelevant. Improper disclosure initiates potential loss of confidentiality. Carelessness cannot be tolerated when dealing with protective orders and confidential information. *In re Baycol Products Litigation*, 2004 WL 1052968 (D. Minn. 2004); *see also Marrocco v. General Motors Corp.*, 966 F.2d 220, 224-225 (7th Cir. 1992)(citing *National Hockey League v. Metropolitan Hockey Club, Inc.*, 427 U.S. 639, 640 (1976) and noting "the Supreme Court has expressly stated that sanctions may be appropriate in any one of three instances--where the noncomplying party acted *either* with willfulness, bad faith *or* fault"). Parties "*must* comply with the terms of [a] protective order or subject themselves to possible sanctions." *American National Bank and Trust Co. of Chicago v. AXA Client Solutions, LLC*, 2002 WL 1067696, *3 (N.D. Ill. 2002) (emphasis added).

Respondents sought a Protective Order for a very specific and definite reason—to protect valuable and confidential trade secrets and work product from being revealed to competitors. Complaint Counsels' improper conduct, whether deliberate or merely the result of gross indifference to the Court's Order, caused the very harm Respondents sought to avoid and the Court sought to shield. Such serious and irreparable breaches should not be taken lightly. Complaint Counsels' multiple violations of the Protective Order justify sanctions, irrespective of whether the breaches were intentional or

incidental. The harm to Respondents is incalculable. The ALJ has the authority to impose sanctions and they are unquestionably appropriate here. 16 C.F.R. § 3.42(h).

Complaint Counsel violated a valid and binding Order that was clear and unambiguous. As a result, Respondents have forever lost the opportunity to object to disclosure of the highly confidential and sensitive documents and materials provided in good-faith to the Commission in response to its demands. Complaint Counsel failed to exercise their responsibility and duty to restrict access to Respondents' Confidential Materials in accordance with the terms of the Protective Order when they provided those materials to Drs. Eckel and Dr. Heymsfield. Moreover, had Complaint Counsel properly complied with the Protective Order, Respondents would have objected to Drs. Eckel and Heymsfield being permitted access to respondents' Confidential Material, as Drs. Eckel and Heymsfield have been and currently are affiliated with Respondents' competitors in the weight loss product industry, including Merck, a pharmaceutical company with an entire division dedicated to obesity.

Due to Complaint Counsels' grievous breaches of the Protective Order, Respondents' product formulations have been provided to at least one direct competitor of Respondent. Of course Complaint Counsels' experts were also in possession of this same material when conducting or assisting in studies for other direct and indirect competitors of Respondents and involving products that may compete directly with Respondents' products. This creates a clear conflict of interest for both Dr. Eckel and Dr. Heymsfield and it is simply impossible to "un-ring the bell."

Even after violating the terms of the Protective Order and providing confidential documents and materials without notice to Respondents, Complaint Counsel clearly

failed to properly educate their own experts concerning the importance of the Order and the gravity of potential breaches. Dr. Heymsfield's testimony is evidence of that very point.

Several potential sanctions are made available to the Court under 16 C.F.R. § 3.38(b) for violations of discovery orders. These provide some guidance to the ALJ, who must ultimately determine exactly which sanctions are suitable here. Striking Drs. Eckel and Dr. Heymsfield and entirely excluding them from these proceedings is appropriate and warranted, as Complaint Counsels' multiple violations and breaches of the Protective Order and other discovery rules specifically relate to these two witnesses. Moreover, Drs. Eckel and Heymsfield cannot be considered truly independent experts given their past and current affiliations with Respondents' direct and indirect competitors—including, as noted above, employment of one of the experts by a direct competitor for the express purpose of getting competing products to market. *See also Beam Sys.*, 1997 WL 364081 (excluding plaintiff's expert witness after disclosure of defendant's Confidential Materials to expert who had a past and ongoing business relationship with plaintiff); *Pride v. BIC* Corp 218 F.3d 566 (6th Cir. 2000) (excluding expert witness testimony and staying discovery due to plaintiff's violation of protective order and late-disclosed expert testimony and staying discovery due to plaintiff's violation of protective order and late-disclosed expert testimony).

Given the gravity of the violations, the seriousness of the injury and the inability to fairly cure the harm, Respondents respectfully submit striking these witnesses is the only remedy adequate here.

B. COMPLAINT COUNSEL SHOULD BE ORDERED TO SUBMIT A SWORN DECLARATION.

The full scope of Complaint Counsels' breaches of the Protective Order is unclear and unknown to Respondents. Respondents were unaware that Drs. Eckel and Heymsfield would be provided access to their Confidential Materials. Additionally, it is unclear exactly which confidential documents and other materials may have been disclosed to them or to other third parties. From the face of Dr. Eckel's report it is clear that Complaint Counsel reproduced at least some of Respondents' confidential documents and materials, also in violation of Paragraph 7 of the Protection Order, which prohibits unauthorized copying or reproduction of the same. Dr. Heymsfield's deposition conclusively establishes the same with respect to other documents, as Dr. Heymsfield acknowledges that he received from Complaint Counsel many documents marked "Confidential" by Respondents. Complaint Counsels' carelessness must not be condoned or ignored. Respondents' confidence in Complaint Counsels' ability to properly handle and safeguard their Confidential Materials must be restored. The Court has and should exercise the authority, under 16 C.F.R. § 3.42(h) to restore that confidence and provide some measure of certainty concerning the scope and breadth of the violations.

In order to determine the extent of Complaint Counsels' breaches of the Protective Order to date, Respondents move that Complaint Counsel be required to submit a sworn declaration detailing in specific terms: (a) their handling of Respondents' protected confidential information; (b) the measures undertaken by Complaint Counsel to safeguard that information and comply with the Court's Protective Order; (c) the identities of all individuals who were provided access to or copies of the protected information; and (d) detailing the full scope of their violations of the Protective Order.

III. CONCLUSION

Complaint Counsel clearly violated the plain, unambiguous language of the Court's Protective Order, and the FTC's experts failed to comply with the discovery rules. As a result, Respondents respectfully request an Order from the Court pursuant to 16 C.F.R. § 3.38(b) and 16 C.F.R. § 3.42(h), that: (1) sanctions Complaint Counsel for their deliberate or grossly indifferent breach of this Court's Protective Order by excluding from these proceedings the two expert witnesses, Dr. Eckel and Dr. Heymsfield, to whom Complaint Counsel improperly provided Respondents' confidential information; (2) commands the return to Respondents from Drs. Heymsfield and Eckel of all documents in their possession marked or designated confidential, together with sworn affidavits affirming that reasonable efforts were made to confirm that no additional copies, either electronic or in hard form, exist, and that to their knowledge no other person has had access to the materials while in their possession; and (3) requires Complaint Counsel to file a declaration or sworn affidavit detailing in specific terms; (a) their handling of Respondents' protected confidential information, (b) the measures undertaken by Complaint Counsel to safeguard that information and comply with the Court's Protective Order, (c) the identities of all individuals who were provided access to or copies of the protected information, and (d) detailing the full scope of their violations of the Protective Order.

Alternatively, should the Court be disinclined to strike Dr. Heymsfield and Dr. Eckel from these proceedings, Respondents request the declaration from Complaint Counsel described above in addition to an Order from the Court commanding: (1) Dr. Heymsfield to make available immediately all documents in his possession responsive to

Respondents' subpoena as well as those documents this Court has required experts in this case to produce; (2) Complaint Counsel to make Dr. Heymsfield available for two days of questioning, following adequate opportunity for Respondents to review and digest the documents we have yet to receive from Dr. Heymsfield or Complaint Counsel, to ensure Respondents have a full and fair opportunity to resume and conclude their deposition; (3) Complaint Counsel to reimburse Respondents' costs and fees for conducting a second deposition; and (4) setting a new date, ten days after completing Dr. Heymsfield's deposition, for Respondents to respond to Complaint Counsels' motions for summary decision, in order to ensure a fair opportunity to respond to the arguments, issues and evidence presented therein, at least some of which will undoubtedly be based upon Dr. Heymsfield's opinions.

The relief requested is necessary to sanction Complaint Counsel for their improper conduct, and to remedy, to the extent possible, the harm and prejudice caused to Respondents by Complaint Counsel and their witnesses.

Respectfully submitted,

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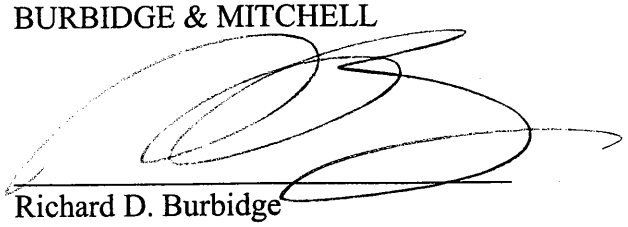
DATED this 26th day of January, 2005.

BURBIDGE & MITCHELL



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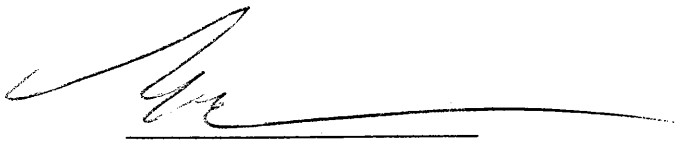

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Pro Se Respondent

CERTIFICATE OF SERVICE

I HEREBY CERTIFY that a true and correct copy of the foregoing *Motion to Strike* was provided to the following parties this 21st day of January, 2005 as follows:

(1) One (1) original and two (2) copies by Federal Express to Donald S. Clark, Secretary, Federal Trade Commission, Room H-159, 600 Pennsylvania Avenue, N.W., Washington, D.C., 20580;

(2) One (1) electronic copy via e-mail attachment in Adobe® ".pdf" format to the Secretary of the FTC at Secretary@ftc.gov;

(3) Two (2) copies by Federal Express to Administrative Law Judge Stephen J. McGuire, Federal Trade Commission, Room H-104, 600 Pennsylvania Avenue N.W., Washington, D.C. 20580;

(4) One (1) copy via e-mail attachment in Adobe® ".pdf" format to Commission Complaint Counsel, Laureen Kapin, Joshua S. Millard, and Laura Schneider, all care of lkapin@ftc.gov, jmillard@ftc.gov; rrichardson@ftc.gov; lschneider@ftc.gov with one (1) paper courtesy copy via U. S. Postal Service to Laureen Kapin, Bureau of Consumer Protection, Federal Trade Commission, Suite NJ-2122, 600 Pennsylvania Avenue, N.W., Washington, D.C., 20580;

(5) One (1) copy via U. S. Postal Service to Elaine Kolish, Associate Director in the Bureau of Consumer Protection, Federal Trade Commission, 600 Pennsylvania Avenue, N.W., Washington, D.C. 20580

(6) One (1) copy via United States Postal Service to Stephen Nagin, Esq., Nagin Gallop & Figueredo, 3225 Aviation Avenue, Suite 301, Miami, Florida 33131.

(7) One (1) copy via United States Postal Service to Richard Burbidge, Esq., Jefferson W. Gross, Esq. and Andrew J. Dymek, Esq., Burbidge & Mitchell, 215 South State Street, Suite 920, Salt Lake City, Utah 84111, Counsel for Dennis Gay.

(8) One (1) copy via United States Postal Service to Ronald F. Price, Esq., Peters Scofield Price, A Professional Corporation, 340 Broadway Centre, 111 East Broadway, Salt Lake City, Utah 84111, Counsel for Daniel B. Mowrey.

(9) One (1) copy via United States Postal Service to Mitchell K. Friedlander, 5742 West Harold Gatty Drive, Salt Lake City, Utah 84111, *Pro Se.*

CERTIFICATION FOR ELECTRONIC FILING

I HEREBY CERTIFY that the electronic version of the foregoing is a true and correct copy of the original document being filed this same day of January, 2005 via Federal Express with the Office of the Secretary, Room H-159, Federal Trade Commission, 600 Pennsylvania Avenue, N.W., Washington, D.C. 20580.

_____

UNITED STATES OF AMERICA

BEFORE THE FEDERAL TRADE COMMISSION

OFFICE OF ADMINISTRATIVE LAW JUDGES

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In the Matter of

BASIC RESEARCH, L.L.C.,
A.G. WATERHOUSE, L.L.C.,
KLEIN-BECKER USA, L.L.C.,
NUTRASPORT, L.L.C.,
SOVAGE DERMALOGIC LABORATORIES, L.L.C.,
BAN, L.L.C.,
DENNIS GAY,
DANIEL B. MOWREY, and
MITCHELL K. FRIEDLANDER,

Docket No. 9318

Respondents.

----------X

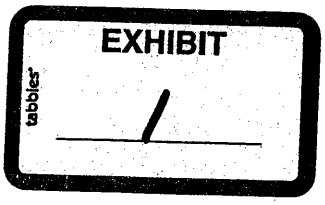
VIDEOTAPED DEPOSITION OF

STEVEN B. HEYMSFIELD

New York, New York

Tuesday, January 11, 2005

Reported by:
Thomas R. Nichols, RPR
Toni Allegrucci
JOB NO. 168691



January 11, 2005

9:25 a.m.

Videotaped Deposition of Expert Witness, STEVEN B. HEYMSFIELD, held at the offices of Esquire Deposition Services, 216 East 45th Street, New York, New York, pursuant to Subpoena, before Thomas R. Nichols, Registered Professional Reporter and Toni Allegrucci Notaries Public of the State of New York.

A P P E A R A N C E S:

UNITED STATES FEDERAL TRADE COMMISSION
BUREAU OF CONSUMER PROTECTION
DIVISION OF ENFORCEMENT
600 Pennsylvania Avenue, NW
Washington, DC 20580
BY: ROBIN M. RICHARDSON, ESQ.
LAUREEN KAPIN, ESQ.
WALTER C. GROSS, III, ESQ.
JOSHUA S. MILLARD, ESQ.

FELDMAN GALE
Attorneys for Respondents
201 South Biscayne Boulevard
Miami, Florida 33131
BY: JEFFREY D. FELDMAN, ESQ.

BURBIDGE & MITCHELL
Attorneys for Respondent Dennis Gay
215 South State Street
Salt Lake City, Utah 84111
BY: ROBERT J. SHELBY, ESQ.

APPEARANCES (CONT'D.):

PETERS SCOFIELD PRICE
Attorneys for Respondent Daniel B. Mowrey
340 Broadway Centre
111 East Broadway
Salt Lake City, Utah 84111
BY: RONALD F. PRICE, ESQ.

ALSO PRESENT:

MITCHELL K. FRIEDLANDER, PRO SE
DANIEL MOWREY

SUBPOENA DUCES TECUM

Issued Pursuant to Rule 3.34(b), 16 C.F.R. § 3.34(b)(1997)

1. TO

Steven B. Heymsfield, M.D.
St. Luke's-Roosevelt Hospital
Obesity Research Center
1090 Amsterdam Avenue #14C
New York, NY 10025

2. FROM

UNITED STATES OF AMERICA
FEDERAL TRADE COMMISSION

This subpoena requires you to produce and permit inspection and copying of designated books, documents (as defined in Rule 3.34(b)), or tangible things - or to permit inspection of premises - at the date and time specified in Item 5, at the request of Counsel listed in Item 9, in the proceeding described in Item 6.

3. PLACE OF PRODUCTION ~~OR INSPECTION~~

Peters Scofield Price
111 East Broadway, Suite 340
Salt Lake City, Utah 84111

4. MATERIAL WILL BE PRODUCED TO

Peters Scofield Price
A Professional Corporation

5. DATE AND TIME OF PRODUCTION OR INSPECTION

Monday, December 6, 2004

6. SUBJECT OF PROCEEDING

In the Matter of Basic Research, LLC, et. al., Docket No. 9318

7. MATERIAL TO BE PRODUCED SEE EXHIBIT A

In lieu of production at the above place, documents may be produced by return mail on or before December 6, 2004, to Ronald F. Price, at Peters Scofield Price, 111 East Broadway, Suite 340, Salt Lake City, UT 84111.

8. ADMINISTRATIVE LAW JUDGE

The Honorable Stephen J. McGuire

Federal Trade Commission
Washington, D.C. 20580

9. COUNSEL REQUESTING SUBPOENA

Peters Scofield Price
A Professional Corporation

DATE ISSUED

10/12/2004

SECRETARY'S SIGNATURE

Donald S. Clark

GENERAL INSTRUCTIONS

APPEARANCE

The delivery of this subpoena to you by any method prescribed by the Commission's Rules of Practice is legal service and may subject you to a penalty imposed by law for failure to comply.

MOTION TO LIMIT OR QUASH

The Commission's Rules of Practice require that any motion to limit or quash this subpoena be filed within the earlier of 10 days after service or the time for compliance. The original and ten copies of the petition must be filed with the Secretary of the Federal Trade Commission, accompanied by an affidavit of service of the document upon counsel listed in Item 9, and upon all other parties prescribed by the Rules of Practice.

TRAVEL EXPENSES

The Commission's Rules of Practice require that fees and mileage be paid by the party that requested your appearance. You should present your claim to counsel listed in Item 9 for payment. If you are permanently or temporarily living somewhere other than the address on this subpoena and it would require excessive travel for you to appear, you must get prior approval from counsel listed in Item 9.

This subpoena does not require approval by OMB under the Paperwork Reduction Act of 1980.

FTC Form 70-B (rev. 1/97)

EXHIBIT 3

RETURN OF SERVICE

I hereby certify that a duplicate original of the within subpoena was duly served: (check the method used)

○ *in person.*

○ *by registered mail.*

○ *by leaving copy at principal office or place of business, to wit:*

---

on the person named herein on:

(Month, day, and year)

(Name of person making service)

(Official title)

EXHIBIT A

1. Your complete file related to this matter.

2. All correspondence with the Federal Trade Commission concerning this matter regardless of whether you were the author, addressee or copy recipient.

3. All correspondence with any individual or entity other than the Federal Trade Commission concerning this matter regardless of whether you were the author, addressee or copy recipient.

4. All reports prepared by you in connection with your work on this matter.

5. All drafts of all reports prepared by you in connection with your work on this matter.

6. All documents reviewed by you in connection with your work on this matter.

7. All materials consulted by you or relied on by you in forming any opinion in connection with this matter.

8. All documents that you have ever authored or contributed to regarding:

a. obesity
b. weight loss
c. fat loss
d. the Federal Trade Commission
e. clinical trial protocol or procedures
f. the definition of "competent and reliable scientific evidence"
g. Federal Trade Commission advertising rules and regulations
h. dietary supplements
i. weight loss or fat loss advertising.

9. All documents relating to lectures, speeches or testimony that you have ever given relating to:

a. obesity
b. weight loss
c. fat loss
d. clinical trial protocol or procedures
e. the Federal Trade Commission

f. the definition of "competent and reliable scientific evidence"
g. Federal Trade Commission advertising rules and regulations
h. dietary supplements
I. weight loss or fat loss advertising.

10 All documents relating to medical or clinical studies or tests that you have conducted or contributed ton or participated relating to or involving:

a. obesity
b. weight loss
c. fat loss
d. dietary supplements

11. All patents and patent applications (whether or not published or pending review by the United States Patent and Trademark Office) in which you are named as an inventor or patent owner or assignee of any invention relating to:

a. obesity
b. weight loss
c. fat loss
d. dietary supplements

12. All documents relating to lawsuits, whether criminal or civil, in which you were named as a party.

13. All documents pertaining to work that you have performed for any company that manufactures, markets or sells pharmaceuticals or dietary supplements relating to:

a. obesity
b. weight loss
c. fat loss

14. All documents relating to weight loss or fat loss advertisements that you have authored, reviewed or approved relating to any weight loss or fat loss product.

15. All documents relating to requests for approval that you have made to the FDA, FTC or any other regulatory body, either on behalf of yourself or some other third party, relating to advertising or package labeling claims that you sought to make in relation to any weight loss or fat loss product.

16. All documents relating to efforts by you, either on your own behalf or on behalf of any other third party or parties, to justify or substantiate

advertising claims made in relation to any weight loss or fat loss product including but not limited to pharmaceutical products or dietary supplements.

17. All documents pertaining to work that you have performed for the Federal Trade Commission, The Food and Drug Administration or any other federal agency, whether as an expert, consultant or in any other capacity, relating to:

a. obesity
b. weight loss
c. fat loss
d. the Federal Trade Commission
e. clinical trial protocol or procedures
f. the definition of "competent and reliable scientific evidence"
g. Federal Trade Commission advertising rules and regulations
h. dietary supplements
I. weight loss or fat loss advertising.

18. All scientific and/or medical testing protocols you have authored.

19. All scientific and/or medical testing protocols on which you have provided comments, including your comments.

20. All documents which the Federal Trade Commission, including Complaint Counsel in this matter, has provided to you in connection with this matter.

21. All documents, including drafts, which you have provided to the Federal Trade Commission, including Complaint Counsel in this matter, in connection with this matter.

22. All notes of any meetings and/or telephone conversations and/or any other communications you have had with the Federal Trade Commission, including Complaint Counsel in this matter, and/or any other entity or person, in connection with this matter.

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23. All records and documents of whatever kind reflecting side effects experienced by subjects in control or placebo groups during the study titled Weight Control and Risk Factor Reduction in Obese Subjects Treated for 2 Years with Orlistat: A Randomized Controlled Trial a copy of which is attached as Exhibit A. You may provide redacted records or documents redacting identifying information concerning the test subjects including but not limited to name, address, telephone number, social security number or similar.

24. All records and documents of whatever kind reflecting comments by subjects concerning or related to any side effects experienced by subjects in the control or placebo group during the study titled Weight Control and Risk Factor Reduction in Obese Subjects Treated for 2 Years with Orlistat: A Randomized Controlled Trial a copy of which is attached as Exhibit A. You may provide redacted records or documents redacting identifying information concerning the test subjects including but not limited to name, address, telephone number, social security number or similar.

REPORT OF ROBERT H. ECKEL, M.D.

To the matter of Basic Research et al, Docket #9318

Robert H. Eckel, M.D. hereby reports as follows:

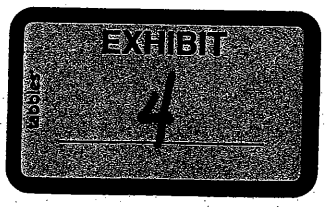
QUALIFICATIONS

1) I am a Doctor of Medicine and am board certified in both Internal Medicine and Endocrinology and Metabolism. I received my M.D. degree from the University of Cincinnati in 1973 graduating as a member of the honorary medical society Alpha Omega Alpha. I am licensed to practice medicine in the state of Colorado.

2) My current appointment is Professor of Medicine in the Division of Endocrinology, Metabolism and Diabetes and in the Division of Cardiology at the University of Colorado Health Sciences Center (UCHSC) in Denver/Aurora, Colorado. I also have a joint appointment in the Department of Physiology and Biophysics at UCHSC and an adjunct appointment as Professor of Food Science and Human Nutrition at Colorado State University in Ft. Collins, Colorado.

3) I received my undergraduate degree (BS) in Bacteriology in1965 (with Honors) from the University of Cincinnati. Following medical school I trained (internship and residency) in Internal Medicine at the University of Wisconsin Hospitals in Madison, Wisconsin (1973-1976) with a research and clinical fellowship in Endocrinology and Metabolism at the University of Washington in Seattle, Washington (1976-1979).

4) My research has focused predominantly on fat metabolism. For almost 30 years of academic pursuit, my science has included studies in the laboratory at the level of:
   a) DNA and RNA
   b) biochemistry
   c) cell biology
   d) animals



e) human subjects and

f) human populations

g) Related studies have included investigations on weight regulation, nutrition, obesity, insulin resistance, lipid and lipoprotein metabolism, and diabetes mellitus.

5) In my years of research, I have been continuously funded by the National Institutes of Health (NIH) in addition to support from other professional organizations (Juvenile Diabetes Foundation, American Heart Association, American Diabetes Association) for 27 consecutive years, trained 29 graduate students or post-doctoral fellows, published 138 peer reviewed articles, numerous editorials, book chapters and reviews, and nearly 200 abstracts. Among the most quoted articles is a paper entitled *Lipoprotein Lipase: A Multifunctional Enzyme Related to Common Metabolic Diseases* that was published as an invited *Seminar from the Beth Israel Hospital* of Harvard Medical School in the *New England Journal of Medicine* in 1989.

6) I presently serve on the editorial boards of the *American Journal of Medicine, Journal of Endocrinology and Metabolism, Arteriosclerosis, Thrombosis and Vascular Biology, The International Journal of Obesity,* and *Obesity Research.* I have reviewed journal articles for 69 medical journals including *Nature, Science, Proceedings of the National Academy of Sciences, Journal of the American Medical Association,* and the *New England Journal of Medicine.* I have been asked to write many editorials, book chapters, and am proud to have recently edited a book on *Obesity: Mechanisms and Clinical Management* published by Lippincott, Williams and Wilkins, 2003. This book has been widely circulated and also received reviews in major medical periodicals, i.e. the *New England Journal of Medicine* and *The Lancet.*

7) Over my career, there have been increasing examples of recognition of my accomplishments in the fields of weight regulation, obesity and nutrition.

a) Again, I have been continuously supported by for the last 25 years by the NIH. This funding has been in the form of at least one, and sometimes up to three R01 grants. R01 grants through the NIH are the major mechanism by

which biomedical research scientists are supported by the Federal government. These grants are highly competitive being peer-reviewed by specific study sections prior to further consideration by one or more of the 27 scientific institutes at the NIH. Typically, award rates are in the range of 20-25%.

b) In 1987, I was promoted to Professor of Medicine at UCHSC and also elected to two very prestigious societies – The American Society for Clinical Investigation in 1987 and The Association of American Physicians in 1999.

c) In 1989, I was asked to join the NIH Nutrition Study Section and became their chairman from 1991-1993

d) Since 1991, I have been the Program Director of the National Center for Research Resources (NCCR) of the NIH funded Adult General Clinical Research Center at the UCHSC.

e) From 1997 to the present, I have been the Associate Director of the UCHSC Center for Human Nutrition.

f) I have been the Vice Chair for Research in the Department of Medicine since 1998.

g) In 2001 I was honored as the recipient of the Charles A. Boettcher II Endowed Chair in Atherosclerosis..

h) In 1996, I joined the Nutrition Committee of the American Heart Association (AHA) and in 1999 became chairman of the committee. In 2002 I would become the second chairperson of the Scientific Council on Nutrition, Physical Activity and Metabolism (NPAM) of the AHA and in July 2004, I assumed the penultimate position as President Elect of the AHA with my Presidency to begin in July 2005.

i) In 2001, I was asked by the National Heart, Lung and Blood Institute (NHLBI) and National Institutes of Diabetes, Digestive and Kidney Diseases (NIDDK) at the NIH to chair a working group on the pathophysiology of obesity-associated cardiovascular diseases (*Circulation*, 2002). In 2002 I joined the NIDDK Advisory Council, a term that lasts until 2006. In this position, I am one of twelve extramural academicians providing advice about

NIDDK grants to be awarded and programmatic development in fields related to the mission of the institute including obesity. In 2002, I was also appointed to the NIDDK Clinical Obesity Research Panel, a working group that helps establish policy relating obesity science to the population.

j) In 1996, I was President of the North American Association for the Study of Obesity (NAASO), an organization that I continue to support with my science.

8) My research has focused on the tissue specific regulation of the uptake and metabolism of circulating fatty acids into tissues including adipose tissue (fat) and skeletal muscle. Once the circulating fatty acids are stored in tissues as triglycerides, these depots of fat can breakdown (lipolysis) and again produce fatty acids. Once generated, fatty acids can again be released into the circulation (from adipose tissue) or burned (from muscle). In my laboratory, we have used cultured fat cells and muscle cells, fat pieces obtained from whole animals (mice and rats) and humans to study this process. In this regard, I feel that I am in a strong position to comment on how body fat is regulated.

9) Perhaps the most exciting observation in my research career was in the 1980s. This related to the important fact that weight loss is often short-lived in animals and humans. When my group examined obese subjects three months after a 13% weight reduction, we discovered that the enzyme that controls the uptake of lipoprotein-derived fatty acids into adipose tissue, lipoprotein lipase (LPL) was not only not decreased, but increased with an exaggerated increase to the infusion of insulin and meal ingestion (*J Clin Invest*, 1987). Subsequent studies would document that LPL in skeletal muscle was decreased in humans after weight reduction (*Eur J Clin Invest*, 1995). These observations would establish one mechanism by which an increase in food intake in the reduced state could lead to expansion of the fat mass and weight regain. These studies complemented existing knowledge from animal experiments and helped to establish the current recognition of obesity as a lifelong disease.

10) For nearly 30 years now, I have designed numerous experiments that are aimed at testing hypotheses. Studies in animals and humans are similar in that control groups are necessary to determine the effect of any diet, drug or other clinical perturbation on the outcome. To be successful, studies must be designed based on the number of animals or humans needed to satisfy statistical significance. Built into this is a pre-existing knowledge of the variability of the measurements that will be employed. Only with careful consideration of all of these scientific principles will hypotheses be rigorously tested.

11) A copy of my most recent Curriculum Vitae is attached (Report 1).

12) In summary, I believe my career in biomedical research provides a strong foundation of education, training, and experience in the area of fat metabolism and weight regulation including the biology of adipose tissue to serve as an expert witness in this case. My science has extended from DNA to molecules to cells to animals to human subjects to populations. The quality of my work has been recognized by years of consecutive funding, numerous peer reviewed publications and leadership roles, and election to a number of prestigious professional societies. My position on the NIDDK Advisory Council and upcoming Presidency of the AHA are recent reflections of this credibility.

ANALYSIS OF PRODUCT INFORMATION

13) I have been asked by attorneys from the Division of Enforcement of the Federal Trade Commission (FTC) to assist them in determining the scientific validity of claims made by Basic Research LLC, a limited liability corporation doing business with other corporations and laboratories, that

a) "Dermalin-Apg causes rapid and visibly obvious fat loss in areas of the body to which it is applied" (Complaint ¶14);

b) "Cutting Gel causes rapid and visibly obvious fat loss in areas of the body to which it is applied" (Complaint ¶17);

c) "Tummy Flattening Gel causes rapid and visibly obvious fat loss in areas of the body to which it is applied" (Complaint ¶20);

d) "Published, clinical testing proves that Cutting Gel causes rapid and visibly obvious fat loss in areas of the body to which it is applied" (Complaint ¶23); and

e) "Published, clinical testing proves that Tummy Flattening Gel causes rapid and visibly obvious fat loss in areas of the body to which it is applied" (Complaint ¶25).

14) The staff of the Federal Trade Commission (FTC) have provided the following materials for my review: Complaint and Exhibits A-L (product advertisements); Respondents' CLAIM SUBSTANTIATION FOR "TUMMY FLATTENING GEL"; Respondents' CLAIM SUBSTANTIATION FOR DERMALIN Apg"; Respondents' CLAIM SUBSTANTIATION FOR "CUTTING GEL"; DermTech report dated 6/11/03 titled "Evaluation of the Percutaneous Absorption of Aminophylline, *in vitro*, Using the Human Cadaver Skin Model"; DermTech report dated 9/01/02 titled "Determination of the Percutaneous Absorption of Aminophylline, *in vitro*, Using the Human Cadaver Skin Model"; and DermTech report dated 12/06/01 titled "Evaluation of the Percutaneous Absorption of Aminophylline, *in vitro*, Using the Human Cadaver Skin Model". A hard copy and CD-Rom were both supplied.

15) The report to follow is based on my review of the materials provided to me by the FTC including documents from Basic Research LLC et al, published articles that I have retrieved following my review of the relevant medical literature through the National Library of Medicine (*PubMed*), and my knowledge and experience in the scientific field of regional and whole body fat metabolism. I reserve the right to review additional documents that become available and modify my report accordingly. My compensation is $500.00 per hour.

Reviewed articles:

- Caruso MK, Pekarovic S, Raum WJ, and Greenway F. Topical fat reduction from the waist. Manuscript status unclear.
- Collis N, Elliot L-A, Sharpe C, and Sharpe DT. Cellulite treatment: A myth or reality: A prospective randomized, controlled trial of two therapies, Endermologie and aminophylline cream. *Plastic and Reconstructive Surg* 104:1110-1114, 1999.
- Eckel RH. Obesity: A disease or a physiologic adaptation for survival? In: *Obesity: Mechanisms and Clinical Management*, Eckel RH, editor; Lippincott, Williams and Wilkins, pp 3-30, 2003.
- Greenway FL and Bray GA. Regional fat loss from the thigh in obese women after adrenergic modulation. *Clin Ther* 9:663-669, 1987.
- Greenway FL, Bray GA, and Heber D. Topical fat reduction. *Obes Res* 3:561S-568S, 1995.
- Lesser T, Ritvo E, and Moy LS. Modification of subcutaneous adipose tissue by a methylxanthine formulation: A double-blind controlled study. *Dermatol Surg* 25:455-462, 1999.
- Mowery D. Evaluation of the percutaneous absorption pf Epidril™ – A lecithin-based aminophylline gel, *in vitro*, using the human skin model: A review of research conducted by Paul Lehman, M.Sc. Manuscript status unclear.
- Muller B, Kasper M, Surber C, and Imanidis G. Permeation, metabolism and site of action concentration of nicotinic derivatives in human skin. Correlation with topical pharmacological effect. *Eur Journal Pharmaceut Sci* 20:181-195, 2003.

SUMMARY OF FINDINGS

16) I find **no evidence to support that**

a) "Dermalin-Apg causes rapid and visibly obvious fat loss in areas of the body to which it is applied";

b) "Cutting Gel causes rapid and visibly obvious fat loss in areas of the body to which it is applied";

c) "Tummy Flattening Gel causes rapid and visibly obvious fat loss in areas of the body to which it is applied";

d) "Published, clinical testing proves that Cutting Gel causes rapid and visibly obvious fat loss in areas of the body to which it is applied"; or

e) "Published, clinical testing proves that Tummy Flattening Gel causes rapid and visibly obvious fat loss in areas of the body to which it is applied".

DETAILED ANALYSIS

17) I'd like to set the stage for my review of whether the evidence supports the claims of Basic Research LLC by an overview of adipose tissue (fat) metabolism. This summary will provide support for the concept that body fat is regulated and defended against depletion.

Adipose Tissue Metabolism

18) Adipose tissue (fat) is formed in the fetus during the 3rd trimester of pregnancy, and throughout life has a number of critical roles. Certain periods of development such as puberty are dependent on the presence and quantity of adipose tissue. The defense of body fat speaks to the importance of adipose tissue fuel reserves in providing the needed energy when food deprivation occurs. Although this role of adipose tissue may be less relevant in the increasingly obese world of the 21st century, even in the 20th century data from the famines of the world wars support the contention that the amount of adipose tissue is a survival advantage (Eckel, In *Obesity*, Eckel, Lippincott, Williams and Wilkins, 2003).

a) Today, adipose tissue is also recognized as an endocrine organ, i.e. an organ that secretes hormones or proteins that have effects on other tissues. Substances that are made by fat that have an impact elsewhere in the body include leptin, adiponectin, angiotensin II, plasminogen activator inhibitor-1 (PAI-1), and cytokines. Leptin communicates information to the brain about the amount of adipose tissue, adiponectin increases the effects of insulin in muscle and liver, angiotensin II produces constriction of blood vessels, PAI-1

impedes the process by which blood clots are dissolved, and cytokines are involved in the inflammatory response to foreign organisms and/or injury. Thus, important aspects of the biology of life relate to the function of adipose tissue. Moreover, when fat mass increases in amount and volume, many of these functions become disrupted and/or contribute to the adverse consequences of obesity including insulin resistance, hypertension, an increased tendency to clot blood, and tissue damage (liver disease, atherosclerosis – coronary heart disease, stroke - as a result of the cytokine burden).

Regionalization of Adipose Tissue

19) The regionalization of body fat is sex dependent and controlled by the relative quantity of sex steroids, i.e. estrogens and androgens. Other hormones may also be influential, e.g. the adrenal hormone cortisol (hydrocortisone). When high levels of cortisol are produced by the adrenal gland or by taking hydrocortisone as a drug, excessive fat is deposited centrally, i.e. around the waist and trunk, with depletion of fat stores in the arms and legs. This type of excessive fat distribution is also seen in other conditions, e.g. in patients with human immunodeficiency virus infection, particularly when treatment with anti-retroviral drugs ensues. Genetics also appear to be involved. Recently, up to 30 genes have been identified in humans that relate to body fat distribution. At present, the genetic influence is at the level of association only, however, I'm sure that in the next few years more science will add to the relevance of many of these genes.

Regulation of fat cells

20) Fat cells are filled with triglycerides, storage molecules produced from fatty acids and glucose. These fatty acids are for the most part delivered to fat from circulating particles called lipoproteins that are produced by the liver and the intestine after eating. This uptake of lipoprotein-derived fatty acids by fat tissue is controlled in part by insulin. During periods without food intake, these triglyceride molecules are acted

upon by the enzyme hormone sensitive lipase that facilitates the breakdown of the triglycerides back to fatty acids. These fatty acids then leave the fat cells and travel to other organs to be utilized for energy. This process is regulated by a number of hormones such as adrenaline (epinephrine).

21) The number of fat cells is determined during critical periods throughout life including *in utero*, during infant feeding, puberty and after large amounts of weight gain. What regulates the number of fat cells is not clear, but when fat cells reach a maximum volume, new cells are formed.

Defense of body fat

22) Body fat is maximized by the important interaction between a man or woman and their environment. Today, the environment has been sufficiently modified to more easily enhance the development of obesity with the appropriate genetic predisposition. The failure of forced overfeeding studies to cause obesity in humans (Bouchard C et al, *New England Journal Med* 322:1477-82, 1990) and the lack of sustained weight loss in most dieters (Miller WC et al, *Int J Obes* 21:941-947, 1997) support the biology of body fat maintenance. Many explanations for this recidivism after weight reduction include the fall in basal metabolic rate, increase in appetite and preference for fat and sugar containing foods, reduction in physical activity, and a change in metabolism that favors carbohydrate (not fat) burning, and therefore fat storage (Eckel, In *Obesity*, Eckel, Lippincott, Williams and Wilkins, 2003). The long-term success of a weight loss program depends on continuous attention to the amount of food intake and physical activity (McGuire MT et al, *Obes Res* 7:334-341, 1999).

23) Alternatively, the surgical removal of adipose tissue in rodents is typically followed by re-accumulation of adipose tissue (Mauer MM et al, *Neurosis Biobehav Rev* 25:15-28, 2001). In humans, it is unclear whether surgical removal of subcutaneous fat has lasting effects, but in studies we did in which regional subcutaneous fat was

removed by liposuction, re-accumulation of subcutaneous fat occurred locally or elsewhere by one year if a lifestyle modification (diet and/or exercise) was not employed (Yost et al, *Plast Reconstr Surg* 92:1101-1108, 1993).

CLINICAL TRIAL DESIGN

24) To determine whether a drug works to favorably modify a disease state or a physiologic parameter, pre-clinical studies in animals need to establish the efficacy of the compound, in addition to a safety profile over a reasonable dosage range. In Phase I clinical trials in humans, drug safety is the focus. Phase I studies also include measurements of drug absorption, distribution, metabolism and excretion over a pre-determined dosage range. Once successfully completed, Phase II studies can be initiated. These studies are intended to provide additional evidence of drug safety, but whether or not the drug works in humans as in animals (efficacy) now becomes relevant. In Phase III studies, randomization of human subjects to a drug group vs no-drug (placebo) group is necessary for valid conclusions to be made. Studies are typically double-blinded, a study design wherein neither the subject nor physician/scientist know to which group the subject has been assigned. This strategy is needed to avoid any bias on behalf of the subject or treating physician/scientist as to whether the drug works. In these types of studies, a placebo arm is expected to determine whether or not simply taking or applying a substance or 'the drug' produces an effect. Data from Phase II studies should be able to allow the Phase III trial to focus in on dosage and treatment interval necessary to produce the desired effect. Studies should be powered based on preliminary data to predict the sample size necessary to achieve the desired response, and investigators should remain blinded to the data until the study is completed or stopped due to an unexpected treatment outcome and/or adverse event.

25) Placebo-controlled trials as just described are lacking for Dermalin-Apg, Cutting Gel, and Tummy Flattening Gel. There are actually no trials of any type presented to indicate Dermalin-Apg, Cutting Gel, and Tummy Flattening Gel work. More

specifically, there is no evidence that Dermalin-Apg, Cutting Gel or Tummy Flattening Gel produce rapid and visibly obvious fat loss in areas of the body to which they are applied.

Products

26) Dermalin-Apg, Cutting Gel, and Tummy Flattening Gel are all aminophylline-containing skin preparations designed for the purposes of removing fat locally, i.e. in the areas to which it is applied. The concentration of aminophylline in all of the

[Redacted]

From this point forward, comments will be based on the scientific evidence that support or fail to support the validity of the claims of Basic Reseaarch LLC et al related to all 3 of the aforementioned products unless specifically otherwise stated (Attachment 2).

Relevant Science

27) The scientific fact that the drug aminophylline (theophylline) results in lipolysis (the release of fatty acids from lipid stores) in adipose tissue and adipocytes (fat cells) outside the body is supported by the medical and pharmacological literature (Manganiello et al *J Cyclic Nucl Prot Phosphor Res* 11:497-511, 1986). This effect is related to the ability of aminophylline to inhibit the breakdown of cyclic-AMP (cAMP), the biochemical mediator of lipolysis, after stimulation of fat cells by appropriate stimulants (β- or α-2 adrenergic agonists). Basic Research LLC has no data to indicate that any of their products stimulates lipolysis and fat cell shrinkage in fat cells *in vitro*. Nor is there any evidence that Dermalin Apg, Cutting Gel or Tummy Flattening Gel cause rapid and visibly obvious fat loss in areas of the body to which they are applied.

28) The effectiveness of aminophylline-containing products to produce regional fat loss in humans *in vivo* exists from peer/reviewed (Greenway and Bray, *Clin Ther* 9:663-669, 1987) and non-peer reviewed publications (Greenway et al, *Obes Res* 3:561S-568S, 1995). These reports indicate that the topical application of a product containing aminophylline cream, not Dermalin Apg, Cutting Gel or Tummy Flattening Gel, reduces thigh circumference in women.

29) In the Greenway and Bray *Clin Ther* (1987) paper, 28 obese women were put on a 600-800 calorie diet and encouraged to engage in a walking program. Five groups were differentially assigned, but only two used aminophylline cream. One of the aminophylline cream groups applied the cream + forskolin/yohimbine to one thigh and placebo to the other 5 days/week for 4 weeks; the other aminophylline cream group applied the cream without forskolin/yohimbine in an identical manner. Measurements were made 2/3 of the distance between the knee and greater trochanter.

30) Variable amounts of weight loss occurred in 9/10 subjects (2 groups combined) whereas all 10 subjects receiving an aminophylline-containing cream lost more circumference in the thigh in which the cream was applied than the one to which placebo was applied. In the study in which aminophylline/forskolin/yohimbine cream was applied, the mean difference between the drug-treated and placebo-treated thighs was 2.03 ± 1.36 cm. The difference was 1.50 ± 0.77 cm when aminophylline cream *only* was compared with placebo. The only adverse effect was a rash in one subject in the aminophylline/forskolin/yohimbine group.

31) This study documents the benefit of aminophylline cream, however, in an extremely small sample. Clearly, validation is needed in larger populations.

32) In the Greenway et al *Obes Res* paper (1995) - which was not peer reviewed, 6 study groups were identified, 3 of which appear to be studies previously reported in the *Clin*

Ther (1987) paper. The 3 additional groups were treated with one of three aminophylline-containing creams, 0.5%, 2% and 10% (5 grams each) to one thigh and placebo to the other. Unlike the previous studies, two measurements were made: one at half the distance between the fibular head and the greater trochanter, and a 2nd 5 cm above the 1st. Only the 10% aminophylline cream group was placed on a 900-1100 calorie diet without encouragement to exercise. In all 3 groups, modest to moderate decreases in thigh circumference were seen. In the 10% aminophylline-treated study wherein 23 of 30 women completed the study, weight loss was 3.3 ± 2.2 kg with a girth loss greater in the aminophylline-treated thigh than in the placebo-treated thigh, 0.77 ± 0.66 cm at the lower girth measurement and 0.78 ± 0.89 cm at the upper girth measurement ($p<0.001$) after 6 weeks of treatment. The 2% aminophylline application study was carried out over 5 weeks in women who claimed their thighs were 'undesirably fat and dimpled'. One subject developed a rash. Again the aminophylline-treated thigh was reduced more in circumference than the placebo-treated thigh, 1.21 ± 0.31 cm ($p<0.01$). Finally, in the 0.5% aminophylline vs placebo cream study, the difference between thighs was 3.08 ± 0.27 cm over 5 weeks.

33) Unfortunately, no analyses were carried out to examine whether or not the apparent greater reduction in thigh circumference occurred on 0.5% aminophylline cream (> 3.0 cm) vs. the other two concentrations (~ 1.5 cm on 10% and ~ 1.3 cm on 2%) was true statistically. Does this suggest that at higher doses the effect of aminophylline is partially lost? This is of particular interest because the 10% aminophylline-treated group was placed on a reduced calorie diet and actually lost weight over the 6 weeks of observation. This relationship between the amount of weight loss and the reduction in thigh circumference with aminophylline requires further study. It is also unclear why the additional data presented in this manuscript vs. the *Clin Ther* 1987 paper were never subjected to a peer-review process. In science and medicine, peer review is accepted as an honored process by which the value of scientific discovery is weighed. Whenever possible, publications that have been peer-reviewed are cited before those that have not been so examined.

34) More recently, I have been provided with a copy of a manuscript from the Greenway group that is either intended to be submitted for publication or has been already submitted (Caruso et al). In this study, 50 overweight and obese men and women with a body mass index (BMI) of > 27 kg/m^2 (mean of 28.2 and 28.5, respectively) between the ages of 21 and 65 years participated. A male pattern fat distribution characterized by a waist to hip ratio ≥ 0.94 was present in the study subjects. The presence or absence of co-morbidities associated with obesity, i.e. diabetes, lipid disorders, and heart disease, or medications that the subjects were taking, were not described. Nor was there any detail provided about the measurement of the thigh circumferences, i.e. what sites (anatomical landmarks) were used, what was the variability of measurement, did one or more individuals carry out these determinations? All subjects were treated with a 1200-calorie diet and were encouraged to follow a walking program throughout the 12 week study. Subjects were either given a 0.5% aminophylline cream to apply vs no topical application to their abdomen twice a day over the study period. This is an inadequate study design because of the absence of a placebo cream application. Thus, neither the subjects nor the investigators were blinded. This raises substantial concern.

a) Athough the data were not provided, the reduction in weight and BMI was stated to be similar between the 2 groups. In the 0.5% aminophylline group, the waist circumference was reduced by 11.0 ± 1.0 cm whereas in the no-treatment group the reduction was 5.0 ± 0.6 cm ($p < 0.001$). Waist circumference reductions were seen in both men and women, but the waist circumference decline was greater in women than men, 11.6 ± 0.6 vs 9.4 ± 0.7 cm, respectively.

35) It is extremely difficult to understand how with this much reduction in waist circumference that weight reduction was not different between the groups. Aminophylline levels were undetected in the plasma of participants, and there were no adverse effects of the cream. With this amount of surface- area of application of aminophylline, it's difficult to believe that no drug was measurable in the plasma.

Unfortunately, the assay utilized is not described. Also noteworthy is the very brief bibliography, with no citations related to the effect of aminophylline cream on regional subcutaneous body fat other than those of the senior author (Greenway) listed.

36) In the study of Lesser et al (*Derm Surg* 25:455-462, 1999), soft supportive evidence of the benefit of a topical caffeine cream is provided. Caffeine is a methylxanthine compound in the same drug class as aminophylline, and therefore could produce fat cell lipolysis. This study was carried out over 2 months in 49 subjects, 41 of whom finished the study. Here, a 1% or 2% cream was applied to one side of the body including abdomen, thighs, triceps and hips with a placebo-containing cream administered to the opposite side. Caliper measurements in addition to circumferences were utilized in the various fat regions to quantify benefit.

a) The 2% caffeine-containing cream reduced caliper thickness in all areas, with a range from 3.0 to 5.4 mm ($p = 0.001$ for all). There was also a benefit using the 1% cream ranging from 2.1-3.4 mm ($p = 0.005$ for the triceps area and $p = 0.001$ for the remainder of regions). Despite the statistical significance of these data, this amount of change in caliper thickness might well be too small to be detected by the subjects. This is supported by the tape measurements that failed to show any differences between the 1% and 2% creams and placebo.

37) Despite the evidence presented from the Greenway and Bray (*Clin Ther*, 1987) and Greenway et al (*Obes Res*, 1995) articles, data are lacking for any clinical benefit of Dermalin-Apg, Cutting Gel and Tummy Flattening Gel. Specifically, there is no indication that any of these products produce rapid and visibly obvious fat loss in areas of the body to which they are applied. Moreover, there is no evidence these products or the aminophylline creams used by Greenway et al affect 'resistant body fat' any differently.

38) Basic Research LLC claims that 'exercise or decreased caloric content *is needed* to burn off released fat': This statement suggests that even if decreases in regional fat did ensue subsequent to the application of Dermalin-Apg, Cutting Gel and Tummy Flattening Gel, that to sustain the effect a diet and/or exercise is needed. Although this statement in general supports the scientific dogma that body fat is regulated and defended against reduction/depletion, there are no data provided to speak to the specific claim of Basic Research, LLC.

39) In the advertisements provided by Basic Research LLC, repeatedly stated is that studies wherein adipocytes are incubated with aminophylline *in vitro*, there is a loss in adipocyte volume. This contention is simply stated; there are no data to support the claim. Even if such data were available, it would be important to know whether these studies were carried out in acutely prepared or cultured isolated fat cells, in 3T3-L1 cells or in other cultured cells after fat cell differentiation? If isolated adipocytes were used, it would be important to know whether these cells were sourced from rodents or humans.

40) With the epidemic of overweight/obesity and the overzealous concern (even among normal weight individuals) about the unacceptable cosmetic effects of excess fat in certain anatomic regions, it is hard to explain the paucity of published science in this area. Why at this time is there only one peer-reviewed report in the literature on the value of topically applied aminophylline-containing products to selected fat-containing regions? Certainly, the development and implementation of scientifically sound randomized double blind controlled clinical studies would appear straightforward. The lack of adventure by Basic Research LLC into this area of opportunity is without explanation. Yet, the Greenway group appears to among the few participants in this area of clinical science. However, still there remains the major issue, that there are no data to support that Dermalin Apg, Cutting Gel or Tummy Flattening Gel cause rapid and visibly obvious fat loss in areas of the body to which they are applied.

41) The only other report to my knowledge provides no evidence to document the benefit of a product containing 2% aminophylline on regional fat (Collis et al, *Plast Reconstr Surg* 104:1110-1114, 1999). In this study, 52 women with 'cellulite' deposits on their thighs and/or buttocks were studied. The age of the study population ranged from 19-70 years, the weight from 53.6-93.7 kg and BMI from 21 –38.5 kg/m^2. Subjects were divided into 3 groups; only one group used a 2% aminophylline cream + 10% glycolic acid which was applied to one thigh/buttock with placebo applied to the other. These applications were carried out twice a day for 12 weeks. Measurements included physical examination, photographs, thigh girth at two points, and thigh fat depth by ultrasound. After 12 weeks of application, there was no statistical difference in any of the measurements between the aminophylline-containing and placebo-containing creams. Only 3 of the 35 research subjects who finished the study believed that their cellulite had been favorably modified at the study's terminus. Of note, skin reactions were seen in 9 subjects treated with aminophylline cream that finished the study and 11 of the 45 subjects so treated if subject withdrawals are included.

42) Overall, the data of Collis et al are in conflict with the data of Greenway et al. An explanation for these differences could be related to study design. Perhaps more disturbing is the absence of any further scientific experiments to validate this area of science.

Drug Absorption

43) Using the report of Mowrey, rates of absorption for aminophylline were carried out in a human skin model using a lecithin-based aminophylline gel -Epidril™. The active ingredient was measured over 48 hours after elution by high performance liquid chromatography (HPLC) in the 'subcutaneous fatty layer' of human cadaver skin. The peak rate of delivery was 0.795 µg/cm^2/hr with a peak time of absorption of 26.3 ± 2.9 hours and total absorption of 48.22 ± 4.16%. In studies described under DP02-618 and DP01-645, rates for the '*Cutting Gel*' were ~0.8 µg/cm^2/hr and for the

'Ripping Gel' 0.379 μg/cm^2/hr. Peak absorption for the *'Cutting Gel'* ranged from a time of 10 to 26.3 hours and for the *'Ripping Gel'* 27.6 hours. In addition, total absorption for the *'Cutting Gel'* was 48-56% and 11.7% for the *'Ripping Gel'*.

44) Despite the potential value of these studies, the human cadaver skin technique may have limitations in assessing the subcutaneous absorption of drugs in living human subjects. First it remains unclear how deeply the products of discussion would have penetrated into human skin in living subjects. The science of skin penetration of drugs topically applied is dependent on the drug itself, its formulation, and the skin to which it is applied. The metabolism of the drug by the skin and the blood supply to the area of application are both important factos in the local effect of drugs and their absorption. Of course both of these issues would not be applicable to cadaver skin. This area of science and medicine is complex, with an optimal system to evaluate drug penetration, metabolism, and absorption still lacking (Muller et al, *Eur J Pharm Sci* 20:181-195, 2003).

45) As noted above, the repeated claims of the Greenway group that no aminophylline was found in the blood stream of subjects to whom the creams were applied is surprising. With greater penetration, the blood supply is more extensive; thus, it would seem likely that levels of aminophylline would be measurable in the blood stream. We have no studies with Dermalin-Apg, Cutting Gel or Tummy Flattening Gel to know whether or not this is true.

Lack of Pharmaceutical Company Activity

46) Even in the absence of much or any principal investigator-initiated pursuit of the efficacy and safety of aminophylline-containing creams or similar products on subcutaneous adipose tissue, the apparent reluctance of industry efforts in this cosmetic arena appears to speak for itself. Since the prevalence of overweight/obesity affects 65% of the US population and more than a few in the 'normal weight group' seek cosmetic surgery for 'cellulite' or related undesirable fat

deposits, there must be some reason for the lack of enthusiasm and development by the pharmaceutical industry. There certainly appears to be a large market place out there waiting for such an innovation. Yet, since the conflicting publications of Greenway and Bray in 1987 and that of Collis et al in 1999, there is no evidence of any further such research and development.

Conclusions

47) Based on my over 25-year experience as a research physician in the area of fat metabolism, I feel strongly that the evidence to support that Dermalin-Apg, Cutting Gel, and Tummy Flattening Gel are effective in reducing undesirable deposits of subcutaneous fat is unfounded. There are no data that speak to the claim that 'Dermalin-Apg', 'Cutting Gel' and 'Tummy Flattening Gel' cause rapid and visibly obvious fat loss in areas of the body to which they are applied. Competent and reliable randomized double blind trials are lacking, and the intended value of these products is based on a limited experience by others using aminophylline-containing creams that differ from those of Basic Research LLC. An additional problem here is that these 'success' stories have been carried out almost entirely by one group (Greenway et al) with validation wanting. Although Dermalin-Apg, Cutting Gel, and Tummy Flattening Gel may not be harmful, there are no data to support even this claim. I believe that the task before Basic Research LLC remains to provide support for what at present appear to be unfounded claims.

Robert H. Eckel

Robert H. Eckel, M.D.

October 19, 2004

REPORT 1

ROBERT H. ECKEL, M.D.

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EDUCATION

1969 Bachelor of Sciences, Bacteriology, University of Cincinnati, Cincinnati, Ohio

1973 Doctor of Medicine, University of Cincinnati College of Medicine, Cincinnati, Ohio

POSTGRADUATE TRAINING

1973-74 Internship in Medicine, University of Wisconsin Hospitals, Madison, Wisconsin

1974-76 Medical Residency, University of Wisconsin Hospitals, Madison, Wisconsin

1976-79 Senior Fellowship in Metabolism and Endocrinology, Department of Medicine, University of Washington School of Medicine, Seattle, Washington

LICENSURE TO PRACTICE

Wisconsin, July 10, 1974, License No. 18866
Washington, July 10, 1976, Certificate No. 25209
Colorado, July 10, 1979, License No. 22425

APPOINTMENTS

1979-85 Assistant Professor of Medicine,
University of Colorado Health Sciences Center, Denver, Colorado

1981-93 Associate Program Director, Adult General Clinical Research Center,
University of Colorado Health Sciences Center, Denver, Colorado

1983-1985 Acting Chairman, Division of Endocrinology,
University of Colorado Health Sciences Center, Denver, Colorado

1984-present Graduate Faculty Appointment,
University of Colorado Health Sciences Center, Denver, Colorado

1985-1989 Associate Professor of Medicine,
University of Colorado Health Sciences Center, Denver, Colorado

1989-90 Faculty Affiliate, Department of Food Science and Human Nutrition,
Colorado State University, Fort Collins, Colorado

1989-present Professor of Medicine,
University of Colorado Health Sciences Center, Denver, Colorado

1989-95 Professor of Biochemistry, Biophysics and Genetics,
University of Colorado Health Sciences Center, Denver, Colorado

1990-present Graduate Faculty Appointment, Department of Food Science and Human Nutrition,
Colorado State University, Fort Collins, Colorado

1991-1997 Co-Director, Center for Human Nutrition,
University of Colorado Health Sciences Center, Denver, Colorado

1993-present	Program Director, Adult General Clinical Research Center, University of Colorado Health Sciences Center, Denver, Colorado
1995-present	Professor of Physiology and Biophysics, University of Colorado Health Sciences Center, Denver, Colorado
1997-present	Co-Director, Clinical Nutrition Research Unit, University of Colorado Health Sciences Center, Denver, Colorado
1997-present	Associate Director, Center for Human Nutrition, University of Colorado Health Sciences Center, Denver, Colorado
1998-present	Vice-Chairman, Research Affairs, Department of Medicine, University of Colorado Health Sciences Center, Denver, Colorado
2000-present	Charles A. Boettcher Endowed Chair in Atherosclerosis, Department of Medicine, University of Colorado Health Sciences Center, Denver, Colorado
2002-present	Professor of Medicine, Division of Cardiology University of Colorado Health Sciences Center, Denver, Colorado

BOARDS

Diplomate, National Board of Medical Examiners, July 1974
Diplomate, American Board of Internal Medicine, June 16, 1976
Diplomate, American Board of Endocrinology and Metabolism, June 19, 1979

HONORS

1969	Bachelor of Sciences Cum Laude in Bacteriology, University of Cincinnati, Cincinnati, Ohio
1973	Alpha Omega Alpha, University of Cincinnati College of Medicine, Cincinnati, Ohio
1987	American Society for Clinical Investigation
1987-88	Full-Time Faculty Housestaff Teaching Award, Department of Medicine, University of Colorado Health Sciences Center, Denver, Colorado
1990	Moses Barron Award, American Diabetes Association, Minnesota Affiliate
1991	Award of Excellence, Colorado Dietetic Association
1994-99, 2001-2004	Best (Top) Doctors in America Recognition
1997	Association of American Physicians
1998	Excellence in Teaching, Medical Student Council
1999	Outstanding Teaching Award, Clinical Science Program, University of Colorado Health Sciences Center
2000	Charles A. Boettcher Endowed Chair in Atherosclerosis, Department of Medicine, University of Colorado Health Sciences Center, Denver, Colorado
2000	Robert H. Williams-Rachmiel Levine Award, Western Metabolism Club
2001	Robert W. Schrier Award of Excellence, Department of Medicine, University of Colorado School of Medicine

2002	The Alexander Marble Lecturer at the Elliott P. Joslin Research Laboratory, Harvard Medical School
2003	Fellow of the American Heart Association and the Council on Nutrition, Physical Activity and Metabolism, April 20, 2003
2004	Frank and Sheila Thompson Lectureship in Endocrinology, Scott & White Clinic, Tempie, Texas, January 9, 2004

GRANTS

7/77-6/79	Juvenile Diabetes Foundation Research Fellowship. Cultured Fibroblasts as a Cellular Model of Diabetes Mellitus and its Complications.
4/79-3/80	University of Colorado Research Assistance, BRS699 Grant: Hormonal Control of Adipose Tissue Lipoprotein Lipase.
12/79-5/05	NIDDK, Grant:DK26356, Hormonal Control of Adipose Tissue Lipoprotein Lipase.
09/80-08/82	Juvenile Diabetes Foundation Research, Grant: 80R055Pathophysiology of Adipose Tissue Lipoprotein Regulation in Diabetes: Use of Cultured Human Preadipocytes.
7/82-6/84	Cambridge Quest Foundation. Adipose Tissue Lipoprotein Lipase Regulation after Weight Reduction in Obese Humans.
11/84-10/86	Mead Johnson, Nutritional Division. Regulation of Adipose Tissue Lipoprotein Lipase Before and After Weight Loss in Obese Subjects. Response to Variable Fatty Acid Chain Length Formula Diets.
04/86-03/89	NIDDK, Grant: AM30747. Diabetes Mellitus in the San Luis Valley, Colorado. Principal Investigator: Richard F. Hamman, MD
02/87-01/90	NICHHD, Grant: HD19547. Physiological Factors Affecting Human Lactation. Principal Investigator: Margaret C. Neville, PhD. A
03/89-02/90	Procter and Gamble Co. Effect of Medium Chain Triglycerides on Glucose and Lipid Metabolism in Type II Diabetes Mellitus.
12/89-11/92	NIDDK, Grant: DK42266. Nutrition, Lipoprotein Lipase and Body Weight Regulation.
09/92-08/94	USDA, Grant: 92-37200-7522. Transcriptional Control of Lipoprotein Lipase by Cytokines.
07/94-06/99	NIDDK, Grant: DK46881. Diet, Lipoprotein Lipase, Insulin Action and Weight Gain.
01/95-09/02	NIDDK, Grant: Clinical Nutrition Research Unit, Metabolic Core Director.
04/95-04/02	NIDDK, Grant: DK42266. Nutrition, Lipoprotein Lipase and Body Weight Regulation.
12/96-06/98	Amgen, The Effect of Leptin on Postprandial Lipid Metabolism.
09/97-present	Merck Unrestricted Grant 97-033, Assessment of Coronary Atherosclerosis in Young Adults with Type 1 Diabetes by Ultrafast Computerized Tomography.
03/98-02/04	Slim-Fast Nutrition Institute, Effects of Weight Loss with Reduced-Weight Maintenance and Subsequent High-Carbohydrate vs. High-Fat Macronutrient Feeding on Neck Morphology, Whole-Body Energy Metabolism, Sleep Dynamics, Pulmonary Function, and Insulin Sensitivity in Severely Obese Humans.

02/99-06/02 Abbott, Effect of Triglyceride-Lowering on Endothelial-Dependent and Endothelial-Independent Arterial Vasodilation, Independent of the Known Effects of Hypercholesterolemia on Endothelial Function.

07/99-06/04 NHLBI, K-30 Grant: Co PI, Advanced Training for Clinical Investigators.

07/00-07/04 American Diabetes Association, Mentor Based Postdoctoral Fellowship, Title??????

12/01-03/04 Merck and Co., PNO111-057 Grant: The Impact of HMG Co-A Inhibitors on C-Reactive Protein in Patients with Type 2 Diabetes.

08/02-07/04 Eli Lilly and Company, The Acute Effects of Feeding High Fat Diets on Insulin Clearance.

08/03-07/08 NIH, DK61668: Grant: Mechanisms Defending Fat Mass in Humans After Lipectomy.

10/03-09/04 National Multiple Sclerosis Society. Lipoprotein Lipase and Nerve Myelination.

TRAINEES

Past Trainees

Name	Pre/Post Doctorate	Dates in Lab	Degree	Current Position
Craig Sadur	Post	07/80-06/82	M.D.	Private practice
Phil Kern	Post	07/81-06/85	M.D.	Prof of Medicine, Univ. of Arkansas, Little Rock, Arkansas
Mary O'Shea	Post	07/84-06/85	M.D.	Private practice
Tammi Gregg	Pre	1988-1989	BS	Dietitian, Madison, Wisconsin
Paul Rudolf	Post	07/85-06/87	M.D.	Private practice
Daniel Rule	Post	09/86-07/87	Ph.D.	Assoc Prof, Animal Sciences Univ. of WY, Laramie, Wyoming
Daniel Bessesen	Post	07/87-12/90	M.D.	Assoc. Prof of Medicine, Div. of Endocrinology, Metab & Diabetes UCHSC, Denver, CO
Mary Kay Rozmyslovicz	Post	07/88-06/90	M.D.	Private Practice
Mary Raynolds	Post	05/88-07/92	.. Ph.D.	Asst. Prof., Division of Cardiology, UCHSC, Denver, CO
Jamie Erskine	Pre	09/90-09/92	.. Ph.D.	Asst. Prof, Human Nutrition Univ of Northern Colorado Greeley, CO
Jackie Berning	Pre	09/90-12/94	..Ph.D.	Asst Prof, Kinesiology Univ. of Colorado, Denver, CO
Sharon Travers	Post	07/92-07/94	..M.D.	Assoc. Prof, Division of Endocrinology, Dept of Pediatrics UCHSC, Denver, CO
Hong Liu	Pre	09/92-06/94	Ph.D.	Graduate Student, Dept. Nutrition Univ. of Texas, Austin, Texas

Carrie Ganong	Post	07/94-1997	.M.D.	Asst. Prof, Pediatrics Ohio State Univ., Columbus, OH
Cathy Morin	Post	09/92-06/98	.Ph.D.	Asst Prof, Preventive Medicine, UCHSC, Denver, CO
Angie McGinnis	Pre	07/96-06/98	BS	Pre doc
Patricia Uelmen Huey	Post	09/96-09/99	.Ph.D.	Assistant Professor of Medicine Emory University, Atlanta, GA
Paul Poirier	Post	02/98-12/99	M.D.	Scientific Director, Cardiovascular Rehabilitation Program Quebec Heart Institute/Laval Hospital Quebec, Canada
Luis Ferreira	Post	11/98 – 10/01	Ph.D.	Instructor of Medicine University of Perth, Perth, Australia
William Troy Donahoo	Post	07/95-02/04	M.D.	Asst. Prof of Medicine University of Vermont

Current Trainees

Lisa Kosmiski	Post	07/93-present	M.D.	Asst. Professor of Medicine, UCHSC, Denver, Colorado
Bakary J. Sonko	Post	11/00-present	Ph.D.	Postdoc
Warren H. Capell	Post	07/01-present	M.D.	Postdoc
Alison M. Morris	Post	09/01-present	Ph.D.	Postdoc
Dean J. Calsbeek	Post	07/02-present	Ph.D.	Postdoc
Linda Barbour	Post	09/02-present	M.D.	Assoc. Professor of Medicine, OB/GYN, UCHSC
Leigh Perreault	Post	11/02-present	M.D.	Assistant Professor of Medicine Boulder GCRC
Paul Wischmeyer	Post	11/02-present	M.D.	Assistant Professor of Anesthesiology
Bryan C. Bergman	Post	04/03-present	Ph.D.	Assistant Professor

PROFESSIONAL ORGANIZATIONS

Adipocytes: An International Journal Devoted to Basic and Clinical Research on Adipocyte and Adipose Tissue Biology, Editorial Board
American College of Physicians, Fellow
American Diabetes Association
American Federation for Clinical Research
American Heart Association, FAHA
Council on Arteriosclerosis, Thrombosis, and Vascular Biology
Council on Nutrition, Physical Activity, and Metabolism
American Society for Clinical Investigation
American Society for Clinical Nutrition
American Society for Nutritional Sciences
Association of American Physicians
Association for Patient-Oriented Research
Endocrine Society
North American Association for the Study of Obesity

Western Association of Physicians
Western Society for Clinical Investigation

COMMITTEES - LOCAL

1. Animal Care Control Committee
   University of Colorado Health Sciences Center, Denver, Colorado 1980-1983
2. Intern Selection Committee, Department of Medicine
   University of Colorado Health Sciences Center, Denver, Colorado 1980-present
3. American Diabetes Association, Colorado Affiliate, Organizational Committee, 1980-1985
4. Juvenile Diabetes Foundation, Denver, Colorado Denver Medical Advisory Board, 1980-1981
5. Attending Physician, Gordon Meiklejohn Service, Department of Medicine
   University of Colorado, Denver, Colorado 1980-1993; Ward Darley Service, 1993-present.
6. Scientific Advisory Committee, Adult General Clinical Research Center
   University of Colorado Health Sciences Center, 1981-present
7. Grant Review Committee, Colorado Heart Association, 1983-1987
8. Dean's Subcommittee on "Centers of Excellence" in Cardiovascular Disease
   University of Colorado Health Sciences Center, Denver, Colorado 1984
9. Faculty Senate
   University of Colorado Health Sciences Center, Denver, Colorado 1985-1987, 1989-present
10. Research and Development Committee, Veterans Administration Medical Center, Denver, Colorado,
    Dean's Committee Representative, 1985-1988
11. Curriculum Review Committee
    University of Colorado College of Medicine 1986-1994.
12. Inter-Departmental Steering Committee on Nutrition, University of Colorado College of Medicine,
    1987-1990.
13. University of Colorado/Colorado State University Consortium for Human Nutrition
    Board Member, 1988-present
14. Nutrition Support Subcommittee, University of Colorado Hospital, Department of Medicine
    Representative, 1990-1992.
15. Search Committee, Executive Director, Barbara Davis Childhood Diabetes Center, 1991-1992.
16. Metabolic Regulation of Fetal Growth Center Grant: Internal Advisory Committee, University of
    Colorado HSC, 1991-1997.
17. Faculty Communications Committee
    University of Colorado College of Medicine, 1991-1993.
18. University of Colorado School of Medicine Faculty Council Representative, 1992-1994.
19. Clinical Trials Office Committee
    University of Colorado Health Sciences Center, Denver, Colorado 1995-1997.
20. Graduate School Governance Committee
    University of Colorado Health Sciences Center, Denver, Colorado 1996-present.
21. Doctor of Philosophy in Biology, Clinical Investigation Track Curriculum Committee
    University of Colorado Health Sciences Center, 1997-present.
22. The Robert W. Schrier Award of Excellence Committee, 1997.
23. University of Colorado Health Sciences Center Committee on Planning and Fiscal Policy, 1997-1999.
24. Denver Metro Physicians Advisory Council, American Heart Association, Colorado Affiliate, 1997-
    1998.
25. American Heart Association of Colorado, Public Affairs Committee, 1998-present.
26. Electives Committee, University of Colorado School of Medicine, 1999-present
27. Clinical/Translational Research Committee, University of Colorado School of Medicine, Co-Chair, 2001
28. Veterinarian Pathologist Search Committee, 2002-2003.
29. University Hospital Research Committee, 2002-present.
30. Co-Chair Advanced Studies Curriculum Committee, University of Colorado College of Medicine, 2004–
    present.
31. Research Advisory Committee, University of Colorado College of Medicine, 2004 – present.

COMMITTEES – NATIONAL/INTERNATIONAL

1. National Eye Institute, Diabetes Retinopathy Study II, Medical Therapy Committee, 1977-1978.
2. Councilor, American Federation for Clinical Research (Western Section), 1982-1984.
3. National Councilor, American Federation for Clinical Research, 1984-1988.
4. Secretary-Treasurer, American Federation for Clinical Research (Western Section), 1985-1988.
5. Councilor, North American Association for the Study of Obesity, 1987-1990.
6. Technical Advisory Board, Institute for Creation Research, El Cajon, California, 1988-present.
7. Program Committee, Council on Nutritional Sciences and Metabolism, American Diabetes Association, 1989-1991.
8. Education Committee, North American Association for the Study of Obesity, 1991-1992.
9. Chairman, Publications Committee, North American Association for the Study of Obesity, 1991-1994.
10. External Advisory Committee, Nutrition Training Grant: University of California, Davis, 1990-1995.
11. Vice-President, North American Association for the Study of Obesity, 1993-1994.
12. Program Committee, International Association for the Study of Obesity, Toronto, August 1994.
13. President, North American Association for the Study of Obesity, 1995-1996.
14. Executive Committee, General Clinical Research Center Program Directors, 1995-present.
15. Nutrition Committee, American Heart Association, 1996-present; Vice-Chairman 1997-1998, Chairman July 1998-present.
16. Co-Chairman, Annual Meeting of the North American Association for the Study of Obesity, 1996.
17. Program Committee, International Association for the Study of Obesity, October 1996.
18. President's Committee, North American Association for the Study of Obesity, 1996-present.
19. Long Range Planning Committee, North American Association for the Study of Obesity, 1996-present.
20. President, Western Society for Clinical Investigation, 1997-1998.
21. Co-Chairman, Planning Committee, General Clinical Research Program Directors Meeting, 1998.
22. Chairman, Nutrition Committee, American Heart Association, July 1998-present.
23. Executive Board, Kern Aspen Lipid Conference, 1997-present.
24. Boston Obesity Nutrition Research Center, Scientific Advisory Committee, 1998-2001.
25. Board of Directors, Association for Patient-Oriented Research, 1998-present.
32. Co-Chairman, Program Committee, Association for Patient-Oriented Research, 1999-2001.
33. Vice-Chairman, Council on Nutrition, Physical Activity and Metabolism, American Heart Association, 2000-2002.
34. Secretary-Treasurer, Association of Patient-Oriented Research, 2001-2003.
35. Chair, Prevention VII: Obesity, A Worldwide Epidemic Related to Heart Disease and Stroke, American Heart Association, Honolulu, Hawaii, April 26-27, 2002
36. Chairman, Council on Nutrition, Physical Activity and Metabolism, American Heart Association, 2002-2004.
37. Chair, Kern Aspen Lipid Conference, Fatty Acid Transport and Metabolism: Impact on Insulin Action/Secretion and Body Weight Regulation, 2002
38. Member, Science Advisory and Coordinating Committee, American Heart Association, 2002-present.
39. University of North Carolina Chapel Hill, Member of External Advisory Committee for Mentored Clinical Research Scholar Program, NIH K-12 Award RR-02-2001, 2002 - present.
38. CNRU NIDDK sponsored "National Task Force on Prevention and Treatment of Obesity," 2003-present.
39. NIDDK Advisory Council, 2003-2006.
40. American Board of Internal Medicine Endocrinology Subspecialty Writing Committee 2003-2006.
41. Chairman, Nutrition, Physical Activity and Metabolism Council, American Heart Association, 2002-present.
42. Keystone Symposia Scientific Advisory Board, 2003-2006.
43. Program Committee, L.J Filer Symposium, American Heart Association, March 2004, San Francisco, CA.
44. President, Kern Aspen Lipid Conference, 2002-present.
45. President-Elect, Association for Patient Oriented Research, 05/03-04/04.
46. President, Association for Patient Oriented Research, 05/04-04/05.
47. President-Elect. American Heart Association, 07/04-06/05.

JOURNAL REVIEWS

Acta Endocrinologica
American Heart Journal
American Journal of Clinical Nutrition, Editorial Board, 1997-1999
American Journal of Medicine, Editorial Board, 1998-present
American Journal of Physiology
- Applied Physiology
- Anthropology
- Endocrinology and Metabolism
- Heart and Circulatory
- Regulatory Integrative Comparative

Annals of Epidemiology
Annals of Internal Medicine
Archives of Biochemistry and Biophysics
Archives of Internal Medicine
Arteriosclerosis, Thrombosis and Vascular Biology, Editorial Board, 2001-present
Arthritis and Rheumatology
Atherosclerosis
Biochimica Biophysica Acta
Chemical Reviews
Circulation
Circulation Research
Cleveland Clinic Journal
Clinical Chemistry
Clinical Endocrinology
Clinical Science
Diabetes, Editorial Board, 1988-1992
Diabetes Care
Diabetes, Obesity and Metabolism
Diabetes/Metabolism Research and Reviews
Diabetologia
Endocrine
Endocrinology
European Journal of Clinical Investigation
Gene
Hepatology
Hormone and Metabolic Research
Human Gene Therapy
Hypertension
In Vitro
International Journal of Obesity, Editorial Board, 1989-present
Journal of the American Society of Nephrology
Journal of Biological Chemistry
Journal of Cardiopulmonary Rehabilitation
Journal of Cardiovascular Research
Journal of Cellular Physiology
Journal of Clinical Endocrinology and Metabolism, Editorial Board, 2003-present
Journal of Clinical Investigation
Journal of Epidemiology and Community Health
Journal of Gerontology
Journal of Hepatology
Journal of Investigative Dermatology
Journal of Laboratory and Clinical Medicine
Journal of Lipid Research
Journal of Nutritional Biochemistry
Journal of Obesity and Weight Regulation, Editorial Board, 1984-present

Journal of Pediatric Gastroenterology
Journal of Pediatrics
Mayo Clinic Proceedings
Mechanisms of Ageing and Development
Metabolism
Metabolic Syndrome and Related Disorders, Editorial Board
Neuroendocrinology
New England Journal of Medicine
Nutrition
Obesity Research, Editorial Board, 1992-present
Obstetrics and Gynecology
Proceedings of the National Academy of Sciences
Proceedings of the Society for Experimental Biology and Medicine
Science
The Online Journal of Current Clinical Trials

INVITED PARTICIPANT

1. Adipose Tissue Development and Metabolism Satellite Symposium to the Third International Congress on Obesity; Gothenburg, Sweden; October 4-6, 1980.
2. First International Symposium on Acarbose; Montreux, Switzerland; October 8-10, 1981.
3. National Institutes of Health Workshop on the Classification of Obesity; Poughkeepsie, New York, October 17-19, 1982.
4. American Diabetes Research Symposium; Denver, Colorado; January 10-12, 1983.
5. National Institutes of Health Special Study Section; Bethesda, Maryland; August 9, 1983.
6. National Institutes of Health Site Visit; East Carolina University; July 23-25, 1985.
7. Joint Conference on Obesity and Non-Insulin Dependent Diabetes Mellitus; Toronto, Ontario, Canada; October 30 - November 1, 1985.
8. National Institutes of Health Reverse Site Visit; Bethesda, Maryland; October 8-10, 1986.
9. General Clinical Research Center Site Visit; Stanford University; December 3-4, 1986.
10. Council on Nutritional Sciences and Metabolism of the American Diabetes Association: Program on Obesity and Exercise in Type II Diabetes; Indianapolis, Indiana; June 6, 1987.
11. American Diabetes Association, Northern Illinois Affiliate, Thirtieth Professional Symposium; Chicago, Illinois; October 14, 1987.
12. Seminars in Medicine, Beth Israel Hospital, Harvard Medical School; Boston, Massachusetts; December 15, 1987.
13. The Endocrine Society National Meeting, Symposium on Hormonal Control of the Adipose Cell; New Orleans, Louisiana; June 9, 1988.
14. American Diabetes Association Symposium on Current Issues in Nutrition and Metabolism; San Francisco, California; October 7, 1988.
15. National Institutes of Health Nutrition Study Section, Special Reviewer; Bethesda, Maryland; October 6-17, 1988.
16. General Clinical Research Center Site Visit; University of Cincinnati; December 1-2, 1988.
17. National Institutes of Health Metabolism Study Section, Special Reviewer; Bethesda, Maryland; December 15, 1988..
18. National Institutes of Health Nutrition Study Section; July 1, 1989 - June 30, 1993; Chairman; July 1, 1991 - June 30, 1993.
19. General Clinical Research Center Site Visit; Brown University; April 5-6, 1989.
20. American Society for Clinical Nutrition Postgraduate Course; Washington, D.C.; April 27-28, 1989.
21. American Diabetes Association National Meeting. Symposium: Issues in the Nutritional Management of Patients with Diabetes; Detroit, Michigan; June 6, 1989.
22. FASEB Summer Conference on the Regulation of Energy Balance; Vermont Academy; July 31 - August 4, 1989.
23. National Institutes of Health Workshop on Basic and Clinical Aspects of Regional Fat Distribution; Bethesda, Maryland; September 9-11, 1989.
24. International Symposium on Lipoprotein Metabolism and Lipid Lowering Agents; Netherlands; February 27 - March 2, 1990.

25. National Institutes of Health, Genetics of Obesity; Phoenix, Arizona; March 26-27, 1990.
26. General Clinical Research Center Site Visit; University of Florida; April 10-11, 1990.
27. The Endocrine Society National Meeting. Symposium on Control of Regional Fat Distribution; Atlanta, Georgia; June 21, 1990.
28. The Aspen Bile Acid/Cholesterol Conference. Hepatic Cholesterol and Postprandial Lipoproteins; August 18-21, 1990.
29. International Symposium on Obesity and Diabetes Mellitus; Nagoya, Japan; October 18-19, 1990.
30. North American Association for the Study of Obesity Symposium on the Treatment of the Patient with Medically Significant Obesity; Atlanta, Georgia; December 1, 1990.
31. Keystone Symposia. The Adipose Cell: A Model for Integration of Hormone Signaling in the Regulation of Cellular Function; Park City, Utah; January 18-24, 1991.
32. The Science of Food Regulation Symposium. Pennington Biomedical Research Center; Louisiana State University; Baton Rouge, Louisiana; March 14-15, 1991.
33. North American Association for the Study of Obesity/Society for the Study of Ingestive Behavior National Meeting; Sacramento, California;' October 20-28, 1991.
34. FASEB Summer Conference on Regulation of Energy Balance: From Organism to Gene; Copper Mountain, Colorado; August 2-7, 1992.
35. North American Association for the Study of Obesity Symposium Obesity Update: Pathophysiology, Clinical Consequences and Therapeutic Options; Atlanta, Georgia; August 31-September 2, 1992.
36. American Heart Association Scientific Conference on Central Obesity/Insulin Resistance Syndrome and Coronary Artery Disease; Washington, DC; April 22-23, 1993.
37. International Symposium on Insulin Resistance as a Risk Factor for Cardiovascular Disease; Ste-Adèle, Québec, June 22-24, 1993.
38. North American Association for the Study of Obesity. National Meeting; Milwaukee, Wisconsin; October 17-20, 1993.
39. International Symposium on Atherosclerosis; Montreal, Ontario; October 9-12, 1994.
40. Master Clinical Dieticians; Key Biscayne; January 14, 1995.
41. NIDDK Program Project on Body Composition Site Visit, St. Luke's-Roosevelt Hospital, Columbia University; New York, New York; April 5-7, 1995.
42. North American Association for the Study of Obesity. National Meeting; Baton Rouge, Louisiana; October 14-17, 1995.
43. NIDDK Program Project on Body Composition, Columbia University; Reverse Site Visit, Washington, D.C.; March 24-25, 1996.
44. NIDDK Symposium on Transgenic Animals in Nutrition Research, FASEB Meeting; Washington, D.C.; April 14, 1996.
45. NIDDK Metabolism Study Section, Special Reviewer; Bethesda, Maryland; July 2, 3, 1996.
46. The Endocrine Society Clinical Endocrinology Update; Chicago, Illinois; October 9, 1996.
47. General Clinical Research Center Site Visit, University of Alabama; Birmingham, Alabama; December 17-18, 1996.
48. Kern Aspen Lipid Conference; Aspen, Colorado; August 15-18, 1997.
49. Satellite Symposium of the 11th Annual International Symposium on Atherosclerosis; St. Malo, France; October 3-5, 1997
50. American Heart Association Annual Sessions, Plenary speaker; Orlando, Florida; November10-13, 1997
51. Pennington Biomedical Research Symposium on Obesity; Baton Rouge, Louisiana; March 1-3, 1998
52. American Heart Association, Obesity: Impact on Cardiovascular;, Amelia Island, Florida; May 22-24, 1998
53. American College of Sports Medicine; Orlando, Florida; June 3-6, 1998
54. American Diabetes Meeting; Chicago; June 13-16, 1998
55. FASEB Summer Conference on Behavioral and Metabolic Sub-phenotypes in Obesities, Genetic and Molecular Aspects, Pathophysiology and Therapeutic Implications; Snowmass, Colorado; June 20-25, 1998
56. NIDDK Metabolism Study Section, Special Reviewer; July1-2, 1998
57. General Clinical Research Center Site Visit, Beth Israel Deaconess, Harvard Medical School; July 13-15, 1998
58. NIDDK Diabetes Complications Special Study Section, Chairman; July 30-31, 1998
59. 8th International Congress on Obesity, Plenary speaker; Paris, France; Aug 29-Sept 3, 1998

60. American Association of Cardiovascular and Pulmonary Rehabilitation Annual Meeting; Denver, CO; Oct 15, 1998
61. Joslin Diabetes Center 100th Anniversary; Boston, Mass; Oct 22-24, 1998.
62. FASEB, 50th Anniversary of NIDDK, San Diego Convention Center, April 17, 2000.
63. NIDDK Special Study Section on Lipids and Lipoproteins, Chair, June 18, 19, 2000
64. North American Association for the Study of Obesity, Women's Health and Obesity Across the Life Span Symposium speaker, Oct 31, 2000
65. American Heart Association Annual Sessions, Cardiovascular Seminar on Secondary Prevention in the Elderly, Nov 13, 2000
66. American Heart Association Annual Sessions, Special Session on It's Time to Treat Obesity, Nov 15, 2000
67. American Heart Association Prevention VI on Cardiovascular Disease and Diabetes Mellitus, Orlando, Jan 18-20, 2001
68. NIDDK Special Study Section on Lipids and Lipoproteins, Chair, Feb 21, 2001
69. NHLBI-NIDDK Working Group on The Pathophysiology of Obesity-Associated Cardiovascular Disease, Chair, May 23, 24, 2001.
70. NIDDK Special Study Section on Lipids and Lipoproteins, Chair, June 18, 2001.
71. The Endocrine Society Annual Meeting, Meet the Professor on Hyperlipidemia, June 20, 2001.
72. The American Dietetics Annual Meeting, 'Beyond the AHA Guidelines', St. Louis, Oct 22, 2001
73. American Diabetes Association Meeting on Insulin Resistance. Tempe, Arizona, Dec 6-8, 2001.
74. American Heart Association Prevention VII: Obesity, a Worldwide Epidemic Related to Heart Disease and Stroke, Honolulu, April 26-28, 2002, Chair.
75. The Kern Aspen Lipid Conference, 'Fatty Acid Transport and Metabolism: Impact on Insulin Action/Secretion and Body Weight Regulation', Aspen, Aug 17-20, 2002, Co-Chair.
76. MSDM (Multiclinical Study for Diabetic Macrovascular Complication) Tokyo, Japan October 11-12, 2002
77. U.S.-Japan Panel on Nutrition and Metabolism Tokyo, Japan October 13, 2002.
78. American Heart Association Scientific Sessions, Moderator "Emerging Epidemics, Obesity, Diabetes Mellitus, Sedentary Lifestyle" and "Curbing the Diabetic Epidemic," Chicago, IL, November 17, 2002.
79. American Heart Association Scientific Sessions, November 18, 2002, Plenary Session "Metabolic Syndrome: Pathogenesis of CVD in Metabolic Syndrome and Diabetes," Chicago, IL, November 17, 2002.
80. American Heart Association Scientific Sessions, Chicago, IL, November 18, 2002, "Role of Diet in the Statin Era".
81. Keystone Symposia's Role in Translational Medicine, January 14, 2003.
82. 2005 Keystone Symposia Planning Committee, January 15, 2003.
83. NPAM and ATVB Councils of American Heart Association "Skeletal Muscle Lipoprotein Lipase, Nutrient Partitioning and Insulin Action." Washington, DC, May, 2003.
84. UCLA SCOR Atherosclerosis Grant Review, Lipoprotein Lipase, Nutrient Partitioning and Insulin Action." Lake Arrowhead, California, September, 2003.
85. Metabolic Syndrome Clinical Conference, Washington, DC. "Obesity and its Metabolic Complications".
86. Frontiers in Cardiovascular Science Meeting, Israel Atherosclerosis Society, "Skeletal Muscle Lipoprotein Lipase, Nutrient Partitioning and Insulin Action." Eilat, Israel, October, 2003.
87. American Heart Association Scientific Sessions "The Skinny on Weight Reduction Diets," Orlando, FL, November 2003.
88. American Heart Association Scientific Sessions, "Postprandial Lipemia and Diet" Orlando, FL, November 2003.
89. Co-Chair, NIH, Adipose Tissue Secretory Function and Its Role in Obesity-Associated Co-Morbidities, Washington, DC, 2003.
90. Duke CRI Conference Medical Problem- Medical Approach, Experience to Date Emerging Therapeutic Targets, McLean, VA, January 2004.
91. NIH/NIDDK, "Lipids and the Pathophysiology of Obesity", Washington, DC, May, 2004.
92. FDA/NIDDK, Joint Symposium on Diabetes", Bethesda, MD, May, 2004.
93. AHA, "Quality of Care and Outcomes Research in Cardiovascular Disease and Stroke", Washington, DC, May, 2004.
94. Endocrine Society, "The Metabolic Syndrome: The Most Important Risk Factor for Coronary heart Disease?", New Orleans LA, June 17, 2004.

95. Oregon Health & Science University, "Muscle Lipoprotein Lipase, Energy Balance and Insulin Resistance" and "The Metabolic Syndrome: Therapeutic Options". Portland OR, June 28-29, 2004.
96. Clinical Science Course Sessions, "General Clinical Research Opportunities for Research". Denver CO, August 7, 2004.
97. Kaplan Lectureship Program: Obesity, "Translation of Basic Science to Clinical Research & Practice in Obesity Management". Cincinnati OH, August 27-28, 2004.
98. American Heart Association: Weight Loss Book, "Weight Loss Principles Used in Practice". Dallas TX, September 3, 2004.
99. American Heart Association: Prevention of Vascular Disease, "The Current National Epidemics of Obesity, Diabetes and the Metabolic Syndrome, Including the Effects and Relationships of These Diseases on Cardiovascular Disease". Detroit MI, September 9, 2004.
100. Fall Symposium on Atherosclerosis Prevention, "The Metabolic Syndrome: Therapeutic Consideration for Major Modifiable Risk Factor(s) for CHD". Blowing Rock NC, October 15-16, 2004.

BIBLIOGRAPHY

PUBLICATIONS

1. **Eckel RH**, Crowell EB Jr, Waterhouse BE, Bozdech MJ. Platelet inhibiting drugs in thrombotic thrombocytopenic purpura. *Arch Int Med* 37:735-737, 1977.
2. **Eckel RH**, Fujimoto WY, Brunzell JD. Development of lipoprotein lipase in cultured 3T3-l1 cells. *Biochem Biophys Res Comm*; 78:288-293, 1977.
3. **Eckel RH**, Fujimoto WY, Brunzell JD. Insulin regulation of lipoprotein lipase in cultured 3T3-l1 cells. *Biochem Biophys Res Comm*; 84:1069-1075, 1978.
4. **Eckel RH**, Fujimoto WY, Brunzell JD. Gastric inhibitory polypeptide (gip) enhanced lipoprotein lipase activity in cultured preadipocytes. *Diabetes*; 28:1141-1142, 1979.
5. **Eckel RH**, Green WL. Postpartum thyrotoxicosis in a patient with Graves disease association with low radioactive iodine uptake. *J Am Med Assoc*; 243:1454-1456, 1980.
6. **Eckel RH**, Albers JJ, Cheung MC, Wahl PW, Lindgren FT, Bierman EL. High density lipoprotein composition in insulin-dependent diabetes mellitus. *Diabetes*; 30:132-138, 1981.
7. **Eckel RH**, Fujimoto WY. Insulin stimulated glucose uptake, leucine incorporation into protein and uridine incorporation into RNA in skin fibroblast cultures from patients with diabetes mellitus. *Diabetologia*; 20:186-189, 1981.
8. **Eckel RH**, Albers JJ, Cheung MC, McLean EG, Bierman EL Plasma lipids and microangiopathy in insulin-dependent diabetes mellitus. *Diabetes Care*; 4:447-493, 1981.
9. **Eckel RH**, Fujimoto WY. Quantification of cell death in human fibroblast by measuring the loss of [14^c] thymadine from prelabeled cell monolayers. *Anal Biochem*; 114:118-124, 1981.
10. **Eckel RH**, Fujimoto WY, Brunzell JD. Effect of *in vitro* lifespan of 3T3-l1 cells on hormonal responsiveness of lipoprotein lipase activity. *Int J Obesity*; 5:571-578, 1981.
11. Brunzell JD, Schwartz RS, **Eckel RH**, Goldberg AP. Insulin and adipose tissue lipoprotein lipase activity in humans. *Int J Obesity*; 5:685-694, 1981
12. Sadur CN, **Eckel RH**. Insulin stimulation of adipose tissue lipoprotein lipase: use of the euglycemic clamp technique. *J Clin Invest*; 69:1119-1125, 1982.
13. Kern PA, Knedler; **Eckel RH**. Isolation and culture of microvascular endothelium from human adipose tissue. *J Clin Invest,* 71(6):1822-1829, 1983.
14. Sadur CN, **Eckel RH**. Insulin-mediated increases in the HDL cholesterol/cholesterol ratio in humans. *Arteriosclerosis*; 3:339-343, 1983.
15. **Eckel RH**, Prasad JE, Kern PA, Marshall S: Insulin regulation of lipoprotein lipase in cultured isolated rat adipocytes. *Endocrinology*; 114:1665-1671, 1984.
16. Kern PA, **Eckel RH**. Absence of lipoprotein lipase in cultured human adipose stromal cells. *Arteriosclerosis,* 4:232-237, 1984.

17. Sadur CN, Yost TJ, **Eckel RH**. Fat feeding decreases insulin responsiveness of adipose tissue lipoprotein lipase. *Metabolism*; 33:1043-1047, 1984.
18. **Eckel RH**, Robbins RJ: Lipoprotein lipase is produced, regulated and functional in rat brain. *Proc Natl Acad Sci USA*; 81:7604-7607, 1984.
19. Sadur CN, Yost TJ, **Eckel RH**. Insulin responsiveness of adipose tissue lipoprotein lipase is delayed but preserved in obesity. *J Clin Endocr Metab*; 59:1176-1182, 1984.
20. Kern PA, Marshall S, **Eckel RH**. Regulation of lipoprotein lipase in primary cultures of isolated human adipocytes. *J Clin Invest*; 75:199-208, 1985.
21. Berr F, **Eckel RH**, Kern F Jr. Plasma decay of chylomicron remnants is not affected by heparin-stimulated plasma lipolytic activity in normal fasting men. *J Lipid Res*; 26:852-859, 1985.
22. Berr F, **Eckel RH**, Kern F Jr. Contraceptive steroids increase hepatic uptake of chylomicron remnants in healthy young women. *J Lipid Res*; 27:645-651, 1986.
23. Garvey WT, Grundy SM, **Eckel RH**. Xanthogranulomatosis in an adult: lipid analysis of xanthomata and plasma. *J Am Acad Derm*; 16:183-187, 24, 1987.
24. Baron AD, **Eckel RH**, Schmeiser L, Kolterman OG. The effect of short term α-glucosidase inhibition on carbohydrate and lipid metabolism in type II diabetics. *Metabolism*; 36:409-415, 1987.
25. Kern PA, Mandic A, **Eckel RH**. Regulation of lipoprotein lipase by glucose in primary cultures of isolated human adipocytes: relevance to the hypertriglyceridemia of diabetes. *Diabetes*; 36:1238-1245, 1987.
26. **Eckel RH**, Yost TJ. Weight reduction increases adipose tissue lipoprotein lipase responsiveness in obese women. *J Clin Invest*, 80:992-997, 1987.
27. **Eckel RH**, Goldberg IJ, Steiner L, Yost TJ, Paterniti JR ,Jr. Plasma lipolytic activity: relationship to postheparin lipolytic activity and evidence for metabolic regulation. *Diabetes*; 37:610-615, 1988.
28. **Eckel** RH, Sadur CN, Yost TJ. Deficiency of the insulin/glucose-mediated decrease in serum triglycerides in normolipidemic obese subjects. *Int J Obesity*; 12:369-376, 1988.
29. Yost TJ, **Eckel RH**. Fat calories may be preferentially stored in reduced-obese women: a permissive pathway for resumption of the obese state. *J Clin Endocr Metab*; 67:259-264, 1988.
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31. Yost TJ, **Eckel RH**. Hypocaloric feeding in obese women: metabolic effects of medium-chain triglyceride substitution. *Am J Clin Nutr*; 49:326-330, 1989.
32. **Eckel RH**, Yost TJ. HDL subfractions and adipose tissue metabolism in the reduced-obese state. *Am J Phys.* 256:E740-746, 1989.
33. Kern PA, Svoboda ME, Graves D, **Eckel RH**, Van Wyk JJ. Insulin-like growth factor action and production in adipocytes and endothelial cells from human adipose tissue. *Diabetes,* 38:710-717, 1989.
34. **Eckel RH**. Lipoprotein lipase: A multifunctional enzyme relevant to common metabolic diseases. *N Engl J Med*; 320:1060-1068, 1989.
35. Samuels MH, **Eckel RH**: Massive insulin. overdosage: Detailed studies of free insulin levels and glucose requirements. *Clin Tox J Tox*; 26:157-168, 1989.
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37. Brass EP, Tserng K-Y, **Eckel RH**. Urinary organic acid excretion during feeding of medium-chain or long-chain triglyceride diets in patients with non-insulin dependent diabetes mellitus. *Am J Clin Nutr*; 52:923-926, 1990.

38. **Eckel RH**, Raynolds MV, Bessesen DH, Farese RV Jr, Ohtake A, Yost TJ. Lipoprotein lipase: hormonal and nutritional regulation. *J Drug Devel*; 3 Suppl 1:91-94, 1990.
39. Raynolds MV, Awald PD, Gordon DF, Gutierrez-Hartmann A, Rule DC, Wood WM, **Eckel RH**. Lipoprotein lipase gene expression in rat adipocytes is regulated by isoproterenol and insulin through different mechanisms. *Mol Endocr*; 4:1416-1422, 1990.
40. Farese RV Jr, Yost TJ, **Eckel RH**. Tissue-specific regulation of lipoprotein lipase activity by insulin in normal weight humans. *Metabolism*; 40:214-216, 1991.
41. Neville MC, Waxman LJ, Jensen DR, **Eckel RH**. Lipoprotein lipase in human milk:compartmentalization and effect of fasting, insulin and glucose. *J Lipid Res*; 32:251-257, 1991.
42. Jensen DR, Bessesen DH, Etienne J, **Eckel RH**, Neville MC. Distribution and source of lipoprotein lipase in mouse mammary glands. *J Lipid Res*; 32:733-742, 1991.
43. Regensteiner JG, Mayer EF, Shetterly SM, **Eckel RH**, Haskell WL, Marshall JA, Baxter J, Hamman RF. Relationship between habitual physical activity and hyperinsulinemia among non-diabetic men and women: The San Luis Valley Diabetes Study. *Diabetes Care*; 14:1066-1074, 1991.
44. Bessesen DH, Robertson AD, **Eckel RH**. Weight reduction increases adipose but decreases cardiac LPL in reduced-obese Zucker rats. *Am J Phys*; 261:E246-E251, 1991.
45. Mayer EJ, Burchfiel CM, **Eckel RH**, Marshall JA, Haskell WL, Hamman RF. The role of insulin and body fat in associations of physical activity with lipids and lipoproteins in a bi-ethnic population: The San Luis Valley Diabetes Study. *Arteriosclerosis and Thrombosis*; 11:973-984, 1991.
46. Yost TJ, **Eckel RH**. Regional similarities in the metabolic regulation of adipose tissue lipoprotein lipase. *Metabolism*; 41:33-36, 1992.
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48. Barchiesi BJ, **Eckel RH**, Ellis PP. The cornea and disorders of lipid metabolism. *Surv Ophthalmol*, 36:1-22, 1991.
49. Glaser DS, Yost TJ, **Eckel RH**. Preheparin lipoprotein lipolytic activities: Relationship to plasma lipoproteins and postheparin lipolytic activities. *J Lipid Res*; 33:209-214, 1992.
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52. Currie RA, **Eckel RH**. Characterization of a high affinity octamer transcription factor binding site in the human lipoprotein lipase promoter. *Arch Biochem Biophys*; 298:630-639, 1992.
53. Yost TJ, Jensen DR, **Eckel RH**. Tissue-specific lipoprotein lipase: Relationship to body composition and body fat distribution in normal weight humans. *Obesity Res*; 1:1-4, 1993.
54. **Eckel RH**. Insulin Resistance: An adaptation for weight maintenance. *Lancet*; 340:1452-1453, 1992.
55. Bessesen DH, Richards CL, Etienne J, Goers JW, **Eckel RH**. The spinal cord is the most abundant source of lipoprotein lipase in the central nervous system. *J Lipid Res*; 34:229-238, 1993.
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63. Travers SH, Jeffers BW, Bloch CA, Hill JO, **Eckel RH**. Gender and Tanner Stage differences in body composition and insulin sensitivity in early pubertal children. *J Clin Endocr Metab*; 80:172-178, 1995.

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65. Bessesen DH, Rupp CL, **Eckel RH**. Trafficking of dietary fat in lean rats. *Obesity Res*; 3:191-204, 1995.

66. Bessesen DH, Rupp CL, **Eckel RH**. Dietary fat is shunted away from oxidation towards storage in obese Zucker rats. *Obesity Res*; 3:179-190, 1995.

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68. Regensteiner JG, Shetterly SM, Mayer EJ, **Eckel RH**, Haskell WR, Baxter J, Hamman RF. Relationship between habitual physical activity and insulin area among persons with impaired glucose tolerance: The San Luis Valley Diabetes Study *Diabetes Care*; 18:490-497, 1995.

69. Yost TJ, Sadur CN, **Eckel RH**. Glycohemoglobin levels relate to the response of adipose tissue lipoprotein lipase to insulin/glucose in obese non-insulin-dependent diabetes mellitus. *Metabolism*; 44:1475-1480, 1995.

70. Yost TJ, Jensen DR, **Eckel RH**. Weight regain following sustained weight reduction is predicted by relative insulin sensitivity. *Obesity Res*; 3:583-587, 1995.

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ARTICLES

Topical Fat Reduction from the Waist

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Running Title: Topical Fat Reduction

Abstract

Objective: Topical fat reduction from the thigh in women using aminophylline cream has been demonstrated, but the local fat reduction in other body areas or in men by lowering the local lipolytic threshold with aminophylline cream has not. This study is designed to test the hypothesis that aminophylline cream application to the waist will reduce waist circumference compared to a control.

Research Methods and Procedures: Fifty men and women 21-65 years of age with a BMI >27 kg/m^2 and a waist to hip ratio $\geq$ 0.94 were randomized in a 1:1 ratio to 0.5% aminophylline cream to the waist twice a day or no treatment to the waist. All subjects were instructed to follow a 1200 kcal balanced diet, participate in a walking program and return biweekly to encourage compliance. A theophylline level was drawn monthly and the waist, BMI and waist to hip ratio were re-measured at 12 weeks.

Results: At week 12 there was a significant reduction in BMI from baseline that was not different between the groups. The reduction in waist circumference was 11 $\pm$ 1.0 cm in the aminophylline cream group and 5.0 $\pm$ 0.6 cm in the control group ($p<0.001$). The reduction in waist circumference was significant for both women and men, but the women lost significantly more waist girth, and the waist to hip ratio also declined significantly. Aminophylline levels were undetectable, and were no adverse events.

Discussion: Aminophylline cream offers a safe and effective alternative to invasive surgical procedures for local fat reduction.

Key Words: Aminophylline, Cellulite, Waist to Hip Ratio

Background

The influence of endogenous stimulators and inhibitors of the lipolytic process determine the threshold for lipolysis at the fat cell in the human body. The relative lipolytic thresholds of the body's fat cells, therefore, determine a person's fat distribution. The greater abundance of lipolytically inhibitory alpha-2-adrenergic receptors on the thigh fat cells of women under the influence of estrogen is felt to be responsible for the characteristic lower body fat distribution typical for women (1). Fat is a dynamic storage organ. When weight is stable, fat is stored in fat cells during the day as people eat and used during the night to sustain the body until breakfast the next morning. Fat is quantitatively mobilized from the individual fat cells in proportion to the individual lipolytic thresholds of the regional fat deposits. It is, therefore, logical to assume that lowering the lipolytic threshold in a local fat deposit will cause its fat stores to be preferentially depleted through dynamic process of body lipid turnover.

There are several methods to lower the local lipolytic threshold at the fat cell. One can inject a lipolytic stimulator locally or deliver the lipolytic stimulator transdermally using ointments or creams (2). Aminophylline 0.5% cream has been shown to reduce thigh girth compared to a vehicle control (2). Aminophylline, two theophylline molecules joined by ethylenediamine, inhibits the breakdown of cyclic-AMP in the fat cell amplifying the lipolytic signal and lowering the lipolytic threshold.

Fat is distributed in two different patterns – "gynoid" and "android". A major cosmetic concern for women with a gynoid fat distribution is the size of their thighs, while for women and men with an android fat distribution, it is the size of their waist. Since thigh fat reduction with aminophylline cream occurs due to a reduction in the local lipolytic threshold, the same principle

should apply to other local body areas. This study is designed to test the hypothesis that 0.5% aminophylline cream applied to the waist will cause preferential fat loss from that location.

Methods

Fifty overweight and obese men and women with a BMI > 27 kg/m^2, between the ages of 21 and 65 years of age and with an android fat distribution characterized by a waist to hip ratio $\geq$ 0.94 were included in this study. Subjects using aminophylline, theophylline or having a known allergy to either were excluded.

At baseline all subjects were instructed to follow a balanced 1200 kcal/d diet and encouraged to follow a walking program throughout the 12-week study. The subjects were randomized with blocking for gender into two groups of 25 subjects, one receiving 0.5% aminophylline cream and the other receiving no topical treatment served as a control. All participants in the 0.5% aminophylline cream group were instructed to rub 15 cc of the cream on their waist twice a day for the duration of the 12-week trial. Subjects were seen every 2 weeks, encouraged to follow their diet, encouraged to continue their walking program, encouraged to apply the cream twice daily and asked about any adverse events. Each month, blood was drawn to measure the theophylline level. The BMI, waist circumference and hip circumference were re-measured at the end of the 12-week study. The BMI, waist circumference and the waist to hip ratio were analyzed by t-test.

Results

Twenty females and 5 males were in both groups, and groups were well matched for body mass index at baseline, 28.2 kg/m^2 vs. 28.5 kg/m^2 (p = NS), for the aminophylline cream and the

control groups, respectively. The average waist circumference was 101 cm confirming that the study population had an android fat distribution. The waist circumference of the two groups was not different at baseline. All 50 subjects completed the study.

At week 12, the BMI in the aminophylline cream group was 26.1 $\pm$ 1.0 kg/m^2 (SEM) and 26.2 $\pm$ 1.0 kg/m^2 in the control group, a significant reduction in BMI from baseline that was not different between the groups. The reduction in waist circumference was 11 $\pm$ 1.0 cm in the aminophylline cream group and 5.0 $\pm$ 0.6 cm in the control group ($p<0.001$). The reduction in waist circumference was significant for both women (11.6 $\pm$ 0.6 cm in the aminophylline group and 5.6 $\pm$ 0.6 cm in the control group) and men (9.4 $\pm$ 0.7 cm in the aminophylline group and 4.7 $\pm$ 0.6 cm in the control group) ($p<0.001$). Women treated with 0.5% aminophylline cream lost more girth (11.6 $\pm$ 0.6 cm vs. 9.4 $\pm$ 0.7 cm) than the men ($p<0.001$). The waist to hip ratio declined more in the group treated with 0.5% aminophylline cream than the control group (0.86 $\pm$ 0.05 vs. 0.92 $\pm$ 0.07, $p<0.001$) see Table 1. All monthly aminophylline levels were undetectable. There were no adverse events or allergic reactions to the cream.

Discussion

This trial demonstrates that reducing the local lipolytic threshold with topical aminophylline cream results in a reduction of waist circumference in both men and women. Local girth reduction of the waist in android body types is consistent with the principle that lowering the local lipolytic threshold causes fat reduction in the area of application. Thus, one can extend the principle of local fat reduction with aminophylline cream to both genders and to a body area different from the thigh.

In developing aminophylline cream it was appreciated that aminophylline, two theophylline molecules joined by ethylenediamine, is a skin sensitizer and chemically reactive due to the ethylenediamine it contains. A standard cream base turned yellow from a chemical reaction with aminophylline. This yellow cream was ineffective and caused rashes in some subjects.

Using a specially formulated cream base to stabilize the aminophylline, the safety and efficacy of local thigh fat reduction was demonstrated (2). The same cream base was used in this study. Not only was the aminophylline cream effective for reduction of waist circumference in this study, but it was also safe. The undetectable aminophylline levels confirmed that the cream was acting locally, and there were no rashes or adverse events during the trial.

Although a placebo cream was not used in the control group, the two groups were well matched at baseline, and the BMI loss was similar in both groups. The purpose of the 1200 kcal/d weight loss diet was twofold: 1) to address the subject's overweight problem and 2) to lower the lipolytic threshold through negative caloric balance.

The waist to hip ratio has been used as a surrogate measure of insulin resistance due to its correlation with visceral fat (3,4). Visceral fat correlates with insulin resistance (5). Since the local fat reduction reduced the subcutaneous abdominal fat, it presumably does not reduce insulin resistance despite the reduction in the waist to hip ratio. The change in waist to hip ratio in this study is confirmation that the fat shifted away from the waist.

The most common cosmetic concern for those with a gynoid fat distribution is the size of their thighs and for those with the android fat distribution is the size of their waist. This study demonstrates that fat can be preferentially and safely mobilized from the waist during weight loss in those with an android fat distribution. Subjects in this study volunteered that they felt

better about themselves after the aminophylline cream allowed them to cause preferential fat loss in their area of maximal cosmetic concern. Since cosmetic surgical procedures like liposuction and abdominoplasty are now used for local fat reduction, aminophylline cream offers a non-surgical alternative that is safe and non-invasive.

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Table 1.

Group	Waist	Male Waist	Female Waist	Waist/Hip
0.5% aminophylline (cm)	-11 ± 1.0^{a}	-9.4 ± 0.7^{c}	-11.6 ± 0.6^{a}	0.86 ± 0.05^{e}
Control (cm)	-5.0 ± 0.6^{b}	-4.7 ± 0.8^{b}	-5.6 ± 0.6^{b}	0.92 ± 0.07^{f}

Legend

Table 1 shows the changes in waist circumference (cm) from baseline in the two groups and separately for men and women. The waist to hip ratio is also shown. Different superscripts show values that are significantly different from one another ($p<0.001$).

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Cellulite Treatment: A Myth or Reality: A Prospective Randomized, Controlled Trial of Two Therapies, Endermologie and Aminophylline Cream

[Cosmetic]

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Outline

Graphics

Abstract

Cellulite is a common phenomenon that particularly affects the thighs and buttocks of women. Little scientific evidence exists to support any of the many advertised treatments for it. A total of

52 of 69 women, who were divided into three groups, completed a 12-week, randomized, controlled trial in which the effectiveness of two different treatments for cellulite was assessed. The patients acted as their own controls. The treatments investigated were twice-daily application of aminophylline cream and twice-weekly treatment with Endermologie ES1. Group 1 (double blind) received aminophylline to one thigh/buttock and a placebo cream to the other. Group 2 (singly blind) received Endermologie to one thigh/buttock. Group 3 received Endermologie to both sides and used the same cream regimen as group 1. Results were assessed subjectively by the patient and by clinical examination and photographic assessment by the surgeon (before and after the trial). Morphologic assessment included body mass index, thigh girth at two points, and thigh fat depth measurement by ultrasound. No statistical difference existed in measurements between legs for any of the treatment groups (paired t test, $p > 0.4$). The best subjective assessment, by the patients themselves, revealed that only 3 of 35 aminophylline-treated legs and 10 of 35 Endermologie-treated legs had their cellulite appearance improved. The authors do not believe that either of these two treatments is effective in improving the appearance of cellulite.

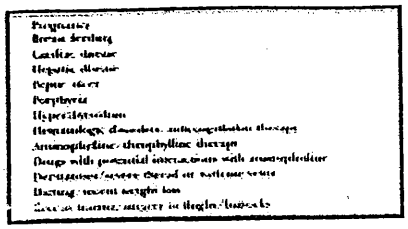
Cellulite is a common phenomenon that causes embarrassment to even the most fit of women. Its cause is unclear, and buttocks and thighs seem to be particularly affected. The scientific and medical press has scant information on cellulite and its treatment; however, women's magazines often feature articles and advertisements advocating therapies that have little, if any, scientific validation, some of which cost several hundred dollars for a course of treatment. The aim of this study was to test the clinical effectiveness of two different methods of treatment for cellulite in a variable, blind, randomized trial in which the patients acted as their own control. Topically applied aminophylline is a pharmacological treatment, and Endermologie ES1 (LPG Systems, Valence, France) is a mechanical method of "aspirated hypodermal mobilization" that claims to mobilize subcutaneous fat and is now licensed for use in the United States.

Although the LS1 machine was purchased for use in the private sector, we felt an ethical obligation to assess its effectiveness before marketing the treatment.

Patients and Methods

Women over the age of 18 with cellulite of the thighs and buttocks were invited to take part in the 12-week trial. A detailed medical history was followed by physical examination. Table I shows patient exclusion criteria. After acceptance onto the trial, a series of morphologic measurements were made on each patient. Body mass index was calculated [weight (kg)/height 2 (m^2)],1 and bilateral thigh circumferences were measured (while standing) at 15 and 25 cm (arbitrarily chosen) above the superior pole of the patella by the first author using a single tape measure. Lateral thigh subcutaneous fat depth was measured midway between these two points using a Toshiba SSA-270A ultrasound machine with a 7.5-MHz linear array probe by the second author. Standardized photographic documentation (anterior, right, left, lateral, and posterior views) of the cellulite was done in a semicircular booth specifically designed for this purpose by LPG Systems. The appearance of the cellulite was best documented without using the camera flash. A total of 69 patients were randomized into one of three groups (23 patients in each) to receive one or both treatments.

TABLE I Patient Exclusion Criteria



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Group 1 patients received two tubs of cream marked left leg and right leg. One contained the glycoaminophylline cream (2% aminophylline with 10% glycolic acid to aid skin penetration), and the other contained a placebo, which did not contain aminophylline but was in every other respect identical to the aminophylline cream. Each was applied twice a day to the respective thigh and buttock for 12 weeks. Using a placebo negated any effect from massaging during application of the creams. This group was double-blind.

Group 2 patients received twice-weekly 10-minute Endermologie sessions for 12 weeks on one thigh/buttock only; the contralateral side acted as a control. Each patient, therefore, received 24 unilateral treatments. This group was single-blind with respect to the investigator.

Group 3 patients received the same creams and instructions as group 1. In addition, they received twice-weekly 10-minute Endermologie sessions to both thighs/buttocks. The same operator was responsible for all of the Endermologie treatment sessions and had attended their training course in France, as recommended by LPG Systems.

Patients were instructed to maintain their lifestyles to minimize any effect of changes in weight and fitness levels on the appearance of the cellulite. After 12 weeks, morphologic measurements and photographs were repeated. In addition, the cellulite was assessed by the first author to discover any difference in the appearance of the cellulite between legs. The patient was then asked whether she thought there had been any improvement in the appearance of the cellulite on either or both legs.

Results

A total of 52 patients completed the 12-week trial, 17 from group 1 (aminophylline cream), 17 from group 2 (Endermologie), and 18 from group 3 (both treatments). Seventeen patients failed to complete the course of treatment (five, seven, and five for groups 1, 2, and 3, respectively). Of these, two developed a dermatologic reaction to the aminophylline cream (not placebo), three found the Endermologie treatment painful, two felt that the treatments were ineffective, and in one patient, Endermologie made the superficial veins in her thigh more prominent. There were also two pregnancies, three unrelated intercurrent illnesses, and four patients for whom the 12-week commitment proved to be too much.

Table II shows the ages and morphologic data for the patient group as a whole, and Table III shows the distribution of the body mass indexes with reference to normal ranges and obesity. Figures 1 and 2 show that when patient photographs were simply ordered according to the severity of cellulite, a spread of body mass indexes and fat depths existed across the range of cellulite, providing evidence that cellulite is not simply related to the amount of subcutaneous fat. Tables IV through VI show the changes in weight, ultrasound-determined fat depth, and thigh girth for each treatment group. There was no significant difference (paired t test, $p = 0.4$ to 0.9) in the fat depth

and thigh girth between legs in any of the three groups. However, the same trend for differences in weight change between the three treatment groups was repeated in the fat depth and thigh girth changes. The results indicate that the changes in these measurements are simply a result of weight loss and not the treatment.

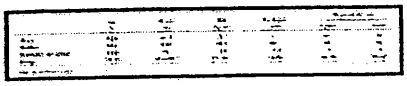

TABLE II Pretreatment Patient Data

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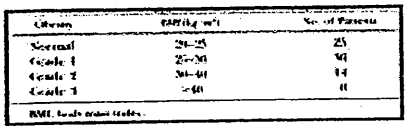

TABLE III Pretreatment Body Mass Index Distribution

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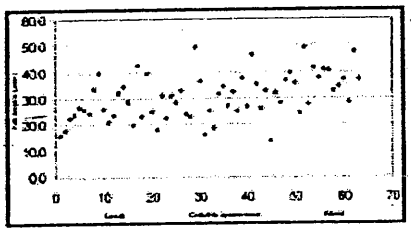

Fig. 1. Fat depth and severity of cellulite appearance. Patients were simply ranked by photographs.

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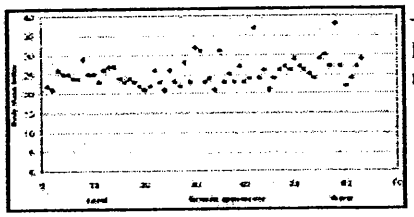

Fig. 2. Body mass index and severity of cellulite appearance. Patients were simply ranked by photographs.

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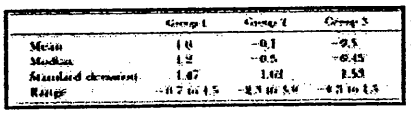

TABLE IV Weight Change (kg)

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TABLE V Ultrasound Fat Depth Changes (mm)

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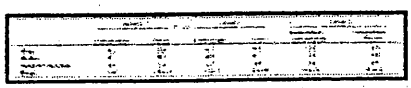

TABLE VI Changes in Thigh Girth (cm)

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Subjective assessment of the cellulite by both the patient and investigator (clinical examination and photographic assessment) revealed poor results from both treatments. Any improvements were slight, and in no patients did the cellulite disappear. Of 17 patients in group 1, only three thought that the appearance of the cellulite in the aminophylline-treated leg had improved. None thought that the placebo leg had improved. The photographs of only two patients showed a slight improvement in the aminophylline-treated leg. The investigator, blind as to which were treatment and placebo legs, could detect no obvious difference between them in any members of this group. In group 2, five patients (of 17) thought that the Endermologie-treated leg improved. The investigator could only detect a similar difference in two patients; photographic assessment discovered a difference in only one. In group 3, five patients (of 18) thought that both legs had improved. None thought that either only one leg, or both differentially, had improved. Clinical examination showed a differential improvement in favor of the aminophylline cream in two cases and the placebo in one case. There was photographic improvement in one patient (both legs). In only one patient was there a consensus of agreement of improvement among the patient, the investigator, and the photographs.

Problems with both treatments were encountered. Nine patients (of 35) developed a dermatologic reaction to the aminophylline cream (none had a reaction to the placebo). This rises to 11 of 45 if patient withdrawals are included. Thread veins developed in two of 35 patients (three of 53 legs) treated with Endermologie. Two patients also withdrew for this reason. Three patients withdrew because the Endermologie treatment was too uncomfortable.

The results, at best, using the patients' own evaluations of treatment efficacy, indicate that only three of 35 legs improved with topically applied aminophylline and 10 of 35 improved with Endermologie.

Discussion

Cellulite is a common and difficult problem that predominantly affects the buttocks and thighs of postpubertal women. Its cause is unclear, although it may result from fatty distension of the superficial fascial system, which connects the dermis to the deep fascia. Points of attachment to the dermis are tethered while surrounding areas bulge, producing the "cobblestone" appearance. Whether the fat storage in these areas is abnormal or merely represents one end of a spectrum is not known. It has also been suggested from ultrasonic analysis of the upper thigh and buttock that there is herniation of subcutaneous fat into the reticular and papillary dermis. Increases in water-binding dermal glycosaminoglycans have also been reported.[2] Cellulite is not exclusively related to obesity but may be accentuated by it. The range of body mass indexes and subcutaneous fat depths across the ranges of cellulite appearance in our study group confirms this observation. Of the various treatments available, only aminophylline and Endermologie have medical literature to support claims for their use. Studies published to date are small, nonrandomized, and often without controls. Results tend to focus on morphologic measurements rather than cellulite appearance. In none of the studies have all the initial patients finished the treatment protocol, and reasons for withdrawal are not given. An unpublished Endermologie study was recently used as evidence to persuade the Food and Drug Administration of the United States to grant a license for "temporary improvement in the appearance of cellulite." There are no published studies to support this claim.

Topically applied aminophylline has been reported to be a safe, pharmacological, and noninvasive treatment for cellulite.[3,4] Aminophylline is an inhibitor of phosphodiesterase, the enzyme responsible for breaking down cyclic adenosine monophosphate. Its use in treating

asthma stems from the bronchial smooth-muscle relaxation that results from the increased levels of cyclic adenosine monophosphate. It also acts as a diuretic by reducing renal tubular reabsorption. Although these and other possible methods of action for its anticellulite activity, including fat lipolysis, have been proposed, none have been proven.5 In 1995, Artz and Dinner 4 showed that systemic absorption of topically applied 2% aminophylline for cellulite treatment was minimal. All 12 patients (no controls) in their study had an improvement in the appearance of their cellulite, and eight showed a thinning of the subcutaneous fat at 3 months. Weight remained static, and thigh circumference decreased by an average of 0.5 cm. However, no actual figures were featured in the results. In 1993, Hamilton et al.3 measured a reduction of between 0.73 and 2.27 cm in all six patients in their study. However, these figures are small in relation to actual thigh girth measurements. Our results do not support these morphologic changes or the effectiveness of aminophylline in cellulite treatment. Adverse reactions to the aminophylline are not mentioned in these or other studies, although the Food and Drug Administration is apparently aware of some patients developing a rash after using the cream; 24 percent of our aminophylline-treated patients developed a rash.

The Endermologie ES1 is a mechanical method of treatment for cellulite. It was developed in France in the 1970s and initially used to relieve muscular aches and to massage and soften burn scars. The proposed method, "aspirated hypodermal mobilization," essentially sucks up a fold of skin and rolls it between two revolving rollers, progressively disorganizing the adipose tissue and gradually smoothing it out over the course of several treatments. This progressive, sublethal damage is similar to the shoulder indentation caused by brassiere straps.6 A nylon body stocking is worn to reduce friction between the rollers and the skin. The Food and Drug Administration recently licensed Endermologie "as effective in the temporary reduction in the appearance of cellulite." Its use has also been recommended as an adjunct to liposuction, particularly after the tumescent technique.7

Ersek et al.6 used Endermologie for "noninvasive body contouring." Only six of their 22 patients completed fourteen 45-minute sessions. All completed at least seven treatments and were encouraged to drink water and maintain a low-fat diet. A mean loss in body circumference measurements was related to the number of treatments, regardless of weight loss or gain, although the results were better if weight was lost. However, no controls were used and the appearance of cellulite was not mentioned. A follow-up study by the same group reported the same conclusion.8 Only 39 of 85 patients in the latter study managed to complete the course of 14 sessions; again, the reasons for withdrawal were not given. Our results suggest that changes in morphologic measurements are related to weight loss alone and not a twice-weekly, localized, 10-minute encounter with an Endermologie machine. Patients willing to complete a 12-week, twice-weekly treatment are much more likely to modify their diet and exercise regimens, consciously or otherwise, to try and gain maximum effect. However, only 10 patients who received Endermologie treatment in the current study perceived any improvement in the appearance of their cellulite, and only three of these patients had lost weight. Adcock et al.9 analyzed the effects of Endermologie in a porcine model and found no decrease in subcutaneous tissue thickness after up to 20 treatments.

We do not believe that either aminophylline or Endermologie is effective in the treatment of cellulite. Most of the benefits are probably derived from the adjuncts of exercise, dietary modification, and increased water intake that most treatments recommend.

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TABLE II

	Age (y)	Weight (kg)	BMI (kg/m^2)
Mean	44.6	67.3	25.7
Median	45.5	64.9	25.3
Standard deviation	11.2	10	3.6
Range	19–70	53.6–93.7	21–38.5

BMI, body mass index.

TABLE II Pretreatment Patient Data
From: Collis: Plast Reconstr Surg, Volume 104(4).September 1999.1110-1114

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TABLE I

Pregnancy
Breast feeding
Cardiac disease
Hepatic disease
Peptic ulcer
Porphyria
Hyperthyroidism
Hematologic disorders/anticoagulation therapy
Aminophylline/theophylline therapy
Drugs with potential interactions with aminophylline
Dermatoses/severe thread or varicose veins
Dieting/recent weight loss
Recent trauma/surgery to thighs/buttocks

TABLE I Patient Exclusion Criteria
From: Collis: Plast Reconstr Surg, Volume 104(4).September 1999.1110-1114

TABLE III

Obesity	BMI(kg/m^2)	No. of Patients
Normal	20–25	25
Grade 1	25–30	30
Grade 2	30–40	14
Grade 3	>40	0

BMI, body mass index.

TABLE III Pretreatment Body Mass Index Distribution
From: Collis: Plast Reconstr Surg, Volume 104(4).September 1999.1110-1114

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Fig. 1

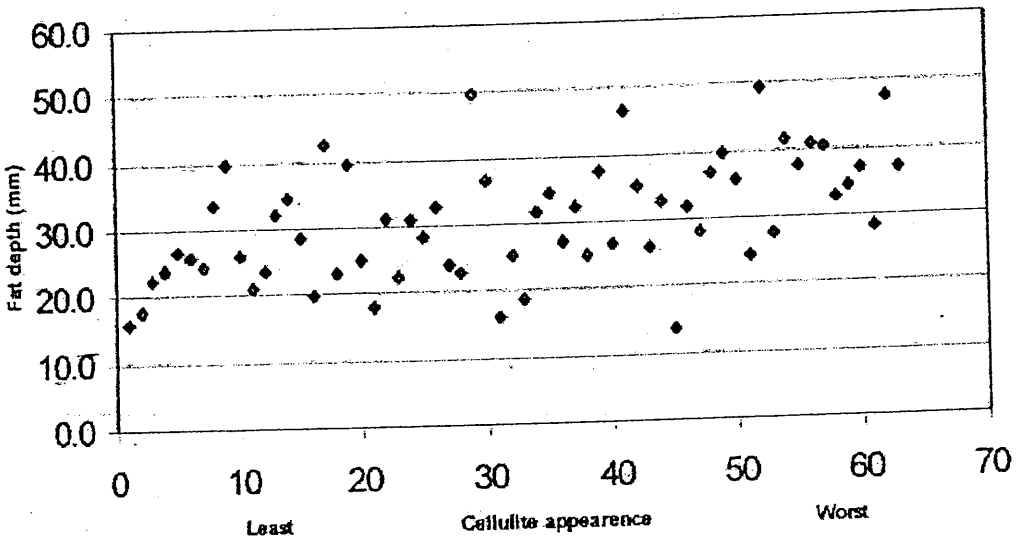

Fig. 1. Fat depth and severity of cellulite appearance. Patients were simply ranked by photographs.
From: Collis: Plast Reconstr Surg, Volume 104(4).September 1999.1110-1114

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Fig. 1

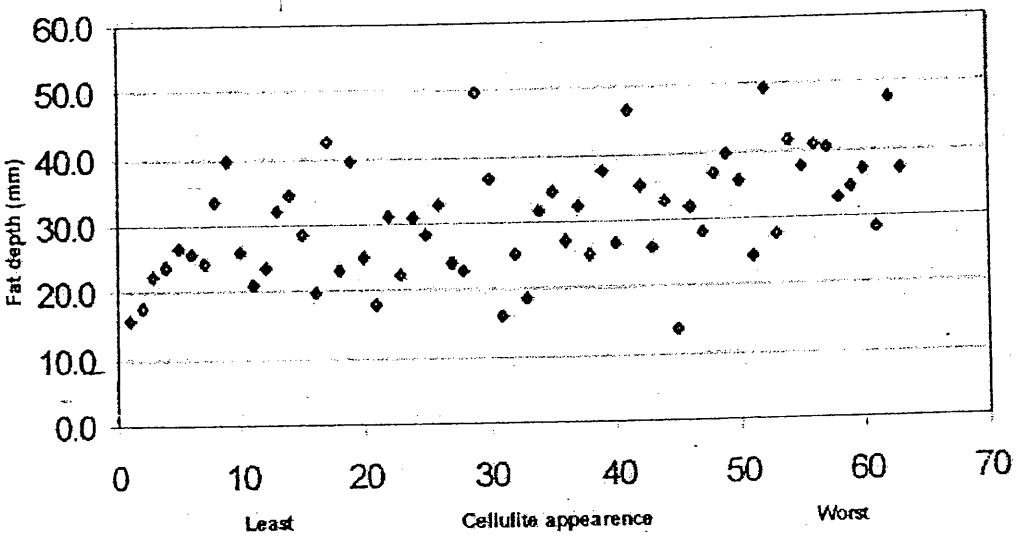

Fig. 1. Fat depth and severity of cellulite appearance. Patients were simply ranked by photographs.
From: Collis: Plast Reconstr Surg, Volume 104(4).September 1999.1110-1114

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Fig. 2

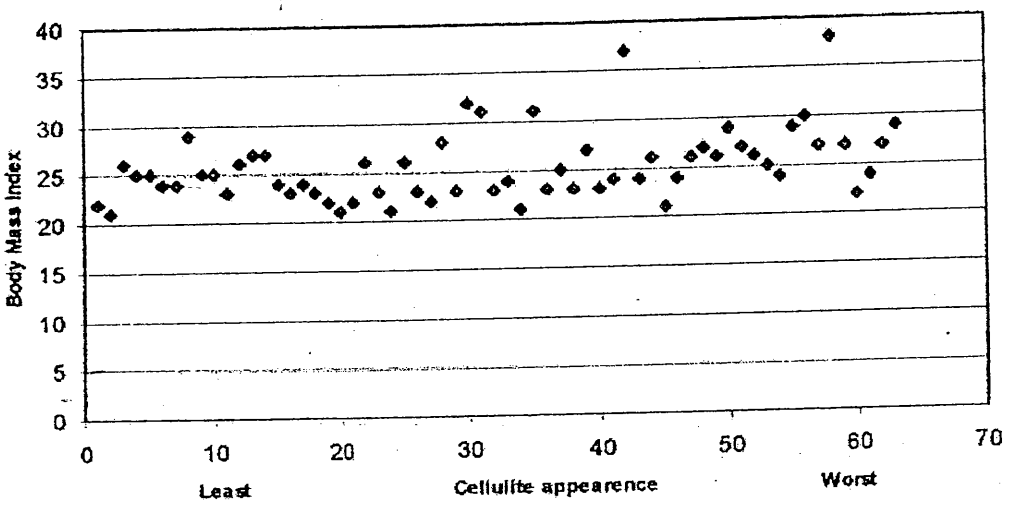


Fig. 2. Body mass index and severity of cellulite appearance. Patients were simply ranked by photographs.
From: Collis: Plast Reconstr Surg, Volume 104(4).September 1999.1110-1114

TABLE IV

	Group 1	Group 2	Group 3
Mean	1.0	−0.1	−0.5
Median	1.2	−0.5	−0.45
Standard deviation	1.47	1.63	1.53
Range	−0.7 to 4.5	−2.3 to 3.9	−4.3 to 1.5

TABLE IV Weight Change (kg)
From: Collis: Plast Reconstr Surg, Volume 104(4).September 1999.1110-1114

TABLE V

	Group 1		Group 2
	Aminophyilline	Placebo	Endermologie
Mean	0.1	0.5	−0.5
Median	−0.2	−0.2	−1.1
Standard deviation	2.6	2.8	3.3
Range	−2.3 to 7.3	−4.3 to 5.9	−7.7 to 5.6

TABLE V Ultrasound Fat Depth Changes (mm)
From: Collis: Plast Reconstr Surg, Volume 104(4).September 1999.1110-1114

TABLE VI

	Group 1		Group 2
	Aminophyilline	Placebo	Endermologie
Mean	0.1	0.5	0.2
Median	0	0.5	0.3
Standard deviation	1.1	1.3	1.7
Range	−3 to 2	−3 to 3	−4 to 3

TABLE VI Changes in Thigh Girth (cm)
From: Collis: Plast Reconstr Surg, Volume 104(4).September 1999.1110-1114

Obesity

Mechanisms and Clinical Management

Robert H. Eckel

LIPPINCOTT WILLIAMS & WILKINS

1

Obesity: A Disease or a Physiologic Adaptation for Survival?

Robert H. Eckel

A disease is a condition of a living plant, animal, or human that impairs normal functioning and implies a condition of ill health. If obesity is a disease—a position that is supported by many, including a number of organizations (1), consensus conferences (2), and experts in the field (3)—it has now reached epidemic proportions. Using the definition of overweight as 25.0 to 29.9 kilograms per meter squared (kg/m^2) and obesity as equal to or more than 30.0 kg/m^2 in adults (4,5), Flegal and Troiano (6) estimate that 26% of the population of the United States is obese and that 61% is overweight. Moreover, the problem of relative adiposity is increasing throughout the world (4). According to Kopelman (4), if the current trend in body mass index (BMI) continues, 40% of the United States population will be labeled obese by 2025.

Adolescents in the 85th and 95th percentiles have been represented as "at risk" and "overweight," respectively (7). However, because the 85th percentile in both children and adolescents approximates a BMI of 25 kg/m^2 and the 95th percentile approximates a BMI of 30 kg/m^2, the most recent recommendation is to use a similar range of BMIs in children as in adults (8). For both adults and children, BMIs of 25 and 30 kg/m^2 are below the mean for many segments of the population, including Hispanics, Native Americans, and African Americans (9,10). If this "disease" were virally induced as was suggested by Dhurandhar et al. (11), by now Congress would have funded research for developing antiviral drugs, analogous to the funds which it provided for human immunodeficiency virus and the related acquired immune deficiency syndrome in the last decade. However, despite the increasing attention to obesity as a "disease" of increasing prevalence, the solutions seem even further away.

OBESITY AS A DISEASE?

Definition of the Disease

Adipose tissue is the body's largest energy reservoir. The triglyceride storage pool in the average normal-weight man and woman with 15% and 25% adipose tissue, respectively, amounts to 10 and 15 kg, respectively. In the average normal-weight man and woman, total body fat stores amount to approximately 88,000 and 132,000 kcal (about 370 and 555 MJ), respectively. This is about three to six times the amount stored as protein (roughly 24,000 kcal or 100 MJ). These fuel depots are used to meet the needs of tissues during exercise, stress, and periods of food deprivation lasting from 60 to 90 days (12,13).

To classify a condition as a disease, typically a pathologic basis is needed. However, unlike pulmonary thromboembolism, rheumatoid arthritis, or hepatitis C, conditions for which criteria for diagnosis are reasonably well established, the definition of obesity is

limited to the BMI and to increases in adipocyte cell size (hypertrophy) and/or cell number (hyperplasia) (3). If an increase in adipocyte volume alone would be considered diagnostic, corresponding criteria have not yet been established. Moreover, even if criteria with an acceptable level of sensitivity were established, increases in adipocyte volume might be present in some regions of adipose tissue even though the individual's BMI is less than 25 kg/m^2. The use of adipocyte number for diagnostic assessment is even more difficult because hyperplasia is typically preceded by hypertrophy (14), a generalization that is open to question. Examples to the contrary from transgenic rodents include mice with adipose tissue–specific overexpression of the insulin-mediated glucose transporter GLUT-4 (15) or the α_2-adrenergic receptor (16). In summary, the pathologic criteria that are needed to label obesity as a disease have not yet been established.

Single Gene Defects

Substantial evidence indicates that obesity rarely results from single gene mutations; it is instead polygenic. However, in this genetic age, an increasing number of single gene defects responsible for the obese phenotype have been identified in humans. As Chapter 2 outlines in more detail, most of the known single gene defects are associated with other phenotypes including mental retardation, endocrine (reproductive) disorders, and/or malformations (see Table 2.4 in Chapter 2). Today, the most common single gene mutation associated with obesity in the absence of mental retardation is that of the melanocortin-4 receptor (MC4R) (17). A number of other mutations have also been identified, and the related hyperphagic obesity can present with either dominant or recessive patterns of inheritance (18). The consequences of mutations in MC4R do not appear to be accompanied by other pathophysiologic defects. Because of the high prevalence of this genetic modification (i.e., represents about 4% of patients with severe obesity [17]) and in the absence of accompanying morphologic and/or functional abnormalities, this mutation could perhaps be viewed as "beneficial" for survival rather than as harmful. Of course, this advantage is appreciated only in environments in which adequate sources of energy intake are not available.

Comorbidities as Diseases

When lifespan continues into the eighth and ninth decade, the consequences of excess body fat are anything but advantageous. As other chapters in this text relate, obesity is either directly or indirectly associated with an increased incidence and prevalence of heart disease and stroke (Chapter 8), obstructive sleep apnea (Chapter 9), type II diabetes mellitus (Chapter 10), dyslipidemia (Chapter 16), hypertension (Chapter 11), hepatobiliary disease (Chapter 12), cancer (Chapter 13), endocrine disorders (Chapter 14), psychosocial disturbances (Chapter 15), and orthopedic complications (Chapter 17). Clearly these outcomes are measurable not only clinically but also pathologically in both gross and microscopic examinations.

Using relative hazards associated with elevated BMI in six United States studies (Alameda Community Health Study, Framingham Heart Study, Tecumseh Community Health Study, American Cancer Society Cancer Protection Study 1, National Health and Nutrition Survey 1 Epidemiological Follow-Up Study 1, and Nurses Health Study), the national distribution of adult BMI, and the estimates of population size and total deaths from the same period, Allison et al. (19) calculated that the annual number of deaths attributable to obesity was 280,000. When hazard ratios were calculated from data for nonsmokers or never-smokers only, this figure was increased by 16% and by 34% in

TABLE 1.1. *Estimates of obesity-attributable mortality in United States adults*

Rank	Cause of death	Number	Role of obesity
1	Diseases of the heart	725,192	+++
2	Malignant neoplasms	549,838	++
3	Cerebrovascular diseases	167,366	++
4	Chronic lower respiratory diseases	124,181	+
5	Accidents	97,860	+
6	Diabetes mellitus	68,399	+++
7	Influenza and pneumonia	63,730	+
8	Alzheimer disease	44,536	0
9	Nephritis, nephrotic syndrome, and nephrosis	35,525	+
10	Septicemia	30,680	+
11	Intentional self-harm (suicide)	29,199	+
12	Chronic liver disease and cirrhosis	26,259	+
13	Essential (primary) hypertension and hypertensive renal disease	16,968	++
14	Assault (homocide)	16,889	0
15	Aortic aneurysm and dissection	15,807	++
16	Other	378,970	+
	TOTAL	**2,391,399**	

The approximate role of obesity, from 0 to ++++, in the pathophysiology for each of the causes of death is simply an estimate.

Data are for the 15 most common causes of death + "Other" in 1999 and the estimated contribution of obesity based on the prevalence of obesity in each of the categories of disease.

Data from Hoyert DL, Arias E, Smith BL, et al. Deaths: final data for 1999. *National Vital Statistics Reports* 2001;49:1–113, with permission.

"ostensibly healthy weight-stable nonsmokers or never smokers." Although these deaths were variably attributable to underlying diseases, such as coronary heart disease, stroke, and diabetes in Framingham (20), obesity was rarely listed as the cause of death; it was more likely to be noted as the associated comorbidity.

When the prevalence of obesity-related comorbidities is examined, age-based and gender-based distributions must be considered. In addition, the criteria used to stipulate the specific comorbidity must be specified. Nevertheless, when estimates of the contributory role of obesity are made for the causes of death in 1999 (Table 1.1), the importance of obesity in contributing to mortality through a number of different pathophysiologic mechanisms may be identified. Recently, criteria for the metabolic syndrome were developed by the National Cholesterol Education Program Adult Treatment Panel III (21). For diagnosis, three or more of the following five components must be present: (a) a waist circumference greater than 102 cm for men and more than 88 cm for women; (b) a fasting triglyceride level higher than 150 mg per dL; (c) a high-density lipoprotein (HDL) cholesterol level less than 40 mg per dL for men and less than 50 mg per dL for women; (d) blood pressure higher than 130/85; and (e) a fasting serum glucose concentration greater than 110 mg per dL. Based on these criteria, the age-adjusted prevalence of the metabolic syndrome in adults 20 years or older is 23.7% (22). Based on data from the 2000 census, 47 million United States citizens are thus afflicted. All of the criteria for the metabolic syndrome point to obesity as an underlying disorder.

Basis for the Definition of Obesity as a Disease

The National Institutes of Health held a Consensus Development Conference on the Health Implications of Obesity in 1985. After presentations by 19 experts in relevant areas of obesity science, a panel of 15 impartial senior-level professionals came to the conclusion that obesity is a disease (2). Although the panelists agreed that the amount of

body fat is a continuum within populations, the conclusion that was reached was that an increase in body weight of 20% or more above the desirable body weight is associated with a plethora of comorbidities in addition to excess mortality and that it thus constitutes a health hazard. The panel did note that the precise determination of body fat requires technically sophisticated methodologies that are not readily available to most clinicians and that BMI as an assessment of body fat has limitations. Despite this limitation, because significant health risks (e.g., diabetes, hypertension, heart disease, and others) can occur in some individuals at lower percentages of increased body fat, clinical concern about excess adiposity was extended to this population. (Even though this manuscript is frequently cited in support of approaching obesity as a disease, the term disease is never mentioned in the manuscript.)

The value of this decision by a group of "unbiased" professionals centers in the current thrust of the "obesity as a disease" argument regarding health care reimbursement for a disorder that was estimated in 1998 to amount to 5.5% to 7.8% of total health care expenditures (23). If obesity were considered a disease, early therapeutic and preventive strategies to diminish this epidemic would be implemented and reimbursed. Presently, reimbursement almost always relates to the comorbidities of obesity, such as hypertension, obstructive sleep apnea, and dyslipidemia. Recently, the metabolic syndrome has been given an *International Classification of Diseases* code (in the ninth revision), providing yet another disease category under which reimbursements for some obese patients may be filed. Moreover, even though the Internal Revenue Service now considers the expenses for obesity evaluation and treatment to be medically related tax deductions (24), the high level of expenditures that is necessary for health claims to receive deductions is unlikely to provide the financial incentive that is necessary for most obese individuals to seek additional attention.

In the United States, the importance of obesity as a health problem was highlighted in *Healthy People 2010,* a comprehensive nationwide health-promotion and disease-prevention program orchestrated by the United States Department of Health and Human Services (25). In late 2001, the Surgeon General's call to action notably highlights the need to prevent an increase in overweight individuals and obesity in late 2001 (26). Clearly, the lobbying effort of both professional organizations and lay groups is directed towards future reimbursement for obesity as a disease. The bigger concern is whether the health care system can absorb these costs.

Presently the health care budget in the United States is about $190 billion (27). Using these figures and an average estimate that 6.7% of this budget is obesity related, obesity-related expenses could result in a cost of $12.7 billion. Of course, some of these expenses are presently covered under obesity-related comorbidities, but many are not. These would then be added to the economic burden indicated by the following figures. Each year, about $33 billion is spent in the United States on weight-loss programs, including dietary, exercise, and behavior modifications (28). In fact, a recent bill to prevent obesity that was proposed by Senators Frist, Bingaman, and Dodd ("Improved nutrition and physical activity act" or "Impact," available in May 2002) estimated the annual cost of obesity in the United States as $117 billion. Although the source for this amount is not stated in the bill, incorporating even a small proportion of such costs into health care reimbursement is unthinkable.

If obesity is not a disease but rather a metabolic adaptation for survival in settings of food deprivation, would this fact affect the view of health care economists? The answer may be yes; however, if a preventive strategy is accompanied by sufficient evidenced-based documentation that obesity prevention or treatment modifies hard outcomes (e.g., death, myocardial infarction, stroke, and type II diabetes), the result may

be not only clinically effective but also cost saving. Ultimately, this may translate into the same type of "quality-adjusted life-year saved" assessments that have been applied in other areas of medicine. Recent examples include $10,983 per life-year saved for repeated colonoscopy for colorectal carcinoma screening beginning at 50 years of age (29) and $22,256 per life-year saved for hepatitis A vaccines administered to patients with chronic hepatitis C at 30 years of age (30). To reach an "acceptable" median level of cost of $42,000 per life-year saved (31) for obesity using the assumption of the present 26% prevalence of obesity, the cost would be about $14,000 for each life-year saved if only 10% of the obese population were benefitted. The debate about whether obesity is a disease may then be irrelevant when this approach to obesity outcome–related expenditures is used.

OBESITY AS A SURVIVAL ADVANTAGE

Despite the magnitude of the health problem of obesity and its comorbidities today, substantial evidence from history illustrates that the consequences of obesity are far from unfavorable. Adipose tissue remains the predominant storage depot of energy as triacylglycerol. As was noted above, if typical fat depots of 10 and 15 kg for the average adult man and woman, respectively, are assumed, the energy stored therein is sufficient for 60 to 90 days of starvation at a level of energy expenditure of 6 MJ a day (12,13). Energy is also available from protein, but the amount of stored protein can provide only approximately one-half of the stored quantity of protein before life-threatening loss of lean tissue ensues (32). However, expanded adipose tissue mass preserves protein mass (33) (Fig. 1.1).

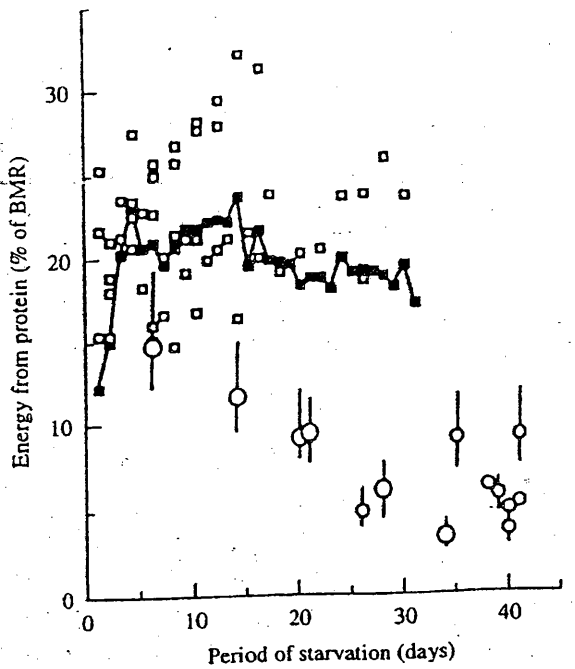


FIG. 1.1. The effect of starvation in lean subjects (*solid squares*) from the Benedict study (179) and from other studies (*open squares*) versus obese subjects (*open circles* from groups of subjects and *small squares* from individual subjects) in the studies of Elia (32) and Elia, Stubbs, and Henry (128). (From Elia M. Hunger disease. *Clin Nutr* 2000; 19:379–386, with permission.)

Adipose Tissue Functions

Adipose tissue has other important functions that are both related to and independent of energy storage (34) (Table 1.2). These include the synthesis and secretion of various proteins that regulate adipose tissue fuel flux, insulin sensitivity, vasomotor tone, cell turnover, inflammation, coagulation, and conversion of androgens to estrogens. Many of these proteins (i.e., those that regulate insulin action, cell proliferation, thrombosis, vascular reactivity, and the inflammatory response) may be related to the survival advantage of obesity.

In addition to periods of food deprivation, other important periods of energy provision include pregnancy and lactation. Low maternal weight before pregnancy and poor weight gain during pregnancy increase the prevalence of low–birth-weight infants (35,36). Although lactation does occur in the absence of abundant adipose tissue stores, energy intakes must increase with a body composition of this type in order to maintain breast milk quantity and quality (37).

In addition, adipose tissue acts as insulation and protects against the adverse effects of cold air or water (38). It also has an important role in fertility. The age at which menarche occurs is at least partially attributable to adipose tissue content (39,40), and, as fat mass decreases with exercise or eating disorders, oligomenorrhea and amenorrhea may result (41). Although the mechanisms for the reproductive function of adipose tissue remain controversial, leptin appears to be important. Not only is leptin able to induce reproductive maturation in female rodents (42), but the decrease in leptin that occurs with weight reduction and loss of adipose tissue mass appears to be partially responsible for the altered function of the hypothalamic–pituitary–ovarian (testicular) axis (43). Bone

TABLE 1.2. *Roles of proteins secreted from human adipose tissue*

Regulation of adipose tissue fuel flux	
Adenosine	Kather et al. (182)
Leptin	Friedman and Halaas (150)
Acylation-stimulating protein	Sniderman et al. (183)
Regulation of insulin action	
Adiponectin	Weyer et al. (184)
TNF-α + soluble receptor	Moller (185)
Regulation of vasomotor tone	
Angiotensinogen	Van Harmelen et al. (186)
Angiotensin-converting enzyme	Gorzelniak et al. (187)
PGI_2	Fink et al. (188)
Regulation of cell turnover	
PGI_2	Negrel et al. (189)
TGF-β	Alessi et al. (190)
IGF-I	Wabitsch et al. (191)
Regulation of coagulation	
Plasminogen activator inhibitor-1	Crandall et al. (192)
PGI_2	McCarty (193)
Regulation of inflammation	
TNF-α + soluble receptor	McDermott (194)
Interleukin-6	Hirano et al. (195)
Adipsin	Esterbauer et al. (196)
Steroid conversion, reproduction, bone mass	
Cytochrome P450–dependent aromatase	Bulun et al. (197)
17β-hydroxysteroid oxidoreductase	Crobould et al. (198)
Other	
Agouti signal protein	Voisey et al. (199)

Abbreviations: IGF, insulin-like growth factor; PGI, prostaglandin I; TGF, transforming growth factor; TNF, tumor necrosis factor.

mass and adipose mass are also highly related (44), and obesity protects against the development of osteoporosis (45). When osteoporosis occurs in obese patients, a workup for Cushing syndrome is mandatory.

Environmental Influences on Adipose Tissue Mass

The present environment of food availability and decreased physical activity favors an increase in adipose tissue mass. Although evidence from adoption and twin studies support a genetic basis for body fatness (46–48), the gene pool has changed little, so this does not explain the epidemic of overweight individuals and obesity that has been encountered in the last two decades. Even if genetics could explain a large percentage of the overweight and obesity epidemic, the literature is mixed regarding which component of energy balance is etiologic. Moreover, variable data on the contributions of alterations in energy expenditure to changes in fat mass over time do not clarify the mechanism of the genetic impact (49). Thus, the environment must be examined as the cause.

Influences of the environment on body fatness could work through increases in energy intake and/or decreases in energy expenditure. As Chapter 3 reviews, data on food intake are contradictory, with some studies showing no change in caloric consumption (50,51) and others demonstrating increases (52,53). Evidence supporting a higher consumption of dietary fat as etiologic is equally as unconvincing as that which demonstrates no relationship between dietary fat and weight change (54,55). Some believe that the overconsumption of dietary carbohydrates resulting from the "fat is bad" mentality of the late twentieth century is etiologic (56,57). However, when examining intakes for populations, caution must be used in making conclusions about cause and effect; for example, using such an approach, the increase in overweight and obese individuals can be attributed to the consumption of diet beverages. Reductions in physical activity also contribute to the positive energy balance that, in turn, results in increases in weight and adipose tissue over time (58). Activity data, which have been collected only in the United States since 1985, reveal that 60% of the United States population has no regular pattern of physical activity and that 25% reports no physical activity (59). Many people in the United States and in the rest of the civilized world are increasingly "desk bound" in their occupations, and this lifestyle is supported by the many advances of the modern world.

Substantial evidence from migratory patterns of populations indicates not only that food is more available and more energy dense but also that the physical activity profiles of past generations have been exchanged for a more sedentary lifestyle. Examples from these include the Samoans within the Samoan archipelago and Hawaii (60), the Pima Indians of Mexico and Arizona (61), the Japanese-American immigrants (62), and the West African Diaspora and its migrations out of Africa to the United Kingdom and the United States (63). In these instances, fatness results when the stresses of life in a more deprived setting are replaced by the conveniences of the modern world. One conclusion that seems tenable is that the body creates an excess adipose tissue reservoir in preparation for less favorable environmental conditions.

Another relatively recent example of the impact of the environment on body weight regulation and obesity prevalence was the experience of the Dutch population during the famine of World War II (1944 to 1945). During this 6-month period of food rationing in the Netherlands, infant size was substantially decreased and infant mortality was significantly increased (64). A similar experience was seen in Leningrad and Odessa at the same time (65). Moreover, when food deprivation in the Netherlands was greatest during the third trimester, the incidence of obesity in the offspring was decreased, whereas food

deprivation in the first trimester produced a much higher rate of obesity in the offspring (66). The authors concluded that the early deprivation likely caused damage to the hypothalamic centers, which regulate food intake and growth; when deprivation occurs later in gestation, a defect in adipogenesis may be responsible. This experience suggests that the regulation of adipose tissue mass at least partially relates to the intrauterine environment and that it may differ during the course of the prenatal period.

Overfeeding and Obesity

The response to forced overfeeding, which is somewhat unrealistic, remains one way to determine the genetic predisposition to overweight and/or obesity. In general, overfeeding rodents results in weight gain and adipose tissue accumulation; however, this response is typically dependent on the rodent strain (67) and likely on the thermogenic response of the rodent to overfeeding (68). In female baboons that are overfed during infancy, hypertrophic obesity develops after puberty (69). However, variable amounts of intragastric overfeeding in adult male rhesus monkeys resulted in weight gain, but this was also accompanied by reductions in *ad libitum* food intake (70). When the intragastric overfeeding period was discontinued in these monkeys, normal energy intake stabilized over a period of several months and their body weights dropped rapidly. Some monkeys returned to their initial body weight, whereas some net weight gain occurred in others.

A number of overfeeding experiments have been performed in humans. Bouchard et al. (71) performed one of the the longest and best studies for determining the genetic basis of the metabolic and anatomic response to excess calories. In his study, 12 pairs of identical twins were overfed by 84,000 kcal over 100 days. The average weight gain was 8.1 kg, but a range of 4.3 kg to 13.3 kg was observed. Although 63% of the excess calories were stored, predicting an increased cost of weight maintenance at an expanded body weight (72), about one-third of the excess calories were not stored, thus implying that thermogenic mechanisms in response to overfeeding also occurred. Twin pairs were similar in their response, with three times more variance in weight gain and in the increase in adipose tissue among pairs than within pairs. An even greater similarity within twin pairs was noted in the changes in regional adipose tissue mass. Moreover, within 4 months of overfeeding, 82%, 74%, and 100% of the overfeeding gain in body weight, fat mass, and fat-free mass, respectively, were lost (72).

The classic overfeeding studies of Sims et al. (73) also demonstrated that massive caloric overfeeding, which, in this experiment, was accompanied by substantial increases in physical activity, resulted in variable weight gain; an increased thermogenic response; and, for most participants, resumption of their initial body weight after overfeeding was discontinued. More recently, research subjects at the Mayo Clinic who were overfed 1,000 kcal per day for 8 weeks experienced increases in fat from 58 g to 687 g and in fat-free mass from 17 g to 78 g (74). Although energy expenditure increased in most subjects, this ranged from −100 kcal to +360 kcal. The greatest predictor of the change in weight and fat mass during this relatively brief period of overfeeding was the individual's increase in non–exercise-associated thermogenesis (NEAT), or fidgeting. Presently the genetic basis of NEAT remains undefined, but NEAT may be extremely important in determining the response to the environmental factors that lead to obesity regardless of whether it results from the expression of one or many genes.

Reduced Obesity Predicts Resumption of the Obese State

Achieving weight reduction is difficult for obese patients, and it may be more difficult to sustain (75–77). The term reduced obesity defines the behavioral and metabolic status of an obese person or animal after weight reduction and isocaloric weight maintenance. Similar responses may also occur in weight-reduced normal-weight organisms. Although these variables and intervals are probably influenced by the amount of weight reduction and the duration of the weight-reduced state among other factors, they are poorly defined and thus they require additional elucidation.

In general, the adaptations of the reduced obese state appear to work in a manner that predicts resumption of the obese weight (Table 1.3). After successful weight reduction and months of maintenance of the reduced obese state, increases in appetite (78,79) and a preference for energy-dense foods (i.e., those containing fat and sugar) (80) are observed. This increase in appetite may be partially related to decreases in leptin, which in at least one study (81) predicted the increase in body weight after weight reduction; other studies (82,83) have not reported the same effect. In a recent report, Cummings et al. (84) suggest that changes in the gastrointestinal hormone ghrelin may also contribute to weight regain after successful diet-induced weight reduction. Normally, levels of ghrelin increase before the meal and fall after the meal; however, Cummings et al. (84) found that during maintenance of the reduced obese state, 24-hour areas under the curve for ghrelin were actually increased, rather than decreased, compared to the baseline.

Regain of weight is also favored by changes in energy expenditure. With weight reduction, the basal metabolic rate falls in proportion to the loss of lean body mass (85). In many subjects, the energy expended in the form of physical activity does not increase (86–88). Klem et al. (89) gathered a nonrandom sample of "successful" reduced obese subjects into the National Weight Loss Registry. In more than 90% of these subjects, a combination of a diet restricted in fat and exercise of more than 500 kcal daily was necessary to maintain a BMI of 25 kg/m^2 (90,91).

Finally, isocaloric maintenance of the reduced obese state modifies the physiologic processes that promote fat storage. This includes increases in insulin sensitivity (92,93), decreases in fat oxidation (94,95), and tissue-specific changes in lipoprotein-lipase (LPL) activity. Although increases in insulin sensitivity after weight reduction and the maintenance of the reduced obese state have been shown to predict weight regain (96), conflicting reports have been made (5,97).

TABLE 1.3. *The reduced obese state: possible predictors of weight regain*

Increased appetite	Doucet et al. (78)
Decreased leptin	Mavri et al. (81)
Increased ghrelin	Cummings et al. (84)
Preference for energy-dense foods	Drewnowski and Holden-Wiltse (80)
Reductions in energy expenditure	
Basal metabolic rate	Astrup et al. (85)
Physical activity	Weigle (87)
Increased insulin sensitivity	Yost et al. (96)
Increased respiratory quotient	Froidevaux et al. (94)
Changes in LPL	
Increased adipose tissue LPL	Schwartz and Brunzell (100)
Decreased skeletal muscle LPL	Eckel et al. (101)

Abbreviation: LPL, lipoprotein lipase.

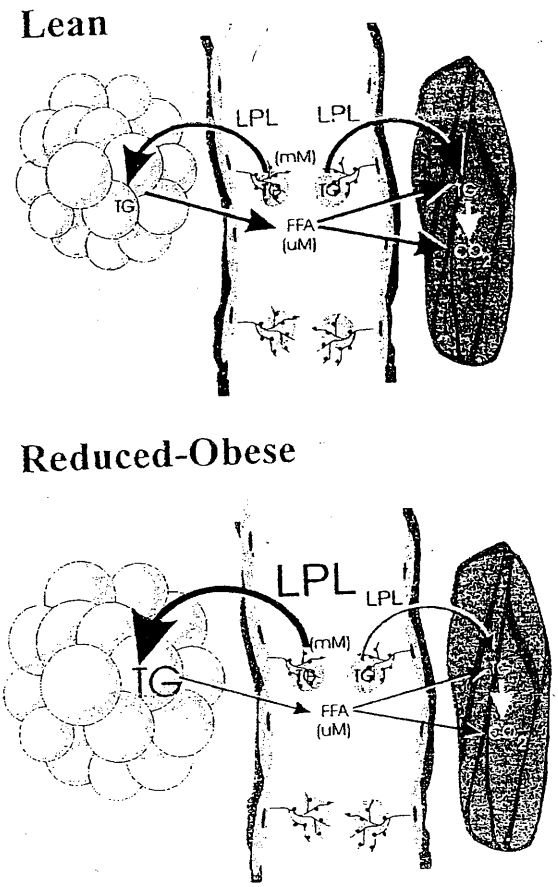


FIG. 1.2. Tissue-specific changes in lipoprotein lipase (LPL) in the reduced obese state. LPL, which is shown bound to the glycocalyx of capillary endothelial cells, hydrolyzes the triglyceride (TG) core of circulating TG-rich lipoproteins, very low density lipoproteins, and chylomicrons, resulting in the production of free fatty acids (FFA) and monoacylglycerol, which are then taken up by adipose tissue and muscle. There the FFAs are either stored (adipose tissue) or stored and/or oxidized (muscle).

LPL hydrolyzes the triglyceride core of circulating triglyceride-rich lipoproteins to provide fatty acid fuels for the LPL-producing tissues of the body, including adipose tissue and muscle. After 3 months of sustained weight reduction, the activity of the fasting enzyme in adipose tissue either remains unchanged or increased (98–100), and the response of LPL to insulin and meals is increased (98). In skeletal muscle, LPL levels are reduced in comparison to the levels that were present before weight reduction (99). These changes in macronutrient partitioning and presumably in storage (Fig. 1.2) do not occur in a vacuum; instead, they are permitted by a setting in which energy intake is greater than energy expenditure.

Overall, these changes in behavior and metabolism are probably important for explaining the relatively low success rate of sustained weight reduction, and they point to the potential role of obesity in defending the organism during food deprivation.

Evidence that Obesity Promotes Survival

Death from starvation is almost always accompanied by marked, if not complete, loss of adipose tissue (102). This is illustrated by adult necropsies performed during the Irish famine of 1847 (103), World War I (104), and World War II (105), as well as in

children who were victims of starvation in Kharkov (106). In general, the loss of subcutaneous adipose tissue precedes the loss of fat located elsewhere or of muscle mass (105) (Fig. 1.3). During prolonged periods of food deprivation, the amount of weight reduction varied from 15% over 5 months (107) to nearly 25% over 3 years (108) in World War I, from 22% to 26% during the Russian famine of 1920 to 1922 (109), and from 9.3% to 13.6% among Parisian civilians during World War II (110). More recent studies have ascertained that, for men, a reduction in body fat to less than 4% and in fat mass to less than 2.5 kg reaches a level that is inconsistent with good health (111). Generally, this results in a BMI of about 13 kg/m^2, a level of estimated fatness that separates survivors from nonsurvivors in men. In women, a much greater variability in BMI is seen in survivors versus nonsurvivors (13) (Fig. 1.4). However, more recent data provided by the famine in Somalia suggest that a BMI of less than 10 kg/m^2 can support life as long as the individual receives specialized care (12). However, in Somalia, starving male patients had more severe edema and a poorer prognosis than females at any given level of severity of starvation.

As the previous statement suggests, *women* appear to withstand semistarvation and starvation better than *men*. In addition to the experience in Somalia, other examples include the 1941 to 1942 famine in the Greek cities of Athens and Piraeus (112) (Fig. 1.5), the German siege of Leningrad from 1941 to 1942 (113), and the Dutch famine in 1945 (114) (Fig. 1.6). Although the data accrued from these unfortunate incidents of history are far from satisfactory, the percentage increase in starvation-related mortality in Greece was lower in women than in men. In Leningrad, the peak incidence of and the rise in death mortality were delayed by 2 to 5 months in women versus men. In the Netherlands, the mortality for men increased by 169% while that for women was only

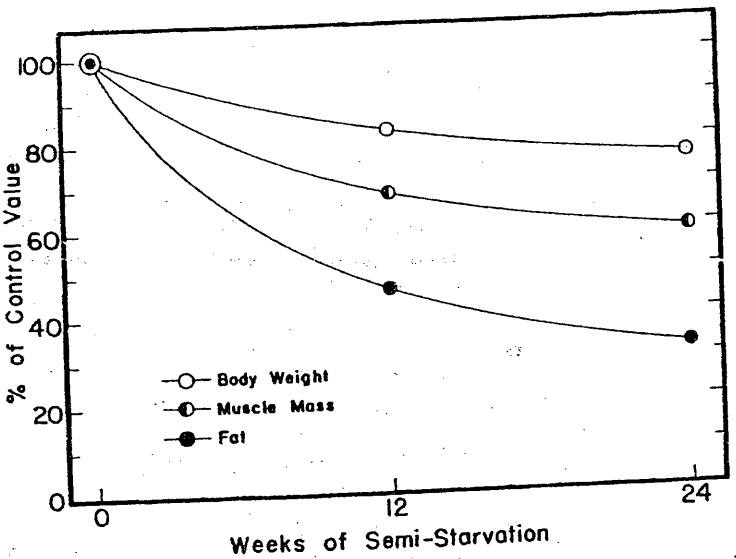


FIG. 1.3. Percentage changes in body weight, muscle mass, and fat during semistarvation in the Minnesota experiment. In this experiment, 32 men weighing 69.3 kg with a body composition of 13.9% fat at baseline voluntarily ingested an average of 1,570 kcal per day for 24 weeks. Body fat was estimated by specific gravity. (From Keys A, Brozek J, Henschel A, et al. *Body fat in biology of human starvation*. Vol. 1. Minneapolis: University of Minnesota Press, 1950:161–183, with permission.)

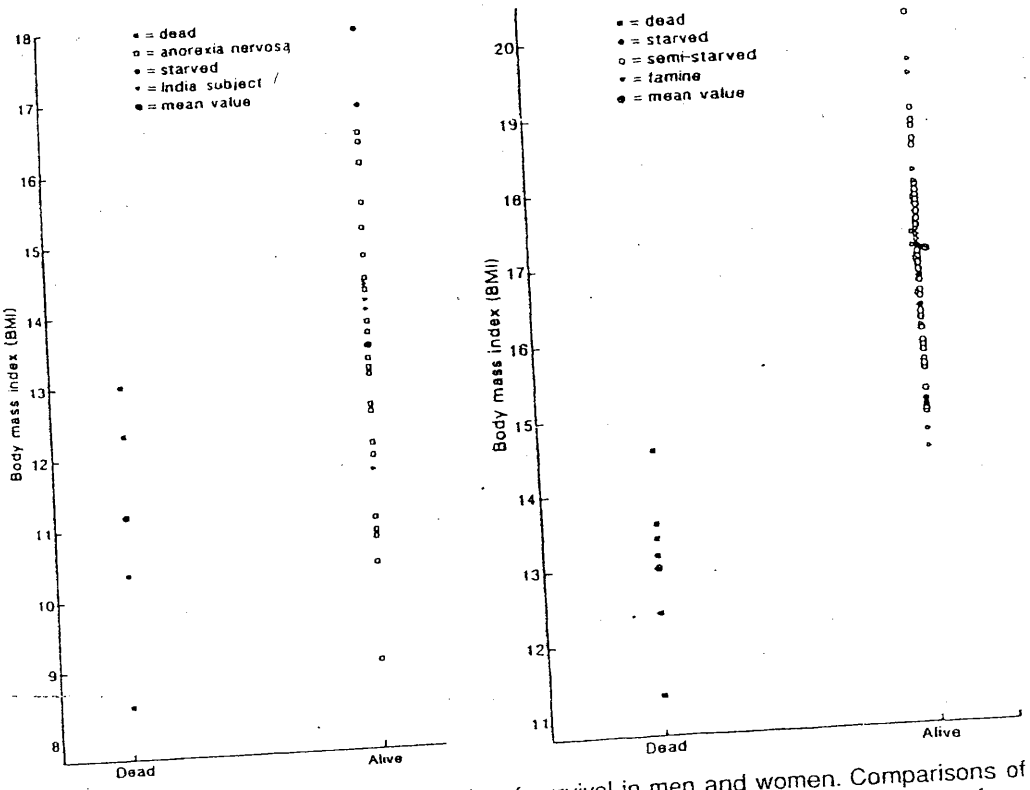


FIG. 1.4. Body mass index (BMI) and limits of survival in men and women. Comparisons of BMIs of male and female survivors versus those of nonsurvivors are portrayed. (Data from Henry CJK. Body mass index and the limits of human survival. *Eur J Clin Nutr* 1990;44: 329–335, with permission.)

72%. One of the factors that most likely contributes to the relative survival advantage of women versus men is the increased fat stores in women. Moreover, the smaller fat cells and reduced metabolic activity of pelvic versus abdominal fat provide a survival advantage in periods of starvation, and they could protect against the pathologic sequelae of central adipose tissue deposition that is more typical in men during periods of caloric excess.

In the "Minnesota Experiment," or semistarvation experiment, of Keys et al. (102), the adipose tissue dramatically responded to refeeding, predicting the following return of body weight (Fig. 1.7). In more recent experiments, the pattern of lean and fat tissue deposition during the refeeding period appears to be due to individual differences in energy partitioning; in other words, the disproportionate gain in fat versus lean tissue is a consequence of a relative greater reduction in thermogenesis that enhances energy efficiency (115). This metabolic efficiency may actually occur in response to low energy intakes (116), although this view is controversial (117,118).

Until the late eighteenth century, individual life expectancy was only 25 to 35 years (119). Even at the beginning of the twentieth century, the average lifespan in the United States was less than 50 years (120). By the early 1900s, survival increased to 55 years (121), and for those born in the G7 countries today (i.e., Canada, France, Germany, Italy, Japan, United Kingdom, United States), the progressive decline in mortality predicts a longevity of nearly 80 years (122). In fact, in a recent and provocative report by Oeppen

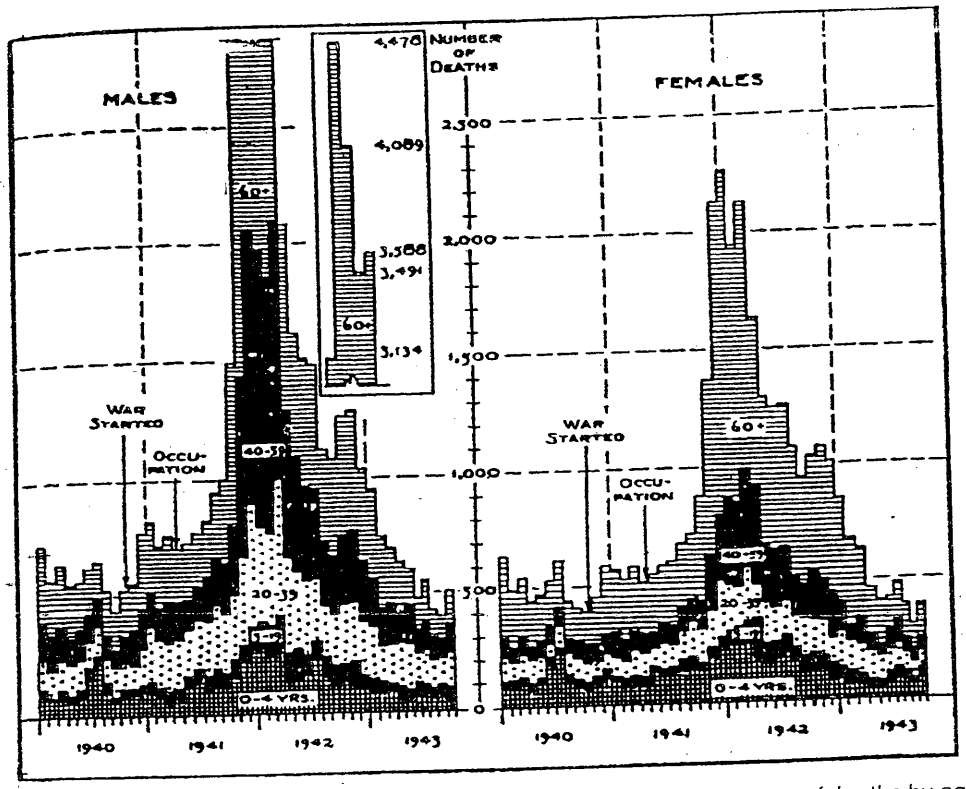


FIG. 1.5. Effects of famine on the population of Greece. Shown is the number of deaths by age for men and women in Athens and Piraeus during the period of World War II, 1940–1943. (From Valaoras VG. Some effects of famine on the population of Greece. *Milbank Memorial Fund Quarterly* 1946;24:215–234, with permission.)

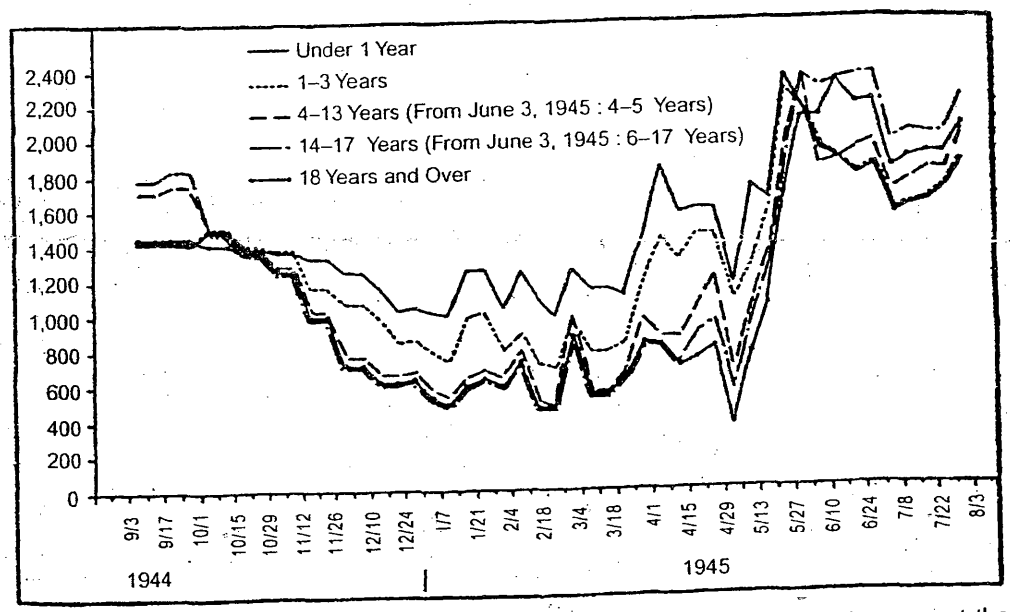


FIG. 1.6. Average calories per day for various age groups. The data portrayed represent the average calories per day by weekly periods for food rations distributed daily to various age groups in the Western Netherlands from September 3, 1944 to August 5, 1945. (From Dols MJL, van Arcken DJAM. Food supply and nutrition in the Netherlands during and after World War II. *Milbank Memorial Fund Quarterly* 1946;24:319–355, with permission.)

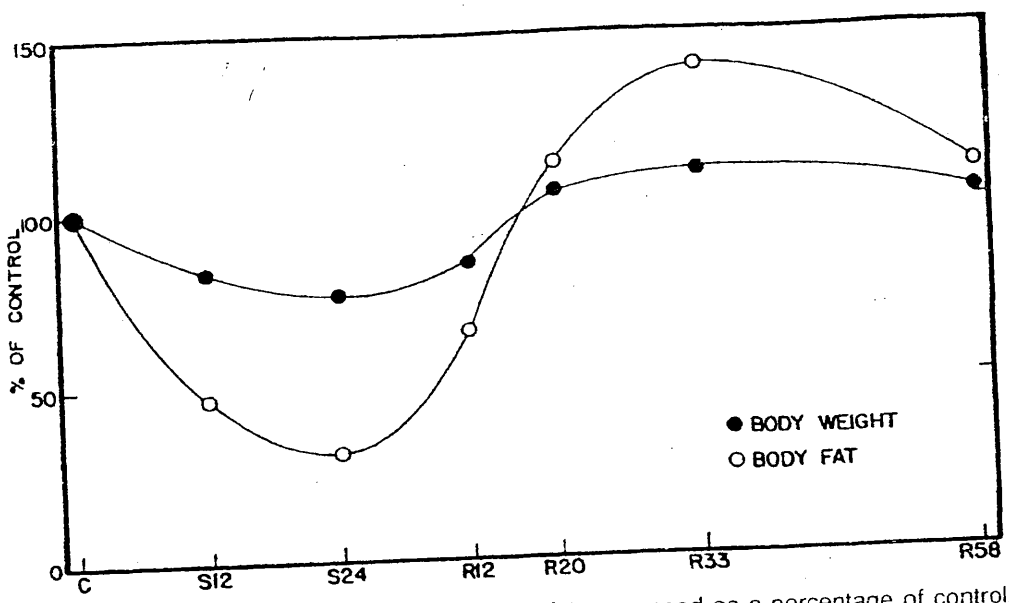


FIG. 1.7. The recovery of body weight and body fat, expressed as a percentage of control, after 24 weeks of semistarvation (1,570 kcal per day) in 32 men in the Minnesota Experiment. During the first 12 weeks of rehabilitation (R1–R12), subjects were divided into four groups consisting of four different caloric levels ranging from 2,378 to 3,392 kcal per day (average 2,896 kcal per day) with or without protein and vitamin supplementation. (From Keys A, Brozek J, Henschel A, et al. *Body fat in biology of human starvation.* Vol. 1. Minneapolis: University of Minnesota Press, 1950:161–183, with permission.)

and Vaupel (123), they indicate that, if the current trends in survival continue, by 2070 the average lifespan could be extended by 12 to 15 years. Although the increasing incidence and prevalence of obesity may ultimately modify this trend, the data available today for obese individuals older than 65 years of age may not indicate an earlier demise (124). However, because a maximum lifespan exists for all mammals (Fig. 1.8), death rarely occurs without it being attributed to one or more "natural or degenerative causes." Although senescence is accepted as the biology of aging, death is still attributed to the failure of one or more organs, and the increased survivorship of these times is largely a function of medical intervention (120).

Evidence indicates that obesity prolongs survival in periods of food deprivation (102,125). In the Irish hunger strikes, the lifespan ranged from 45 to 73 days (32), in which longevity could be predicted by a normal amount of fat mass before starvation. However, prolonged fasts of up to 400 days have been accomplished by obese subjects who fasted for therapeutic reasons (126,127). A number of metabolic factors explain these results (Table 1.4). As has already been noted and portrayed (Fig. 1.1), obese individuals have more body protein (fat-free mass) than lean individuals, but during starvation they excrete less nitrogen than their lean counterparts (128). In addition, within weeks of the onset of starvation, the energy contribution from protein remains the same in lean subjects, but it progressively decreases in obese individuals (33). Other metabolic differences observed in the obese versus the lean include a decreased rate of protein oxidation (128), a decreased rate in the rise of circulating ketone bodies and of the indices of the mitochondrial redox state (129), a greater production of glucose from gluconeo-

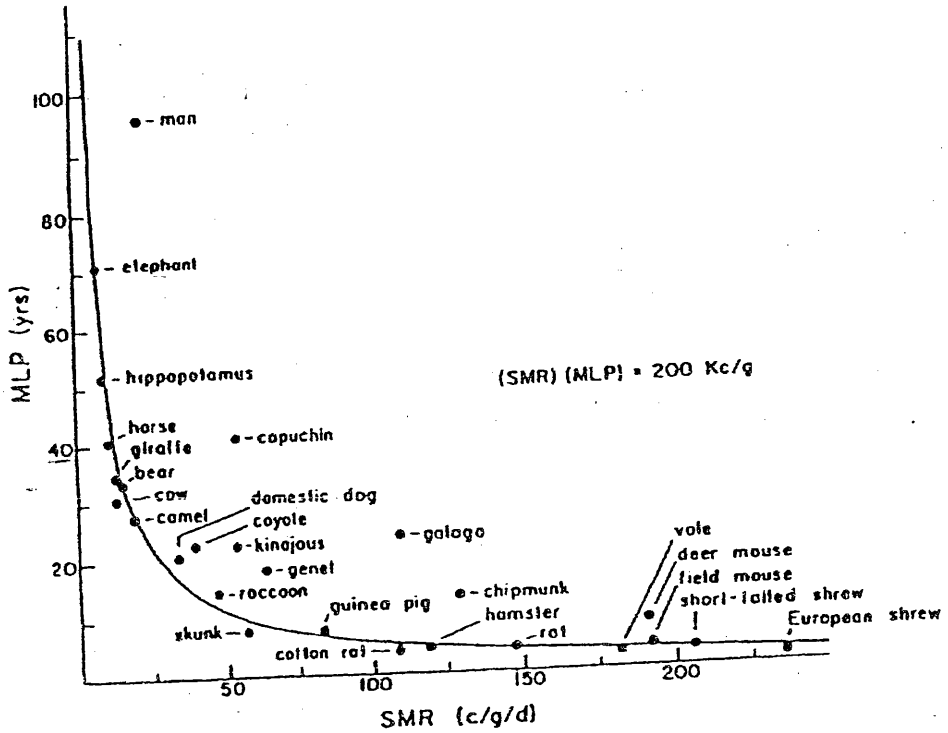


FIG. 1.8. Relationship between maximum lifespan potential *(MLP)* and specific metabolic rate *(SMR)* expressed as calories consumed per gram of body weight per day in a variety of mammalian species (180). (From Weiss KM. Are the known chronic diseases related to the human lifespan and its evolution? *Am J Hum Biol* 1989;1:307–319, with permission.)

genesis by the kidney (130), and a lower deterioration in glucose tolerance (131). The reduced rise in ketogenesis and in the indices of the mitochondrial redox state indicate the relative preservation of glucose as the dominant energy source for the brain in the obese individual. The experiments of Elia et al. (128) portray this nicely (Fig. 1.9). Overall, these differences in the metabolic response to starvation in obese versus lean individuals are not trivial, and they most likely contribute to the prolonged survival that is seen in those with expanded adipose tissue mass.

TABLE 1.4. *Metabolic differences between lean and obese individuals during starvation*

Rate of rise of ketone bodies over 3–4 days	Lean > obese
Ketone body concentration at 3–4 days	Lean > obese
Rate of rise of 3-hydroxybutyrate/acetoacetate ratio	Lean > obese
Concentration of 3-hydroxybutyrate/acetoacetate ratio	Lean > obese
Deterioration of glucose tolerance	Lean > obese
Protein oxidation at 60 h	Lean > obese
Nitrogen excretion	Lean > obese
Renal gluconeogenesis	Lean < obese

From Elia M. Hunger disease. *Clin Nutr* 2000;19:379–386, with permission.

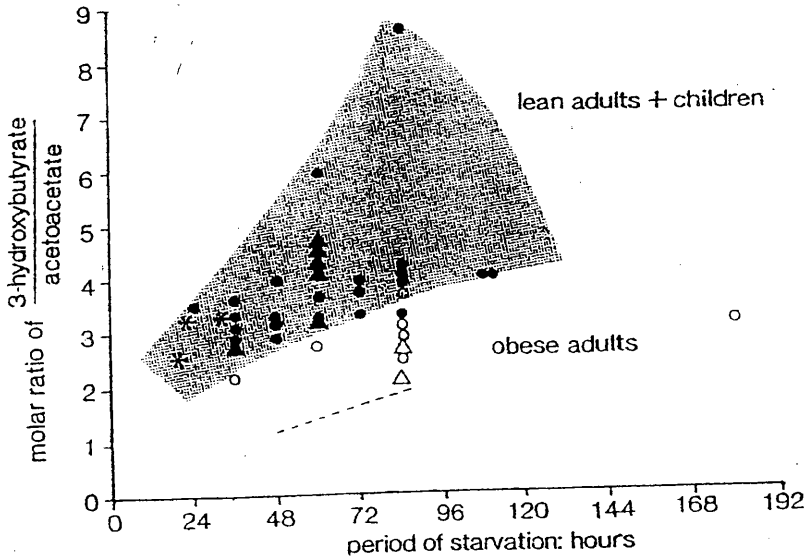


FIG. 1.9. The effect of total starvation on the plasma molar ratio of 3-hydroxybutyrate to acetoacetate in children (*) and lean (*solid symbols*) and obese (*open symbols*) adults. (From Elia M, Stubbs R, Henry CJK. Differences in fat, carbohydrate, and protein metabolism between lean and obese subjects undergoing total starvation. *Obes Res* 1999;7:597–604, with permission.)

The Thrifty Genotype

As Bray (132,133) has repeatedly pointed out in historical reviews, obesity has been exemplified since the Paleolithic period, approximately 23,000 to 25,000 years ago (134). As Fig. 1.10 demonstrates, Venus figurines portraying female obesity have been located in many sites throughout Europe and the Middle East. The most famous of these is the "Venus of Willendorf" made of limestone, which is the only one that has been discovered in Austria. These figurines have been viewed as representing primordial deities reflecting the bounty of the earth. These artistic records of history may also represent the longevity associated with obesity at a time when the lifespan was only two decades (125).

The concept of the "thrifty genotype" was initially proposed by Neel (135) in 1962 and was revisited in 1982 (136) and 1998 (137). Although his initial interpretation of genes beneficial to survival in a "hunter–gatherer" type of environment related more to diabetes than to obesity, the most recent view focuses more on obesity and it also includes hypertension (137). The putative genes would operate such that carriers are predisposed to extract scarce resources more efficiently from the environment. In the modern world in which the "hunter–gatherer" concept is fading and the lifespan extends far past the reproductive age, these genes are no longer beneficial and they can, in fact, be detrimental. Now called *pleiotropic*, these genes may also predispose their carriers to physiologic degeneration or loss of function during their middle or later years of life (138). This model thus provides a theoretical basis for explaining aging and the many degenerative diseases that accompany the extended lifespan.

As Gerber and Crews (121) outline, the "thrifty and/or pleiotropic" model of degenerative diseases with aging can be applied at two levels. First, specific risk factors associated with

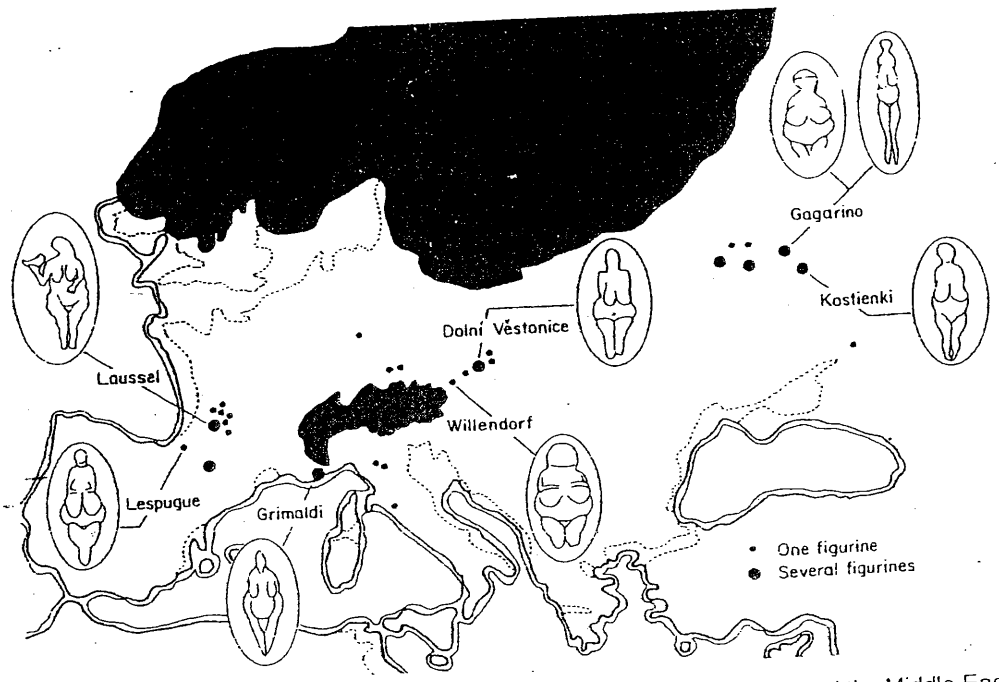


FIG. 1.10. Approximate location of Paleolithic "Venus" figurines in Europe and the Middle East about 22,000 BC. (From Bray G. Historical framework for the development of ideas about obesity. In: Bray GA, Bouchard C, James WPT, eds. *Handbook of obesity.* New York: Marcel Dekker Inc, 1998:1–29, with permission.)

degenerative diseases follow either the accumulation (thrifty) or the scarcity or loss (non-thrifty) of specific nutrients, metabolites, stores, or deposits. Second, some degenerative diseases result from the antagonistic effects of thrifty genotypes that have high selective value during development. These effects then predispose their carriers to the risk factors associated with degenerative diseases. The transition from obesity to type II diabetes and then to the additional risk factors for cardiovascular disease, such as dyslipidemia, the prothrombotic state, and hypertension, is a well-documented example of the proposed model.

Biologic Considerations of the Thrifty or Pleiotropic Genotype

A gene or genes that favorably modify energy storage during periods of food deprivation and that could ultimately result in degenerative diseases during midlife and late life generally are genes that at least have some influence on energy balance. Ideally, candidate genes would operate to enhance energy intake and metabolic efficiency in a way that partitions fuels for storage rather than for oxidative metabolism. Because of the limited capacity for carbohydrate storage, these genes would need to influence lipid uptake and storage mechanisms. The plethora of metabolic effects of insulin satisfy these criteria, so a gene or genes that enhance insulin sensitivity in the periphery is worthy of serious consideration.

In an insulin-sensitive environment, anabolism is the rule. This is related to the multiple effects of insulin that promote fuel uptake and storage in peripheral tissues (Table 1.5). In protein metabolism, insulin increases amino acid uptake and protein synthesis

TABLE 1.5. *Insulin action in insulin-sensitive tissues*

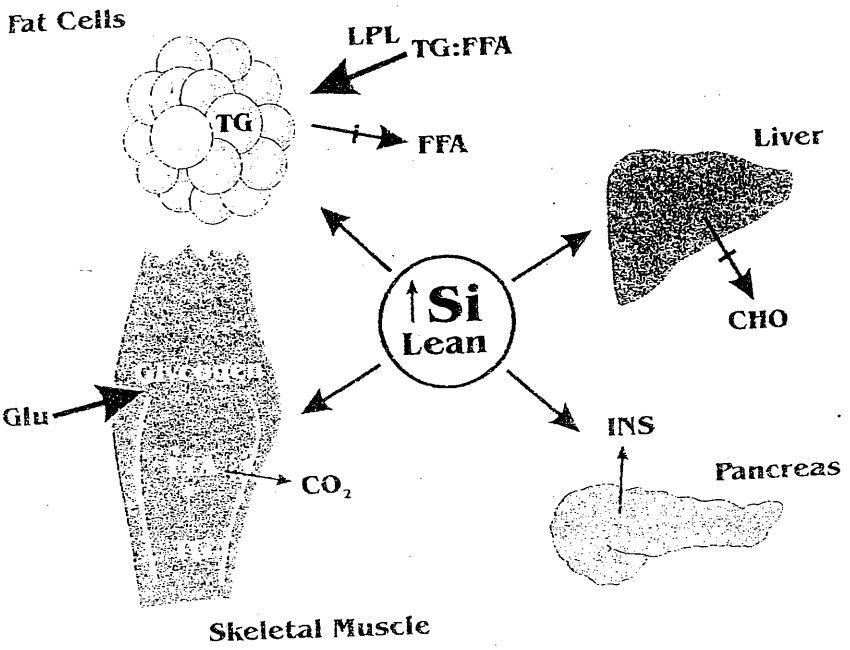
Protein metabolism	Lerner (200)
Increases amino acid uptake	Miers et al. (201)
Inhibits proteolysis	Kimball et al. (202)
Increases protein synthesis	
Glucose metabolism	Ryder et al. (203)
Increases glucose transport (adipose tissue, muscle)	Srivastava and Pandey (204)
Increases glycogen synthesis	Cherrington et al. (205)
Inhibits hepatic and renal glucose production	
Lipid metabolism	Bergman (206)
Inhibits lipolysis	Sparks and Sparks (207)
Increases fatty acid and triglyceride synthesis (liver)	Sadur and Eckel (208)
Increases adipose tissue lipoprotein lipase	Farese et al. (209)
Inhibits skeletal muscle lipoprotein lipase	Lewis and Steiner (210)
Inhibits very low density lipoprotein secretion	

and inhibits proteolysis. Insulin modifies carbohydrate metabolism by increasing glucose uptake and glycogen synthesis in muscle and adipose tissue and by increasing glycogen synthesis and inhibiting glucose production in the liver and kidney. Finally, in lipid metabolism, insulin inhibits adipose tissue lipolysis of stored triacylglycerol (the most sensitive parameter of insulin action); increases lipoprotein-derived fatty acid uptake by its effect on adipose tissue LPL; inhibits the secretion of very low density lipoprotein from the liver; and inhibits skeletal muscle LPL modestly, thus decreasing the availability of circulating lipoprotein-derived fatty acids to muscle. All of these effects of insulin work to enhance energy storage and thus to protect the organism against periods of nutrient deprivation. However, insulin is also known to inhibit food intake when it is infused into the third ventricle in baboons and rodents (139,140). How this parameter of insulin action relates to energy balance in the periphery remains unclear.

A substantial amount of evidence provides support for the concept that insulin sensitivity promotes weight gain and insulin resistance protects against weight gain (141–144). Some epidemiologic studies (145,146) indicate that fasting hyperinsulinemia—a marker of insulin resistance—or more likely insulin resistance itself predicts weight maintenance; however, not all studies (147,148) support this. Nevertheless, several recent studies have demonstrated that increased insulin sensitivity is a predictor of weight (fat) gain or of the rebound of obesity after successful weight reduction (96). In these cases as well, alternative data do exist (97).

Graphical depictions (Fig. 1.11) indicate that, when the thrifty gene effects of insulin sensitivity are manifested, increases in adipocyte number and/or size and insulin resis-

FIG. 1.11. The progression of insulin sensitivity in leanness **(A)** to obesity **(B)**, which represents the intermediate metabolic paradigm, to the insulin resistance of type II diabetes mellitus **(C)**. In the setting of insulin sensitivity, insulin-mediated glucose uptake and glycogen deposition in skeletal muscle is enhanced with relative sparing of fatty acid oxidation and the uptake of lipids with storage in adipose tissue. The liver remains responsive to insulin-mediated suppression of hepatic glucose production. In obesity, defects in all aspects of insulin action ultimately result; however, insulin secretion increases, resulting in hyperinsulinemia but preservation of glucose tolerance. With persistence of insulin resistance and islet failure, insulin secretion fails. In this metabolic setting, insulin resistance increases, including resistance to insulin suppression of hepatic glucose production, and further defects in insulin-mediated glucose disposal and hyperglycemia result. Because of persistent and worsening insulin action in adipose tissue, fat mass fails to increase further.



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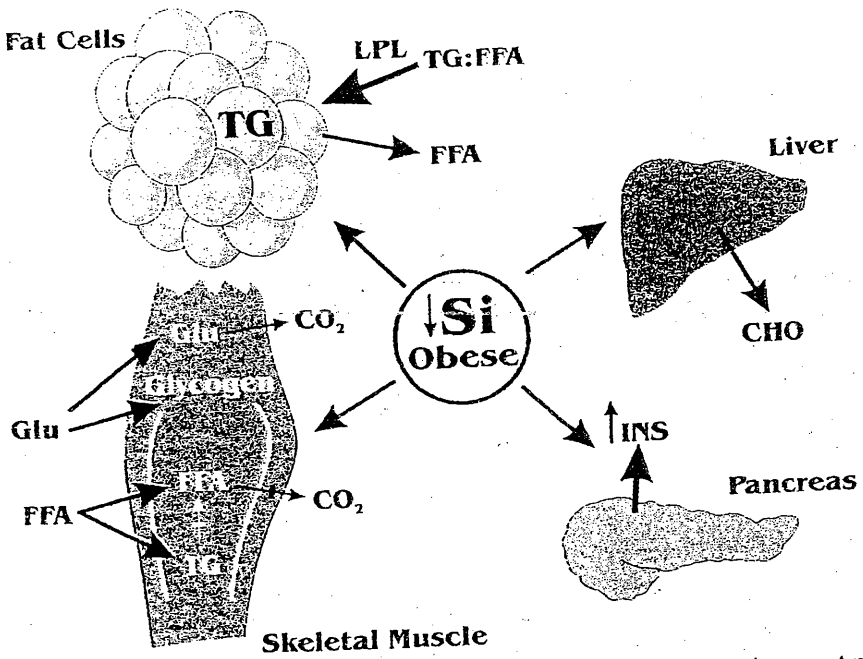

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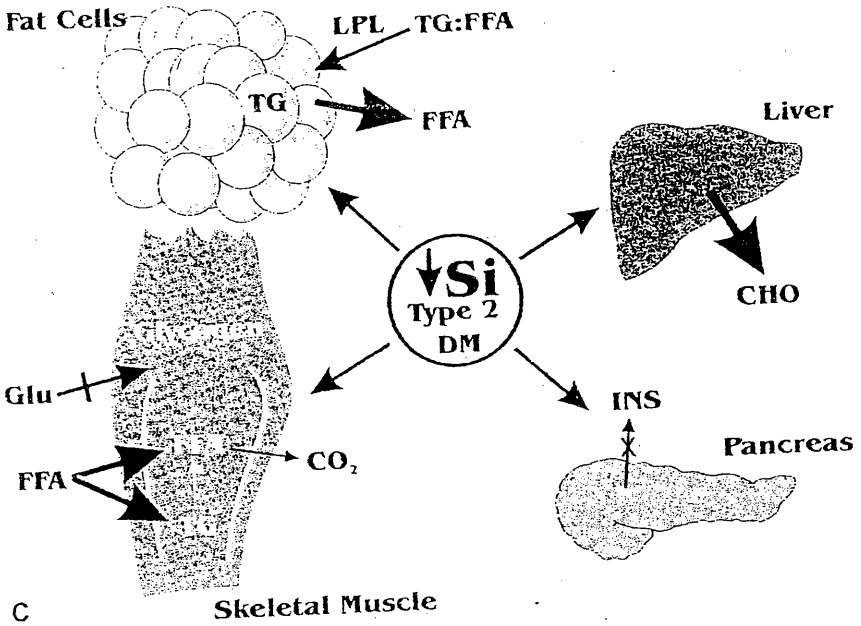

FIG. 1.11. *Continued.*

tance, as manifested by hyperinsulinemia, ultimately result. In adipose tissue, insulin resistance to the antilipolytic effects of insulin and the insulin stimulatory effects on LPL become apparent. Moreover, with the development of obesity, insulin resistance in tissues also results in defects in insulin-mediated glucose transport and glycogen synthesis, with increases in triacylglycerol accumulation and fatty acid oxidation in muscle and lack of suppression of glucose production by the liver and kidney. Over years, the continued stress of the insulin-resistant environment on the pancreas produces an inability to maintain insulin secretion, and type II diabetes results. Of interest is recent evidence from insulin receptor knockout mice suggesting that the islet defect may also be a consequence of insulin resistance (149). Whether the propensity to develop diabetes relates to the function of the same gene, thus demonstrating its pleiotropic qualities, or to a separate gene must still be elucidated.

Several genes have been entertained as candidates for the thrifty or pleiotropic gene hypothesis or at least for the "thrifty" component. Leptin is a cytokine secreted by adipocytes that regulates energy balance at the levels of intake and expenditure (150). Moreover, although leptin appears to be the long-explored factor that communicates between the periphery (adipose tissue) and the hypothalamus regarding fat mass (adipostat), "favorable" mutations of leptin result not only in obesity but also in reproductive incompetence and infertility (151).

MC4R is expressed in hypothalamic nuclei and is involved in the regulation of energy balance (152,153). Indirect evidence for this was provided when the agouti obesity syndrome in mice was found to be a consequence of chronic inhibition of MC4R signaling (152). Furthermore, mice with a deletion of the MC4R gene have a phenotype indistinguishable from the agouti, and mice with heterozygous knockouts of MC4R have an intermediate phenotype (154). A recent discovery indicates that up to 4% of obesity in

some populations may be related to mutations in MC4R (17). Moreover, those with heterozygous mutations of MC4R are predisposed to obesity, likely as a result of haploinsufficiency (155,156). The variable penetrance of obesity in heterozygotes indicates that other genes and/or environmental influences are important in the development of the obese phenotype.

Presently, the gene that best meets the qualifications for the thrifty or pleiotropic gene is the nuclear receptor peroxisome proliferator-activated receptor γ (PPARγ). This gene product is activated by fatty acids or fatty acid derivatives and forms heterodimers with other nuclear receptors, such as the retinoid X receptor (RXR), to modify the gene transcription of numerous target genes with specific PPAR response elements (157). The PPARγ gene product is particularly relevant because one of the ligands for its activation is the class of drugs called thiazolidinediones, which are currently used to improve insulin sensitivity in the treatment of type II diabetes and other insulin-resistant states (158). Studies in cultured adipocytes show that thiazolidinediones enhance adipocyte differentiation (159) and that *in vivo* they produce weight gain in both animals and humans (160–162). Although some of the weight gain can be attributed to fluid retention (161), the predominant explanation is an increase in adipose tissue.

PPARγ action is not only reflected by increasing adipocyte differentiation and insulin action but also by a number of other effects (Table 1.6). These include its influence on insulin secretion, cell cycle control, immunomodulation, inflammation, atherosclerosis, myocardial function, and carcinogenesis (157). Of added relevance is the fact that naturally occurring mutations in PPARγ and induced haploinsufficiency in mice heterozygous for PPARγ produce interesting modifications in receptor biology. For example, the Pro12Ala PPARγ mutation in humans has variably been associated with obesity (163); diabetes (164); protection from diabetes (163,165); reductions in glucose and arginine-mediated insulin secretion (166); and diverse effects on atherosclerotic risk, including a lipid and lipoprotein phenotype consistent with familial combined hyperlipidemia (167), reductions in total and non–high-density lipoprotein cholesterol (168) and postheparin LPL (169), and reductions in coronary heart disease (170). Alternatively, this mutation has had no metabolic effect in some populations (171,172). However, other mutations in PPARγ have also been linked to human obesity, either directly (173) or via their influence on other obesity-related proteins, such as leptin (174).

Serendipitously, mice heterozygous for PPARγ (PPARγ +/–) or normal mice treated with PPARγ or RXR antagonists are spared adipocyte hypertrophy, they are more insulin sensitive, they are relatively hyperleptinemic with reductions in muscle and liver triacylglycerol levels and increases in fatty acid oxidation, and they have enhanced immune (T-cell and B-cell) function (175–178). When PPARγ +/– mice are treated with a PPARγ or a RXR antagonist, a dramatic metabolic effect characterized by marked reductions in white adipose tissue, leptin, and energy expenditure; increases in triacylglycerol accu-

TABLE 1.6. *Paroxisome proliferator-activated receptor γ actions*

Increased adipocyte differentiation	Fajas et al. (211)
Increased insulin sensitivity	Sood et al. (212)
Increased insulin secretion	Kawai et al. (213)
Cell cycle regulation	Altiok et al. (214)
Immunomodulation	Clark et al. (215)
Reduced atherosclerosis	Molavi et al. (216)
Enhanced myocardial function	Khandoudi et al. (217)
Carcinogenesis	Fajas et al. (211)

mulation in muscle and liver; and decreases in insulin sensitivity is observed (178). A role for RXR in this response is supported by the relative leanness and the increased energy expenditure found in mice with a knockout of RXRγ (90).

Together these data suggest that PPARγ has a pivotal role in adipose tissue biology and insulin action that is modified by the number of PPARγ alleles. The critical role of this gene is also exemplified by embryonic lethality in its absence (175). With a lifespan that extends past the "beneficial" effects of PPARγ gene expression on survival, the pleiotropic downside may be metabolic disorders, atherosclerosis, or malignancy. Much about this fascinating candidate for the thrifty or pleiotropic gene remains to be learned. Moreover, the hope is that, with the human genome sequence now in hand, many more genes that act alone or in concert to produce obesity will likely be uncovered.

CONCLUSIONS

Is obesity a disease or an adaptation for survival? Does this even make a difference? The answer is yes. The impact of the environment is the same with either view; however, the atmosphere in which health care professionals and their patients operate may be profoundly and differentially influenced by the opinion of science, government, health care reimbursement organizations, and the marketplace, which includes pharmaceutical companies, weight loss programs, and over-the-counter products. Although referring to obesity as a disease is politically correct, the criteria for obesity to be called a disease are insufficiently delineated; moreover, abundant evidence indicates that, when famine or semistarvation occur, obesity could be a participant's best friend. Without question, the complications of obesity are diseases. However, an acknowledgment of the importance of obesity over the history of humankind helps the health care professional and the patient to develop an understanding about the facts of excess body fat. This newfound knowledge can modify the behavior of both so that guilt is removed and both can now approach the difficulties of treatment with a greater appreciation of the barriers. With some degree of ambivalence, what was once "thrifty" and beneficial must now be accepted as "pleiotropic" and potentially harmful.

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Regional Fat Loss from the Thigh in Obese Women after Adrenergic Modulation

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ABSTRACT

Beta-adrenergic stimulation and alpha$_2$-adrenergic inhibition increase lipolysis from fat cells. Twenty-eight obese women were placed on a calorie-restricted diet and one of five treatments was applied to one thigh three to five times per week for four weeks: (1) isoproterenol injections; (2) cream containing colforsin (forskolin), aminophylline, and yohimbine; (3) yohimbine cream; (4) colforsin cream; or (5) aminophylline cream. The opposite thigh was treated with a placebo (injection or cream). The treated thighs lost significantly more girth after treatment, both by injection and by cream. No adverse reactions were attributable to either the cream or the injections. It is concluded that local fat reduction from the thigh can be safely accomplished.

INTRODUCTION

Fat is lost more rapidly from the abdomen than from the thigh after intestinal bypass surgery.[1] Studies of fat cells have shown that beta-adrenergic agonists can directly increase cyclic adenosine monophosphate levels and stimulate lipolysis. There is a regional difference in this responsiveness to beta-agonists.[2] Fat cells from the abdomen are more sensitive to the lipolytic effects of beta-adrenergic stimulation than are those from the thigh.[2] This appears to be because there are more alpha$_2$-receptors on fat cells of the thigh than of the abdomen.[3,4] Raising the norepinephrine concentration in the medium suspending the fat cells from the thigh increases in vitro lipolysis from these cells.[2] The maximal lipolytic rate in fat cells from the two locations is equal, but higher concentrations of norepinephrine are needed to reach that maximum when fat cells from the thigh are used. Blocking the alpha$_2$-receptors on fat cells from the thigh enhances lipolysis in the presence of norepinephrine.[2,4]

We hypothesized that by increasing the local concentration of beta agonists or by inhibiting phosphodiesterase or

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alpha$_2$-adrenergic receptors to fat cells in the thigh, fatty acids could be released more readily. We report the results of a series of small trials to test this hypothesis.

MATERIALS AND METHODS

Study 1

Five women who were more than 20% above their desirable body weight (mean weight, 209 lb) and wished to lose weight from their thighs were recruited and placed on a diet of 600 kcal daily and encouraged to engage in a walking program. They received injections of 0.2 ml of 10^{-5} mol/L isoproterenol at intervals of 4 cm around the circumference of one thigh, two thirds of the way from the knee to the greater trochanter, three days per week for four weeks. The 4-cm interval was chosen because calculations from the area of vasodilation on the skin suggested that the spheres of diffusion would overlap when this distance was kept. The opposite thigh was treated similarly, but using normal saline in a double-blind design.

Study 2

Five women (mean weight, 182 lb) who were more than 20% above desirable body weight and wished to lose weight from their thighs were recruited and placed on a diet of 600 kcal daily and encouraged to engage in a walking program. They were seen five days a week for four weeks. At each visit, warm wraps of 600 to 900 mosm/L of magnesium sulfate solution were applied to each thigh for 30 minutes, followed by an application of cream and plastic wrap. The cream applied to one thigh contained 1.2×10^{-5} mol/L colforsin (forskolin), 2.5×10^{-4} mol/L yohimbine, and 1.3×10^{-2} mol/L aminophylline in xipamide (aquaphor) base. The other thigh received xipamide base only in a double-blind design.

Study 3

Eighteen women (mean weight, 197 lb) who were more than 20% above desirable body weight and wished to lose weight from the thighs were recruited and placed on a liquid formula diet of 800 kcal daily and encouraged to engage in a walking program. They were seen five days per week for four weeks. At each visit, warm wraps of 600 to 900 mosm/L of magnesium sulfate solution were applied to each thigh for 30 minutes, followed by cream application. One thigh was treated in each patient with one of the three creams in xipamide: colforsin, 25×10^{-5} mol/L (six patients); yohimbine, 5×10^{-4} mol/L (six patients); or aminophylline, 1.3×10^{-2} mol/L (six patients). The other thigh in each subject was treated with xipamide only, in a double-blind design.

In each study, treatment effectiveness was judged by measuring the thigh circumference two thirds of the way from the knee to the greater trochanter, using the saline- or xipamide-treated thigh as the control. Differences were compared with Student's t test for paired observations.

RESULTS

In Study 1, all five women completed the four weeks of treatment and all but one lost weight. The four subjects who lost weight lost more girth from the thigh treated with isoproterenol injections than

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Study 3

Eighteen women (mean weight, 197 b) who were more than 20% above lesirable body weight and wished to lose veight from the thighs were recruited nd placed on a liquid formula diet of 00 kcal daily and encouraged to engage ı a walking program. They were seen ve days per week for four weeks. At ich visit, warm wraps of 600 to 900 ıosm/L of magnesium sulfate solution ere applied to each thigh for 30 minutes, llowed by cream application. One thigh ıated in each patient with one of ee creams in xipamide: colforsin, $\times 10^{-5}$ mol/L (six patients); yohim- ıe, 5×10^{-4} mol/L (six patients); or ıinophylline, 1.3×10^{-2} mol/L (six tients). The other thigh in each subject s treated with xipamide only, in a ıble-blind design.

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tudy 1, all five women completed the weeks of treatment and all but one weight. The four subjects who lost ht lost more girth from the thigh ed with isoproterenol injections than from the control thigh (Figure 1). In the total group, the thighs treated with isoproterenol lost a mean of 1.8 ± 0.89 cm more than the control thighs ($P < 0.05$). No changes in pulse rate, blood pressure, or other clinical parameters were noted.

In Study 2, all five women completed the four weeks of treatment and all but one lost weight. The five subjects lost a mean of 2.03 ± 1.36 cm more from the thighs treated with cream containing colforsin, yohimbine, and aminophylline than from the control thighs ($P < 0.05$) (Figure 2). The only adverse effect was a pruritic rash that occurred on both thighs in one subject, which was judged to be related to the occlusive plastic-wrap dressing.

In Study 3, 13 of the 18 women completed the trial. In the four subjects who received yohimbine (Figure 3), all lost weight and all but one lost more girth from the thigh treated with yohimbine cream than from the control thigh. The woman who lost more girth from the thigh treated with placebo was the first subject to enter the trial. After her first visit, it was found that having subjects support their body weight on the thigh being measured increased reproducibility. Unfortunately, this was not done in the first patient, and this may have been responsible for the aberrant results in her case. The group as a whole lost a mean of 0.75 ± 0.35 cm more from the yohimbine-treated thighs than from the control

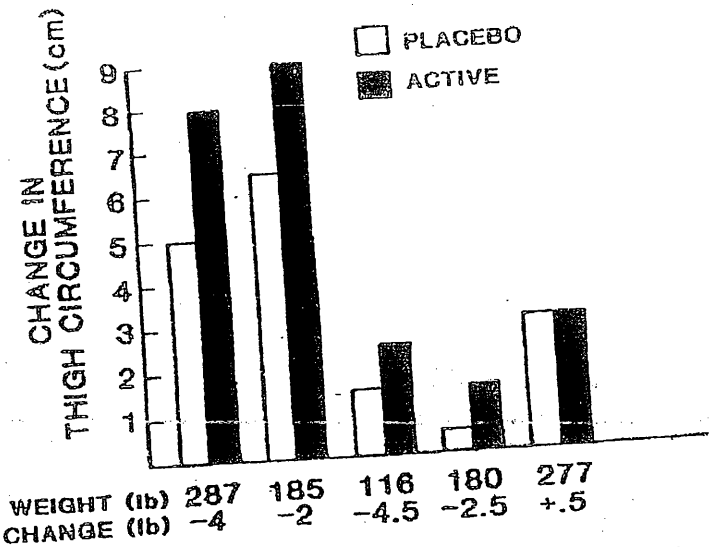

Figure 1. Changes in thigh circumference and total body weight in five subjects after treatment with isoproterenol injection or placebo.

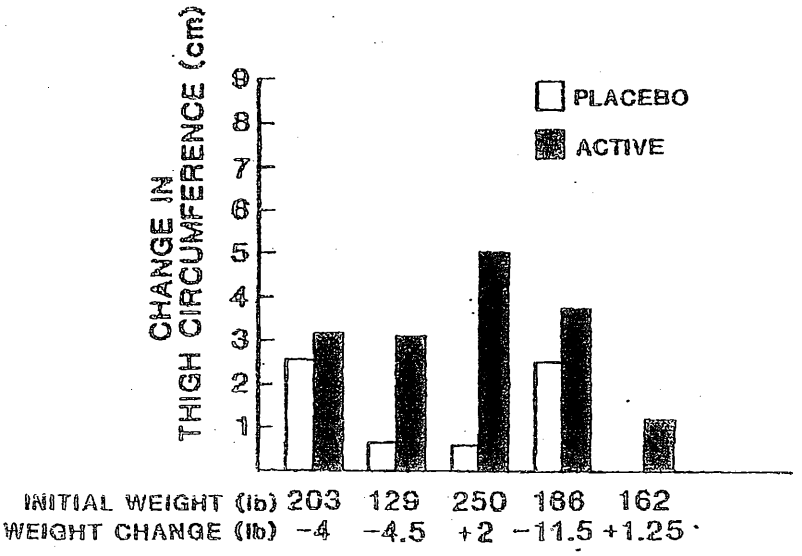

Figure 2. Changes in thigh circumference and total body weight in five subjects after treatment with a cream containing colforsin, aminophylline, and yohimbine or placebo.

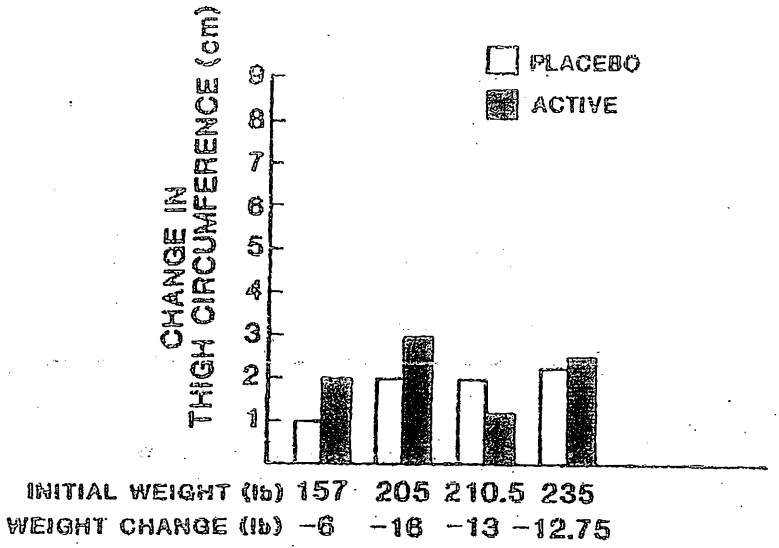

Figure 3. Changes in thigh circumference and total body weight in four subjects after treatment with yohimbine cream or placebo.

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All four subjects who completed the four weeks of treatment with colforsin lost weight. They lost a mean of 1.0 ± 0.61 cm more girth from the thighs treated with colforsin cream than from the control thighs ($P < 0.05$) (Figure 4).

All five subjects who completed the four weeks of treatment with aminophylline lost weight. They lost a mean of 1.5 ± 0.77 cm more girth from the thighs treated with aminophylline cream than from the control thighs ($P < 0.02$) (Figure 5).

No adverse reactions were noted in the three parts of Study 3. There were no changes in blood pressure or pulse, nor any evidence of skin rash. The five patients who dropped out did so after seven, eight, ten, six, and three days of treatment, respectively. Four of the five dropouts had lost more girth from the treated thigh than from the control thigh by the time of departure from the study. The fifth dropped out on the third day of treatment, at which time there was no change in thigh circumference. All five dropouts had lost weight at the time of their departure.

When girth loss from the treated thighs is compared with loss from the untreated thighs in all three studies combined, the treated thighs lost an average of 1.33 ± 1.12 cm more than did the control thighs ($P < 0.001$).

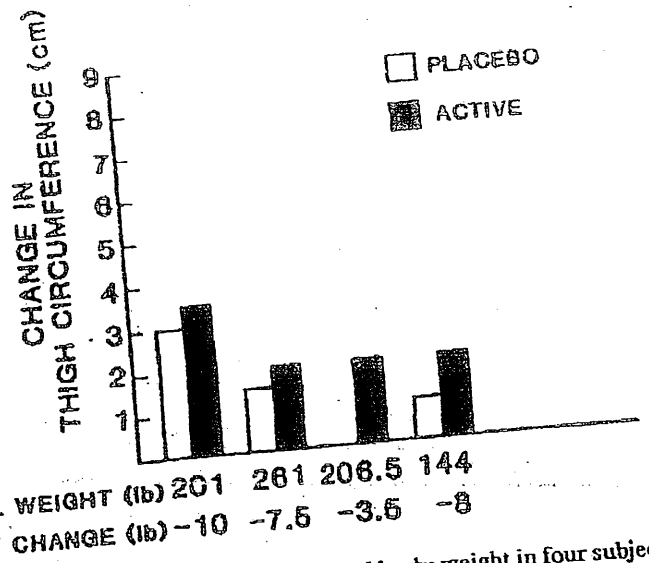
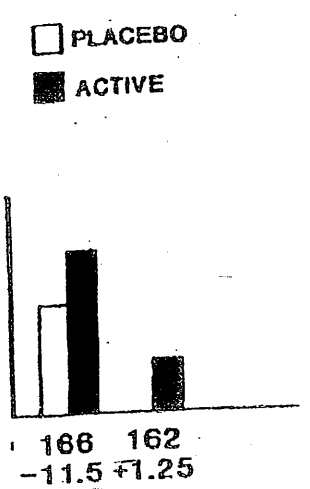


Figure 4. Changes in thigh circumference and total body weight in four subjects after treatment with colforsin cream or placebo.



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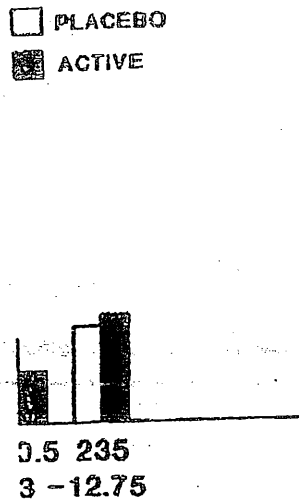

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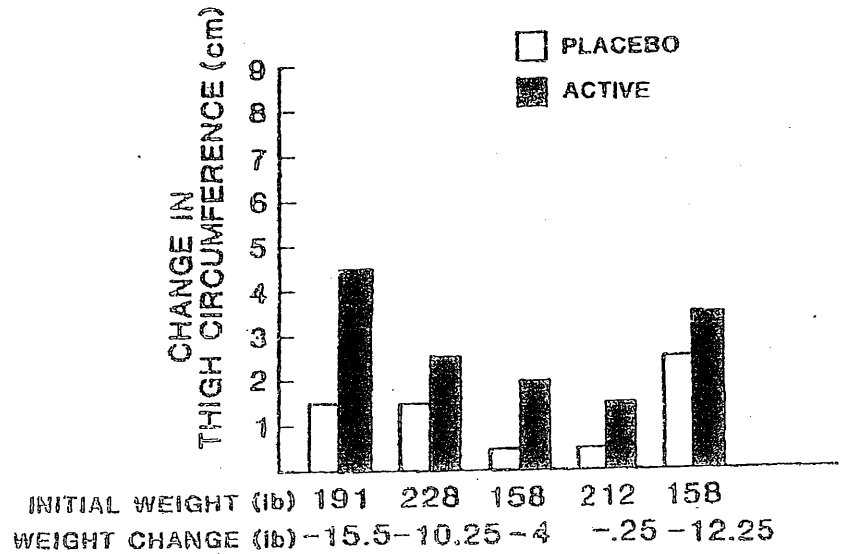

Figure 5. Changes in thigh circumference and total body weight in five subjects after treatment with aminophylline cream or placebo.

DISCUSSION

It has been generally believed for some time that thigh fat in women is hard to mobilize.[5] Others, however, have been reluctant to accept this concept, believing that all fat cells are metabolically the same. In vitro work, however, has suggested that the adrenergic thresholds to lipolysis are indeed different in different sites, and that thigh fat is more difficult to mobilize than abdomen fat.[2,4]

Many attempts have been made to achieve local or spot fat reduction through nonsurgical means but none have succeeded.[6] The use of injections in Study 1, although clinically impractical, did support the concept of local adrenergic modulation of lipolysis in vivo and encouraged us to try the topical application of adrenergic modulators. The results of the first study demonstrated that local fat reduction can be accomplished safely and effectively.

Studies 2 and 3 support the concept that topical application of adrenergic modulators can enhance lipolysis. It might have been postulated that yohimbine, the alpha$_2$-inhibitor, would have been the most effective agent, since the higher threshold for lipolysis in thigh fat cells appears to be a result of a higher concentration of inhibitory alpha$_2$-receptors. No naturally occurring alpha$_2$-inhibitors circulate in human systems, but there are always beta-stimulating catechols present. In these two studies, data on the average loss of thigh circumference suggest that the cream containing a beta-stimulator with a phosphodiesterase

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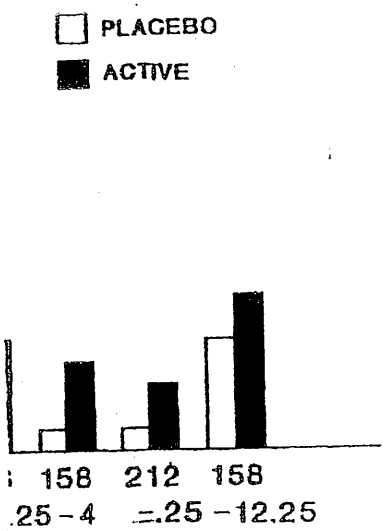

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inhibitor and an alpha$_1$-inhibitor is the most effective. Of these three components, the phosphodiesterase inhibitor was most effective, followed by the beta-stimulator, and then the alpha$_2$-inhibitor. The number of patients in each trial, however, was small, and thus conclusions about the relative potency of the three components of the combined cream are tenuous. The only side effect seen in any of the trials was a skin rash on both thighs in one woman during Study 2 when plastic wrap was used as an occlusive dressing. This complication was not seen in Study 3 when no plastic wrap was used. Clearly, the plastic wrap was not essential to the effectiveness of the treatment. The safety of this treatment was not unexpected since very small amounts of adrenergic modulators could be absorbed into the bloodstream.

CONCLUSIONS

It is concluded that results of the three studies demonstrate that local fat can be reduced with a cream both safely and effectively.

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Topical Fat Reduction

*Frank L. Greenway, George A. Bray, David Heber**

Abstract

GREENWAY, FRANK L, GEORGE A BRAY AND DAVID HEBER. Topical fat reduction. *Obes Res.* 1995;3(Suppl 4):561S-568S.

The fat on women's thighs is more difficult to mobilize due to increased α−2 adrenergic receptor activity induced by estrogen. Lipolysis can be initiated through adipocyte receptor stimulation (β adrenergic) or inhibition (adenosine or α−2 adrenergic) or by inhibition of phosphodiesterase. Since many women desire regional thigh fat loss, a series of clinical trials were initiated using one thigh as a double-blinded control. Trial #1: Five overweight women had injections of isoproterenol at intervals around the thigh three times a week for 4 weeks with diet and walking. Trial #2: Five overweight woman had ointment containing forskolin, yohimbine and aminophylline applied to the thigh five times a week for 4 weeks after hypertonic warm soaks with a diet and walking. Trial #3: Eighteen overweight women were divided into three groups of six and trial #2 was repeated with each agent alone vs. placebo using forskolin, yohimbine or aminophylline in separate ointments. Trial #4: Thirty overweight women had 10% aminophylline ointment applied to the thigh five times a week for 6 weeks with diet and walking. Chemistry panel, theophylline level and patch testing were performed. Trial #5: Twelve women had trial #4 repeated with 2% aminophylline cream without a diet or walking. Trial #6: Trial #5 was repeated with 0.5% aminophylline cream. All trials except yohimbine ointment gave significantly more girth loss from the treated thigh ($p<0.05$ to $p<0.001$). Chemistry panel showed no toxicity. Theophylline was undetectable and patch testing was negative. We conclude that topical fat reduction for women's thighs can be achieved without diet or exercise.

Key words: obesity, adipocyte, aminophylline, lipolysis, adrenergic receptors

Introduction

Many people would like to selectively lose fat from a specific area of their body. The thighs and buttock area seem to be the area of most frequent concern for women. This desire for cosmetic change has been the basis for much popular and professional writing (10). Ronsard (13) popularized the term "cellulite" to describe the dimpling and orange skin look of the thighs (peau d'orange). Bayard (3) attributed it to the aging process. Regardless of the cause, these cosmetic concerns are widespread. As Ohrbach says, "Our images of womanhood are almost synonymous with thinness" (11).

The structure of the subcutaneous adipose tissue accounts for the development of the peau d'orange appearance. Fibrous connective tissue septae surround groups of fat cells and attach to the underside of the dermis. As fat cells enlarge, these septae are stretched and pull down on the overlying skin. The result of this process is an indentation or dimpling of the skin over the thigh and buttock area in women to which Ronsard has given the name cellulite (13). Although Ronsard has proposed treatment procedures for cellulite, no experimental evidence has been published to support the efficacy of her suggestions.

Several studies have examined spot reducing. Early studies suggested that massage might have effects on local fat distribution (5,14). A carefully controlled study by Kalb (7), however, demonstrated that massage was unable to achieve local fat reduction. In his study, 40 patients were placed on an 800 calorie diet with 20 subjects receiving massage and 20 subjects serving as unmassaged controls. All patients lost weight and their arms and legs became smaller, but there were no differences between the massaged group and the unmassaged control group in the loss of arm or leg fat.

Adipose tissue metabolism varies from one region of the body to another. Smith et al. (15), and Kral et al. (8), demonstrated that fat was absorbed more slowly in the femoral region than from the abdominal region in women losing weight after the jejuno-ileal bypass operation for severe obesity. These observations suggested regional differences in the lipolytic processes that might respond to the local application of lipolytic agents. The lipolytic process has been described in great detail in the past 20 years (16). Lipolysis, the process of hydrolyzing triacylglycerol into glycerol and fatty acids, is mediated by the enzyme hormone sensitive lipase. Hormone sensitive lipase is active in the phosphorylated form. This activation is produced by protein kinase-A which is activated by cyclic AMP. Membrane-bound adeny-

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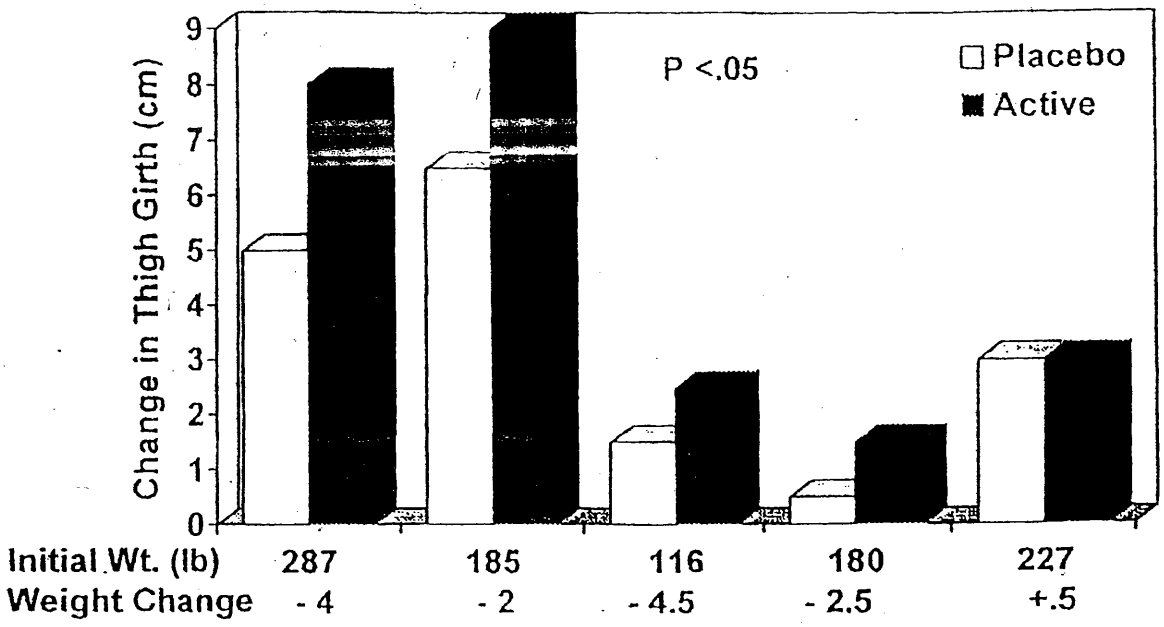

Figure 1: Changes in thigh circumference and total body weight in five subjects after treatment with isoproterenol injection or placebo.

late cyclase can be inhibited or stimulated by the action of inhibitory or stimulatory GTP binding proteins (Gs-proteins) acting on adenylate cyclase. A number of hormones react with cell surface receptors on the adipocyte to influence lipolysis. Stimulation of the β−2 adrenergic receptor stimulates the GTP stimulatory binding protein (G_s protein) which activates adenylate cyclase which, in turn, activates cyclic AMP. The α−2 adrenergic receptor and the adenosine receptor, on the other hand, stimulate GTP inhibitory binding proteins (G_i proteins) which inhibit adenylate cyclase and thus inhibit the lipolytic process. The relative number of β and α−2 adrenergic receptors on the surface of the fat cells determine the lipolytic balance of those cells. Hormones can have long-term effects on the lipolytic processes by influencing the number of α−2 and β receptors on the fat cell. Thus by controlling lipolysis, hormones can determine body fat dis-

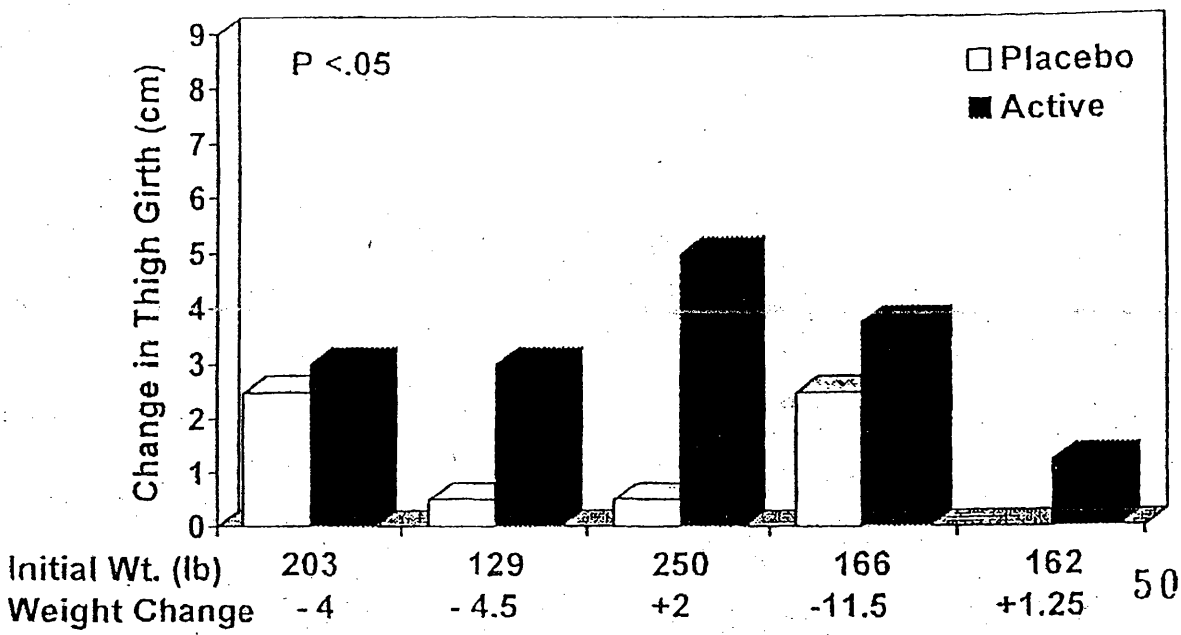

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Figure 2: Changes in thigh circumference and total body weight in five subjects after treatment with ointment containing forskolin, aminophylline and yohimbine or placebo.

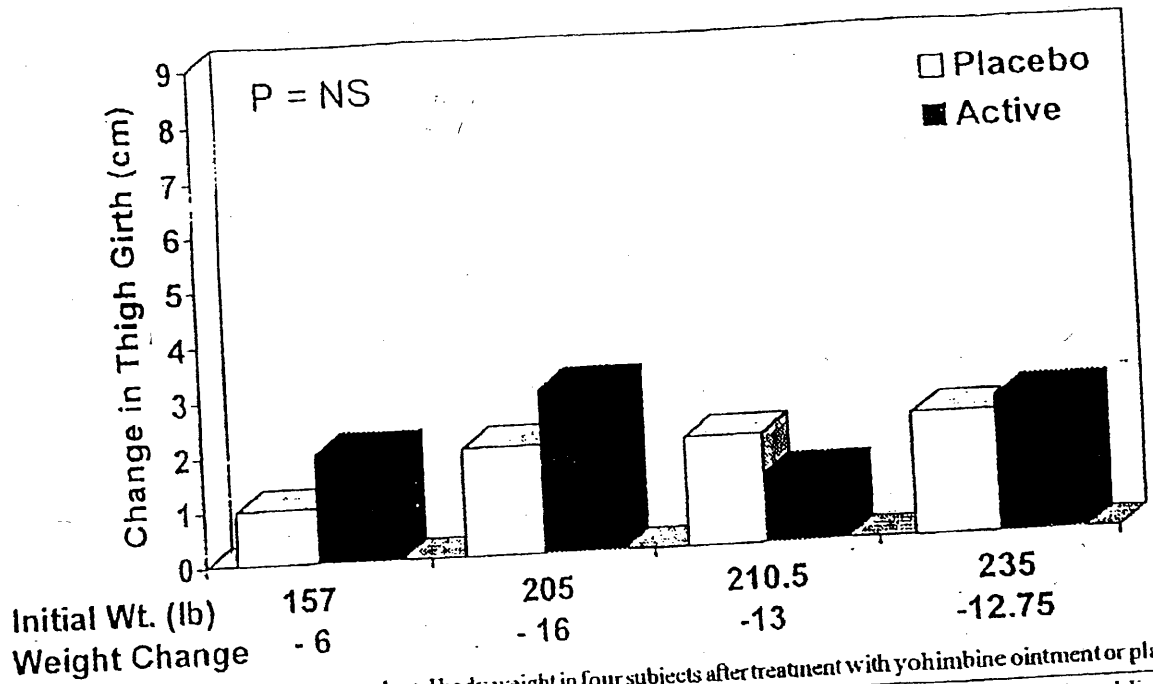

Figure 3: Changes in thigh circumference and total body weight in four subjects after treatment with yohimbine ointment or placebo.

tribution. Women, through the effect of estrogen, have more α-2 receptors on the fat cells of their hips and thighs. This gives a higher lipolytic threshold and causes the concentration of fat in that area in women (1,2,9,12).

Many women are distressed as they lose weight that their hips and thighs remain undesirably fat. To test the hypothesis that local fat reduction could occur by modulating normal fat cell function, we chose women's thighs as the test area for two reasons. First, thighs offer a key advantage in that each woman has a matched set so that one can be used as a control and the other treated. Second, reduced lipolytic receptor activity made this an easy area to test.

Methods and Experimental Protocols

Study 1

The first study compared injections of a β adrenergic agonist against placebo (4,6). Isoproterenol (10^{-5} mM = 2 p moles), a highly selective β agonist, was injected in 0.2 mL of physiological saline at 4 cm intervals around the circumference of the thigh two-thirds of the way from the knee to the

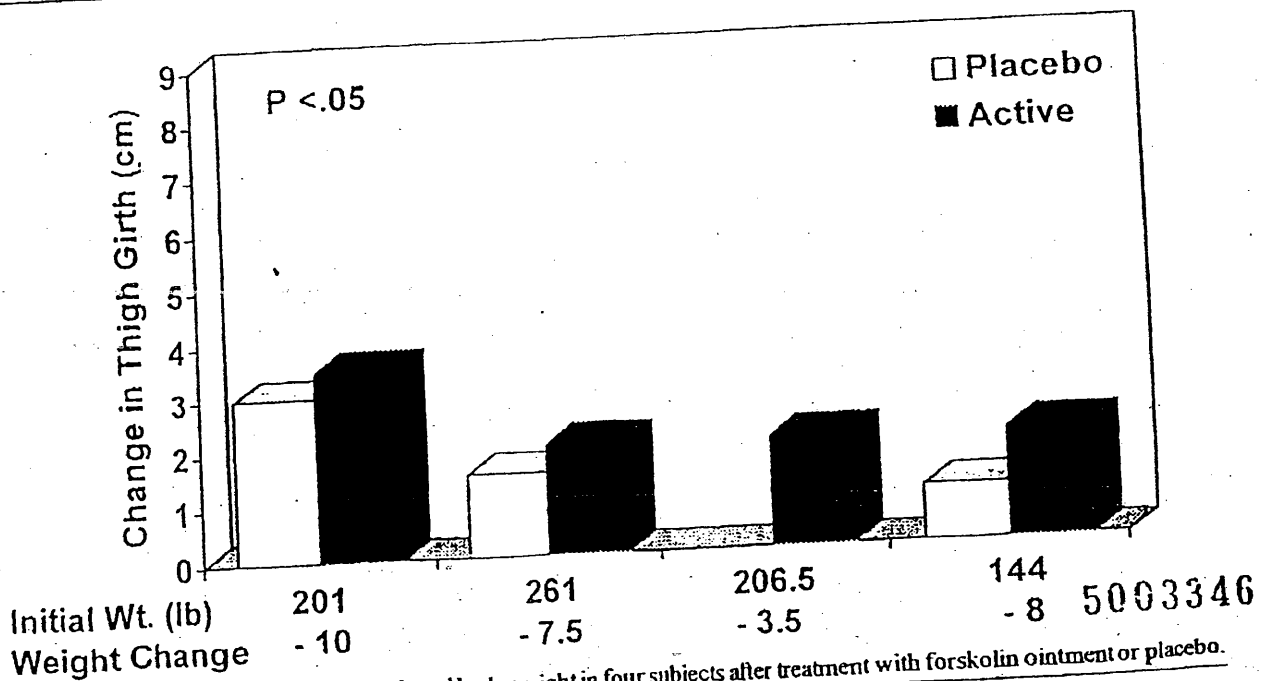

Figure 4: Changes in thigh circumference and total body weight in four subjects after treatment with forskolin ointment or placebo.

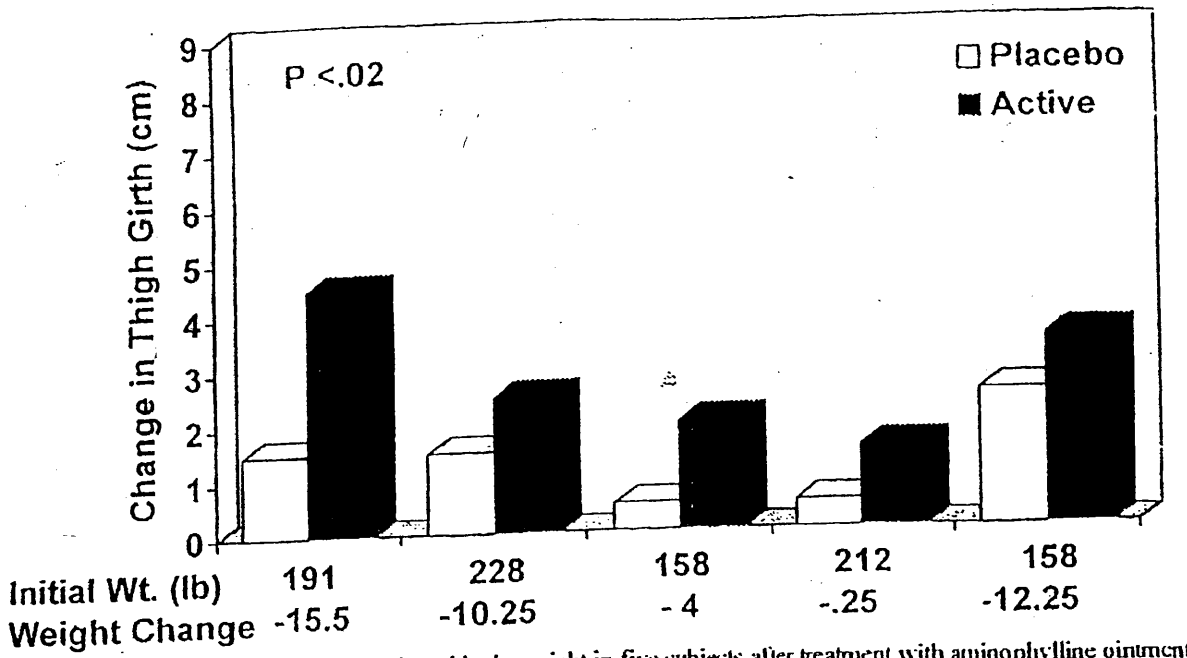

Figure 5: Changes in thigh circumference and total body weight in five subjects after treatment with aminophylline ointment or placebo.

greater trochanter. This dose of isoproterenol was selected since it gives maximal lipolysis in vitro. The spheres of diffusion of the isoproterenol overlapped as judged by the vasodilatation at the skin surface. Five women participated in this single-blind study. The participants were more than 20% above their desirable weights (mean weight 95 kg) and were placed on a 600 kcal/day diet and asked to participate in a walking program. Injections were given three times a week for 4 weeks using one thigh as a saline-injected control. The subjects had their thigh girths measured weekly.

Study 2

The second study tested whether fat loss could be produced using a topical preparation (4,6). In this study, women more than 20% above their desirable weights were given a 600 kcal/day diet and encouraged in a walking program. A combination of compounds that could affect the lipolytic process by different mechanisms was compounded.

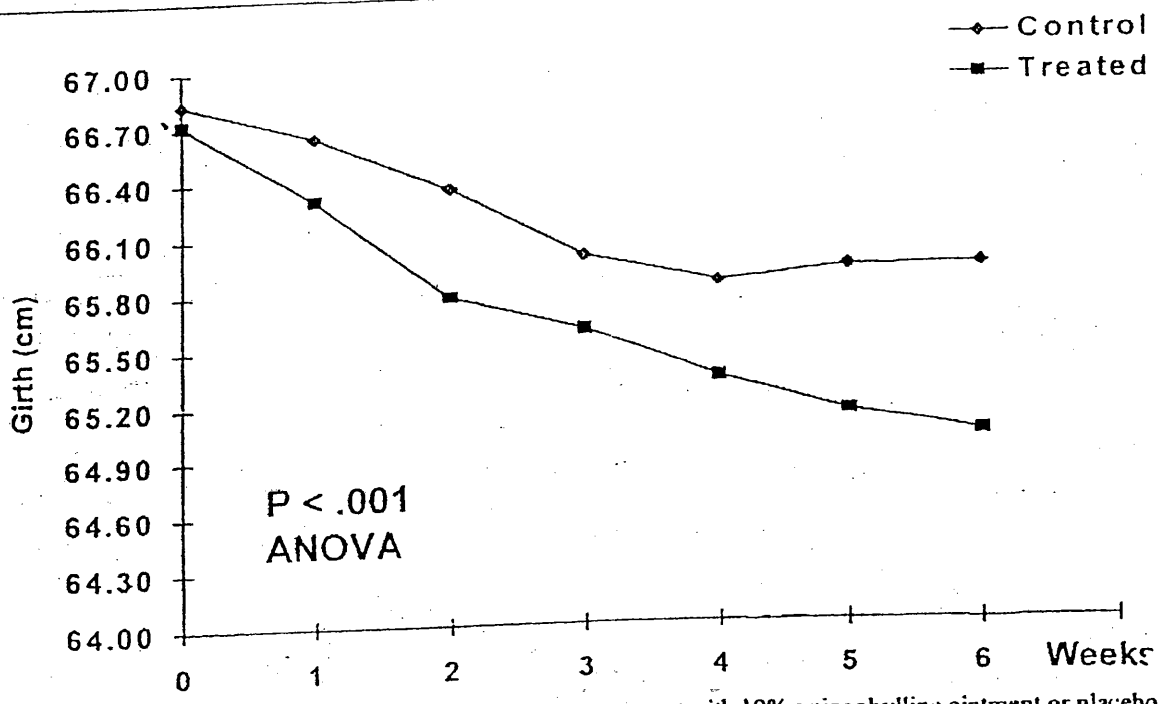

Figure 6: Changes in thigh circumference in 23 subjects after treatment with 10% aminophylline ointment or placebo.

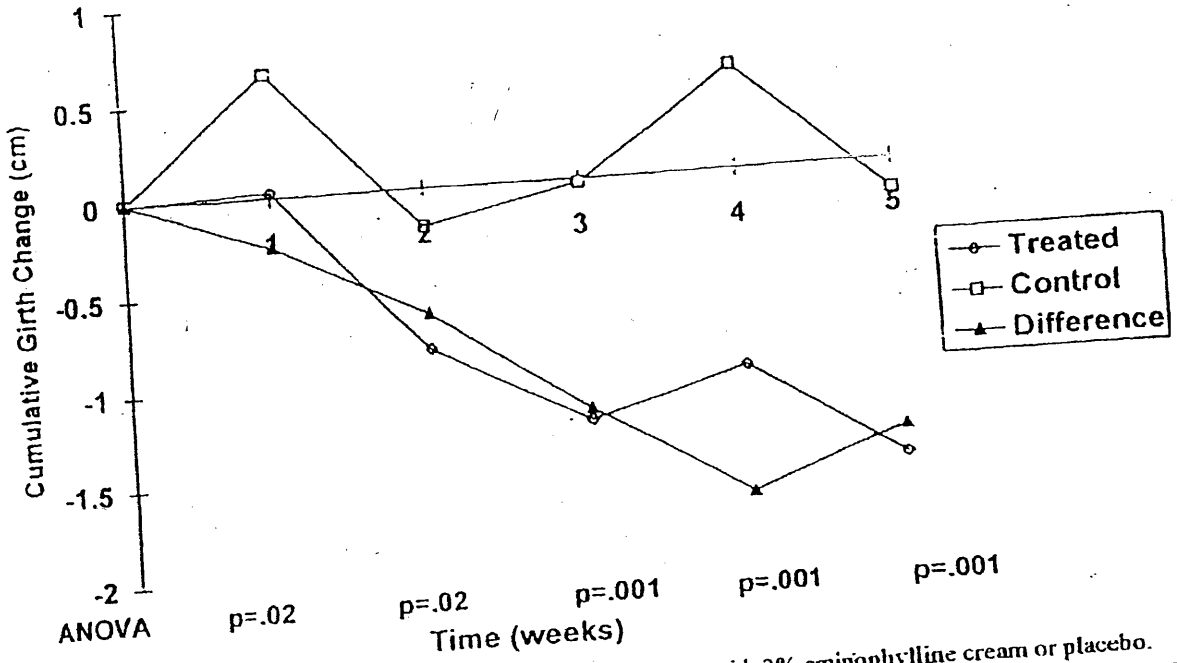

Figure 7: Changes in thigh circumference in 11 subjects after treatment with 2% aminophylline cream or placebo.

This necessitated using a carrier that would accept compounds with diverse solubility characteristics. The final concentrations in the aquaphor base were 1.2×10^{-5} mol/L forskolin (a β stimulator), 2.5×10^{-4} mol/L yohimbine (an α-2 antagonist), and 1.3×10^{-2} mol/L aminophylline (an adenosine receptor antagonist and an inhibitor of phosphodiesterase). In this double-blind study, the subjects' thighs were wrapped with warm 600 to 900 mOsm/L magnesium sulfate solutions for 30 minutes prior to each of the five day/week ointment application to maximize transcutaneous absorption. An occlusive plastic wrap was placed over the area to which the ointment was applied throughout the 4-week study period. Measurements of girth two-thirds of the way between the knee and the greater trochanter were used to judge local fat loss of the thigh treated with the aquaphor vehicle and compared to the thigh receiving active treatment. Five women greater than 20% over weight (mean weight 83 kilograms/182 pounds) participated in the study.

Study 3

Study 3 was divided into three smaller studies which tested the effect of each component of this combination ointment used in Study 2 to determine whether they were effective individually (4,6). A total of 18 women greater than 20% overweight participated in these three studies (mean weight 90 kilograms). The women were placed on an 800 kcal/day diet and encouraged to engage in a walking program. Warm wraps with 600 to 900 mOsm/L of magnesium sulfate solution were again applied to the thighs for 30 minutes five times per week before application of the ointment. An occlusive plastic wrap was not used. Six women had one thigh treated with an ointment containing 25×10^{-5} mol/L forskolin, six women had one thigh treated with an ointment containing 5×10^{-4} mol/L yohimbine, and six women had one thigh treated with ointment containing 1.3×10^{-2} mol/L aminophylline. The other thigh was treated with aquaphor vehicle as the control in a double-blind fashion. Evidence of local fat loss was again taken to be a significant ($p<0.05$) loss of thigh girth at two-thirds the distance between the knee and the greater trochanter on the treated versus the untreated thigh.

Study 4

The fourth study used a 10% aminophylline ointment and was performed at the UCLA Medical Center. Thirty women, who were more than 20% overweight, participated in this 6-week trial. They were placed on a 900 to 1,100 kcal/day diet without any specific recommendations regarding exercise. Warm wraps were omitted in this study. Five grams of the 10% aminophylline in aquaphor was applied to one thigh and an equal amount of aquaphor vehicle was applied to the other thigh as a control. The trial was double-blinded and counterbalanced so that 50% of the subjects had active ointment to the right thigh and 50% to the left. Thigh circumferences were measured weekly with weight supported on the measured thigh. Two measurements were taken, one at half the distance between the fibula head and the greater trochanter, a second 5 cm above the first. Body weight, blood pressure, and pulse were recorded weekly. Patch tests for allergy of the active ointment and the vehicle were done during the last week of the study. A chemistry panel was drawn at the beginning and end of the study. Theophylline levels were measured during the third week of the study. At the end of the study, theophylline levels were drawn at either 30 and 60 minutes or at 60 and 120 minutes after the application of the active ointment to both thighs. Patients were seen 5 days per week during the study at which time ointments were

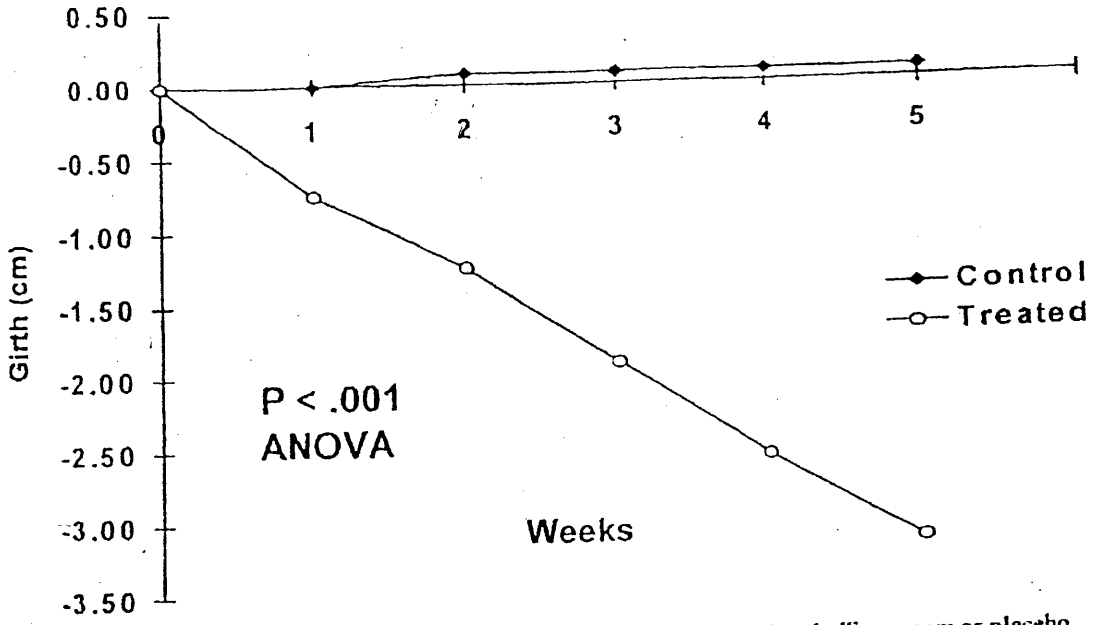

Figure 8: Changes in thigh circumference in 12 subjects after treatment with 0.5% aminophylline cream or placebo.

applied to the thighs by study personnel.

Study 5

A 2% concentration of aminophylline in a cream base was used. Twelve women who felt that their thighs were undesirably fat and dimpled were entered into the study. Some women wanted to lose weight while others were satisfied with their present weight. Thus no specific diet was recommended. A chemistry panel was drawn at the beginning and end of the study. Skin patch testing was done during the last week of the study and theophylline levels were drawn on the final day, 90 minutes after the cream containing aminophylline had been applied to both thighs. An aliquot of 5 g of cream was applied by study personnel in a double-blind and counterbalanced fashion so that 50% of the subjects had active cream applied to the right thigh and 50% had active cream applied to the left thigh. Subjects were seen five days a week for six weeks. Thigh circumference was measured at the beginning of each study week with weight supported on the measured thigh. Circumference was measured at one-half the distance from the fibular head to the greater trochanter.

Study 6

Twelve women volunteered for a study using 0.5% aminophylline cream. This clinical trial used the same metholodology as Study 5 with only the concentration of the cream being changed.

Results

Study 1

All five women completed this study and four lost weight (Figure 1). The women who lost weight lost more girth from the treated than the control thigh. For the group as a whole, there was a 1.8± 0.89 cm greater girth loss on the isoproterenol-treated thigh than on the control thigh ($p<0.05$ by paired t-test). This result was encouraging, although not many women wanted to have multiple injections three times a week around the entire circumference of their thighs.

Study 2

All five participants lost more girth on the treated thigh than the control thigh with a mean ± SEM difference of 2.03 ± 1.36 cm ($p<0.05$ by paired t-test) (Figure 2). This study showed that local fat reduction could be produced without weight loss. During this study, which was conducted during the summer, one of the women developed a heat rash under the occlusive plastic wrap on both legs that disappeared with discontinuation of the plastic wrap.

Study 3

Four of the five subjects completed the yohimbine trial. All lost weight and all but one lost more girth on the treated than on the control thigh (Figure 3). The woman who lost more girth on the placebo thigh was the first entrant into the study. After her first visit, it was found that girth measurements were more reproducible if the subject supported her weight on the thigh being measured so as to give a reproducible muscle tension. Since this was not done in this one patient, the omission may account for her unexpected result. The yohimbine group lost more on the treated thigh than the control thigh. 0.75 ± 0.35 cm, although this did not reach statistical significance due to the small numbers. The four subjects who completed the forskolin trial all lost weight and lost more girth on the treated than the control thigh, 1.0 ± 0.61 cm ($p<0.05$ by paired t-test) (Figure 4). The five subjects who completed the aminophylline trial all lost weight and lost 1.5 ±0.77 cm more girth on the treated thigh than the control thigh ($p<0.02$ by paired t-test) (Figure 5). There were no rashes or

other adverse events. There were no changes in blood pressure or pulse. The five patients who dropped out of the study did so after 3, 6, 7, 8 and 10 days. Four of the five patients had lost more girth from the treated than the placebo thigh at the time that they left the study. The fifth patient dropped out on the third day of the study and there had been no change in her thigh girth. On the basis of these studies, it was concluded that each agent could produce a change in thigh girth. Aminophylline was selected for further studies for two reasons. First, it is more soluble than other xanthines. Second, there has been a long-term experience with aminophylline as a drug for treating asthma in which its low toxicity has been well defined.

Study 4

Twenty-three of the 30 women completed this 6-week trial (Figure 6). No significant changes were seen in the chemistry results except for a reduction in triglycerides from 245 ± 121 mg/dL to 139 ± 57 mg/dL ($p<0.05$) and an increase in free fatty acids from 0.3 ± 0.24 mEq/L to 0.52 ± 0.35 mEq/L ($p<0.05$) which could have been due to the effect of the calorie restricted diet and weight loss. No theophylline could be detected at any time point, and patch testing showed no sensitivity. Weight declined by 3.3 ± 2.2 kg over the course of the six-week study. There were no significant changes in pulse rate or blood pressure. Thigh girth loss was greater in the treated than in the control thigh at the end of the study (Figure 6), 0.77 ± 0.66 cm for the lower girth measurement and 0.78 ± 0.89 cm for the upper girth measurement ($p<0.001$ by ANOVA).

Study 5

Eleven women completed this study (Figure 7). There was no skin irritation with patch testing. Three weeks after the start of the study, one woman developed a rash on the leg being treated with active cream. The cream was stopped and the rash resolved. This patient was then re-entered into the study using 0.5% aminophylline cream. She did not redevelop the rash and lost 2 cm more in girth on the treated than on the control thigh after five weeks of treatment. Problems with freezing and thawing made chemistry panel analysis unreliable. Theophylline levels were undetectable except for one subject who violated protocol and took theophylline for an asthma attack on the night prior to her theophylline level being drawn. The group of 11 women lost more girth from the treated than from the control thigh, 1.21 ± 0.31 cm ($p<0.01$ ANOVA) (Figure 7). Ten of the 11 women lost more girth on the treated than the control thigh at the end of the study. The one woman who, at the end of the study, lost more girth on the placebo thigh had lost more girth on the treated thigh the week before, and the final measurement could have been an error.

Since the 2% aminophylline cream caused a rash in one subject and that subject showed a 2 cm girth differential at the end of five weeks of treatment without a rash on 0.5% aminophylline cream, it seemed prudent to investigate further the lower cream concentration in Study 6.

Study 6

All 12 subjects lost more girth on the treated thigh than the control thigh at 5 weeks of treatment. 3.08 ± 0.27 cm ($p<0.001$ by ANOVA) (Figure 8). The chemistry panel showed significant decreases in ALT, LDH, globulin and creatinine ($p<0.01$ by paired t-test), but these were felt to be clinically insignificant since the changes were within the normal range for the test. There were no rashes, patch testing was negative, and theophylline levels were below the detectable threshold.

Discussion

These six trials demonstrate that local fat reduction as measured by thigh girth, can be produced by topical application of compounds that stimulate lipolysis. Although we made no direct measurement of fat loss, it seems unlikely that the girth change could be attributable to any other component of the thigh, since the physiology of these compounds is known to affect the lipolytic process, and fat cells are directly under the skin where the active ingredient could modulate normal rates of lipolysis.

Differential thigh girth loss with topical lipolytic treatment does not require weight loss, warm hypertonic saline soaks, occlusive dressings, or an exercise program. Since the fat cells directly under the skin are stimulated first, the thigh skin loses its dimpling as tension is relieved on the subcutaneous tissue that attaches to the undersurface of the dermis. This occurs even before significant girth changes can be measured. Without weight loss, the possibility that under topical treatment fat may be redistributed from the thighs to the intra-abdominal area seems unlikely. Lipolysis and lipogenesis are ongoing processes, and the topical treatment only contacts the cells immediately below or in the dermis. The amount of fat mobilized would presumably redistribute through all other fat depots. Thus, the amount partitioned to any single area is likely to be vanishingly small. Definitive answers to these questions, however, will require CT or MRI scans.

The indication for topical lipolytic treatment is cosmetic. Many women are concerned about the appearance of their thighs whether or not they are obese. The women receiving this cream experienced an improved self-image which can result in tangible improvements in their perceived quality of life. They were offered 2 to 3 months of free active cream after they completed the study and all wanted it. It is easier to measure the benefit to women who are so distressed about the appearance of the fat on their thighs that they resort to a surgical procedure such as liposuction for therapy. Topical lipolysis is almost certainly safer and does not carry with it the attendant risks of a surgical procedure with its scarring and risks of infection as well as the risks of anesthesia.

Attempts have been made over many years to affect local fat reduction by a variety of nonsurgical methods. Of these methods, none have been shown to be effective. Now there is an effective method to achieve local fat reduction topically by manipulating the lipolytic mechanism and obviating the need for more risky surgical interventions.

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Permeation, metabolism and site of action concentration of nicotinic acid derivatives in human skin Correlation with topical pharmacological effect

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Abstract

A novel methodology for establishing a pharmacological dose–effect relationship of methyl nicotinate, hexyl nicotinate and nicotinic acid acting as peripheral vasodilators in the skin following topical application is investigated. This methodology involves the estimation of the unbound drug concentration in the aqueous compartment at the site of action in tissue, termed C^*, which was evaluated as the pertinent concentration responsible for the pharmacological effect. Blood capillaries next to the epidermis–dermis boundary were postulated to be the relevant site of action. C^* was estimated from drug transport parameters for different layers of human cadaver skin determined in vitro. Immunohistochemical studies showed that the plane of separation of skin achieved by heat treatment was between the basal cells of the epidermis and the lamina lucida, confirming the integrity of the epidermis and the dermis used in the experiments. The permeation rate for epidermis increased drastically with increasing lipophilicity of the drug. Dermis permeability was roughly the same for all three compounds. The epidermis represented the major transport barrier in vitro for methyl nicotinate and nicotinic acid but not for hexyl nicotinate. The esters were metabolised to nicotinic acid during tissue permeation to an extent that was rather limited for the epidermis but very pronounced for the dermis. Nonspecific α-naphthylacetate-esterase activity was predominately located in the dermis, which was in agreement with the metabolism results. The drugs were applied each at three different concentrations in vivo to the ventral forearm of healthy human volunteers and vasodilation was evaluated based on skin erythema which was quantified by measuring colour change of reflected light. Area under the curve of the change of colour co-ordinates as a function of time was used as a measure of pharmacological effect. The pharmacological effect of all three drugs was comparable when similar C^* values were considered, even though the concentrations applied to the skin differed by orders of magnitude. The effect showed a strong positive dependence on C^*. Methyl and hexyl nicotinate showed identical, nearly sigmoidal effect/C^*-profiles, while the profile for nicotinic acid was linear, suggesting a possible difference in the intrinsic pharmacological potency between the esters and the acid. These results demonstrate the validity of C^* as the relevant drug concentration for the cutaneous pharmacological effect of the topically applied drugs and underline the usefulness of the presented methodology for establishing dose–response relationships in dermal therapy and expressing bioavailability.

Keywords: Nicotinic acid esters; Transdermal permeation; Metabolism; Topical application; In vivo pharmacological dose–effect relationship; Site of action drug concentration

1. Introduction

Skin penetration and permeation of drug after topical administration depend on the physicochemical properties of the drug molecule as well as on the function of the skin as a transport barrier and can be influenced by the applied formulation (Leopold and Maibach, 1996; Bach and Lippold, 1998). These factors, along with skin first-pass metabolism and haemodynamic parameters of the cutaneous tissue, determine the bioavailability of topically applied drugs. The experimental determination of bioavailability and, hence, bioequivalence of dermal formulations presents a formidable challenge because of the difficulty entailed in the measurement of drug concentration in a compartment of cutaneous

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tissue that is relevant for the pharmacologic action of the drug (Code of Federal Regulations, 1999). The site of pharmacologic activity of the drug may vary in terms of depth within the skin and the subcutaneous tissue depending on the treatment. Also, specifying a mode of administration with 100% dermal bioavailability to be used as a reference, in analogy to the intravenous bolus administration in systemic delivery, is not straightforward, neither is the definition of the dose of a dermal formulation given as the amount of drug released by the vehicle and taken up by the skin or as the total drug amount applied with the formulation to the skin surface. Determination of bioavailability based on pharmacologic effect is complicated by the fact that the relationship between drug concentration and pharmacologic effect is not linear.

These issues are related to the difficulty of establishing meaningful pharmacologic dose–response relationships in dermal drug therapy. Approaches taken to this end include using (i) the entire drug amount applied topically (Ryatt et al., 1986; Realdon et al., 1995), (ii) the drug amount (flux) which is released by the formulation in vitro (Realdon et al., 1996) or in vivo (Leopold and Maibach, 1999) and (iii) the drug amount measured in stratum corneum by tape stripping (Pershing et al., 1994) considered to correspond to the drug amount in plasma in case of transdermal medication (Shah and Maibach, 1993), as the dose to be related with the pharmacological effect. While topical bioavailability may correlate with the applied drug amount or the amount released from the formulation when dealing with the same drug, and the amount determined by tape stripping may give a good indication of skin permeation of the drug, bioavailability in general may be influenced by processes at the lower tissue layers, skin first-pass metabolism and haemodynamic effects. Thus, linear multi-compartment models were proposed to describe the distribution and micropharmacokinetics of drug in skin. One model encompassing dermis, subcutis, underlying tissue layers, blood microcirculation and contralateral tissue (Singh and Roberts, 1993; Singh et al., 1998) was validated for the steady state situation using hairless mice with and without blood supply and analysing dermatomed tissue layers. Differences were identified between lipophilic compounds, which were found to penetrate by direct diffusion into subcutaneous tissue located below the application site and hydrophilic compounds, which were taken up by the peripheral blood circulation. Another model describing the interaction between skin and topically applied formulation and drug biotransformation during percutaneous absorption was experimentally validated using the isolated perfused porcine skin flap model (IPPSF) combined with tape stripping and sectioning of skin punch biopsies for determining drug concentration in the skin (Riviere et al., 1995).

The goal of the present work is to evaluate a novel method for establishing a dose–response relationship for topically applied dermatological formulations in humans in vivo. This method entails that the drug concentration at the site of action in the skin is related to the pharmacological effect of the drug. Nicotinic acid derivatives are used as model drugs. The estimation of the drug concentration at the site of action is based on the C^* concept that was proposed earlier. This utilizes a mechanistic model to describe drug transport through successive tissue layers and permeation parameters of the drug for these layers for calculating drug concentration at the interface between epidermis and dermis (Su et al., 1991; Imanidis et al., 1994). This concept was applied to the estimation of the active site concentration of Acyclovir used in the treatment of cutaneous Herpes Simplex Virus-1 infections in mice. As outlined below, this concept allows the estimation of the free, i.e. unbound (rather than the total) drug concentration in the tissue which represents the pharmacologically active fraction and takes into account that a concentration gradient prevails as one moves from the surface to deeper skin layers. These characteristics are not afforded by other techniques involving, for example, punch biopsy, tape stripping, dermatomed layers, suction blister and microdialysis (Surber, 1996) used partly in conjunction with the multicompartment models mentioned above. These techniques yield total tissue drug amount with the exception of microdialysis and suction blister and have all a limited spatial resolution, which for microdialysis specifically is related to the positioning of the tube. Implications of the present study for the assessment of the bioavailability of dermal formulations are discussed with respect to estimating active site drug concentration utilising the outlined method as compared with other proposed methods.

The pharmacological effect of topically applied nicotinic acid derivatives is a vasodilation of the peripheral blood capillaries which are located in the dermal papillae of upper dermis layers adjacent to the epidermis–dermis junction. The mechanism of this action involves the release of prostaglandin D_2 as an important step. It is still uncertain which cell type of the skin exactly is responsible for prostaglandin release. However, prostaglandins have a very short half-life being rapidly metabolised and therefore act strictly locally on cells by which they are released or on neighbouring cells (Robertson, 1995). Considering this, it is postulated that the C^* concentration of nicotinic acid derivatives estimated at the epidermis–dermis interface, which is in the immediate vicinity of the blood capillaries, represents the active site drug concentration.

Nicotinic acid and its hexyl and methyl esters were chosen as drug compounds because of the differences in their lipophilicity which should provide different in vitro and in vivo permeabilities through skin (Dal Pozzo et al., 1991; Le and Lippold, 1995; Guy et al., 1986). Metabolism of the drug esters during permeation through different skin layers resulting in nicotinic acid is investigated since it may affect bioavailability. Cutaneous esterases were reported to be mostly located in the epidermis and in skin associated glands such as hair follicles and perspiratory glands. It was shown that total esterase activity for hydrolysing esters of corticosteroids was equal between the epidermis and the dermis, but it was 20 times higher in the epidermis than in the dermis

when referred to tissue weight (Täuber and Rost, 1987). There was no esterase activity in the stratum corneum. Investigations with mono- and diesters of salicylic acid in cryøstat sections of human skin transplanted onto hairless mice and of human cadaver skin have shown a higher activity of esterases in the upper skin layers (Guzek et al., 1989). Further, ethyl nicotinate was shown to be incompletely metabolised to nicotinic acid during skin transport (Rittirod et al., 1999a).

Permeation parameters required in the C^* approach are determined in vitro using human cadaver skin. Permeation and simultaneous metabolism of the three drugs is studied in the epidermis, the dermis and full thickness skin and permeability parameters of parent drug and, where applicable, metabolite for the different skin layers are determined. The exact plane of separation between epidermis and dermis achieved experimentally by heat treatment is determined histochemically. This is crucial for accurately assigning the determined parameters to tissue layers and forms the basis for the estimation of the active site drug concentration using these parameters. Furthermore, localisation of esterase activity in the epidermis and the dermis is carried out histochemically and is used to interpret the transport and metabolism results.

The pharmacological effect is measured in vivo in healthy human volunteers. The skin erythema caused by vasodilation is scaled using the tristimulus skin colour reflectance (SCR) technique (Wilhelm and Maibach, 1989). This technique is well qualified for quantification of effects on the peripheral blood circulation in comparison to other non-invasive methodologies such as laser Doppler velocimetry, photopulse plethysmography and visual scoring (Guy et al., 1983; Wilhelm et al., 1989).

The employed derivatives have all the same pharmacological effect (Roberts and Morrow, 1997) but they should differ greatly in their skin permeability which expectedly affects the concentration level they reach at the site of their pharmacological action. Each of them was applied to the skin at different concentrations. This made possible to study the relationship between pharmacological effect and concentration at the site of action with the same compound but also for different compounds.

2. Materials and methods

2.1. Drugs and solutions

Nicotinic acid (NA) and its methyl and hexyl esters (MN and HN, respectively) (Sigma, St. Louis, MO, USA) were used as received. For the in vitro permeation experiments, donor solutions were prepared in phosphate buffer (pH 6.8, $\beta = 0.03$) in concentrations of 365 mM and 1 mM for methyl nicotinate, 145 mM for nicotinic acid and 0.7 mM for hexyl nicotinate. The osmolarity of the solutions was adjusted to the physiological level of 285 mosm/l with NaCl except for the 365 mM methyl nicotinate solution which was adjusted to 400 mosm/l. The above phosphate buffer with appropriately adjusted osmolarity to match that of the drug solution was used as receiver solution.

2.2. Human skin for in vitro studies

Cadaver skin from the abdominal region of female donors with no skin diseases or serious internal illnesses (Institute of Pathology of the University of Basel) was isolated within 24 h from death, freed of subcutaneous fat, placed in an air-tight container and immediately stored in a freezer at −70 °C. Before use, skin specimens with no visible hair were thawed at room temperature and soaked in receiver solution for 30 min. When the epidermis or the dermis were investigated separately, they were separated from each other by heat treatment of the skin for 60 s in receiver solution at 60 °C and subsequent mechanical peeling (Kligman and Christophers, 1963) and then they were soaked in receiver medium for 30 min. Intactness of the barrier function of prepared tissue was verified in previous studies by transepidermal water loss (Betz et al., 2001) and membrane electrical resistance measurements (Kochhar and Imanidis, 2003).

2.3. In vitro permeation experiments

A novel glass diffusion cell was developed (Fig. 1). In this, the skin membrane was clamped between donor and receiver compartment against rims with ground glass surface that were spherical in form (rather than plane) with a radius of curvature of 14.3 mm. With this geometry, the membrane was positioned far below the fluid level, preventing the accumulation of gas bubbles at the membrane upon withdrawal of sample volumes as large as one third of the volume of the receiver solution. It was therefore possible, by varying sampling volume and sampling frequency over a wide range and taking advantage of the fairly small volume of the receiver solution, to attain a high sensitivity of flux measurement while maintaining sink conditions. The highly flexible sampling regimen made possible to imitate conditions prevailing in flow-through arrangements. The surface area of diffusion was 2.69 cm^2.

Two ml of drug solution were added occlusively to the donor compartment and 14 ml of receiver solution were used, providing identical fluid levels in both compartments. Occlusion was used in order to eliminate effects arising from the evaporation of vehicle and simulate the conditions of the in vivo study in which drug was applied in Finn chambers. Three diffusion cells mounted on a support base were kept in a water bath at 37 °C and run simultaneously, the receiver solutions stirred at 500 rev./min with Teflon paddles interconnected with a bead string. The materials comprising the cells were inert, showing a maximal concentration decrease for a 0.2 mM solution of hexyl nicotinate of 22% over a 48-h period due to sorption on the Teflon paddle.

Samples of 1–4 ml were drawn from the receiver compartment at predetermined time intervals and replaced with

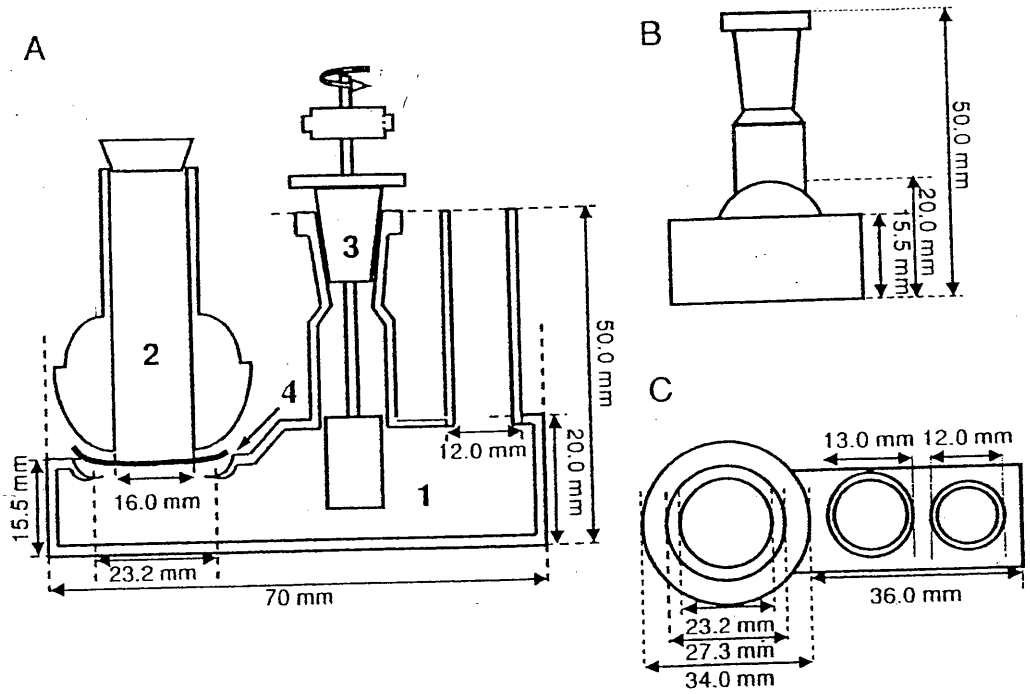

Fig. 1. (A) Cross section of the diffusion cell; 1, receiver compartment; 2, donor compartment; 3, mechanical stirrer; 4, membrane. (B) Frontal view of the receiver compartment; donor compartment in the foreground not shown. (C) Top view of the receiver compartment.

fresh buffer. Samples from the donor solution were drawn in the beginning and the end of the permeation experiment. Chemical stability of hexyl and methyl nicotinate was investigated in separate experiments under conditions identical to those of the permeation studies with samples taken at 1, 2, 4, 6, 8, 10, 12, 24 and 48 h. All samples were analysed for nicotinic acid, hexyl or methyl nicotinate by HPLC.

2.4. HPLC assay

Samples were centrifuged for 10 min (3500 to 15 800×g) and the supernatant was analysed at room temperature by HPLC (Hewlett Packard, series 1050, Hewlett Packard, Waldborn, Germany). A reversed-phase RP-18 column (Spherisorb ODS2, 5 μm, 125×4 mm) with precolumn was used and a flow rate of 1 ml/min. For analysis of nicotinic acid alone, the injection volume was 100 μl, the mobile phase was phosphate buffer (pH 7.5, $\beta = 0.06$) containing 5 mM tetrabutylammonium-hydrogensulphate and detection was performed UV-spectrophotometrically at $\lambda = 219$ nm. For simultaneous assay of methyl nicotinate and nicotinic acid, the injection volume was 10 μl, the mobile phase was a mixture of aqueous phosphate buffer (pH 7.5, $\beta = 0.06$ with 5 mM tetrabutylammonium-hydrogensulphate) and acetonitrile at a ratio of 97:3 (v/v) and detection was performed spectrophotometrically at $\lambda = 219$ nm. For simultaneous assay of hexyl nicotinate and nicotinic acid, the injection volume was 100 μl and the composition of the mobile phase was varied in a gradient mode as follows: 0 to 2 min, phosphate buffer (pH 3.6, $\beta = 0.01$); 2 to 4 min, linear change to a mixture of this phosphate buffer and acetonitrile with a ratio of 50:50 (v/v); 4 to 14 min, phosphate buffer–acetonitrile 50:50 (v/v); 14 to 16 min, linear change to phosphate buffer (pH 3.6, $\beta = 0.01$); stop time = 20 min. Nicotinic acid was detected at $\lambda = 262$ nm and hexyl nicotinate at $\lambda = 219$ nm.

2.5. Histochemical investigations of skin

To determine the exact plane of separation of the skin after heat treatment, pieces of skin were fixed in formaldehyde, embedded in paraffin and sections with a thickness of 10 μm were prepared. A general background staining with haematoxylin–eosin and with the triple dye of van Giessen (Romeis, 1989) was applied first. Subsequently, sections were preliminarily treated with pronase type 14 for 10 min and the protein collagen IV and the glycoprotein laminin, which are present in the basal membrane of the epidermal–dermal junction, were stained immunohistochemically. For this purpose, the Vectostain Elite® ABC (Avidine Biotin Complex) standard kit (Vector Laboratories, Burlingame, CA, USA) was used. Laminin was labelled using a polyclonal IgG antibody from rabbits (Eurodiagnostica, Arnhem, The Netherlands) as a primary antibody, an antirabbit IgG antibody from goats (Vector Laboratories) associated with biotin as secondary antibody, a chromogen converting biotinylated peroxidase complexed with avidin, and diaminobenzidine (Vector Laboratories) as chromogen. Collagen IV was revealed using an IgG antibody from mouse (Daco, Glostrup, Denmark) as a primary antibody,

an antimouse IgG antibody from goat (Vector Laboratories) associated with biotin as secondary antibody, a chromogen converting biotinylated peroxidase complexed with avidin, and diaminobenzidine (Vector Laboratories) as chromogen. The same methods without primary antibody were used as control.

To investigate the distribution of α-naphthylacetate esterase (ANAE), skin samples were fixed in 4% formaldehyde–sucrose for 4 h followed by 12 h in 30% sucrose–phosphate buffered saline. Cryostat sections with a thickness of 8 to 10 μm were prepared and air-dried. The sections were incubated at room temperature under the microscope for 5 to 30 min with a mixture of (I) 40 ml of a 0.1 M phosphate buffer pH 7.5 containing 0.5% of a 4% pararosaniline solution and 0.5% of a 4% sodium nitrite solution and (II) 1 ml of a 1% α-naphthylacetate solution in acetone. Subsequently the sections were rinsed under running water for 5 min, air-dried, and incubated with Harris haematoxylin for 4 min to stain the nuclei of the cells. After 12 h the sections were covered with a cover glass using Eukitt®. The incubation medium without α-naphthylacetate was used as a control (Leder, 1967).

2.6. Analysis of the permeation data

Normalised skin fluxes ($\hat{J}$) into the receiver compartment were calculated for the parent drug, i.e. the drug initially added to the donor compartment and, where applicable, the metabolite, i.e. nicotinic acid and their sum using the steady state portion of the permeation curve according to Eq. (1)

$$\hat{J} = \frac{\Delta m/\Delta t}{C_{D,\Delta t} \cdot S} \quad (1)$$

where $\Delta m/\Delta t$ is the slope of the amount versus time curve obtained by linear regression, $C_{D,\Delta t}$ is the mean donor concentration of the parent drug in the time interval $\Delta t = t_2 - t_1$, S is the surface area of diffusion, t_1 is lag time and t_2 marks the end of the linear segment of permeation. Using a longer time interval and non-linear fitting to calculate normalised fluxes introduced a larger variability to the results. $C_{D,\Delta t}$ was calculated from the initial drug amount and the transport data for parent drug and degradation product based on mass balance considerations.

For the back diffusion of metabolite in the donor compartment, a normalised flux ($\hat{J}_{NA,D}$) was calculated using Eq. (2) assuming that the flux remained constant over the entire duration of the experiment

$$\hat{J}_{NA,D} = \frac{\Delta m_{NA}/t_{tot}}{C_{D,t} \cdot S} \quad (2)$$

where Δm_{NA} is the difference of nicotinic acid amount in the donor between the end and the beginning of the experiment, t_{tot} is the duration of the experiment and $C_{D,t}$ is average donor concentration of the parent drug between time zero and t_{tot} that was calculated in analogy to $C_{D,\Delta t}$.

The degradation rate of the drug during the permeation experiment was defined as the normalised flux of metabolite into the receiver compartment expressed in percent of the sum of normalised fluxes of parent drug and metabolite in the same compartment.

If only chemical hydrolysis in the donor and the receiver solutions of the permeation cell were considered, then the ratio of nicotinic acid (degradation product) to parent drug in the receiver compartment would be:

$$\frac{C_{NA,R}}{C_{MN/HN,R}} = \frac{kt + \exp(-kt) + \dfrac{P_{NA}}{P_{MN/HN}} \dfrac{k^2t^2}{2} - 1}{1 - \exp(-kt)} \quad (3)$$

where $C_{NA,R}$ is nicotinic acid concentration in the receiver compartment, $C_{MN/HN,R}$ is concentration of methyl nicotinate or hexyl nicotinate in the receiver compartment, k is first order chemical degradation constant, t is time, P_{NA} is skin permeability coefficient of nicotinic acid and $P_{MN/HN}$ is skin permeability coefficient of methyl nicotinate or hexyl nicotinate. For the derivation of Eq. (3) insignificant depletion of nicotinic acid and of parent drug in the donor solution was assumed.

2.7. Selection of volunteers for in vivo study

The in vivo investigation was carried out with 20 healthy volunteers, seven males and 13 females aged between 23 and 37 years. The volunteers had neither florid skin diseases nor severe internal illnesses, were not smokers and were not treated by corticosteroids. No extraneous local therapy of any kind on the application site was allowed. One week before starting the investigation, no treatment with nonsteroidal anti-inflammatory drugs was accepted. The consumption of coffee or black tea and the washing of the application site with detergents or the use of skin care products, like lotions or cremes, on the application site was not allowed for 12 h before starting the experiment. Lack of effect of the smallest applied concentration led to the drop out of the particular subject. The volunteers participated in the study without financial compensation and signed a written informed consent.

2.8. In vivo study design

Solutions of hexyl nicotinate (HN), methyl nicotinate (MN) and nicotinic acid (NA) were prepared in the same buffer used for the in vitro experiments. The solutions were applied to skin on the ventral side of the forearm of the subjects occlusively in order to minimise environmental influences using Finn Chambers on Scanpor® application systems (Epitest, Tuusula, Finland). Each chamber contained a filter paper disk with a diameter of 8 mm (application surface area 0.5 cm^2) which was soaked with 20 μl of drug solution. Buffer was used as placebo.

The vasodilatation of peripheral blood vessels after topical application of nicotinates follows a circadian rhythm, the

maximal effect being observed during the day with peaks around noon and the minimal at night (Reinberg et al., 1995). For this reason, the investigations were carried out consistently within a short time period in the morning hours (08:00 to 11:00 h) guaranteeing reproducibility of the results and a nearly maximal pharmacologic response. The volunteers were acclimatised to the room conditions (20–23 °C, 50–60% relative humidity) for half an hour prior to the experiment. Each volunteer participated twice in the study, the first time he/she received hexyl nicotinate and the second time nicotinic acid and methyl nicotinate. A washout interval of at least 2 weeks was applied between the first and the second time. Three different concentrations of HN and a placebo solution were applied in duplicate randomly on each forearm. NA and MN were applied each on a forearm at three different concentrations along with a placebo solution in triplicate in a random fashion. HN included more test points than the other two drugs because of its rather low concentration. To exclude differences between right and left forearm, the test solutions were applied with equal frequency on both forearms among all the subjects.

Drug solutions were applied for 20 min. For HN, which had the highest skin permeation rate, the filter paper disc was replaced every 5 min to prevent drug depletion. After removing the application system, the remaining test solution drops were dabbed off with paper tissue. Twenty-five minutes after drug application commenced, the first reading of skin colour was taken. Measurements of skin colour continued every 10 min for the following 120 min. Measurements were made at the application points and at four control points located distally, proximally, and laterally of the area containing the test points. These controls were used to correct for skin colour changes during the test time. Prior to the application of the drug solutions, the application and control points were marked and a baseline measurement of skin colour was made.

2.9. Recording and analysis of the in vivo data

The measurement of skin colour was carried out using a Minolta Chroma Meter CR-300 (Minolta Camera, Osaka, Japan) with a circular measurement field with a diameter of 8 mm and the data were processed online. The colour of light reflected by the skin after emission of white light was recorded three-dimensionally based on the $L^*a^*b^*$ system (Commission International d'Eclairage, 1976) with L^* (luminance) expressing the relative brightness, a^* the colour range from green to red and b^* the colour range from blue to yellow, all on a −100 to +100 scale. The absolute colour change, ΔE^*, was calculated as follows:

$$\Delta E^* = \sqrt{\Delta L^{*2} + \Delta a^{*2} + \Delta b^{*2}}. \quad (4)$$

The change of colour coordinate a^* for each application point and at each time point, denoted as $\Delta a^*(t)$, was calculated as follows:

$$\Delta a^*(t) = \frac{a^*(t) \cdot a^*_{m,C}(t=0)}{a^*_{m,C}(t)} - a^*(t=0) \quad (5)$$

where $a^*(t)$ is the reading of coordinate a^* at the time point t and $a^*(t=0)$ is the reading of the same coordinate at time zero for the same application point. Natural changes of the skin colour during the experiment were corrected for using the mean value of the four control points at the time t, $a^*_{m,C}(t)$, and at the beginning of the experiment, $a^*_{m,C}(t=0)$. The changes of the colour coordinates L^* and b^* were calculated in an analogous manner.

The arithmetic mean of the colour change of all application sites with the same drug concentration was calculated at each time point. The AUC (cumulative area under the curve) of the skin colour change versus time curve was represented graphically for Δa^* and ΔE^* as a function of time. An example is shown in Fig. 2. The AUC of the placebo was subtracted from the AUC of the test solutions and the resulting corrected AUC was used for further analysis. In this work, the AUC between 0 min and 95 min ($AUC_{95\,min}$) after removal of the drug solution from the skin was used as a measure of pharmacological effect because in more than 80% of the measurements a maximum AUC was reached at this time point (Chan and Li Wan Po, 1993). After discarding dropouts, outliers in each concentration group, both low and high responders, were eliminated by a statistical t-test (Sokal and Rohlf, 1981). The differences in colour change between the means of the different concentration groups were tested for significance by a one-way ANOVA using $AUC_{95\,min}$ as a factor and a two-sided t-test (computer software: SPSS 8.0 for windows, SPSS, Chicago, IL, USA).

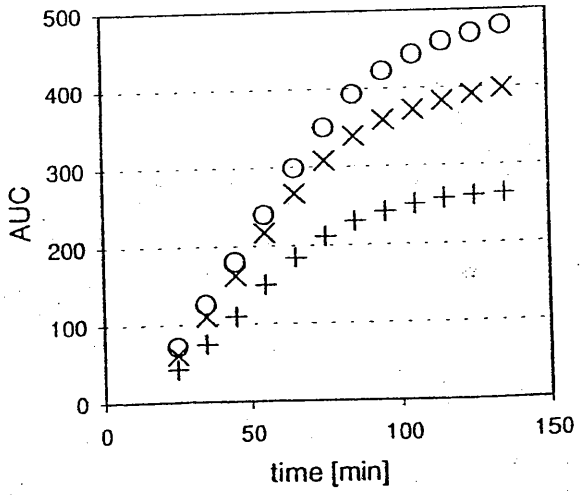

Fig. 2. Typical AUC versus time curve of the absolute colour change ΔE^* after 20 min occlusive application of 100 mM (○), 50 mM (×), and 5 mM (+) of nicotinic acid (NA) solutions.

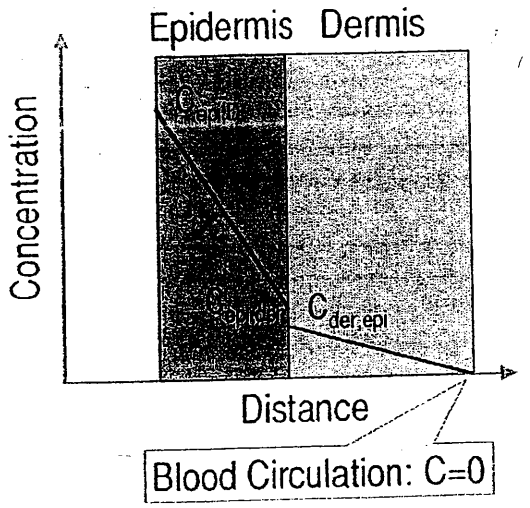

Fig. 3. Schematic representation of concentration profile and relevant concentrations during steady state flux of drug across skin.

2.10. Calculation of the drug concentration at the site of action and of the application time in vivo

The epidermal–dermal junction constitutes the site of cutaneous pharmacological activity of topically applied nicotinic acid derivatives. For the calculation of drug concentration at this interface, the permeation of drug through the epidermis and the dermis, i.e. the tissue layers bordering on this interface, at steady state conditions is considered. Assuming that the drug permeability differs between the two layers, the concentration profile of drug depicted in Fig. 3 will result. The steady state flux, J, for both tissue layers is given by Eq. (6)

$$J = \frac{D_{epi}}{h_{epi}} \cdot (C_{epi,D} - C_{epi,der}) = \frac{D_{der}}{h_{der/20}} \cdot C_{der,epi} \quad (6)$$

where $C_{epi,D}$ and $C_{epi,der}$ is the drug concentration at the borders of the epidermis facing the donor compartment and the dermis, respectively, $C_{der,epi}$ is the drug concentration in the dermis at the boundary to the epidermis, D_{epi} and D_{der} is drug diffusion coefficient in the epidermis and the dermis, respectively, and h_{epi} and $h_{der/20}$ is diffusion path length in the epidermis and the dermis, respectively.

h_{epi} corresponds to diffusion through the entire thickness of the epidermis. In the dermis, the drug enters the systemic circulation where sink conditions prevail, i.e. where the drug concentration is practically equal to zero. Since entering of the drug into the highly branched network of the systemic blood capillaries represents a random process, an effective length of its diffusion pathway in the dermis prior to reaching the sink of the circulation is defined. Previous estimates (Imanidis et al., 1994) have indicated that for drugs with intermediate hydrophilicity/lipophilicity balance, this length ($h_{der/20}$) is approximately equal to one twentieth of the anatomical thickness of the dermis.

In Eq. (6) (and Fig. 3), two tissue layers are considered for the sake of simplicity although the stratum corneum represents a distinct layer in terms of permeability properties within the epidermis. For the ensuing calculations, however, which deal with the concentration at the epidermis–dermis interface, the presence of additional permeation barriers between the 'viable' epidermis and the donor compartment is inconsequential.

With respect to the drug concentration at the epidermis–dermis interface, Eq. (7) holds

$$\frac{C_{der,epi}}{C_{epi,der}} = K_{der/epi} \quad (7)$$

where $K_{der/epi}$ is drug partition coefficient between dermis and epidermis.

The pharmacologically active, i.e. unbound drug concentration, C^*, at the site of action is calculated as follows:

$$C^* = \frac{J}{\hat{J}_{der/20}} \quad (8)$$

where J is the flux through the skin at steady state and $\hat{J}_{der/20}$ is the normalised flux for isolated dermis corresponding to the effective in vivo diffusion path length in the dermis.

The steady state mass flux in vivo may be controlled according to Eq. (6) by permeation through both the epidermis and the dermis. The in vitro skin permeation experiments demonstrate that the permeation rate of nicotinic acid and methyl nicotinate is controlled by the transport through the epidermis. This is even more so in vivo if one considers that only one part of the dermis thickness contributes to diffusional transport. For hexyl nicotinate, flux in vivo is shown to be controlled by diffusion within the donor reservoir when the shorter diffusion path length in the dermis compared to its anatomical thickness is considered. Therefore, mass flux in vivo was calculated according to Eq. (9)

$$J = C_D \cdot \hat{J}_{epi} \quad (9)$$

where $\hat{J}_{epi}$ is normalised flux determined in vitro for the epidermis and C_D is donor drug concentration.

Normalised flux for the dermis calculated according to Eq. (1) from in vitro data and corrected for the difference between the anatomical thickness and the effective in vivo diffusion path length can be expressed by Eq. (10)

$$\hat{J}_{der/20} = \frac{D_{der} \cdot K_{der/D}}{h_{der/20}} \quad (10)$$

where $K_{der/D}$ is the partition coefficient between dermis and donor solution.

By substituting the last term of Eq. (6) and Eq. (10) into Eq. (8) and taking into account firstly, Eq. (7) and secondly, that $K_{der/epi} \times K_{epi/D} = K_{der/D}$, where $K_{epi/D}$ is the partition coefficient between 'viable' epidermis and donor, the following expression for C^* results:

$$C^* = \frac{C_{epi,der}}{K_{epi/D}} \quad (11)$$

Table 1
Normalised fluxes into the receiver compartment for different skin layers

Drug[a]	Flux[b]	Epidermis	Dermis	Full thickness skin
NA	$\hat{J}_{NA}$	$1.97 \times 10^{-8} \pm 1.22 \times 10^{-8}$	1.14×10^{-5}	$2.13 \times 10^{-8} \pm 9.38 \times 10^{-9}$
MN	$\hat{J}_{MN}$	$2.12 \times 10^{-6} \pm 2.04 \times 10^{-7}$[c]	$1.08 \times 10^{-5} \pm 2.43 \times 10^{-6}$[c]	$1.79 \times 10^{-6} \pm 2.58 \times 10^{-7}$[c]
		$1.68 \times 10^{-6} \pm 8.25 \times 10^{-8}$[d]		
	$\hat{J}_{NA}$	8.07×10^{-9}[c]	$5.71 \times 10^{-7} \pm 3.46 \times 10^{-9}$[c]	$8.21 \times 10^{-7} \pm 3.52 \times 10^{-8}$[c]
		$2.98 \times 10^{-8} \pm 2.52 \times 10^{-9}$[d]		
	$\hat{J}_{tot}$	$2.12 \times 10^{-6} \pm 2.06 \times 10^{-7}$[c]	$1.10 \times 10^{-5} \pm 2.04 \times 10^{-6}$[c]	$2.50 \times 10^{-6} \pm 2.90 \times 10^{-7}$[c]
		$1.73 \times 10^{-6} \pm 8.44 \times 10^{-8}$[d]		
HN	$\hat{J}_{HN}$	$2.36 \times 10^{-5} \pm 4.45 \times 10^{-6}$	[e]	[e]
	$\hat{J}_{NA}$	$8.79 \times 10^{-7} \pm 4.48 \times 10^{-7}$	$1.30 \times 10^{-5} \pm 7.53 \times 10^{-7}$	$1.57 \times 10^{-5} \pm 3.39 \times 10^{-6}$
	$\hat{J}_{tot}$	$2.57 \times 10^{-5} \pm 4.68 \times 10^{-6}$	$1.30 \times 10^{-5} \pm 7.53 \times 10^{-7}$	$1.57 \times 10^{-5} \pm 3.39 \times 10^{-6}$

[a] Drug applied in the donor compartment; NA, nicotinic acid; MN, methyl nicotinate; HN, hexyl nicotinate.
[b] Normalised fluxes, mean ± S.D. in cm/s ($n = 2$–7) of NA ($\hat{J}_{NA}$), MN ($\hat{J}_{MN}$), HN ($\hat{J}_{HN}$) and the total of parent drug plus NA ($\hat{J}_{tot}$).
[c] Drug donor concentration, 365 mM.
[d] Drug donor concentration, 1 mM.
[e] Concentration in the receiver was not detectable.

Eq. (11) shows that C^* calculated according to Eq. (8) provides the drug concentration in a location of the epidermis immediately bordering the epidermis–dermis boundary. When an aqueous donor solution is used, $K_{epi/D} \neq 1$ will be due to drug partitioning in lipid domains of the epidermis or binding to tissue components. Consequently, C^* provides the free drug concentration in the aqueous compartment of the epidermis and, sensibly, of the dermis at the epidermis–dermis interface. Thus, C^* corresponds to the free drug concentration at the site of action of the nicotinic acid derivatives which is considered, in analogy to systemic pharmacodynamics, to be responsible for pharmacologic activity. C^* can be calculated from quantities that are experimentally accessible in vitro.

The time required to reach steady state flux across epidermis, which is relevant for the calculation of C^* in the in vivo experiments, was estimated as follows. The mass per unit area, Q_t, that flows at the epidermis–dermis interface as a function of time is given by Eq. (12), considering that initially the concentration throughout the membrane is equal to zero (Crank, 1975)

$$\frac{Q_t}{h_{epi} \cdot C_{epi,D}} = \frac{D_{epi} \cdot t}{h_{epi}^2} - \frac{1}{6} - \frac{2}{\pi^2} \sum_{n=1}^{\infty} \frac{(-1)^n}{n^2} \cdot \exp\left(\frac{-D_{epi} \cdot n^2 \pi^2 t}{h_{epi}^2}\right) \quad (12)$$

$C_{epi,D}$ is related to C_D through the partition coefficient $K_{epi/D}$. Eq. (12) was differentiated numerically with respect to time (computer software: Maple V Release®, Waterloo Maple Ontario, CA, USA) in order to calculate the mass flux at the epidermis–dermis interface as a function of time. Steady state conditions were defined to be reached when flux exceeded 80% of its maximum (asymptotic) value (Fig. 6).

3. Results and discussion

3.1. Normalised fluxes and metabolism in vitro

Normalised fluxes into the receiver compartment for the three drugs and different skin layers are given in Table 1. When methyl and hexyl nicotinate were applied, both the parent drug and nicotinic acid were detected in the receiver solution. The conversion rate of the esters to nicotinic acid is given in Table 2. Nicotinic acid was also detectable in the donor solution when methyl and hexyl nicotinate were used as drugs (Table 3). No other by-product was identified by HPLC. Under the conditions of the permeation experiments,

Table 2
Mean degradation rates during permeation for different skin layers

	Epidermis	Dermis	Full thickness skin
Methyl nicotinate	0.4%[a,b]	5.2%[b]	32.8%[b]
	1.7%[c]		
Hexyl nicotinate	3.4%	100%	100%

[a] Amount within chemical degradation range.
[b] Donor concentration, 365 mM.
[c] Donor concentration, 1 mM.

Table 3
Normalised fluxes of metabolite into the donor compartment for different skin layers

Drug[a]	Flux[b]	Epidermis	Dermis	Full thickness skin
MN	$\hat{J}_{NA,D}$	1.04×10^{-7}[c,e]	2.73×10^{-7}	9.60×10^{-8}[e]
		6.18×10^{-7}[d]		
HN	$\hat{J}_{NA,D}$	1.40×10^{-7}[e]	3.52×10^{-6}	1.90×10^{-7}

[a] Drug applied in the donor compartment; MN, methyl nicotinate; HN, hexyl nicotinate.
[b] Mean normalised fluxes in cm/s ($n = 2$–6) of nicotinic acid ($\hat{J}_{NA,D}$).
[c] Drug donor concentration, 365 mM.
[d] Drug donor concentration, 1 mM.
[e] Amounts in the range of chemical degradation.

chemical hydrolysis over a 24-h period amounted to 2% for methyl nicotinate and 1.5% for hexyl nicotinate. Based on Eq. (3), for which the normalised fluxes of Table 1 are used as approximation for permeability coefficients and the chemical stability data are used to estimate the first order chemical hydrolysis constant, a nicotinic acid fraction of 0.5% in the receiver solution is found to be attributable to chemical hydrolysis for $t = 12$ h.

The degradation rate measured in the receiver solution generally exceeded chemical hydrolysis, evidencing that enzymatic hydrolysis of the nicotinic acid esters takes place during permeation through cutaneous tissue. Back fluxes of metabolite into the donor compartment also yielded levels above chemical hydrolysis in some instances although the high concentration of parent drug produced accordingly high levels of nicotinic acid by chemical hydrolysis which interfered with the determination of small back fluxes.

For permeation of methyl nicotinate through isolated epidermis, enzymatic hydrolysis was measurable at a donor concentration of 1 mM but not of 365 mM, probably because of a saturable system of esterases in the tissue in the concentration range used. Accordingly, the methyl nicotinate flux was at 1 mM smaller than at 365 mM in agreement with the higher metabolic rate at the low compared to the high concentration, while the smaller variability of the flux values at 1 mM compared to 365 mM is likely related to the fact that the former were determined with skin specimens from the same subject compared to different subjects used for the latter. For permeation of hexyl nicotinate through isolated epidermis, enzymatic hydrolysis was detectable from the flux into the receiver solution but not from the back flux.

The enzymatic degradation rate of methyl and of hexyl nicotinate during permeation through isolated dermis was much larger than during permeation through epidermis as evidenced by both forward and back fluxes of metabolite. This can be related to the larger thickness and a higher enzymatic activity of the dermis compared to the epidermis. These factors overshadow the effect of the larger diffusivity of drug in the dermis (see below) which acting alone would have caused a reduction of degradation rate.

The largest rate of metabolite appearance in the receiver solution for methyl nicotinate was found with full thickness skin. This is the result of the lower permeation rate and, secondarily, the larger thickness of full thickness skin compared to the isolated skin layers. Thus, transport through the epidermis, while it does not contribute markedly to metabolism, reduces the drug concentration reaching the dermis where degradation mainly takes place. Consequently, this concentration is lower than the one obtained for the isolated dermis, leading to a higher percentage of degradation in the full thickness skin compared to isolated dermis due to saturable enzyme kinetics. For hexyl nicotinate, complete metabolism was obtained during permeation through full thickness skin as well as through isolated dermis.

Back flux of metabolite into the donor compartment for full thickness skin was not measurable for methyl nicotinate and roughly 1% of the forward flux for hexyl nicotinate. This is because back flux entails that nicotinic acid, which is mostly generated in the dermis, transverses the epidermis which represents a major transport barrier for this compound as outlined below. Back fluxes in permeation experiments through isolated dermis were considerable in relation to the forward fluxes for both esters, which is consistent with the absence of the epidermal transport barrier.

Enzymatic degradation of hexyl nicotinate was more pronounced than that of methyl nicotinate which is probably related to the higher concentration of methyl nicotinate compared to hexyl nicotinate used in most experiments. In addition, however, studies in hairless mice have shown that esterases had a higher affinity for lipophilic than for hydrophilic nicotinic acid esters (Rittirod et al., 1999a). The difference in the activity of cutaneous esterases between mice and humans found during transport of ethyl nicotinate through skin was much smaller than between other species such as rats and humans (Rittirod et al., 1999b).

The degradation rates given in Table 2 are time-averaged values derived from normalised fluxes corresponding to the linear part of the permeation curve. For methyl nicotinate, degradation rate decreased from 37% to 32% for full thickness skin and from 8% to 5% for dermis during this permeation time. For permeation through epidermis, degradation rates were rather constant. The observed changes are relatively small but may reflect some change in the enzymatic activity of the dermis, in particular, during the experiment. When skin was subjected to the heat treatment protocol used for skin separation and then used in full thickness for the permeation experiment, a degradation rate of 32% was obtained for methyl nicotinate throughout. This indicates that the applied treatment did not seriously impair this particular esterase activity of the skin. It is possible, however, that a small fraction of enzymes is susceptible to heat inactivation as a function of time, leading to the observed reduction of degradation rate.

3.2. Skin permeation control

The permeation rate of nicotinic acid through full thickness skin is controlled by the permeation through the epidermis. The difference between the $\hat{J}_{NA}$ values for the two tissue membranes was not statistically significant. Similarly, no statistical difference was found between the $\hat{J}_{tot}$ values of epidermis and full thickness skin for methyl nicotinate. Here, the total forward flux values are used since there was no measurable back diffusion for these tissue membranes. Hence, the epidermis controls skin permeation rate of methyl nicotinate. Dermis flux values of nicotinic acid and methyl nicotinate were considerably higher than flux values for the other tissue membranes. For hexyl nicotinate, on the other hand, $\hat{J}_{tot}$ for epidermis was higher than the corresponding flux for full thickness skin indicating that dermis played a rate controlling role in skin permeation. Here, $\hat{J}_{tot}$ for full thickness skin is calculated from the influx of nicotinic acid

into the receiver and reflects the amount of parent drug permeating through the epidermis in the full thickness skin arrangement. The smaller $\hat{J}_{tot}$ of the dermis compared to full thickness skin is because of the much greater back flux of metabolite observed for the dermis.

Comparable normalised fluxes ($\hat{J}_{tot}$) of the three compounds were obtained for the dermis. The total dermis flux reflects permeation of the parent drug for nicotinic acid and, to a very large extent (95%), for methyl nicotinate, while for hexyl nicotinate it reflects the permeation of the parent drug and of the resulting metabolite. The fact that $\hat{J}_{tot}$ is almost the same in all cases suggests the three compounds have comparable permeability coefficients for the dermis, which is probably because of their similar molecular size resulting in roughly the same diffusion coefficient, while their different lipophilicities do not play a role since the partition coefficient between the donor solution and the predominantly aqueous dermis tissue should for all of them be around unity.

In contrast, normalised fluxes for the epidermis varied considerably between the three drugs. This is attributed to differences in partitioning between the donor solution and the lipid domains of the stratum corneum. Thus, the ~100-fold smaller flux of nicotinic acid compared to methyl nicotinate is in tandem with the lipophilicity of the compounds which, in terms of the isopropyl myristate/water partition coefficient, differs by a factor of 65 ($K_{IPM/H_2O} = 0.035$ and 2.29 for nicotinic acid and methyl nicotinate, respectively; Dal Pozzo et al., 1991). Hexyl nicotinate, on the other hand, showed only a 10-fold higher $\hat{J}_{tot}$ than methyl nicotinate even though its partition coefficient ($K_{IPM/H_2O} = 2089$; Dal Pozzo et al., 1991) is ~1000 times greater. In the following, it is examined whether this could be related to a shift of the control of the permeation rate from the lipid membrane to the diffusion boundary layer as this was reported elsewhere for nicotinates with increasing lipophilicity (Le and Lippold, 1998). Assuming for the sake of the argument that the epidermis permeability coefficient of hexyl nicotinate is entirely due to the diffusion boundary layer in the donor compartment, a thickness of this layer of 2.8 mm is calculated using an aqueous diffusion coefficient of hexyl nicotinate (D_{HN,H_2O}) at 32 °C of 7.22×10^{-6} cm^2/s (Le and Lippold, 1998). This is realistic since the total height of the solution column in the donor compartment is 9.9 mm. The release of drug from an unstirred donor solution in the direction of the skin corresponds to a flux (J_t) which is expressed as a function of time, t, by Eq. (13) assuming an infinite donor volume and sink conditions in the skin (Crank, 1975)

$$J_t = \frac{D_{HN,H_2O} \cdot c_{D,0}}{\sqrt{\pi \cdot D_{HN,H_2O} \cdot t}}. \quad (13)$$

For an initial donor concentration, $c_{D,0}$, of 0.7 mM, fluxes of 4.3×10^{-5} μmol/(cm^2 s), 2.5×10^{-5} μmol/(cm^2 s) and 1.8×10^{-5} μmol/(cm^2 s) are calculated for $t = 10$, 30 and 60 min, respectively. In the in vitro experiments, a constant flux of 1.8×10^{-5} μmol/(cm^2 s) was obtained during 3 to 4 h of permeation. This calculation demonstrates that the experimentally determined permeability of hexyl nicotinate for the epidermis is predominately controlled by diffusion in the donor compartment. Even if one considers that the values calculated with Eq. (13) may underestimate the true values because of agitation taking place in the donor solution due to thermal convection and mechanical vibration, the contribution of transport across the tissue to the overall permeation rate of hexyl nicotinate measured for the epidermis over 3 to 4 h is still expected to be negligible because of the overwhelming effect of the high lipophilicity of the compound.

3.3. Immunohistological investigations of skin separation plane and esterase localization

The epidermal–dermal junction is made of three different components: (i) the basal cell plasma membrane, (ii) the basal lamina composed of lamina lucida and lamina densa and (iii) fibres of connective tissue below the basal lamina. The lamina lucida contains the glycoprotein laminin and the lamina densa the protein collagen IV (Weiss, 1983; Woodley et al., 1983). Immunohistochemical staining of laminin (Fig. 4) and collagen IV (not shown) showed that following skin separation by heat treatment these remained on the outermost surface of the dermis. Consequently, the separation plane is located between the basal lamina and the basal cell membrane of the stratum basale of the epidermis. This result is in agreement with that of studies in which a different protocol for heat separation of skin was used (Woodley et al., 1983). Thus, the in vitro determined drug fluxes for epidermis and dermis can be safely assigned to these tissue layers.

Esterase activity was localised predominately in the dermis as evidenced by the red–brown granules surrounding the nuclei of histeocytes and cells which are in contact with

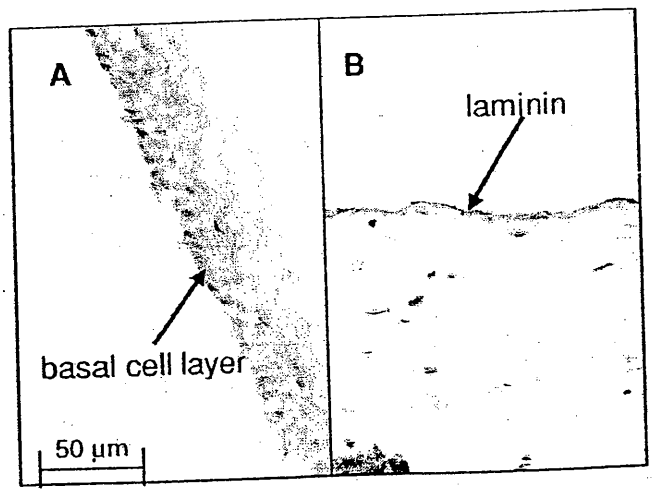

Fig. 4. Immunohistochemical preparation of epidermis (A) and dermis (B) after heat separation of skin. Laminin is stained on the dermis surface facing the epidermis.

Fig. 5. Distribution of α-naphthylacetate esterase (ANAE) in skin. (A) and (C) are controls of dermis and epidermis, respectively. (B) is the stained full-thickness skin. → shows the ANAE positive granules (red–brown staining).

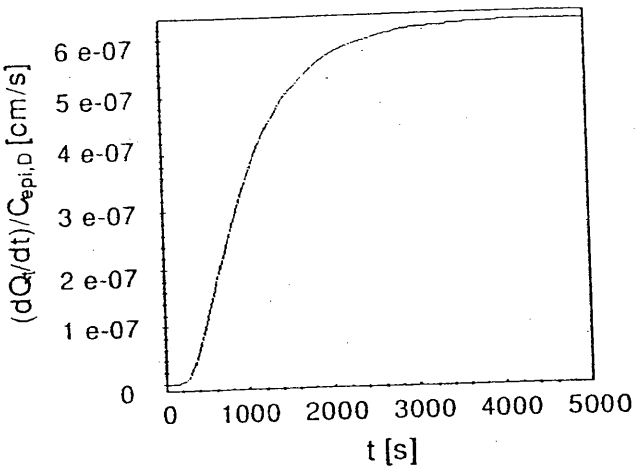

Fig. 6. Computer simulation of normalised mass flux over time at the epidermis–dermis interface for NA based on numerical differentiation of Eq. (12).

peripheral vessels (Fig. 5). No such granules were found in the epidermis. This enzymatic activity reflects the non-specific α-naphthylacetate esterase bound to cell constituents while dissolved enzymes may be released during preparation. No differences in the enzyme localisation were seen between freshly thawed skin and skin subjected to wet heat treatment for 1 and 3 min at 60 °C (not shown). Hence, this histochemical study is in full agreement with the permeation and metabolism experiments with respect to the localisation of and the effect of heat on enzymatic activity in the tissue. It further supports the notion that the increased enzymatic degradation rate of methyl and hexyl nicotinate found for the dermis may, in addition to the difference in tissue mass, be due to a higher abundance of enzymes in the dermis compared to the other tissue layers. It should be noted, however, that these results may be unique to the system studied here since for corticoid esters and a rather different skin treatment protocol, esterase activity was detected in the epidermis as well as in the dermis and heat was found to have an adverse effect on the enzymes (Täuber and Rost, 1987).

3.4. Application time, applied concentration and C^* in vivo

The in vivo application time of the drug was determined based on the time required to attain steady state flux across the epidermis. This was estimated using Eq. (12) which gives the amount of drug crossing the epidermis–dermis interface, i.e. the site of pharmacologic action, as a function of time. The diffusion coefficient of drug for the epidermis, D_{epi}, was calculated from the total normalised flux for epidermis, $\hat{J}_{epi,tot}$ (Table 1; 1 mM for MN), using the isopropyl myristate/water partition coefficient of the compounds as an approximation of the partition coefficient between epidermis and donor solution and an epidermis thickness of 4×10^{-3} cm which was determined experimentally. The use of total normalised flux introduces only a small inaccuracy since it is demonstrated in the in vitro studies that enzymatic degradation during epidermis permeation amounts to a few percentage points and that back flux of metabolite is barely detectable. D_{epi} values for NA and MN of 2.5×10^{-9} and 3×10^{-9} cm^2/s, respectively, were obtained. This calculation is not applicable to HN because the $\hat{J}_{epi,tot}$ measured for this compound is due to the diffusion boundary layer in the donor compartment rather than permeation through epidermis. From the numerical differentiation of Eq. (12), flux as a function of time is calculated and plotted for NA, as an example, in Fig. 6. The time required to reach steady state flux was defined as the time at which at least 80% of the maximum flux value corresponding to $t \rightarrow \infty$ was reached. This was around 20 min for NA and MN. Therefore, the drug application time in vivo was fixed at 20 min. This was expected to be suitable also for HN which has a similar size and should therefore have a comparable diffusivity in the epidermis as the other compounds. It is noted that the lag time of permeation does not correspond to the lag time of the pharmacological effect of nicotinates (Guy et al., 1984) and, therefore, cannot be deduced from it. The fraction of the applied dose that is released from the donor solution during the 20 min of application can be calculated using Eq. (9) and the $\hat{J}_{epi,tot}$ values of Table 1 and taking into account the applied solution volume and the surface area of the application device. This fraction was 0.06% for NA, 5.7% for MN, and 77.1% for HN. Therefore, in order to prevent excessive depletion, the applied HN dose was renewed every 5 min.

Range finding of the in vivo applied drug concentration was carried out in preliminary studies with five male volunteers aged between 26 and 29 years complying with the inclusion criteria of the in vivo investigation. The AUC of skin colour change over 100 min after application was used as a scale of the pharmacological effect. Test solutions were applied for 2.5, 10, 20 and 180 min. During the 180 min application, skin colour was measured and fresh drug solution applied every 15 min. For MN, concentrations of 10, 1 and 0.1 mM were used. Both drug concentration and application

Table 4
Applied and site of action drug concentration

Drug	$\hat{J}_{epi,tot}$ (cm/s)	$\hat{J}_{der/20,tot}$ (cm/s)	C_D (μmol/cm³)	C^* (μmol/cm³)
NA	1.97×10^{-8}	2.28×10^{-4}	100	8.00×10^{-3}
			50	4.30×10^{-3}
			5	4.30×10^{-4}
MN	1.73×10^{-6}	2.20×10^{-4}	1	7.86×10^{-3}
			0.5	3.93×10^{-3}
			0.1	7.86×10^{-4}
HN	2.57×10^{-5}	3.30×10^{-4}	0.15	1.17×10^{-2}
			0.06	4.67×10^{-3}
			0.015	1.17×10^{-3}

NA, nicotinic acid; MN, methyl nicotinate; HN, hexyl nicotinate. $\hat{J}_{epi,tot}$ is the total normalised flux for epidermis in vitro. $\hat{J}_{der/20,tot}$ is the total normalised flux for dermis corresponding to the reduced diffusion path length in vivo. C_D is the applied concentration in vivo. C^* is the unbound drug concentration in the aqueous compartment of the site of pharmacologic activity.

time affected the pharmacological response in a saturable and interdependent fashion. The difference of the effect between 0.1 and 1 mM was reduced as the application time increased. For concentrations above 1 mM, no increase of the pharmacological effect was observed for application times ≥2.5 min. This finding is confirmed by studies in which a substantially higher concentration of 100 mM was applied for 5 to 30 s (Guy et al., 1984). This saturation of the pharmacological effect is probably related to the mechanism of the vasodilatation of the peripheral blood vessels involving the secretion of prostaglandin D_2 as a primary transmitter and possibly nitrogen monoxide as a secondary transmitter (Roberts and Morrow, 1997) and the nature of the effect itself. A clear concentration dependence of the pharmacological effect for the employed application time of 20 min was observed for concentrations between 0.1 and 1 mM for MN and 10 and 100 mM for NA.

The concentrations of the different drugs applied in vivo were defined based on this preliminary range finding study and taking into account the pharmacologically active drug concentration at the site of action, C^*.C^* is calculated using Eq. (8). For all three drugs the applied concentrations were chosen in a way that they produced values of C^* lying in the same range. These concentrations are shown in Table 4. This choice of concentrations served the purpose of testing the hypothesis that C^* represents a relevant concentration parameter that correlates with the pharmacologic effect, resting on the background that the different nicotinic acid derivatives have the same mechanism of action (Roberts and Morrow, 1997).

It was shown in the in vitro experiments using drug concentrations of the same order of magnitude as in the in vivo study, that metabolic degradation of nicotinic esters was quite small in the epidermis and took place predominately during permeation through the dermis. Thus, the drug concentration reaching the site of action at the epidermis–dermis interface is not greatly affected by metabolism. In addition, the resulting metabolite has reportedly the same pharmacological effect as the parent compounds (Roberts and Morrow, 1997). Therefore, for the calculation of C^* total normalised fluxes for the epidermis, $\hat{J}_{epi,tot}$, were used. Even if the potency of pharmacological effect of these compounds were not the same, which is not known with certainty, the introduced inaccuracy would be rather small. The determination of normalised flux for isolated dermis, which is also required for calculating C^*, is affected by metabolism, quantitatively not so much for MN, for which roughly 5% of metabolic conversion was measured, but markedly more for HN, for which degradation was 100%. In case of simultaneous transport and metabolism, the normalised flux of drug, corresponding to a combined transport–bioconversion permeability, is equal to the sum of fluxes of parent drug and metabolite both in the receiver and the donor compartments. Therefore, for the calculations with Eq. (8), the total normalised fluxes for isolated dermis, $\hat{J}_{der,tot}$, considering flux in both compartments were used (Table 4). The values in Table 4 are additionally adjusted to an in vivo diffusion path length equal to one twentieth of the anatomical thickness of the dermis (see also Section 2).

3.5. Pharmacological response and correlation with C^*

Table 5 shows the results of the measurement of the pharmacological effect expressed by the colour coordinate a^* and the absolute colour E^* for different applied concentrations of the three drugs. The difference of the effect between 5 and 50 mM for NA and between 0.015 and 0.06 mM for HN was highly significant ($P<0.005$ and $P<5 \times 10^{-10}$, respectively). The difference between 50 and 100 mM for NA and between 0.06 and 0.15 mM for HN was significant ($P<0.05$). Solely, the difference of the effect between 0.5 and 1 mM of MN was not statistically significant ($P=0.11$). Thus, overall, a strong positive dependence of the pharmacologic response on the applied concentration is evident. The low concentrations were close to the minimal effective concentrations while towards the high concentrations a levelling off of the effect seemed to take place. The concentration range in which a clear concentration dependence of the effect could be measured was rather narrow. The colour co-

Table 5
Cumulative area under the colour change versus time curve ($AUC_{95\,min}$) of the colour coordinate a^* and the absolute colour E^*

Drug and number of volunteers	C_D (mM)	Δa^* $AUC_{95\,min}$ Mean±S.E.M.	ΔE^* $AUC_{95\,min}$ Mean±S.E.M.
Nicotinic acid	100	239.0 ± 28.2	279.5 ± 33.3
$n=10$	50	160.2 ± 21.6	189.4 ± 25.5
	5	70.5 ± 15.0	92.6 ± 18.6
Methyl nicotinate	1	304.3 ± 13.9	371.8 ± 18.5
$n=13$	0.5	277.3 ± 15.2	341.4 ± 17.8
	0.1	162.8 ± 16.8	221.0 ± 22.0
Hexyl nicotinate	0.15	365.1 ± 18.6	424.9 ± 21.7
$n=13$	0.06	302.2 ± 19.8	356.2 ± 22.1
	0.015	49.0 ± 4.6	70.3 ± 7.1

n is the number of data sets without dropouts and outliers.

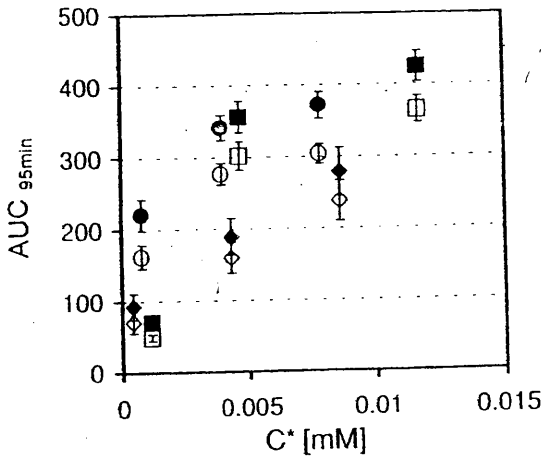

Fig. 7. Pharmacological effect expressed as $AUC_{95\,min}$ as a function of drug concentration at the site of action, C^*. Filled and empty symbols correspond to ΔE^* and Δa^*, respectively. Key (◆, ◇) NA; (■, □) HN; (●, ○) MN. Points and bars denote mean and S.E.M.

ordinate a^* corresponding to the red–green dimension was the most sensitive measure of pharmacological effect reflecting obviously the increased presence of red blood cells in the skin, while no response of the colour coordinate b^* was recorded. Colour brightness L^* was also influenced by the drugs giving rise to the observed difference between Δa^* and $\Delta E^*.a^*$, however, seemed to be more sensitive to drug activity than the absolute colour E^*.

In Fig. 7 the pharmacological effect is plotted against the concentration at the site of action, C^*. For the same value of C^*, the same or a very similar effect is obtained for all three drugs. Also, a similar effect/C^* relationship results for these drugs. This is remarkable considering that the applied concentrations varied between the drugs by orders of magnitude and demonstrates that C^* is the relevant parameter that correlates with the efficacy of the topically applied nicotinic acid derivatives. This further implies the potential utility of the model approach yielding C^* for estimating bioavailability as well as establishing dose–response relationships of topical formulations.

Validation of the estimated absolute values of C^* as the concentration that is responsible for the pharmacological effect in this study represents an unanswered challenge. No independent data about the concentration of pharmacologic action of nicotinic acid derivatives on blood capillaries are available. Direct intradermal injection of nicotinates produced a vasodilatatory effect at drug concentrations of the order of 0.1 μM (Stoughton et al., 1960). The concentration of the injected solution, however, cannot be converted reliably to a site-of-action concentration because of an unknown volume of distribution of the tissue. Therefore, this concentration cannot be compared to C^*. Furthermore, the experimental determination of drug concentration at the site of action in the skin with sufficient resolution is with our present means not possible. Nevertheless, in a previous study that dealt with the topical antiviral effect of Acyclovir in cutaneous Herpes Simplex Virus-1 infections in mice, independent validation of C^* was possible (Imanidis et al., 1994). In that study, the estimated active C^* was in good agreement with the antiviral IC_{50} of the drug determined in cell culture in vitro. Also, the topically attained C^* was consistent with free serum concentrations of the drug showing a systemic antiviral effect.

The data points of MN and HN in Fig. 7 follow an identical pattern indicating that the intrinsic pharmacologic activity of these compounds is the same. This is in agreement with suggestions made earlier according to which all nicotinic acid esters have the same pharmacological potency (Roberts and Morrow, 1997). The $AUC_{95\,min}$ versus C^* curve levels off for MN and HN at high concentrations, which is typical for a saturable pharmacologic response. The NA response curve runs somewhat lower than that of MN and HN and seems to be linear. Comparing the activity at the highest used concentrations between NA and MN, a significant difference was found ($P<0.05$). These data indicate a lower intrinsic potency of NA compared to MN and HN. Whether the maximal attainable effect of NA at higher concentrations would be comparable to that of the two esters is rather difficult to predict. Overall, these results demonstrate that the presented model can be used as a tool for comparative studies of topical bioavailability and efficacy of drugs with very different physicochemical properties such as NA, MN and HN.

In addition to the quantitative evaluation, a qualitative difference of the colour change reaction between NA on one hand and MN and HN on the other was observed. MN and HN caused a regular and on the application area limited colour change while for NA, colour change was irregularly dotted and extended over the application area in some of the subjects (Fig. 8). Also, in 11 out of 20 volunteers, a secondary colour reaction occurred 24 h after the end of NA application, the colour change re-emerging and lasting for 2 to 3 days followed by a brown coloration resembling a pigmentation, possibly due to increased melanin resulting

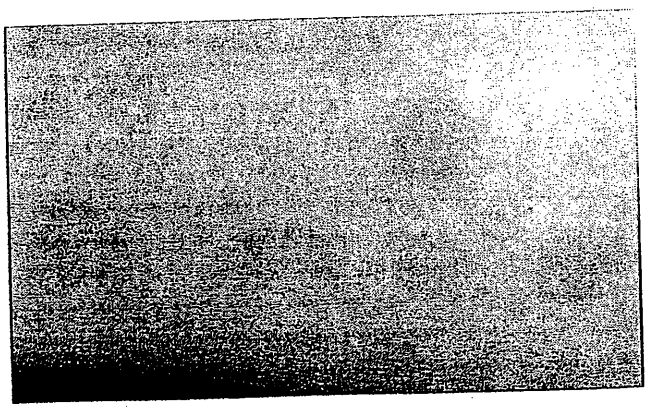

Fig. 8. Photograph of the ventral side of the lower arm of a subject showing change of skin colour in vivo after drug application. Dots correspond to application areas of (from left to right and top to bottom) NA 5 mM, placebo, MN 0.5 mM, placebo, NA 100 mM, NA 50 mM, MN 0.1 mM, MN 1 mM.

from inflammatory response, that was visible for 1 to 2 weeks. It appears therefore, from the quantitative as well as the phenomenological effect assessment that the pattern of pharmacological activity of nicotinic acid differs from that of its esters, this being in contrast with the commonly accepted notion to date.

To fully appreciate the meaning of the correlation between pharmacologic effect and site of action concentration, C^*, found for the three nicotinic acid derivatives, the assumptions involved in setting up this correlation should be discussed. C^* is calculated on the basis of steady state flux across the skin. The effect of the drugs, on the other hand, is evaluated not only at one time point after steady state flux is established but cumulatively for 95 min post-removal of the drug, that is, until the effect completely receded. These measurements provided the most reproducible results in the present study, while momentary measurements for example at the time point of removal of the application device did not provide useful data. This may be because the pharmacologic effect of nicotinates recorded here, i.e. vasodilatation, is primarily brought about by intermediate events of their own time scale involving the action of prostaglandins (see above). Thus, recording of the full effect elicited by the drug may better be achieved with a prolonged rather than a momentary measurement. Moreover, drug reaching the site of action prior to the establishment of steady state flux and drug still present at the site of action after removal of the application device may also elicit an effect if its concentration exceeds the minimal active level. With the present measurement protocol, an integral effect is recorded. An attempt to correlate the measured integral effect with an 'integral concentration' over time at the site of action would entail the assumption that the effect of progressively increasing or decreasing concentration is additive. In view of the mechanism of action of the nicotinates and the fact that the pharmacologic response of a pulse delivery of drug is not known, the validity of this assumption is difficult to ascertain. Therefore, this approach was not taken in the present work. The approach of correlating the cumulative drug flux with the pharmacological effect (Albery and Hadgraft, 1979) is confounded by the difficulty of converting flux values into tissue concentration and by the fact that it disregards the removal of drug by the systemic circulation. C^* used here to establish an effect–concentration relationship is a representative concentration reflecting the drug level reached in deeper tissue layers, notably at the site of pharmacologic action. Its use is in line with the emphasis widely placed on concentration, rather than dose, as the relevant predictor of pharmacologic effect. This simplifying approach is shown to provide very good results in terms of establishing an effect–concentration relationship in topical drug therapy.

Acknowledgements

Support of this project by scholarships to B.M. from the 'Stiftung zur Förderung des pharmazeutischen Nachwuchses in Basel (SENGLET)' and the 'Stipendienfonds der Basler Chemischen Industrie zur Unterstützung von Doktoranden auf dem Gebiete der Chemie, der Biotechnologie und der Pharmazie' is thankfully acknowledged. The authors wish to thank Prof. Fred Gudat, Institute of Pathology, University of Basel for his support with the histochemical investigations. Supply of human cadaver skin by the Institute of Pathology of the University of Basel, Prof. M.J. Mihatsch, was greatly appreciated.

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UNITED STATES OF AMERICA
FEDERAL TRADE COMMISSION
WASHINGTON, D.C. 20580

Bureau of Consumer Protection
– Laura Schneider
Attorney

Direct Dial
202-326-2604

July 1, 2004

Via Federal Express
Robert H. Eckel M.D.
University of Colorado Health Sciences Center
Division of Endocrinology, Metabolism, and Diabetes
4200 East Ninth Avenue, B-151
Denver, Colorado 80262

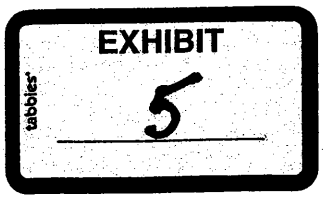
– Re: Basic Research LLC, FTC Matter No. 002-3300

Dear Dr. Eckel:

Pursuant to your conversation with Jonathan Cowen of our office, thank you for agreeing to assist the Division of Enforcement staff in the above matter. Enclosed for your review please find the following materials:

- Complaint and Exhibits A-L (product advertisements)
- Respondents' CLAIM SUBSTANTIATION FOR "TUMMY FLATTENING GEL"
- Respondents' CLAIM SUBSTANTIATION FOR "DERMALIN Apg"
- Respondents' CLAIM SUBSTANTIATION FOR /CUTTING GEL"
- DermTech report dated 6/11/03 titled "Evaluation of the Percutaneous Absorption of Aminophylline, *in vitro,* Using the Human Cadaver Skin Model."
- DermTech report dated 9/1/02 titled "Determination of the Percutaneous Absorption of Aminophylline, *in vitro,* Using the Human Cadaver Skin Model."
- DermTech report dated 12/6/01 titled "Evaluation of the Percutaneous Absorption of Aminophylline, *in vitro,* Using the Human Cadaver Skin Model."

We have supplied both a hard copy and a copy of all of the above on CD-Rom. Please note that there is a certain amount of overlap among the claim substantiation that Respondents submitted for each of the gels.



I will contact you in two weeks to discuss these materials. Please let me know via email or telephone if there are any particular convenient times for you to discuss this matter. Thank you for your assistance.

Sincerely,

Laura Schneider
Attorney
(202) 326-2604
Lschneider@ftc.gov

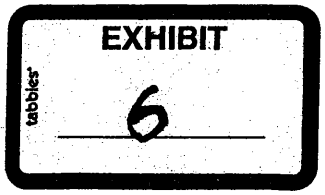
enc.

UNITED STATES OF AMERICA
BEFORE THE FEDERAL TRADE COMMISSION
OFFICE OF ADMINISTRATIVE LAW JUDGES

In the Matter of BASIC RESEARCH, LLC, a limited liability company; A.G. WATERHOUSE, LLC, a limited liability corporation; KLEIN-BECKER USA, LLC, a limited liability company; NUTRASPORT, LLC, a limited liability company; SOVAGE DERMALOGIC LABORATORIES, LLC, a limited liability company; BAN, LLC, a limited liability corporation, also doing business as BASIC RESEARCH, L.L.C., OLD BASIC RESEARCH, L.L.C., BASIC RESEARCH, A.G. WATERHOUSE, KLEIN-BECKER USA, NUTRA SPORT, and SOVAGE DERMALOGIC LABORATORIES, DENNIS GAY, individually and as an officer of the limited liability corporations, DANIEL B. MOWREY, Ph.D., Also doing business as AMERICAN PHYTOTHERAPY RESEARCH LABORATORY, and MITCHELL K. FRIEDLANDER, Respondents.	Docket No. 9318 **PUBLIC DOCUMENT**

RESPONSE TO COMPLAINT COUNSEL'S FIRST SET OF INTERROGATORIES

Pursuant to Rule 3.35 of the Federal Trade Commission's Rules of Practice, Respondents Basic Research, LLC, A.G. Waterhouse, LLC, Klein Becker usa, LLC, Nutrasport, LLC, Sovage



Dermalogic Laboratories, LLC, Ban, LLC (collectively, "Respondents") object and respond to Complaint Counsel's First Set of Interrogatories ("Request") as follows:

General Objections

A. Respondents object to the Interrogatories as overbroad and unduly burdensome on the grounds and to the extent that they call for responses that are neither relevant to the subject matter of the pending action nor reasonably calculated to lead to the discovery of admissible evidence.

B. Respondents object to the Interrogatories on the grounds and to the extent that they seek responses that are subject to (i) the attorney-client privilege; (ii) the attorney and/or party work product immunity, and (iii) any other privilege or immunity, including common law and constitutional right of privacy and/or trade secret protection. Respondents hereby claim such privileges and immunities. Any disclosure of any such privileged or immunized information is inadvertent and is not, and is not intended, as a waiver of those privileges and immunities.

C. Respondents object to the Interrogatories and to the Definitions and Instructions on the grounds and to the extent that they are overbroad, unduly burdensome and oppressive, and purport to impose obligations on Respondents that are beyond the scope of the Rules of Practice or other applicable law.

D. Respondents object to the Interrogatories on the grounds and to the extent that they are vague, ambiguous and unintelligible, particularly in light of the inherent vagueness and ambiguity in the standards employed by the Commission as well as in the charges that have been levied in this matter, which is the subject of Respondents' pending motion for an interlocutory appear and more definite statement by the Commission.

E. Respondents incorporate by this reference Respondents' Motion to Quash in Part and to Limit Subpoenas on Non-Parties and each response, objection and basis therefore in the motion, and further objects to each Interrogatory on those grounds.

F. Respondents' objections and responses to the Interrogatories are not intended to waive or prejudice any objections that Respondents may assert now or in the future, including,

without limitation, objections as to the relevance of the subject matter of any interrogatory, or of the admissibility of any response or document or category of responses or documents, at hearing, trial or any other time. Respondents expressly reserve any and all rights and privileges under the Rules of Practice, applicable evidentiary rules, and any other law or rule, and the failure to assert such rights and privileges or the inadvertent disclosure by Respondents of information protected by such rights or privileges shall not constitute a waiver thereof, either with respect to these responses or with respect to any future discovery responses or objections.

Specific Objections and Responses

Based on, subject to, and without waiving its General Objections, Respondents specifically and additionally respond to each of the Specifications contained in Complaint Counsel's Interrogatories as follows:

Interrogatory No. 1:

Identify and **describe** in detail the current and former duties, responsibilities, or work performed by each **person relating to** the **promotional materials** for each of the **challenged products**. (This request **includes**, but is not limited to, the creation, development, evaluation, approval, modification, and dissemination of **promotional materials**.)

Response:

Respondents incorporate by reference each General Objection as set forth here in full. Respondents further objects to this interrogatory on the following grounds: (a) it is vague and ambiguous; (b) it is overly broad and unduly burdensome; (c) it seeks irrelevant information and information not reasonably calculated to lead to the discovery of admissible evidence; and (d) it seeks information protected from disclosure by the attorney-client privilege, work product doctrine, and/or right of privacy. Based on, subject to, and without waiving the foregoing responses and objections, Respondents responds as follows:

Respondents interpret this interrogatory as requesting the identity of persons and descriptions of duties, responsibilities and work performed. In providing the following response,

Respondents do not discuss or imply, or intend to discuss or imply, any relationship between any of the parties and/or any of the persons identified below:

1. Dan Mowrey, Ph.D, researched and developed product ideas, concepts and ingredients; performed ad substantiation research, and reviewed ads for substantiation;
2. Mitch Friedlander, determined commercial viability of products, wrote copy, directed ad layout, and assisted with marketing;
3. Gina Gay, placed advertisements with media;
4. Jeff Davis, proof read advertisements;
5. Brett Madsen, assisted with copy layout;
6. Ned Simpson, assisted with copy layout;
7. John Swallow, reviewed ad copy;
8. Nathalie Chevreau, Ph.D., PediaLean project director; assisted with website development for PediaLean; performed PediaLean safety tests;
9. Carla Fobbs, facilitated and obtained substantiation review from outside counsel;
10. Dennis Gay, final approval of products and advertisements; and
11. Stephen Nagin, Esq., performed substantiation review.

Interrogatory No. 2:

Identify and **describe** in detail the current and former duties, responsibilities, or work performed by each **person** consulted by you, or upon who advise, opinion, or expertise you relied in the production of each of the **challenged products**. (This request **includes**, but it not limited to, the creation, development, evaluation, approval, and manufacture of the **challenged products**.)

Response:

Respondents incorporate by reference each General Objection as set forth here in full. Respondents further objects to this interrogatory on the following grounds: (a) it is vague and

ambiguous; (b) it is overly broad and unduly burdensome; (c) it seeks irrelevant information and information not reasonably calculated to lead to the discovery of admissible evidence; and (d) it seeks information protected from disclosure by the attorney-client privilege, work product doctrine, and/or right of privacy. Based on, subject to, and without waiving the foregoing responses and objections, Respondents respond by referring to their Response to Interrogatory No. 1, which includes the persons consulted.

Interrogatory No. 3:

Describe in detail the composition of each of the **challenged products**. (This request **includes**, but is not limited to, the identity of each ingredient and the amount of each ingredient contained in a single capsule, application, and serving. If any **challenged product** has been reformulated, provide a separate answer for each version of the product that has been marketed and sold, **identifying** the time period(s) in which each version was marketed and sold.

Response:

Respondents incorporate by reference each General Objection as set forth here in full. Based on, subject to, and without waiving the foregoing objections, Respondents refer to Attachment "A," which has been designated pursuant to the Protective Order as "Restricted Confidential, Attorney Eyes Only—FTC Docket No. 9318."

Interrogatory No. 4:

Disclose the total amount of sales, in terms of units and dollars that each Respondent has achieved for each of the **challenged products** for each year from 2001 to the present.

Response:

Respondents incorporate by reference each General Objection as set forth here in full. Respondents further objects to this interrogatory on the following grounds: (a) it is vague and ambiguous; (b) it is overly broad and unduly burdensome; (c) it seeks irrelevant information and information not reasonably calculated to lead to the discovery of admissible evidence (the

requested information has no relationship to the alleged false or misleading advertising claims that Complaint Counsel pursues in this matter); and (d) it seeks information protected from disclosure by the attorney-client privilege, work product doctrine, and/or right of privacy, including financial privacy.

Interrogatory No. 5:

To the extent a **challenged product** is a **substantially similar product** to others products, identify each other product.

Response:

Respondents incorporate by reference each General Objection as set forth here in full. Respondents further objects to this interrogatory on the following grounds: (a) it is vague and ambiguous; (b) it is overly broad and unduly burdensome; (c) it seeks irrelevant information and information not reasonably calculated to lead to the discovery of admissible evidence (the requested information has no relationship to the alleged false or misleading advertising claims that Complaint Counsel pursues in this matter); and (d) it seeks information protected from disclosure by the attorney-client privilege, work product doctrine, and/or right of privacy, including financial privacy.

Interrogatory No. 6:

Disclose all payments that each **Respondent** has received, directly or indirectly, in connection with the advertising, marketing, promotion, and sale of ach of the **challenged products** for each year from 2001 to the present. (This request **includes** the total dollar amount and source of all payments. For consumer sales, it is not necessary to disclose names, addresses, or telephone numbers.)

Response:

Respondents incorporate by reference each General Objection as set forth here in full. Respondents further objects to this interrogatory on the following grounds: (a) it is vague and ambiguous; (b) it is overly broad and unduly burdensome; (c) it seeks irrelevant information and

information not reasonably calculated to lead to the discovery of admissible evidence (the requested information has no relationship to the alleged false or misleading advertising claims that Complaint Counsel pursues in this matter); and (d) it seeks information protected from disclosure by the attorney-client privilege, work product doctrine, and/or right of privacy, including financial privacy.

Interrogatory No. 7:

Disclose the total amount of dollars that each **Respondent** has spent to advertise, market or otherwise promote each of the **challenged products** for each year from 2001 to the present, broken down by each medium used (*i.e.*, television, print, internet, radio, or other means). (This request **includes**, but is not limited to, all expenditures attributable to the creation, development, evaluation, approval, modification, and dissemination of **promotional materials**).

Response:

Respondents incorporate by reference each General Objection as set forth here in full. Respondents further objects to this interrogatory on the following grounds: (a) it is vague and ambiguous; (b) it is overly broad and unduly burdensome; (c) it seeks irrelevant information and information not reasonably calculated to lead to the discovery of admissible evidence (the requested information has no relationship to the alleged false or misleading advertising claims that Complaint Counsel pursues in this matter); and (d) it seeks information protected from disclosure by the attorney-client privilege, work product doctrine, and/or right of privacy, including financial privacy.

Interrogatory No. 8:

Provide a **dissemination schedule** that **describes** in detail how each item **of promotional materials** submitted in response to the **Requests for Production** was disseminated or otherwise exposed to consumers.

Response:

Respondents incorporate by reference each General Objection as set forth here in full. Respondents further objects to this interrogatory on the following grounds: (a) it is vague and

ambiguous; (b) it is overly broad and unduly burdensome; (c) it seeks irrelevant information and information not reasonably calculated to lead to the discovery of admissible evidence (the requested information has no relationship to the alleged false or misleading advertising claims that Complaint Counsel pursues in this matter); and (d) it seeks information protected from disclosure by the attorney-client privilege, work product doctrine, and/or right of privacy, including financial privacy.

Interrogatory No. 9:

Describe in detail the actions each **Respondent** has taken to comply with the U.S. Food and Drug Administration's prohibition on the sale of dietary supplements containing ephedrine alkaloids, effective April 12, 2004. (This request **includes**, but is not limited to, **identification** of any product formulations that have been created, modified, or removed from distribution, **identification** of any **promotional materials** that have been created, revised, or removed from dissemination, and the date(s) on which all of the actions described in your answer took place; and how orders for Leptoprin or Anorex or in response to existing **promotional materials** Leptoprin or Anorex have been fulfilled.)

Response:

Respondents incorporate by reference each General Objection as set forth here in full. Respondents further objects to this interrogatory on the following grounds: (a) it is vague and ambiguous; (b) it is overly broad and unduly burdensome; (c) it seeks irrelevant information and information not reasonably calculated to lead to the discovery of admissible evidence (the requested information has no relationship to the alleged false or misleading advertising claims that Complaint Counsel pursues in this matter); and (d) it seeks information protected from disclosure by the attorney-client privilege, work product doctrine, and/or right of privacy, including financial privacy.

Based on, subject to, and without waiving the foregoing responses and objections, Respondents state that during the first part of April 2004 Basic Research started the process of identifying all products that considered to be "adulterated" according to 21 CFR Part 119, which

states, "*all dietary supplements containing ephedrine alkaloids are adulterated under the Federal Food, Drug, and Cosmetic Act.*"

April 1st through April 6th of 2004 all products and raw materials containing a source of ephedrine alkaloids, such as ephedra, Ma huang and pinellia were gathered together and quarantined along with all boxes, labels, advertising brochures and other artwork containing information relative to ephedrine containing products.

On April 7th, 2004 Basic Research prepared Material Destruction Forms, which contained all necessary information for tracking the materials through all steps of the destruction process. The Material Destruction Forms included approval signatures, raw material lot numbers, finished good lot numbers, label revision numbers, persons responsible for destruction and all other pertinent information required to conform to the regulation.

On April 8th, 2004 Basic Research conducted one last search throughout the facility to ensure that every product considered adulterated by the FDA, had been properly identified and quarantined. All adulterated materials discovered during this comprehensive search were quarantined and Material Destruction Forms filled out.

On Friday the 9th of April 2004, all adulterated materials were destroyed prior to the April 12, 2004 deadline. During the destruction process, each item was verified by two separate individuals who immediately thereupon affixed their signatures to the Material Destruction Forms.

In accordance with 21 CFR part 119, Basic Research immediately destroyed every product containing a source of ephedrine alkaloid (such as ephedra, Ma huang and pinellia) returned to our facility by customers of Basic Research subsequent to the April 12, 2004 deadline.

Interrogatory No. 10:

Disclose the total amount of refunds to consumers, in terms of units and dollars, that each Respondent has made for each of the **challenged products** for each year from 2001 to the present.

Response:

Respondents incorporate by reference each General Objection as set forth here in full. Respondents further objects to this interrogatory on the following grounds: (a) it is vague and ambiguous; (b) it is overly broad and unduly burdensome; (c) it seeks irrelevant information and information not reasonably calculated to lead to the discovery of admissible evidence (the requested information has no relationship to the alleged false or misleading advertising claims that Complaint Counsel pursues in this matter); and (d) it seeks information protected from disclosure by the attorney-client privilege, work product doctrine, and/or right of privacy, including financial privacy.

Respectfully submitted this 11th day of August, 2004

JEFFREY D. FELDMAN

CERTIFICATE OF SERVICE

I HEREBY CERTIFY that a true and correct copy of the foregoing was provided to the following parties this 6 day of August, 2004 as follows:

(1) One (1) copy via e-mail attachment in Word document format to Commission Complaint Counsel, Laureen Kapin, Joshua S. Millard, and Laura Schneider, all care of lkapin@ftc.gov, jmillard@ftc.gov; rrichardson@ftc.gov; lschneider@ftc.gov with one (1) paper courtesy copy via U. S. Mail, First Class Postage Prepaid to Laureen Kapin, Bureau of Consumer Protection, Federal Trade Commission, Suite NJ-2122, 600 Pennsylvania Avenue, N.W., Washington, D.C., 20580;

(2) One (1) copy via U. S. Mail, First Class Postage Prepaid to Stephen Nagin, Esq., Nagin Gallop & Figueredo, 3225 Aviation Avenue, Suite 301, Miami, Florida 33131.

(3) One (1) copy via U. S. Mail, First Class Postage Prepaid to Richard Burbidge, Esq., Jefferson W. Gross, Esq. and Andrew J. Dymek, Esq., Burbidge & Mitchell, 215 South State Street, Suite 920, Salt Lake City, Utah 84111, Counsel for Dennis Gay.

(4) One (1) copy via U. S. Mail, First Class Postage Prepaid to Ronald F. Price, Esq., Peters Scofield Price, A Professional Corporation, 340 Broadway Centre, 111 East Broadway, Salt Lake City, Utah 84111, Counsel for Daniel B. Mowrey.

(5) One (1) copy via U. S. Mail, First Class Postage Prepaid to Mitchell K. Friedlander, 5742 West Harold Gatty Drive, Salt Lake City, Utah 84116, Pro Se.

Jeffrey D. Feldman

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ATTORNEYS EYES ONLY
FTC DOCKET NO. 9318

EXHIBIT A

Cutting Gel™

Ingredient	% per Formula
[Redacted]	

Dermalin-APg™:

Ingredient	% per Formula

[Redacted]

Tummy Flattening Gel:

Ingredient	% per Formula
[Redacted]	

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Anorex™:

Raw ingredient	Raw ingredient mg/serving for production	raw ingredient mg/capsule	Amount of Elemental Ingredients (caffeine, ephedrine alkaloids, vitamin & mineral) per serving (mg) for label	% Daily Value
[Redacted]				

LeptoPrin[10]:

Raw ingredient	Raw ingredient mg/serving	raw ingredient mg/capsule	Amount of Elemental Ingredients (caffeine, ephedrine alkaloids, vitamin & mineral) per serving (mg)	% Daily Value
[Redacted]				

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PediaLean®:

Raw ingredient	mg/serving	mg/cap

[Redacted]

VERIFICATION

I, Carla R. Fobbs, being duly authorized to execute the aforesaid Answers to Complaint Counsel's First Set of Interrogatories on behalf of Basic Research, LLC, A.G. Waterhouse, LLC, Klein-Becker usa, LLC, Nutrasport, LLC, and Sovage Dermalogic Laboratories, LLC, having read and reviewed said answers, hereby state that foregoing answers are true and correct to the best of my knowledge and belief.

FURTHER AFFIANT SAYETH NAUGHT.

Carla R. Fobbs

CARLA R. FOBBS

STATE OF UTAH)
:ss.
COUNTY OF SALT LAKE)

SWORN TO AND SUBSCRIBED before me this 16th day of August, 2004 by CARLA R. FOBBS, who is personally known to me / has produced DRIVERS LICENSE # 146319375 as identification.

O. H. Michaelis

Notary Public, State of Utah

My Commission Expires: 09/08/07

NOTARY PUBLIC
O. H. MICHAELIS
4108 SPLENDOR WAY
SALT LAKE CITY, UT 84124-2156
MY COMMISSION EXPIRES
SEPTEMBER 8, 2007
STATE OF UTAH